Transcriptional and translational regulation of the expression of the major outer surface proteins in Lyme disease *Borrelia* strains

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The major outer surface proteins of Lyme disease spirochaetes are differentially expressed in different isolates. *Borrelia afzelii* strain F1 expresses none, or very low amounts, of the OspA and OspB proteins. To elucidate the mechanisms that control the expression of these abundant surface proteins the *ospAB* operon of *B. afzelii* F1 was cloned, sequenced and compared to the previously sequenced *ospAB* operon of *B. afzelii* ACAI and *Borrelia burgdorferi* B31. The two *B. afzelii* strains showed almost 100% identity at the DNA level, although Coomassie-stained gels and Western blot analyses showed significant variation in the Osp protein content. Transcriptional analysis revealed that the amount of *ospAB* mRNA produced in *B. afzelii* F1 varies more than the amount of protein, suggesting that the expression of OspA and OspB proteins is regulated at both the transcriptional and the translational level. Furthermore, the inverse relationship between the transcription of *ospC* and the *ospAB* operon could indicate coregulation of these separately encoded operons.

Keywords: *Borrelia afzelii*, *Borrelia burgdorferi*, outer surface proteins, OspA, OspB and OspC

INTRODUCTION

The multisystem disorder Lyme disease can be caused by three spirochaetal species, *Borrelia afzelii*, *Borrelia burgdorferi* and *Borrelia garinii* (Burgdorfer *et al.*, 1982; Baranton *et al.*, 1992; Canica *et al.*, 1993). Lyme disease is characterized by a variety of different symptoms, which can develop into a chronic syndrome. Several reports indicate that the spirochaetes can survive for a prolonged period of time in the human host. They have been isolated from an acrodermatitis chronica atrophicans (ACA) patient 10 years after the onset of the disease (Åsbrink & Hovmark, 1985). It has further been reported that some patients with Lyme disease developed new antibodies a year or more after the initial infection (Craft *et al.*, 1986). This indicates that the borreliae may escape the human immune defence system by some kind of antigenic variation or modulation of expression of one or several outer surface molecules. However, this is contradicted by recent studies of *Borrelia* infections in mice, which indicate that the spirochaetes are antigenically stable, and may

survive by sequestration in parts of the body which are inaccessible to the immune system (Barthold, 1993; Barthold & Bockenstedt, 1993; Jonsson *et al.*, 1995).

Most Lyme disease *Borrelia* strains express two major outer surface lipoproteins, OspA and OspB. In the American *B. burgdorferi* reference strain B31, the molecular masses of these proteins are 31 and 34 kDa, respectively (Howe *et al.*, 1985; Bergström *et al.*, 1989). The OspA and OspB proteins from different species and isolates vary both in molecular mass and immunological reactivity with different monoclonal antibodies (mAbs) (Barbour, 1984; Barbour *et al.*, 1985; Barbour & Schrupf, 1986; Wilske *et al.*, 1988b; Bundoc & Barbour, 1989). Some strains appear to lack the expression of both OspA and OspB, but instead express a smaller 21–25 kDa protein, OspC. In addition, a fourth outer surface protein, the 28 kDa OspD, has been reported. This protein was first shown to be expressed only in low passage, virulent strains (Norris *et al.*, 1992). However, more recent data contradict the influence of passages on expression of the OspD protein (Marconi *et al.*, 1994). The OspA, OspB and OspC proteins are all highly immunogenic. Despite this immunogenicity, only antibodies directed against OspC, and not against the OspA and OspB proteins, are usually

Abbreviations: mAb, monoclonal antibody; ACA, acrodermatitis chronica atrophicans.

found in sera from patients with early Lyme disease (Wilske *et al.*, 1986, 1988a). This indicates differences in the expression or exposure of the proteins within the human host.

The *ospA* and *ospB* genes are located in one operon, separated by a few bases, and transcribed as one transcriptional unit from a linear plasmid about 50 kb in size (Howe *et al.*, 1986; Barbour & Garon, 1987; Bergström *et al.*, 1989; Jonsson *et al.*, 1992). The *ospC* gene is not part of the *ospAB* operon, but is encoded on one of the small circular plasmids (Marconi *et al.*, 1993; Sadziene *et al.*, 1993). The expression of the OspA and OspB proteins and their regulation have been studied. Eiffert *et al.* (1992) published the nucleotide sequence of the *ospAB* operon from a *B. burgdorferi* strain that expressed OspA but not OspB. Analysis of the sequence revealed that the *ospB* gene could not be expressed because it lacked a start codon in the correct reading frame. This was probably due to a frameshift mutation caused by a deletion. Rosa *et al.* (1992) have shown that new *osp* loci can be formed *in vitro* by homologous recombination and point mutations. This can result in the loss of expression of the OspB protein, or the loss of both OspA and OspB in favour of the expression of a chimeric OspA/OspB protein (Rosa *et al.*, 1992). Moreover, Margolis & Rosa (1993) have published a study of a *B. burgdorferi* strain which does not express the OspA or OspB proteins, even though it contains an intact *ospAB* operon capable of expression. In that study, it was suggested that the expression of the OspA and B proteins is regulated at the transcriptional level, as no transcript was detected.

The regulation of OspC expression has also been studied. *B. burgdorferi* B31 usually lacks the expression of OspC. However, mutant clones of B31 which had lost a 16 kb linear plasmid resumed expression of the OspC protein. This, together with the finding that only strains lacking DNA homologous to the 16 kb plasmid express the protein, suggests that OspC is negatively regulated by a factor encoded by this plasmid (Hinnebusch & Barbour, 1992; Sadziene *et al.*, 1993). This is different from the situation found in another study, where OspC-expressing clones were obtained from a non-expressing isolate of *B. burgdorferi* Pk2 upon culture on solid media (Wilske *et al.*, 1993). This could, however, be due to loss of the 16 kb plasmid during subsequent cultivation in liquid medium.

In the present work, we studied the expression of Osp proteins of the Swedish tick isolate *B. afzelii* F1. This strain was previously reported to lack both OspA and OspB in favour of the smaller OspC protein (Barbour & Schrupf, 1986; Shoberg *et al.*, 1994). To gain understanding of the regulatory mechanisms involved in the variable expression of the Osp proteins, we studied the expression of these proteins, and cloned and sequenced the *B. afzelii* F1 *ospAB* operon. By comparison with the *ospAB* operon of *B. afzelii* ACAI we hoped to obtain an indication as to which mechanisms are involved in the regulation of outer surface protein expression. We demonstrated that the nucleotide sequence of the *ospAB* operon of *B. afzelii* strains F1 and ACAI are almost identical, the

transcripts are similar in size and the *ospAB* operons in both strains use the same transcriptional starting point. The amount of transcript detected in different F1 populations varies, indicating that the level of mRNA produced can be modulated. The relative amount of *ospAB* transcript produced fluctuates more than the amount of protein produced, suggesting that the expression of OspA and OspB may be regulated not only at the transcriptional level, but also at the translational level. Moreover, there is an inverse relationship between the transcription of the *ospC* gene and the *ospAB* operon.

METHODS

Bacterial strains. The *Borrelia* strains used in this study were the North American *B. burgdorferi* reference strain B31 (ATCC 35210), the B31 mutant B313, which lacks the 49 kb linear plasmid and thereby the whole *ospAB* operon (Sadziene *et al.*, 1993), and the two Swedish *B. afzelii* isolates, ACAI (Åsbrink *et al.*, 1984) and F1 (Barbour & Schrupf, 1986). The borreliae used were all high passage strains. Different cultures of F1 are denoted F1a1 etc., where F1a2 is inoculated from culture F1a1 and so on. F1a, b and c are lineages inoculated from different frozen cultures. B31 and F1 are both tick isolates, while ACAI was cultured from the biopsy of a patient with ACA. The *Borrelia* strains were cultivated in BSKII medium as previously described (Barbour, 1984). The *Escherichia coli* strain DH5 α (BRL) was used for the propagation of recombinant plasmids.

Preparation of an anti-OspA rabbit serum. Rabbit antibodies were raised by repeated subcutaneous injections of 85 μ g recombinant ACAI OspA protein (kindly provided by Dr M. Strömqvist, Symbicom, Umeå, Sweden) with Freund's incomplete adjuvant. Serum was collected at various intervals after immunization, and the titre of OspA-specific antibodies was determined by Western blotting (data not shown).

Extraction of *Borrelia* proteins, SDS-PAGE and Western blotting. Whole cell lysates were obtained by sonication of approximately 10^{10} *Borrelia* cells, and the amount of protein was determined as previously described (Jonsson *et al.*, 1992). Proteins were separated on SDS-PAGE gels essentially as described previously (Barbour *et al.*, 1985) in 12.5% acrylamide separation gels and 4% acrylamide stacking gels. The gels were either fixed and stained with Coomassie brilliant blue (Sigma), or transferred to Fluorotrans transfer membranes (Colly, Stockholm, Sweden) by electroblotting as described previously (Jonsson *et al.*, 1992). In SDS-PAGE gels processed for immunoblotting, 5 μ g protein of whole cell lysate was used in each lane, and 50 μ g of total protein was used in gels for visualization by staining. The molecular mass standards included proteins in the size range of 14.4–94 kDa (Pharmacia).

The mAbs used in these studies were the genus-specific anti-flagellin mAb H9724 (Barbour *et al.*, 1985), anti-OspA mAb 184.1 (a kind gift from Dr B. Luft, Stony Brook, New York, NY, USA), and the anti-OspB mAb 84C (kindly provided by Dr D. D. Thomas, San Antonio, TX, USA). The preparation of polyclonal anti-OspA serum is described above, and the polyclonal anti-OspC serum was a kind gift from Dr A. G. Barbour, San Antonio, TX, USA. The Western blot filters were blocked by incubation with 5% (w/v) non-fat milk powder in PBS (0.15 M sodium chloride, 0.05 M sodium phosphate, pH 7.4) for 2 h or overnight. The filters were then incubated with the antibodies diluted in 2.5% (v/v) milk/PBS (1:40, v/v, for mAb H9724; 1:25, v/v, for mAb 184.1; 1:1000, v/v, for mAb 84C and polyclonal anti-OspA serum; and 1:200, v/v,

Fig. 1. Genetic map showing the sequence and location of the oligonucleotides used in Northern blots, primer extension and PCR amplification. The boxes represent the *fla*, *ospC*, *ospA* and *ospB* genes, the arrows indicate the location and direction of the oligonucleotides and the solid lines below the *ospAB* genes represent PCR fragments used as probes for Southern blotting.

for polyclonal anti-OspC serum) for 1 h at room temperature, washed four times for 5 min each with PBS/0.05% Tween 20, and then incubated with the appropriate secondary antibody for 1 h at room temperature. Detection of binding of mAb 184.1 was performed using horseradish peroxidase-linked secondary antibodies and the Amersham ECL Western blotting analysis system as recommended by the manufacturer. For the polyclonal anti-OspA and anti-OspC sera, and for mAbs H9724 and 84C, ^{125}I -labelled species-specific F(ab')_2 (Amersham) were used at a concentration of 1 mCi (37 MBq) ml^{-1} PBS/0.05% Tween 20. The filters were washed once for 15 min, four times for 5 min each in PBS/0.05% Tween 20, rinsed in PBS and then air-dried prior to autoradiography.

Electron microscopy. Cells were fixed with 4% paraformaldehyde/0.3% glutaraldehyde in PBS for 1 h, centrifuged and postfixed in 4% paraformaldehyde overnight. Small pieces of the pellet were infused with 20% polyvinylpyrrolidone in 2.3 M sucrose for 2 h and subsequently frozen in liquid nitrogen. Ultrathin sections were cut and mounted on formvar-coated nickel grids. For the antibody reactions, the grids were floated on drops of: (a) 0.12% glycine-PBS/foetal calf serum (FCS) for 10 min, (b) affinity-purified (Sambrook *et al.*, 1989) polyclonal anti-OspA or anti-OspC sera diluted 1:10, v/v, in PBS/FCS for 1 h, (c) PBS/FCS for 30 min, (d) goat anti-rabbit immunogold (GAR 10) for 1 h, (e) PBS for 30 min. The grids were then rinsed in distilled water and embedded in methylcellulose before microscopic analysis.

Quantification of proteins. Quantification of the Western blot signals was performed using a PhosphorImager (Molecular Dynamics). The flagellin signal was used as an internal standard to normalize for the amount of protein loaded in each lane. After this normalization, the amount of OspA, OspB and OspC was expressed relative to the highest value of the respective protein.

DNA techniques. Restriction enzymes, T4 DNA ligase (BioLabs, Finnzymes OY) and the T7 sequencing kit (Pharmacia) were used as recommended by the manufacturers. *Borrelia* DNA was prepared as previously described (Barbour, 1988; Barbour & Garon, 1987; Howe *et al.*, 1985).

Construction and screening of pUC18 plasmid gene libraries. *B. afzelii* F1 DNA was completely digested with *Hind*III,

separated on a 0.7% agarose gel and transferred to a Nylon filter (Hybond-N, Amersham) by capillary blotting overnight. The filter was hybridized with two [α - ^{32}P]dATP oligolabelled *osp*-specific fragments. One fragment, 1151 bp long, covers the whole *ospA* gene and the beginning of *ospB*, while the other, 254 bp long, was synthesized from the middle of the *ospB* gene. The fragments were obtained by PCR amplification of *B. afzelii* F1 DNA using the primer pairs B1-B3 and B6-B9 (Fig. 1), and the same reaction conditions as described previously (Jonsson *et al.*, 1992). The areas corresponding to the signals were cut out from a 0.7% low melting point agarose gel (LMP Sea Plaque GTG, FMC, In vitro AB) DNA was extracted using Magic PCR Preps DNA Purification System (SDS Promega), ligated into a *Hind*III-digested pUC18 plasmid screen, and then transformed into competent *E. coli* DH5 α . To screen for clones containing the *osp* genes, colony hybridization was performed according to standard methods (Ausubel *et al.*, 1992), both with the mixed PCR probe and the *ospA*-specific oligonucleotide B2.

Nucleotide sequence analysis. The clones containing the *osp* genes were sequenced by the Sanger dideoxy sequencing method (Sanger *et al.*, 1977) using vector-specific and internal *osp*-specific oligonucleotides as primers (Jonsson *et al.*, 1992). The sequences obtained were assembled using the GENEUS (Harr *et al.*, 1986) software for VAX computers (Digital Equipment).

RNA techniques. RNA was extracted from late exponential phase *Borrelia* cultures, i.e. dense cultures with motile spirochaetes, using hot phenol extraction (von Gabain *et al.*, 1983). Total RNA (10 μg) was loaded in each lane and separated on a 1% (v/v) formaldehyde agarose gel at 100 V for 6 h. The gel was neutralized in 1 $\times$ HEPES (Ausubel *et al.*, 1992), and the RNA was transferred to a Nylon filter (Hybond-N, Amersham) by capillary blotting overnight. The filter was hybridized at high stringency with [γ - ^{32}P]ATP end-labelled oligonucleotides. B2 was used for *ospA*, FB5 was used for *fla*, and C3 for *ospC* (Fig. 1). Quantification of the Northern blot signals was performed as described above for Western blot quantification.

Transcriptional analysis was performed on total RNA prepared as described above. Total RNA (5 μg) was dried together with 0.5 pmol [γ - ^{32}P]ATP end-labelled oligonucleotide A1 (Fig. 1), and then dissolved in 8 μl annealing buffer (50 mM Tris/HCl, pH 8.3, 60 mM NaCl and 10 mM DTT). The mixture was denatured at 80 $^{\circ}\text{C}$ for 5 min, and then immediately transferred onto ice for 5 min. Eight μl extension buffer (25 mM Tris/HCl, pH 8.3, 30 mM NaCl, 15 mM MgCl_2 , 1.25 mM DTT, 1 mM of each dNTP, and 7 units of avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim) were added. The samples were incubated at 42 $^{\circ}\text{C}$ for 1 h. Formamide sample buffer (16 μl) from the T7 sequencing kit (Pharmacia) was added. Samples (2 μl) were loaded following denaturation at 80 $^{\circ}\text{C}$ for 3 min. Only full-length extension product was synthesized, and the size of the transcript was compared with that of a regular Sanger dideoxy [^{32}S]dATP α S DNA sequence on plasmid pMJ08 for strain F1, or pMJ05 for strain ACAI (Jonsson *et al.*, 1992) with the same oligonucleotide primer.

Expression of OspA in *E. coli*. The plasmid pMJ05 was digested with *Hind*III and the 1.5 kb fragment containing the *ospA* gene was isolated and ligated into pUC18. A clone, pMJ06, having the *ospA* gene in the opposite direction to the *lacZ* gene in pUC18, was isolated. *E. coli* DH5 α containing pUC18, pMJ05, pMJ06 and pMJ08 was grown in L-broth and harvested at a cell density of 4×10^8 cells ml^{-1} by centrifugation. Western blotting with the polyclonal anti-OspA rabbit serum and secondary peroxidase-labelled anti-rabbit serum was performed as described above.

RESULTS

Biochemical and immunochemical characterization of the outer surface proteins

Whole cell protein extracts prepared from *B. burgdorferi* B31, *B. afzelii* ACAI and different *B. afzelii* F1 cultures were separated on a 12.5% SDS polyacrylamide gel (Fig. 2). Preparations of strain B31 contained a 31 kDa OspA and a 33.5 kDa OspB (Fig. 2). *B. afzelii* ACAI expressed an OspA of about 32.5 kDa and a 34.5 kDa OspB. In *B. afzelii* F1, the dominant band was a 25 kDa protein. However, bands of 32 and 35 kDa were also seen, indicating low but detectable expression of the OspA and OspB proteins in this strain.

Western blot analysis was performed to confirm the expression of OspA, OspB and OspC. Two mAbs, 184.1 and 84C, directed against the OspA and OspB proteins, respectively, and polyclonal anti-OspA and anti-OspC sera, were used. 184.1 is an anti-OspA mAb (Jiang *et al.*, 1990) which reacts only with the B31 and ACAI proteins, and not with those of the F1 strain (Fig. 3a). The polyclonal anti-OspA serum raised against the *B. afzelii* strain ACAI reacts with all three strains. The sequence heterogeneity between the OspA of *B. burgdorferi sensu stricto* and the *B. afzelii* strain is probably the reason for the apparently weaker reactivity to the *B. burgdorferi sensu stricto* B31 OspA protein (Fig. 3b). mAb 84C has been shown to react with OspB of most Lyme disease *Borrelia* strains that have been tested (Shoberg *et al.*, 1994) and reacted with all three strains used in this study (Fig. 3c). Western blot analysis using the polyclonal anti-OspA

Fig. 2. Whole cell lysates of *Borrelia* strains separated on a 12.5% SDS polyacrylamide gel and stained with Coomassie brilliant blue. Lanes: 2, *B. burgdorferi* B31; 3, *B. afzelii* ACAI; 4, *B. afzelii* F1c1; 5, *B. afzelii* F1b1. Lane 1 contains molecular mass standards labelled in kDa.

Fig. 3. Western blot analysis of 10 µg whole cell lysate from *Borrelia* strains separated on 12.5% SDS polyacrylamide gels and treated with (a) OspA-specific mAb 184.1, (b) polyclonal anti-OspA serum, (c) OspB-specific mAb 84C or (d) polyclonal anti-OspC serum. Lanes: 1, *B. burgdorferi* B31; 2, *B. afzelii* ACAI; 3, *B. afzelii* F1c1; 4, *B. afzelii* F1b1. In (a) the antibodies were detected with the ECL Western blotting detection system and in (b), (c) and (d) with iodinated secondary F(ab')₂ fragments. Molecular mass standards are shown on the left in kDa.

serum shows that the F1 strain expresses the OspA protein, but at a much lower level than strains B31 and ACAI (Fig. 3b). There is only 77% identity at the amino acid level of the OspA protein in B31 compared to both ACAI and F1 (Jonsson *et al.*, 1992; this study). The heterogeneity between B31 and F1 in combination with the low level of expression could explain the lack of reactivity of mAb 184.1 to the OspA of strain F1 (Fig. 3a). No signal could be detected with the OspAB-less mutant *B. burgdorferi* B313, demonstrating the specificity of the polyclonal serum (data not shown). A slight cross reactivity to the B31 OspB protein could be seen, but not to the ACAI or F1 OspB proteins. A Western blot experiment was also performed with a polyclonal OspC-specific antiserum. This reacted strongly with a protein of about 25 kDa in the F1 strains, and very weakly with a protein of similar size in the ACAI strain (Fig. 3d).

Quantification of the outer surface proteins

The relative amounts of OspA, OspB and OspC proteins in ACAI and three different cultures of F1 are shown in Fig. 4, together with the relative amount of *ospAB* transcript. In ACAI the OspA and OspB proteins were expressed at a high level. In the three preparations of F1 lysates shown, the amounts of OspA and OspB protein differ, but the level is much lower than in ACAI. In all three lysates, less OspB than OspA protein was made. The signals corresponding to the OspB proteins in all F1 strains were above the detection limit, but, as the OspB signal of strain ACAI was much stronger, the relative amounts of OspB in the F1 cultures was very low (Fig. 4). The opposite was observed with the OspC protein. The

Fig. 4. The relative amounts of *ospAB* transcript and of OspA, OspB and OspC proteins in *B. afzelii* ACAI (■) and *B. afzelii* F1b2 (□), F1c1 (▒) and F1c2 (░). The amount of transcript or protein was first normalized to the amount of flagellin transcript or protein in the preparation and then expressed relative to the highest value of the respective transcript or protein.

OspC signal from strain ACAI was just on the limit of detection, but the polyclonal anti-OspC serum gave a weaker signal compared to the other Osp antibodies, resulting in an apparently higher relative amount of OspC protein in strain ACAI. Less material was applied on the protein gel from culture F1c2, increasing the statistical error of the Osp protein signals.

Electron microscopy

Representative photomicrographs of the dramatic differences in protein expression in *B. afzelii* ACAI and *B. afzelii* F1 are shown in Fig. 5. Considerable OspA, but very little OspC was found on the surface of strain ACAI. In contrast, OspC was clearly an abundantly expressed outer surface protein of strain F1.

Genetic characterization of the *osp* operon

Separation of total DNA prepared from *Borrelia* strains B31, ACAI and F1 by pulsed field gel electrophoresis showed that the DNA content of F1 is very similar to that of ACAI, except for the existence of one small (25 kb), additional plasmid in F1 (data not shown). Southern blot hybridization using an OspA-specific oligonucleotide, showed that F1 has its *ospAB* operon localized in a 50 kb linear plasmid, similar to that in B31 and ACAI (Jonsson *et al.*, 1992).

Southern blot hybridization of *Hind*III-digested *B. afzelii* F1 DNA using two PCR fragments covering both the beginning of the *ospAB* operon and the middle of the *ospB* gene as probes, was performed. Two hybridization signals were observed, indicating that there is a *Hind*III site in the *B. afzelii* F1 *ospAB* operon. The signals correspond to fragments of about 0.9 and 1.5 kb. Other restriction enzymes with sites in suitable cloning vectors were tested, but none gave a single fragment. The 0.9 and 1.5 kb

Fig. 5. Electron micrographs of *B. afzelii* ACAI (a, c) and *B. afzelii* F1 (b, d) treated with affinity-purified polyclonal anti-OspA (a, b) or anti-OspC (c, d) sera. Bar, 1 µm.

fragments were isolated and ligated into the *Hind*III site of pUC18. The whole DNA sequence was determined using vector-encoded and internal *osp*-specific oligonucleotides as primers. A comparison of the *ospA* and *ospB* nucleotide sequences of strain F1 with the previously published sequences from *B. afzelii* ACAI and *B. burgdorferi* B31 showed that the F1 sequence is almost identical to the ACAI sequence (data not shown). Only two base pairs differ within the coding region. There is an A to G transition in position 264, i.e. at the beginning of *ospA*, giving a glycine instead of a glutamate in the putative amino acid sequence. It is possible that this amino acid is part of the epitope for mAb 184.1, as the F1 OspA is not recognized by this mAb. The second difference is an A to C exchange at the end of *ospB*, in position 1419. This change gives a leucine instead of an isoleucine. Furthermore, in the region upstream of the -35 hexanucleotide of the promoter, strain ACAI has a stretch of ten Ts, whereas strain F1 has only nine Ts in this area (Fig. 6).

To determine the transcriptional start site, a primer extension analysis on RNA from both ACAI and F1 was

		-35
B31	TT-G--CC--A-TTA----A-ACC-A--T---G--G-----C-----	
ACAI	CAATAAAAAATCCATATTAGATTTATACCTAATTAAAAATTATTATCATTTTATTTTTTTTAAATTTCTATTGTTA	
F1	-----	
	-10 +1 RBS <i>ospA</i> →	
B31	---G--A-----A-----	
ACAI	TTTATTGATCTTATACTATAATTATACTTGTATTAAAGTTATATTAATATAATATAAAAAAGGAGAATATATTATGAAAAA	
F1	-----	

Fig. 6. Comparison of the nucleotide sequence upstream of the *ospAB* operon in *B. burgdorferi* B31, *B. afzelii* ACAI and *B. afzelii* F1. The entire ACAI sequence is shown. In the other two sequences, hyphens indicate identical bases, letters indicate different bases and gaps indicate missing (or inserted) bases. The -35 and -10 regions of the promoter, ribosome-binding sites (RBS) and the transcriptional start point (+1) are indicated.

Fig. 7. The relative amount of *ospAB* transcript in *B. burgdorferi* B31, *B. afzelii* ACAI and different cultures of *B. afzelii* F1. F1b3* had been stored at 4 °C for a number of weeks before RNA preparation. The amount of transcript was measured by scanning Northern blots with a PhosphorImager. To correct for different amounts of mRNA loaded in the lanes on the gel, the *ospAB* signals were first normalized to the amount of flagellin transcript in the preparation and then expressed relative to the highest value of *ospAB*.

performed (data not shown). The transcriptional start point of the *ospAB* operon in B31 was previously determined to be at the G denoted +1 in Fig. 6. Primer extension analysis showed that the transcripts from both F1 and ACAI start at the same G nucleotide (data not shown).

The amount of *ospAB* transcript detected in different preparations of total RNA from strains ACAI and B31 was consistently very high. The amount of *ospAB* mRNA found in different cultures of F1 varies quite dramatically, as can be seen in Fig. 7. The highest amount of this transcript was found in culture F1b3 which had been stored at 4 °C for a number of weeks before the RNA was prepared. This suggests that the *ospAB* transcript is probably very stable. It is also possible that the transcription of the *ospAB* operon is induced or derepressed by either stationary phase growth or low temperatures.

The amount of *ospC* transcript was determined in B31, ACAI and the two F1 cultures expressing the lowest and highest amounts of *ospAB* transcript, F1c1 and F1b3. As

Fig. 8. The relative amount of *ospC* transcript from *B. burgdorferi* B31, *B. afzelii* ACAI and two different cultures of *B. afzelii* F1. The relative amounts of transcript were calculated as described in the legend to Fig. 7.

can be seen in Fig. 8, the F1c1 culture, which did not contain *ospAB* transcript, expressed very high amounts of *ospC* transcript, whereas F1b3, which contained the most *ospAB* transcript, expressed the least *ospC*.

Expression of *OspA* in *E. coli*

To investigate the difference in expression of the *OspA* proteins from *B. afzelii* strains ACAI and F1 in further detail, the expression of the cloned *ospA* genes was studied in *E. coli*. The plasmid pMJ05 is a pUC18 derivative with a 5 kb *EcoRI* fragment which contains the whole *osp* operon from strain ACAI including a 1.5 kb upstream sequence. Plasmid pMJ06 is a subclone with a 1.5 kb *HindIII* fragment obtained from pMJ05 containing the *ospA* gene cloned into pUC18 with a 0.5 kb upstream sequence. pMJ06 is a homologue of the pMJ08 clone which contains the F1 *ospA* gene. The clones have the *ospA* gene in the same orientation opposite to the *lacZ* promoter in pUC18. All *ospA* expression should therefore be directed from its own promoter. Western blot analysis using the polyclonal anti-*OspA* serum shows that similar

Fig. 9. Western blot analysis of lysates of *E. coli* DH5 α containing pUC18 (lane 1), pMJ05 (lane 2), pMJ06 (lane 3) and pMJ08 (lane 4) with polyclonal anti-OspA serum. The bound antibody was detected with the ECL Western blotting detection system.

expression levels were obtained from all three constructs (Fig. 9). The specificity of the signal is confirmed by its absence in the lane with pUC18 alone.

DISCUSSION

In the present study, we show that the expression of the outer surface proteins OspA, OspB and OspC differs considerably between the three Lyme disease *Borrelia* strains F1, ACAI and B31. This was shown previously by Barbour & Schrumph (1986). However, studies of the expression of the OspA proteins in *E. coli* showed that both the F1 and the ACAI *ospA* genes can be transcribed and translated. This result indicates that there are factors in the *Borrelia* strains which modulate the expression of OspA which are not present in *E. coli*. Surrounding DNA seems to have little, if any, influence on expression, at least in *E. coli*, since no significant difference in expression between pMJ05 and pMJ06 can be seen. Nothing is known about DNA binding proteins and the effect of DNA topography on gene expression in *B. burgdorferi sensu lato*. Therefore, it remains possible that differences in the control region of the *ospAB* operon are important for expression in the borreliae. To study whether differences in the turnover rate of the OspA proteins in ACAI and F1 could to some extent explain the various amounts of protein found, a pulse-chase experiment was performed (data not shown). This showed that the OspA proteins in both ACAI and F1 are extremely stable, with half-lives of more than one generation. Therefore, the differences in amounts of proteins are not due to variations in protein stability.

Eiffert *et al.* (1992) studied *B. burgdorferi* strain GÖ2 which expresses OspA, but not OspB. The lack of OspB expression was probably due to a point mutation, which introduced a stop codon into the beginning of the *ospB* gene. Margolis & Rosa (1993) recently published a study demonstrating that the expression of the OspA and OspB proteins are regulated at the transcriptional level. In that study, expression of the OspA and OspB protein genes was shown to be regulated by turning on and off the transcription of the whole *ospAB* operon. Padula *et al.* (1993) analysed the expression of the *ospC* gene and

identified a region upstream of the promoter that affects the level of transcription of the *ospC* gene. *B. burgdorferi* B31 has a 51 bp deletion in this control region which explains the lack of *ospC* expression in this strain. Collectively, these results suggest that Osp gene expression is essentially regulated at the transcriptional level.

Northern blot analysis with probes directed against either *ospA* or *ospB* gave the same result (data not shown), indicating that the *ospAB* operons of both strains ACAI and F1 are transcribed as one transcriptional unit as previously shown for the *B. burgdorferi* B31 *ospAB* operon (Howe *et al.*, 1985; Bergström *et al.*, 1989). This is supported by the fact that the transcripts in all three strains are of the same molecular mass and have the same transcriptional start point. The amount of *ospAB* transcript found is consistently high in strains B31 and ACAI, while it varies in different cultures of strain F1 (Fig. 8). This suggests that transcription is fully induced in B31 and ACAI, while it is only partially induced in F1. Alternatively, transcription in F1 is partially inhibited by some repressor which is inactive or absent in the other two strains. The translation of the *ospAB* operon also seems to be repressed to some extent in F1, as the relative amount of protein expressed is apparently low with respect to the amount of transcript produced. This is particularly true for F1b2, which has relatively high amounts of *ospAB* transcript, but very low amounts of OspA and OspB proteins. Thus, this result is different from that previously shown by Margolis & Rosa (1993) for the *ospAB* operon of *B. burgdorferi* clone CA-11 2A from the tick isolate CA-11-90. In this clone no mRNA was detected, although the *ospAB* operon was present and intact.

As Lyme disease *Borrelia* species move between ticks and mammals in their life cycle, they must be able to adapt to differences in their surroundings. Mammalian blood is very low in purines, while guanine is one of the primary waste products in ticks. The *guaA* and *guaB* genes are involved in purine biosynthesis, and are therefore very important for the borreliae when they are living in a mammalian host. Interestingly, the *guaAB* operon has been mapped to the same 26 kb circular plasmid which encodes the *ospC* gene (Margolis *et al.*, 1994). The two operons are separated by approximately 400 bp and are transcribed in opposite directions. It can be speculated that activation of the *guaAB* operon may simultaneously activate the *ospC* gene. It is also possible that activation of the 26 kb circular plasmid somehow represses the *ospAB* operon resulting in the inverse relationship between OspAB and OspC expression observed in this study.

Our data demonstrate a new form of regulation of OspA and OspB proteins that has not been observed before. We suggest that expression of OspA and OspB can be regulated not only at the transcriptional level, but also at the level of translation. The factors involved in this regulation remain unknown and are currently under investigation.

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