Oligopeptide permease in *Borrelia burgdorferi*: putative peptide-binding components encoded by both chromosomal and plasmid loci

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To elucidate the importance of oligopeptide permease for *Borrelia burgdorferi*, the agent of Lyme disease, a chromosomal locus in *B. burgdorferi* that encodes homologues of all five subunits of oligopeptide permease has been identified and characterized. *B. burgdorferi* has multiple copies of the gene encoding the peptide-binding component, OppA; three reside at the chromosomal locus and two are on plasmids. Northern analyses indicate that each *oppA* gene is independently transcribed, although the three chromosomal *oppA* genes are also expressed as bi- and tri-cistronic messages. Induction of one of the plasmid-encoded *oppA* genes was observed following an increase in temperature, which appears to be an important cue for adaptive responses *in vivo*. The deduced amino acid sequences suggest that all five borrelial OppA homologues are lipoproteins, but the protease-resistance of at least one of them in intact bacteria is inconsistent with outer-surface localization. Insertional inactivation of a plasmid-encoded *oppA* gene demonstrates that it is not essential for growth in culture.

Keywords: oligopeptide permease, *oppA*, plasmids, Lyme disease, spirochaetes

INTRODUCTION

Borrelia burgdorferi, the spirochaetal agent of Lyme disease (Burgdorfer *et al.*, 1982), is maintained in nature by an infectious cycle between tick vectors and small rodent hosts (Lane *et al.*, 1991). Like many bacterial pathogens, *B. burgdorferi* must cope with an array of changing environmental conditions to successfully persist and proliferate in each location and to ensure subsequent transmission between hosts. To accomplish this, *B. burgdorferi* presumably must regulate gene expression in response to environmental cues. It is unknown how *B. burgdorferi* senses environmental changes and orchestrates the appropriate adaptive responses. In other bacteria, oligopeptide permeases can be involved in sensing pertinent environmental signals in the form of peptides, culminating in diverse responses such as expression of virulence determinants, sporulation, conjugation or competence (Clewell, 1993; Leonard *et al.*, 1996; Perego *et al.*, 1991; Rudner *et al.*,

1991). In addition to these signalling roles, the major role for most bacterial oligopeptide permeases is in nutrient uptake, by recycling cell wall and external peptides and transporting them into the cell, where they can be degraded into constituent amino acids for protein synthesis (Goodell & Higgins, 1987; Payne & Gilvarg, 1968).

We are interested in the potential role of oligopeptide permease in the transmission of *B. burgdorferi* between ticks and mammals. To initiate this investigation, we have identified genes that probably encode the *B. burgdorferi* oligopeptide permease, including homologues of the peptide-binding component OppA, the transmembrane components OppB and OppC, and the associated ATPases OppD and OppF. A locus on the *B. burgdorferi* linear chromosome contains three *oppA* gene homologues, located adjacent to single copies of *oppB*, *oppC*, *oppD* and *oppF* gene homologues. Two plasmid loci, one on a 26 kb circular plasmid (cp26) and the other on a 54 kb linear plasmid (lp54), carry individual *oppA* gene homologues. We have characterized the transcripts from all five *oppA* genes and have demonstrated that a protein product from the *oppA* gene on cp26 can be detected in *B. burgdorferi* lysates,

The GenBank accession numbers for the *opp* nucleotide sequences reported in this paper are AF000365 (*oppAV*), AF000366 (chromosomal *opp* locus) and AF000948 (*oppAIV*).

but is not surface-exposed on intact bacteria. We have begun a genetic investigation of the biological roles of the individual *oppA* genes and oligopeptide permease in *B. burgdorferi* by inactivating the *oppA* gene on cp26.

METHODS

***B. burgdorferi* strains and growth conditions.** *B. burgdorferi* B31 (ATCC 35210), the prototype strain for *B. burgdorferi sensu stricto*, was originally isolated from a tick collected on Shelter Island, NY (Burgdorfer *et al.*, 1982). Wild-type B31 used in RNA and protein analyses was low passage and infectious. $\Delta oppAIV::gyrB^r$ mutant B31-82 was derived from high passage, non-infectious B31 by allelic exchange with recombinant plasmid pKK74, as described below. Cultures were grown in BSK-H medium (Sigma) supplemented with 6% (v/v) rabbit serum at 34 °C (Barbour, 1984), unless indicated otherwise. Single colonies in solid medium were obtained as described previously (Rosa *et al.*, 1996).

Cloning and sequencing of the *opp* loci. While characterizing positive clones from a library of *B. burgdorferi* B31 DNA that had been screened with a *guaA* probe (Margolis *et al.*, 1994), we identified an open reading frame homologous to *OppA* (probability = 5.6×10^{-14} that the observed similarity to *S. typhimurium* *OppA* arose by chance), adjacent to *guaB* (Rosa *et al.*, 1996). Sequence comparisons to protein sequence databases with this sequence (subsequently designated *OppAIV*) were performed using the BLAST network service (National Center for Biotechnology Information) and a BLASTP search (Altschul *et al.*, 1990). A closely related sequence from *B. coriaceae* was also found; this sequence was a chromosomally encoded gene that was used as a PCR target sequence for the detection of *B. coriaceae* (Zingg & LeFebvre, 1994).

An alignment of the *B. burgdorferi oppAIV* and *B. coriaceae* sequences permitted the identification of conserved amino acid residues, and a pair of degenerate oligonucleotides designed from these regions (Table 1, primers *oppA.7* and *oppA.4*) was used in PCR amplification with total genomic B31 DNA. Although the amplification product migrated as a single band on an agarose gel, restriction digests indicated that it contained a mixture of DNA sequences. The undigested PCR fragments were cloned into the pCRII vector (Invitrogen) according to the manufacturer's instructions and restriction digests identified a clone with a novel insert. Sequence analysis indicated that it was homologous to, but distinct from, the *oppAIV* gene on cp26. Rehybridization of a Southern blot with a probe derived from this new *oppA* gene (subsequently designated *oppAII*) indicated that it hybridized most strongly with the chromosome in uncut DNA. The *oppAII* and *oppAIV* probes hybridized to the same set of restriction fragments in digested DNA (Fig. 1), but with different relative intensities (data not shown), with the *oppAII* probe binding strongly at high stringency to the same 6.5 kb *EcoRI* fragment as the *oppD* probe (described below, Fig. 1b and data not shown).

A genomic library of B31 DNA constructed in Lambda Zap II (Stratagene) (Margolis *et al.*, 1994) was screened with the *oppAII* gene probe and eight positive clones were characterized in detail. Portions of five different *oppA* genes were identified (these included *oppAIV* and *oppAII*). A combined strategy of PCR, Southern blotting and sequencing linked three *oppA* genes at a single chromosomal locus, upstream of *oppBCDF* homologues (described below) and located a single *oppA* gene on lp54, approximately 10 kb 3' of the *ospAB*

locus, in addition to *oppAIV* on cp26 (shown schematically in Fig. 2).

Degenerate primers for *oppD* and *oppF* (Table 1, primers *oppD/F5'*, *oppD3'*, *oppF3'*) were designed to conserved residues based on an alignment of the deduced amino acid sequences of ATP-binding proteins from various oligopeptide transport systems (Hyde *et al.*, 1990; Mimura *et al.*, 1991) and used in PCR with *B. burgdorferi* DNA. Amplification products were cloned into the pCRII vector (Invitrogen) and sequence analysis (see below) confirmed that the cloned segments of borrelial DNA were derived from *oppD* and *oppF* homologues. PCR fragments (Table 1, primers *oppD/F5'–oppD3'* and *oppD/F5'–oppF3'*) were used as probes in Southern blots to map the borrelial *oppD* and *oppF* genes to a chromosomal locus that is linked to the *oppA* homologues (Fig. 1b and data not shown), and to screen the genomic library of B31 DNA. Sequence analysis of the genomic clones identified borrelial *oppB* and *oppC* homologues immediately upstream of *oppD* and *oppF* genes (see Fig. 2).

The nucleotide sequences of all *opp* genes were obtained from both strands using either Sequenase 2.0 (US Biochemicals) or a 373A automated DNA sequencer (Applied Biosystems). The nucleotide sequences of *B. burgdorferi opp* genes and flanking DNA on cp26 (3.5 kb), lp54 (2.0 kb) and the chromosome (12.2 kb) have been deposited in GenBank (accession numbers AF000948, AF000365 and AF000366, respectively). Our sequence for the *opp* loci differs from unedited genomic sequence determined by The Institute for Genomic Research and available at <http://www.tigr.org> as follows: four nucleotide substitutions in *oppAI* resulting in four amino acid changes; four nucleotide substitutions in *oppAII* resulting in two amino acid changes; two nucleotide substitutions in *oppAIII* resulting in one amino acid change; one nucleotide substitution in *oppC* resulting in an amino acid change and one nucleotide insertion in *oppC* resulting in an altered reading frame for 76 amino acids and truncation of 87 amino acids; no nucleotide differences in *oppD* and *oppF*; one nucleotide difference in *oppAIV* that did not alter the amino acid sequence; and two nucleotide differences in *oppAV* that resulted in two amino acid changes. There were also four nucleotide differences (one substitution, three insertion/deletions) in the *oppAII–oppAIII* intergenic region.

Southern blot analysis of the *opp* loci. Total genomic DNA was isolated from *B. burgdorferi* as previously described (Rosa & Schwan, 1989). Total plasmid DNA, including both linear and circular molecules, was isolated from *B. burgdorferi* with Qiagen columns following the manufacturer's instructions. Southern blot analyses (Southern, 1975) were performed after DNA was separated on a 0.8% (w/v) agarose gel by field inversion electrophoresis for 24 h at 7 V cm⁻¹ with program 3 of a PPI-200 programmable power inverter (MJ Research). DNA was transferred to Biotrans nylon membranes (ICN), prehybridized and hybridized with radioactive probes in 6 × SSC (where 20 × SSC is 3 M sodium chloride, 0.3 M sodium citrate), 0.1% (w/v) SDS, 0.5% (w/v) non-fat dry milk, 1 mM sodium pyrophosphate at 55 °C (high stringency) or 45 °C (low stringency) in rotating bottles in a hybridization oven (Bellco). Probe fragments were generated by PCR amplification and radiolabelled with [α -³²P]dATP (Du Pont) by random priming (Life Technologies). Blots were washed in 0.2 × SSC, 0.1% (w/v) SDS at 55 °C (high stringency) or 2 × SSC, 0.1% (w/v) SDS at room temperature (low stringency) and visualized by autoradiography.

Northern blot analysis of the *opp* genes. Total borrelial RNA was extracted from exponential-phase cultures using the

Table 1. Oligonucleotide primers used in this study

Our designation	Sequence (5'→3')	Gene	Use for oligonucleotides
U157F + <i>HpaI</i>	ACCGTTAACTTTTAAACATTATATAGTAT	5' <i>gyrB</i>	Mutant construction
1905R + <i>BclI</i>	ACCTTGATCATTACACATCAAGATTAATTAC	3' <i>gyrB</i>	Mutant construction
gb.10	ATTACCTAGTGAGGTTAGTT	5' <i>guaB</i>	Probe
gb.32	TAAATCAGATATTGTTGCTG	3' <i>guaB</i>	Probe
gb.19	GAAAGATGCTTAAGAACGTC	5' <i>oppAIV</i>	Probe
gb.29	CAGGGATTGTAAGCCAAATGT	3' <i>oppAIV</i>	Probe
oppB.1	GAGGAGCAATGTTGCCTGTA	5' <i>oppBC</i>	Probe
oppD.2	CTCCTGAAAATTGATGTGGG	3' <i>oppBC</i>	Probe
gspI.1	ATAGTTGTTAGTGGCGCATAC	5' <i>oppAI</i>	Specific probe
oppA.50	ATTGAATGCTATGTATGCCATTCCG	3' <i>oppAI</i>	Specific probe
gspII.1	AATGAAGTAGAATTAGAAGAG	5' <i>oppAII</i>	Specific probe
gspII.2	GTTGTCAAGTGGTTTGATGTG	3' <i>oppAII</i>	Specific probe
gspIII.1	AACGAACGTTATTATAATGCA	5' <i>oppAIII</i>	Specific probe
gspIII.2	ATAAATTGCATTACTTTTGTTGG	3' <i>oppAIII</i>	Specific probe
gspIV.1	GATGTTGTTCTTGACAGTATT	5' <i>oppAIV</i>	Specific probe
gspIV.2	AAGCGGTTTTACTTTTCATGTTT	3' <i>oppAIV</i>	Specific probe
gspV.1	AATGAAAAATTCTATGATGCT	5' <i>oppAV</i>	Specific probe
gspV.2	AGGTTTAAACATTTGTGTTTAGTG	3' <i>oppAV</i>	Specific probe
oppA.7*	CAAACHTTYATHCCWGTWCC	5' <i>oppA</i>	Degenerate primer
oppA.4*	KTCCAYTTRTCRTKYCTRAA	3' <i>oppA</i>	Degenerate primer
oppD/F 5'*	GTWGGWGARWSHGGWWSHGG	5' <i>oppDF</i>	Degenerate primer
oppD 3'*	ARWGCWGTWGTWGGYTCTTC	3' <i>oppD</i>	Degenerate primer
oppF 3'*	WAYWGAWAVRTCWARWGC	3' <i>oppF</i>	Degenerate primer

*IUB Group Codes identifying base redundancies in the degenerate primers: R = A + G, Y = C + T, M = A + C, K = G + T, S = G + C, W = A + T, H = A + T + C, D = G + T + A, N = A + C + G + T, V = G + A + C.

RNAgents Total RNA isolation system (Promega) or the Ultraspec RNA isolation system (Biotecx) according to the manufacturer's instructions. For temperature shift experiments, borreliae were grown at 23 °C to mid-exponential phase, diluted 1:100 in fresh medium, and grown to mid-exponential phase at 35 °C. RNA was denatured with glyoxal and dimethyl sulfoxide and electrophoresed in a 1% (w/v) agarose gel in 10 mM sodium phosphate buffer, pH 7.0 (Sambrook *et al.*, 1989). The RNA was transferred to nylon membranes (Micron Separations), cross-linked with ultraviolet light and air dried. Prehybridization and hybridization were at 55 °C in 1% (w/v) BSA, 7% (w/v) SDS, 0.5 M sodium phosphate, pH 7.0, and 1 mM EDTA in rotating bottles in a hybridization oven (Bellco). Probe fragments were generated by PCR amplification and radiolabelled with [α -³²P]dATP (Du Pont) by random priming (Life Technologies). Membranes were washed at 55 °C in 0.2 × SSC, 0.1% (w/v) SDS.

Construction of *oppAIV* mutant. The plasmid construct used to inactivate the *oppAIV* gene was generated in several sequential cloning steps. First, the *gyrB*^r gene from the coumermycin-resistant B31 variant NGR (Rosa *et al.*, 1996) was amplified using primers U157F/*HpaI* and 1905R/*BclI* (Table 1). The amplification product was ligated into the pCRII vector and transformed into *Escherichia coli* strain INV α F' as recommended by the manufacturer (Invitrogen), and then moved into *E. coli* strain GM119 (Arraj & Marinus, 1983), which lacks both *dam* and *dcm* methylases. Plasmid DNA from the GM119 background was digested with *HpaI* and *BclI*, and the *gyrB*^r-containing fragment purified from an agarose gel using NA45 membrane (Schleicher and Schuell).

This fragment was ligated with *HpaI*/*BglII*-digested pDH63 (Rosa *et al.*, 1996), a genomic clone isolated from the *B. burgdorferi* B31 DNA library whose 3.5 kb insert is derived from cp26 and spans the *oppAIV* and *guaB* genes. The resultant plasmid, pKK73, contains a *gyrB*^r insertion and corresponding deletion of *oppAIV*, between the *HpaI* site located approximately 400 bp within the gene to the *BglII* site 30 bp beyond the 3' end of the gene (Fig. 2). pKK73 contains only a small segment of *oppAIV* upstream of the *gyrB*^r insertion, so those sequences were replaced with the 2 kb *PstI*-*HpaI* fragment from pDH62, another cp26-derived genomic clone whose insert extends approximately 1.5 kb 5' of *oppAIV*, to create pKK74. This construct has approximately 2 kb and 1.4 kb of cp26 sequence flanking *gyrB*^r on the 5' and 3' ends, respectively. pKK74 was digested with *PvuI* to inactivate the *bla* gene (which removes the ability of this plasmid to confer ampicillin resistance) and the large fragment was purified from an agarose gel using a Unidirectional Electroelutor (model UEA, IBI). This DNA was used to transform *B. burgdorferi* B31 by electroporation as previously described (Rosa *et al.*, 1996; Samuels *et al.*, 1994).

Coumermycin-resistant colonies were screened by PCR to identify transformants with targeted insertion of *gyrB*^r in *oppAIV*. The structure of mutant *oppAIV* on cp26 was confirmed by PCR with five different primer pairs between *gyrB* and cp26 flanking sequences and by Southern blot analysis of plasmid DNA from wild-type and *oppAIV* mutant borreliae with both *oppAIV* and *gyrB* probes. The results were consistent only with the deletion-insertion structure of the anticipated *oppAIV* mutant (Δ *oppAIV*::*gyrB*^r cp26). Although

plasmids can be present at multiple copies per spirochaete (Kitten & Barbour, 1992), we have found no examples of transformants in which the selectable marker was present in only some copies of the plasmid (Rosa *et al.*, 1996; Tilly *et al.*, 1997).

PCR conditions for cloning, generating probes and analysing recombinants. Oligonucleotides used as PCR primers for cloning and generating probes are described in Table 1. Coumermycin-resistant colonies were screened for the presence of the *gyrB*^r gene within *oppAIV* by PCR in a 9600 DNA Thermal Cycler (Perkin Elmer/Applied Biosystems) as previously described (Rosa *et al.*, 1996). PCR conditions for cloning, generating probes and analysing recombinants were 25 cycles of 94 °C for 1 min, 50 °C for 0.5 min and 70 °C for 3 min. Probe fragments were amplified through two rounds of PCR with a 1:100 dilution of the first reaction to minimize carryover of the template genomic DNA in the final PCR product. PCR conditions with degenerate primers were 30 cycles of 94 °C for 1 min, 50 °C for 2.0 min and 65 °C for 2 min, using 1 µg (150 pM) primer per 100 µl reaction mixture.

SDS-PAGE and Western blotting of proteins. *B. burgdorferi* extracts were prepared from equal culture volumes by pelleting the bacteria, washing twice in PBS, and resuspending in sample buffer (Sambrook *et al.*, 1989). After boiling for 5 min, lysates were separated by electrophoresis through 12.5% (w/v) or 10% (w/v) polyacrylamide gels (King & Swanson, 1978) in a Mini-Protean II cell (Bio-Rad). Proteins were blotted to nitrocellulose (Life Technologies), probed with polyclonal serum (anti-*OppAIV*, see below, or anti-*OspC*, Schwan *et al.*, 1993) or hybridoma supernatant (anti-*OspB* monoclonal H4610, Rosa *et al.*, 1992), and antibody-bound proteins were detected as previously described (Tilly *et al.*, 1997) using enhanced chemiluminescence reagents as recommended by the manufacturer (Amersham). *OppAIV* was cloned, expressed and purified as a fusion protein with maltose-binding protein (MBP), according to the manufacturer's instructions (New England Biolabs). Polyclonal rabbit antiserum was raised against the purified recombinant *OppAIV*-MBP fusion protein. *OppAI-V* were individually cloned and expressed in the pCRII vector (Invitrogen). The proteinase K sensitivity of borrelial proteins on viable spirochaetes was assayed by a modification of the protocol described by Barbour *et al.* (1984). Briefly, spirochaetes were pelleted and washed once in PBS-Mg²⁺, resuspended at a concentration of 2×10^9 cells ml⁻¹ and incubated at room temperature for 40 min with varying amounts of proteinase K (0, 0.04, 0.2 and 1 mg ml⁻¹). After inhibiting proteinase K with PMSF, bacteria were pelleted, washed twice in PBS-Mg²⁺, resuspended in sample buffer and boiled for 5 min before analysis by SDS-PAGE and immunoblotting as described above.

RESULTS

Plasmid and chromosomal copies of *oppA*

We initially identified a homologue of *oppA* on cp26 of *B. burgdorferi*, located approximately 300 bp downstream of the *guaAB* locus and in the opposite orientation (Rosa *et al.*, 1996). No open reading frames homologous to other components of oligopeptide permease were located in either the upstream (1.6 kb) or downstream (3.1 kb) flanking regions. Additional copies of *oppA* were subsequently identified elsewhere in the genome by a Southern blot of total undigested borrelial DNA hybridized at low stringency with a probe derived

Fig. 1. Southern blot analysis of *B. burgdorferi* B31 *opp* and *guaA* genes. Blots containing uncut (a) and *EcoRI*-digested (b) DNA from strain B31 were sequentially hybridized to *guaA* and *oppAIV* probes (a), or *oppAIV* and *oppD* probes (b). Hybridizations were done at 45 °C and blots were washed at low stringency. Radioactivity on blots was allowed to decay to undetectable levels between hybridizations. The mobilities of size standards (in kb) are indicated. Chrom, chromosome.

from the cp26 *oppA* gene (Fig. 1a). In addition to the supercoiled and nicked forms of cp26 (indicated by hybridization with a *guaA* probe), this probe also hybridized to the sheared chromosomal DNA and to a 54 kb linear plasmid (lp54, Fig. 1a). Southern blots of total genomic DNA digested with restriction enzymes that did not cut within the cp26 *oppA* gene contained multiple fragments that hybridized to the *oppA* probe at low stringency. For example, the *oppA* probe hybridized with four *EcoRI* fragments (Fig. 1b); washing this blot at increased stringency led to greatly reduced hybridization with the three larger fragments (not shown). These results suggested the presence of additional *oppA* genes on the chromosome and lp54.

Chromosomal locus encoding all five components of oligopeptide permease

Since many bacteria have the genes encoding all five components of oligopeptide permease at a chromosomal locus, we looked for a similar organization in *B. burgdorferi*. Alignments of the amino acid sequences of the ATPase subunits, *OppD* and *OppF*, from a diverse group of bacteria have identified several highly conserved regions (Higgins *et al.*, 1986; Hyde *et al.*, 1990; Mimura *et al.*, 1991). Degenerate oligonucleotides from these regions (Table 1) were used in PCR with *B.*

Fig. 2. Schematic representation of the *B. burgdorferi* *opp* loci. Arrows indicate the direction of transcription. *Bgl*II, *Hpa*I and *Eco*RI sites are indicated by the letters B, H and R, respectively. The *ospA/B* (Bergstrom *et al.*, 1989) and 2H14 (Rosa & Schwan, 1989) loci are located approximately 10 kb 5' and 6 kb 3', respectively, of *oppAV* on lp54.

burgdorferi DNA. Sequence analyses of PCR products of the anticipated sizes confirmed their identities as portions of *oppD* and *oppF*. An amplification product spanning *oppD* and *oppF* was also obtained, indicating that these genes are adjacent in *B. burgdorferi*, as is true in other bacteria. The Southern blot of *Eco*RI-digested DNA hybridized with the *oppA* probe was rehybridized with an *oppD* probe, after the *oppA* signal had decayed (Fig. 1b); 6.5 kb and 9 kb *Eco*RI fragments were recognized by both probes. Similar co-hybridization of *oppA* and *oppD* probes was seen with borrelial DNA digested with several other enzymes (not shown). The *oppD* probe only hybridized to chromosomal DNA in undigested DNA (not shown), suggesting the presence of a chromosomal locus in *B. burgdorferi* with homologues of the *oppA*, *oppD* and *oppF* genes.

To more fully characterize this chromosomal locus, *oppA* and *oppD* probes were used to screen a *B. burgdorferi* genomic library (described in Methods). Sequence analysis of a number of positive clones defined a 10 kb chromosomal locus that contained seven open reading frames (Fig. 2), whose deduced amino acid sequences included homologues of three substrate-binding OppA proteins, the integral membrane proteins OppB and OppC, and the ATPases OppD and OppF. In addition to the previously characterized *oppA* gene on cp26, an *oppA* homologue unlinked to other permease components was identified on lp54 (Fig. 2). We designated the chromosomal *oppA* homologues as *oppAI*, II and III, the cp26 homologue as *oppAIV* and the lp54 homologue as *oppAV* (Fig. 2). An open reading frame encoding a homologue of enolase resides approximately 1 kb downstream of *oppF*; there are no other readily identifiable sequences within approximately 1 kb upstream of the chromosomal locus or 0.6 kb on either side of *oppAV* on lp54.

The three loci shown diagrammatically in Fig. 2 account for all the *Eco*RI fragments that hybridized to the *oppA*

probe (Fig. 1b); the smallest fragment is from cp26, the 6.5 kb fragment is part of the chromosomal locus (*oppAII*–III), and the remaining two fragments of approximately 5 and 9 kb are from lp54 or the remainder of the chromosomal locus (*oppAI*–II). Although the *oppD* probe also weakly hybridized to a 9 kb fragment (Fig. 1b), the location of *Eco*RI sites in the chromosomal locus indicates that this fragment is not derived from the *oppD* gene at this site; a related gene may be further upstream of *oppAI*. Alternatively, the 9 kb *oppD* and *oppA* fragments could be unrelated, co-migrating fragments. From this analysis (and digests with other restriction enzymes) there is no indication of additional *oppA* genes in the *B. burgdorferi* genome.

Characteristic sequence motifs and predicted structures

The linkage of three *oppA* homologues to homologues of *oppBCDF* at the chromosomal locus strongly suggests that they collectively encode a borrelial oligopeptide permease. In addition to overall sequence similarity, the deduced borrelial Opp proteins have predicted structural features and sequence motifs that are consistent with their proposed transport functions and inclusion in the ABC (ATP-binding cassette) transporter family (Higgins, 1992; Higgins *et al.*, 1986).

Like many nucleotide-binding proteins, the *B. burgdorferi* OppD and OppF homologues contain two short sequence motifs known as Walker boxes that are proposed to contribute to the nucleotide-binding fold in the predicted structure (Walker *et al.*, 1982). ATP-binding components of ABC transporters (such as OppD and OppF) also exhibit a highly conserved sequence known as the loop 3 region, which is thought to interact directly with the transmembrane components (OppB and OppC), and couple ATP hydrolysis to the transport process (Higgins, 1992; Hyde *et al.*, 1990; Mimura *et al.*, 1991); this region is also found in the *B. burgdorferi* OppD and OppF homologues.

Hydropathy analyses of the deduced borrelial OppB and OppC homologues indicate five to six potential membrane-spanning domains (not shown), consistent with the proposed structures of these integral membrane components in other bacteria (Dassa & Hofnung, 1985; Higgins, 1992; Pearce *et al.*, 1992). A unique feature of the *B. burgdorferi* OppC sequence relative to OppC of other bacteria is an additional 53 residues within the predicted periplasmic loop between the first and second membrane-spanning regions.

The solute-binding components confer high-affinity substrate specificity upon the ABC transport systems with which they interact. Tam & Saier (1993) have defined eight families or clusters of bacterial solute-binding proteins based on amino acid sequence similarity. The deduced borrelial OppA sequences are most similar to members of cluster 5, which includes the peptide- and nickel-binding proteins, and each OppA homologue contains a good match to the consensus

OppAI	M-----KYIKI-ALMLIIF---SLIACISNAK-KEKIVFRVSNLSEPSLDPQLSTDPYGSNIITNLFGLAVKDSQT
OppAII	M-----LQRSLE.IIFFL---TFLC.NNKKER-.GVS.KI.LGA.....AE.NVA.KM.DTM.R.IVTG.PN.
OppAIII	MSFNKTK.IG.KIKIVTLLMLAV.....NN.SE-.LA.K.YIGGA.....H.VDETI.AR.LEQI.S.LTLNLT.K.
OppAIV	M-----ILIKKLKVVLFNLILL.S.VNESN-RN.L.KLNIG..AT.A..IN.TV..G.VSQM...ILDG.PR.
OppAV	M-----IIRKRL.I.G.A--TV.S.SAMS.P.DD..G.GIGN.T.....FCS.RL.NL..NE..V.LRG.PK.
St	MSNITKSL.AAGI.TAL.AATPTAADVPAGVQLAD.QLTVRN.G..VQ.....HKIEGVPE.VSRD..E.LIS.V-E
OppAI	GKYKPLAKSNISEGGLIYTFNLRBEDIWSDGVAITAEIKKSYLRILNKKTAAMYANLIKS-TIKNAQEFDETVE
OppAII	.GN.....G.D.S..TV.....K.T.....G.R.....E.GSK.VEMV--V..G.K...GQ.TD
OppAIII	.L.....N.EA.K.KKT.Q.Y..DNLF.....E.....G.R.....E.GSTNVDML.-I..G...GK.SD
OppAIV	.G.R.....D.D..V.V..H.DNL.....S.....G.R.....D.E.GSSFV.M..-V...E...GKAN.
OppAV	.G.R.....N.D..Y.NTC..H.D..Y...IK...ST.RD..IMA.ENELDEPHI..LT.-K.....FYETKLN
St	.HPS..V.EK.EN.K.FKVV..H..NAK...TFV..HDFVY.WQ.LADPN..SP..SYLQYGH.A.JDDIAGKK.A
OppAI	SELGKAIDSKTLEITLTSPPKPYFDMLTSAIIPVPMHIVEQYGENWNTNPENIVSGAYLKERSINDKIVIEKNEY
OppAII	...R...E...E...I...V.QSF...V.VT.K.Q...S...M.T..PF...IP.E.Y.F...N...
OppAIII	...L...E...A...L...L.Y.FM...I.VI.K.KG...S...M.TC.PF...K.LP.E..IF...R...
OppAIV	...L...E...K...L...V.QTF...VI.K.QR...D...M...PF...S.VL.E.V.L...N...
OppAV	D.V.V.V.NEQ...SR.AR..L...PIF..I.T...KKF.NK.SS..M.T..PF...R.IL.EE.SL...F
St	TD..V..L.DH.F.V..SE.V..YKL.V.PSVS...KSA..KF.DK..Q.A...TN.....NWV.ER..L.R.EQ.
OppAI	YNKKNVEIDVIFPTGEGSV-AYNMYINGELDFLQGAEK-NNLEIKIRD--DYSSGLKNGMAYIAFNITIKPLDNLKV
OppAII	.DSNE..LE.IT..T.ND.ST..K..E.E..AIF.SIPPDILKNL.L.S--...SAV.AIFYF...H...V.I
OppAIII	...E..L..LVYITSDNLT.V...K.N.I.AIFNSIPPDIVN..LQK--...QHKS.AIYLYS..K...DAR.
OppAIV	..S.D.VL.SI..FV.DN.IT...L.D..AIFKNVPPDL.KDL.L..--MGI.STSFYSL.MKV...V..
OppAV	.D...A.N.L..VTINDTRRI.S.EHN.I.AIFNIPPLSLIN.LIL.E--F.ADI.QIGFSSL.NV..N.V..
St	WDNAKT.V.NQ.TYL.ISSE.TDV.R.RS..I.MTYNNMPELFQKLKEIPNEVRVDPYLCY.YEI.NOKA.FNDVR.
OppAI	RQAISLAIDRETITKVLKSGSDPTRNLTTPKFDDYSYGKNIILFDP-----ENAKKLAEAGYPDGKGF--TLKYKI
OppAII	.K.LT.....YK..DNGT...RA..N.SS..A.S.E..N.....I..A.....N.N...ILK...NT
OppAIII	.E.LF.....K..NDGTV...EI..DLKN.N..K.A...KS.....D...N...ML...NT
OppAIV	.K.L.F...K...ES..ND..I...RA..DYI...KS..S...A-----M.....D...N.NM..LLKV..NT
OppAV	.E.LA..V.....YR..DNNFKA..KIS.NINN.N..K.KPYN--RK.E.....N..TL.I..NK
St	T.L.K..L..DIIVNK.KNOGDL.AYSY..PYT.GAKLVEPEW.KWSQOKRN.E.....FTAD.PLT-FD.L.NT
OppAI	SEGRPTAEFLQEQFKILNINLEIENEWTFGLSRRGTQYQMSVGVIGDYFNPLTFLDSLETTENHFLGAYKYSNK
OppAII	NGANKKIC..I.N.W..N...DV.L...Y.NTKAN...EIARA.....AD.....S-I..QGYTQFSSH...P
OppAIII	N.THKKI.A.I.N.W...MLT..N.PVLTN..N..FEIR..R..E.LD.H.YFT-I..R..SQ.AS.G...L
OppAIV	.DSQRK...I.N.W..N...VQL...S.YIN..VN...EIIRS..S..AD.M..S-I.Q...TSFSS.G...S
OppAV	NLEKEV.S.I.S.W..V...DV...P.T.VNHISNLIK.Q.EI.VRS.Q...LD.MA.FN-I.E.K.SHPAS.G...P
St	.DLHKKL.TAVASIW..N.GV.VNL..Q..K...DT.HQ.TFDVARA..CA..NE.TS..N--TMLSDSSNNTAH.KSP
OppAI	EYDALIKKSNFELDPKRRDILRQAEIIEAKDFPMAPLYIPKSHYLFNDKWTGWVFNIA--ESLYEDIKTKK
OppAII	..NE...DL...Q.....I.....I...I..YGNL.....NT..L--RFDLSQL.L.NK
OppAIII	.F.K..RE.DL.K...KQL.K.S..I...A..I..YSG.....N..VS--V.YLSEL.PI.NAKHN
OppAIV	.L.E.LI..DN.R.IP..QE..KK..A..I.R...AVF.N.TS.S.....K.E..S--RFNLSE.PI
OppAV	.L.E..I..DL.E.EKL.LE..KKI...I.N.Y.II.I.SVAGN..K...L..NT.NS--RFALSE.PIEE
St	AF.K..ADTLKVA.DTQ.SELYAK..QQLD--SAIV.V.YVYNAR.VK--P.V.GYTGKDPDNIYVKNLYII.H

Fig. 3. Alignment of deduced amino acid sequences of *B. burgdorferi* OppA homologues. The region homologous to the cluster 5 signature sequence is underlined. OppA domains I (■), II (□) and III (▨), as defined by the three-dimensional structure of the protein (Tame et al., 1994), are indicated below the *S. typhimurium* (St) (Hiles et al., 1987a) sequence. Residues with ≥66% identity among the six sequences are indicated (*). The presumed acylation site of the *B. burgdorferi* OppA proteins is indicated by a box above the amino acid residue.

Table 2. Identity of the deduced amino acid sequences of OppA homologues

Comparisons were performed of OppA homologues from *Borrelia burgdorferi* (Bb), *Bacillus subtilis* (Bs) (Perego et al., 1991), *Salmonella typhimurium* (St) (Hiles et al., 1987a), and *Enterococcus faecalis* TraC (Ef) (Tanimoto et al., 1993) without leader sequences, using the CLUSTAL V program with the following alignment parameters: fixed gap penalty, 10; floating gap penalty, 10; protein weight matrix, PAM250. Values indicate percentage of identical residues.

Gene	Bb I	Bb II	Bb III	Bb IV	Bb V	Bs	St	Ef
Bb I	100							
Bb II	56	100						
Bb III	52	60	100					
Bb IV	54	64	59	100				
Bb V	44	51	50	52	100			
Bs	22	27	26	29	24	100		
St	26	27	28	28	25	29	100	
Ef	23	22	23	23	22	26	22	100

signature sequence for cluster 5 solute-binding proteins (underlined in Fig. 3).

OppA sequence comparisons

B. burgdorferi OppA homologues share approximately 25% identity with OppA proteins from *Bacillus subtilis* (Perego et al., 1991; Rudner et al., 1991) and *Salmonella*

typhimurium (Hiles et al., 1987a), similar to the degree of sequence identity shared between the OppA proteins of the latter two, and with TraC from *Enterococcus faecalis*, a plasmid-encoded OppA homologue that is a receptor for a peptide pheromone (Tanimoto et al., 1993) (Table 2). Relatedness among the five *B. burgdorferi* OppA homologues ranges from 44% to 64% identity and does not correlate with genomic location

(Table 2). Hydropathy plots of all five deduced *B. burgdorferi* OppA proteins were very similar to each other and to those of other bacterial OppAs (not shown).

Aligning the deduced amino acid sequences of *B. burgdorferi* OppAI–V with OppA from *S. typhimurium* indicated conserved residues throughout (Fig. 3). Although all of the OppA proteins contain hydrophobic leader sequences, and each of the deduced *B. burgdorferi* OppA proteins contains a consensus signal peptidase II cleavage/lipidation site (LXYC or LXYZC) (Wu & Tokunaga, 1986), the amino-terminal regions of the proteins are the most divergent. The crystal structure of *S. typhimurium* OppA defines three domains (Tame *et al.*, 1994); the deduced *B. burgdorferi* OppA gene products are homologous to *S. typhimurium* OppA in all three domains (Fig. 3).

Expression of *oppA* genes

The organization of the chromosomal *opp* genes suggests that *oppAI*, II and III could be individually transcribed, with *oppBCDF* forming an operon. Sizeable intergenic regions separate the *B. burgdorferi* *oppA* genes (*oppAI*–II, 119 bp; *oppAII*–III, 139 bp; *oppAIII*–*oppB*, 355 bp), whereas only a few bases separate the *oppB*, *oppC*, *oppD* and *oppF* genes (*oppB*–*C*, 12 bp; *oppC*–*D*, 11 bp; *oppD*–*F*, 3 bp; see gene diagram in Fig. 2). An alignment of approximately 100 nucleotides upstream of the initiation codon for each *oppA* gene indicated very little conservation of sequence in the potential promoter regions (not shown). Computer analysis of these regions using the program MACTARG SEARCH did not identify any sequence that had greater than 60% identity with that of a consensus sigma 70 promoter from *E. coli*, in contrast to several other borrelial genes, which have sigma 70-like promoters (Bergstrom *et al.*, 1989; Reindl *et al.*, 1993; Simpson *et al.*, 1994; Stevenson *et al.*, 1996; Wallich *et al.*, 1993; Zhang *et al.*, 1997). The regions between the chromosomal *oppA* genes contain short, imperfect inverted repeats, but no sequences resembling rho-independent terminators.

Since the *B. burgdorferi* *oppA* genes share significant sequence identity, probes encompassing them cross-hybridize (Fig. 1 and data not shown), so developing gene-specific probes was a necessary prelude to undertaking studies of *oppA* gene expression. Within the most divergent portion of each gene (encoding amino acid residues 230–300, Fig. 3), the *oppA* genes share less than 60% nucleotide identity with each other. Probes from these regions were shown to be specific for the *oppA* gene from which they were derived by hybridization to plasmid DNA containing cloned segments from individual *oppA* genes (Fig. 4a). The probes did not cross-react with other *oppA* genes, even when the blots were overexposed.

Expression of *B. burgdorferi* *opp* genes in mid-exponential-phase cultures was monitored by Northern blot analysis of total RNA with probes for *oppAI*–V, *oppBC*

and *oppD*. Three transcripts were detected when a probe specific for *oppAII* was used (Fig. 4b). The sizes of these transcripts correspond to those predicted for messages that encompass one (1.8 kb), two (4.1 kb) or three (6.2 kb) *oppA* genes (Fig. 4b, *oppAII*, arrows 1, 2 and 3, respectively). Similar results were obtained using the *oppAI* and *oppAIII* probes (not shown); the large 6.2 kb transcript was the least abundant, but detectable with all three probes. These results suggest that transcription initiates at each *oppA* gene, but can also proceed through the intergenic regions and downstream *oppA* genes at the chromosomal locus. Alternatively, processing of a single large transcript initiating upstream of *oppAI* could result in a similar banding pattern. Only a 1.8 kb transcript was detected for each of the plasmid-located *oppAIV* and *oppAV* genes (Fig. 4b). As a preliminary step toward assessing the functional significance of the five *B. burgdorferi* OppA proteins, we inactivated the *oppAIV* gene by allelic exchange (described in Methods). No *oppAIV* transcript was detected in the *oppAIV* mutant, although it did contain transcripts of the three chromosomal *oppA* genes (Fig. 4b).

Different preparations of RNA yielded varying proportions of mono-, bi- and tri-cistronic transcripts from the chromosomal *oppA* genes, but their relative abundances did not correlate with known culture variables. Transcripts of the three chromosomal *oppA* genes and of *oppAIV* (on cp26) were not more abundant in *B. burgdorferi* cultures that were grown at 23 °C and then shifted to 35 °C (data not shown), but the transcript from *oppAV* (on lp54) was consistently increased in cultures shifted to 35 °C (Fig. 4b, *oppAV*). The *oppAV* transcript was also present at a much lower level than those of the chromosomal *oppA* genes or *oppAIV*, even after induction at 35 °C; the *oppAV* blot was exposed for approximately 6 d, compared to 6 h exposures for the *oppAII* and *oppAIV* blots (Fig. 4b). Hybridization of the same RNA preparations with a probe to the constitutively expressed flagellin gene indicated comparable amounts of RNA in all samples (Fig. 4c) and the quality of each RNA was demonstrated by the integrity of the rRNA bands in ethidium bromide-stained gels (Fig. 4b and data not shown).

No distinct transcripts were detected with probes from the *oppB*, *oppC* and *oppD* genes; instead, a broad band was visible after relatively long exposures with the *oppD* probe (45 h, Fig. 4b) or a larger probe spanning *oppB* and *oppC* (not shown). No *oppA* or *oppD* probe detected a transcript that was long enough to extend from *oppAI* through *oppD*.

Synthesis of OppAIV

To begin studies of OppA protein levels and location, OppAIV was synthesized as a recombinant fusion with maltose-binding protein in *E. coli* and a rabbit polyclonal serum was raised against the purified recombinant protein. Only faint reactivity was evident when an immunoblot containing a protein lysate from the

Fig. 4. (a) High-stringency Southern blot analysis of cloned *oppA* genes with specific probes. Recombinant plasmids containing individual *B. burgdorferi* *oppA* genes were digested with *EcoRI* (except *oppAIV*), separated by agarose gel electrophoresis, blotted and hybridized at 55 °C with *oppA* gene-specific probes, as indicated at the top of each panel. Plasmid DNA was as follows: lane 1, *oppAI*; lane 2, *oppAII*; lane 3, *oppAIII*; lane 4, *oppAIV*; lane 5, *oppAV*. (b) Analysis of *opp* transcripts. Left panel, ethidium-bromide-stained gel of RNA from *B. burgdorferi* indicating the mobilities of 5S, 16S and 23S rRNAs relative to the hybridizing bands on the blots. Remaining panels, Northern blots were hybridized with probes as indicated above each panel. The *oppAII* and *oppAIV* probes were hybridized with RNA from *B. burgdorferi* *oppAIV* mutant (mutant) and wild-type (WT) bacteria. Arrows indicate the mobilities of mono- (1), bi- (2) and tri-cistronic (3) *oppA* transcripts hybridizing with the *oppAII* probe. We assume that the hybridizing band (*) below the 23S rRNA band results from smaller RNA species being pushed in front of the rRNA and is not a discrete *oppA* transcript. The *oppAV* probe was hybridized with RNA from wild-type *B. burgdorferi* grown at 23 °C or shifted to 35 °C. The *oppD* probe was hybridized with RNA from wild-type *B. burgdorferi* grown at 35 °C. Mobilities of size standards (0.24–9.5 kb RNA ladder, Life Technologies) are indicated on the left of the ethidium-stained gel and first Northern blot panel. (c) The blots described above were allowed to decay and hybridized with a flagellin probe to assure equivalent loading of RNA in each lane.

Fig. 5. Immunoblot analysis of wild-type and *oppAIV* mutant *B. burgdorferi*. Lysates of *oppAIV* mutant B31-82 (lane 1) and wild-type *B. burgdorferi* grown at 23 °C (lane 2) or shifted to 35 °C (lane 3) were separated by SDS-PAGE and identical blots were incubated with (a) OppA- or (b) OspC-specific sera. Mobilities of protein size standards (kDa) are indicated. The 35 kDa protein recognized by the anti-OspC antiserum in all samples has been noticed previously and is unrelated to OspC (Norris *et al.*, 1992; Schwan *et al.*, 1993; Tilly *et al.*, 1997).

Fig. 6. Proteinase K sensitivity of OppAIV on intact *B. burgdorferi*. Live, unfixed bacteria were treated with different concentrations of Proteinase K: 1 mg ml⁻¹ (lane 1), 0.2 mg ml⁻¹ (lane 2), 0.04 mg ml⁻¹ (lane 3), and 0.0 mg ml⁻¹ (lane 4). Lysates of treated bacteria were separated by SDS-PAGE and either stained with Coomassie brilliant blue (a), or analysed by immunoblot with OspB (b) and OppAIV (c) antisera. The mobility of proteinase K (pk) is indicated on the Coomassie-stained gel. The mobilities of protein size standards (kDa) are indicated.

oppAIV mutant was probed (Fig. 5a, lane 1), indicating that the majority of the protein recognized by this antiserum in wild-type *B. burgdorferi* lysates (Fig. 5a, lanes 2 and 3) is the *oppAIV* gene product. The other four *B. burgdorferi* OppA proteins were also synthesized as recombinant proteins in *E. coli* and bacterial lysates were tested by immunoblotting; the OppAIV antiserum cross-reacted only very weakly with OppAI, II, III and V (data not shown).

Lysates of wild-type *B. burgdorferi* grown at 23 °C and then shifted to 35 °C were probed by immunoblotting to determine if there were an increase in OppAIV, as had been found for OspC (Schwan *et al.*, 1995). Bacteria grown at both temperatures contained similar amounts of OppAIV (Fig. 5a, compare lanes 2 and 3), consistent

with *oppAIV* transcript levels, whereas OspC was only detected in the culture shifted to 35 °C (Fig. 5b, compare lanes 2 and 3).

Surface inaccessibility of OppA

To determine if the borrelial OppA proteins were surface-exposed, intact borreliae were treated with varying amounts of proteinase K and bacterial lysates were immunoblotted with OppAIV and OspB polyclonal sera (Fig. 6). Whereas only a slight diminution in OppAIV was seen at even the highest concentration of proteinase K (1 mg ml⁻¹; lane 1, Fig. 6), OspB, a surface-exposed lipoprotein, was completely degraded at the lowest concentration of proteinase K (0.04 mg ml⁻¹; lane 3, Fig. 6), relative to the control lysate without

proteinase K treatment (lane 4, Fig. 6). Analysis of the same bacterial lysates by Coomassie blue stain indicated that two other surface-exposed lipoproteins, OspA and OspC, were also degraded by proteinase K treatment, although OspA was somewhat resistant at the lowest concentration (0.04 mg ml^{-1} ; lane 3, Fig. 6). We conclude that, in contrast with several borrelial outer-surface lipoproteins, OppAIV of intact organisms resists digestion by proteinase K and may have a periplasmic location.

DISCUSSION

Multiple substrate-binding components in *B. burgdorferi*

Typically, the genes encoding all the components of oligopeptide permease are present at a single chromosomal locus. An interesting feature of the putative *B. burgdorferi* transporter is the presence of multiple substrate-binding components (OppA homologues), encoded by both plasmid and chromosomal loci. Some other bacteria have additional substrate-binding components encoded on plasmids or elsewhere on the chromosome (Alloing *et al.*, 1994; Jenkinson *et al.*, 1996; Leonard *et al.*, 1996; Ruhfel *et al.*, 1993; Tanimoto *et al.*, 1993). It is intriguing to consider the possibility that the *B. burgdorferi* plasmid-encoded OppA homologues recognize peptide signals that induce an adaptive response, since these plasmids also carry outer-surface protein genes that are differentially expressed during transmission from ticks to mammals (de Silva *et al.*, 1996; Schwan *et al.*, 1995).

In addition to multiple peptide-binding components for a single oligopeptide permease, separate transport systems can exist for peptides of different sizes or characteristics (Hiles *et al.*, 1987b; Koide & Hoch, 1994). Components of the putative *B. burgdorferi* peptide permease are most similar to oligopeptide (Opp) transport components. However, substrate specificities cannot be assigned on the basis of sequence similarity alone, and biochemical and genetic studies are necessary before the physiological role and peptide specificity of this system in *B. burgdorferi* can be determined. Borreliae grow in the laboratory only in a complex, undefined medium, hence complicating *in vitro* studies to directly characterize peptide transport in these bacteria. Expression of *oppAIV* in a *Bacillus subtilis* *oppA* mutant did not restore general peptide transport, as assayed by resistance to the toxic peptide bialaphos and failure of a methionine auxotroph to grow in medium in which peptides were the sole source of amino acids (unpublished data). These data suggest that OppAIV does not bind peptides non-specifically, but are inconclusive due to the nature of negative complementation results in a heterologous system.

OppAIV not essential for *in vitro* growth

As an initial step toward understanding the role of oligopeptide permease in the transmission and adap-

tation of *B. burgdorferi* between the arthropod and mammalian environments, we have inactivated *oppAIV*, the *cp26*-encoded gene. We suspect that the *oppAIV* product could play an important role in sensing environmental signals in the form of peptides. *oppAIV* was inactivated in a non-infectious, laboratory-adapted *B. burgdorferi*, with the intention of subsequently moving this mutation into an infectious isolate. However, we have not succeeded in transforming low-passage infectious *B. burgdorferi* and thus have been unable to directly test the effect of this mutation in the infectious cycle. Clearly, *B. burgdorferi* does not require OppAIV for growth in culture medium.

Homologue of OppAII not essential for *in vivo* infectivity

Das *et al.* (1996) previously described a 30 kDa protein (P30) from *B. burgdorferi* isolate N40 that is homologous to periplasmic substrate-binding proteins of Gram-negative bacteria. Extensive sequence similarity strongly suggests that *p30* and *oppAII* are alleles of the same gene from two different *B. burgdorferi* isolates. The disparity in size between P30 and OppAII (30 kDa versus 58 kDa, respectively) derives from a base deletion in the *p30* gene, which results in a frame shift and premature stop codon. P30 probably is not a functional substrate-binding protein because much of the peptide-binding pocket (Tame *et al.*, 1994) is missing. Despite this mutation, *B. burgdorferi* N40 maintains a mammal-tick infectivity cycle (Fikrig *et al.*, 1992). These data suggest that *p30* represents a naturally occurring *oppAII* mutant and that there may be functional redundancy among the five *B. burgdorferi* OppA proteins such that they are not all essential *in vivo*.

Cellular location of substrate-binding components

The substrate-binding components of high-affinity transport systems are located in the periplasm of Gram-negative bacteria. In Gram-positive bacteria, which lack an outer membrane, these 'periplasmic binding proteins' such as OppA, are surface-exposed lipoproteins that are anchored to the cytoplasmic membrane by an amino-terminal lipid moiety (Gilson *et al.*, 1988; Perego *et al.*, 1991). The deduced amino acid sequences of the *B. burgdorferi* OppA homologues suggest that they are membrane-bound lipoproteins, yet the surface-inaccessibility of one of these proteins (OppAIV) is consistent with a periplasmic location. The borrelial substrate-binding proteins may be periplasmic, but associated with the cytoplasmic membrane by a lipid moiety, thus combining features of both Gram-positive and Gram-negative bacteria. In another spirochaete, *Treponema pallidum*, a protein with homology to the substrate-binding components of ABC transporters is primarily associated with the cytoplasmic membrane (Akins *et al.*, 1997).

Concluding remarks

In conclusion, we have characterized a chromosomal locus and two plasmid loci in *B. burgdorferi* that encode homologues of oligopeptide permease components. To determine the function of this transporter we are pursuing biochemical and genetic studies to investigate the peptide-binding characteristics and biological roles of the five different substrate-binding components. We speculate that *B. burgdorferi* might utilize peptide pheromones to elicit a concerted adaptive response to changing environmental conditions, such as those that the bacteria encounter as they cycle between ticks and mammals.

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