Structure and Expression of the FlaA Periplasmic Flagellar Protein of Borrelia burgdorferi

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The spirochete which causes Lyme disease, Borrelia burgdorferi, has many features common to other spirochete species. Outermost is a membrane sheath, and within this sheath are the cell cylinder and periplasmic flagella (PFs). The PFs are subterminally attached to the cell cylinder and overlap in the center of the cell. Most descriptions of the B. burgdorferi flagellar filaments indicate that these organelles consist of only one flagellin protein (FlaB). In contrast, the PFs from other spirochete species are comprised of an outer layer of FlaA and a core of FlaB. We recently found that a flaA homolog was expressed in B. burgdorferi and that it mapped in a fla/che operon. These results led us to analyze the PFs and FlaA of B. burgdorferi in detail. Using Triton X-100 to remove the outer membrane and isolate the PFs, we found that the 38.0-kDa FlaA protein purified with the PFs in association with the 41.0-kDa FlaB protein. On the other hand, purifying the PFs by using Sarkosyl resulted in no FlaA in the isolated PFs. Sarkosyl has been used by others to purify B. burgdorferi PFs, and our results explain in part their failure to find FlaA. Unlike other spirochetes, B. burgdorferi FlaA was expressed at a lower level than FlaB. In characterizing FlaA, we found that it was posttranslationally modified by glycosylation, and thus it resembles its counterpart from Serpulina hyodysenteriae. We also tested if FlaA was synthesized in a spontaneously occurring PF mutant of B. burgdorferi (HB19Fla−). Although this mutant still synthesized flaA message in amounts similar to the wild-type amounts, it failed to synthesize FlaA protein. These results suggest that, in agreement with data found for FlaB and other spirochete flagellar proteins, FlaA is likely to be regulated on the translational level. Western blot analysis using Treponema pallidum anti-FlaA serum indicated that FlaA was antigenically well conserved in several spirochete species. Taken together, the results indicate that both FlaA and FlaB comprise the PFs of B. burgdorferi and that they are regulated differently from flagellin proteins of other bacteria.

Lyme disease is a tick-transmitted illness caused by the spirochete Borrelia burgdorferi. It is the most prevalent vector-borne bacterial disease in the United States. In 1996, there was a 41% increase in confirmed cases over the previous year (12). B. burgdorferi is morphologically similar to other spirochetes; within the outer membrane sheath, several periplasmic flagella (PFs) wrap around the protoplasmic cell cylinder (5, 30). These PFs have an essential role in motility and cell morphology (30, 31, 63). One striking feature of B. burgdorferi and other spirochetes is their capacity to efficiently swim in a viscous gel-like medium such as connective tissue where other bacteria are slowed down or immobilized (31, 40); this invasive attribute may facilitate their passage through the extracellular matrix and cell junctions in infected tissues (40, 63).

The spirochete PF apparatus is similar to the flagellum of other bacteria; it has a filament, hook, and basal body (5, 6, 8, 14, 33, 34, 44). The PFs of most spirochetes are comprised of a class of core proteins referred to as FlaB and a class of outer layer proteins referred to as FlaA (7, 8, 14, 15, 41, 42, 46, 54, 55, 61, 69). Both FlaA and FlaB are among the most abundant cell proteins (42, 55). Depending on the species, the PFs consist of one to two different FlaA proteins and two to four different FlaB proteins (8, 14, 42, 46, 54, 55, 69). FlaB proteins show sequence similarity to the flagellin proteins from other bacteria and are evidently excreted by the flagellin-specific pathway (9, 14, 54, 55). On the other hand, FlaA proteins show no homology to other proteins and are most likely excreted to the periplasm by the secA-mediated pathway (9, 35, 41, 54). In addition, there is some evidence for glycosylation of PFs. Based on lectin binding results, Brahmsha and Greenberg suggest that a minor FlaB protein may be glycosylated in Spirochaeta aurantia (8). Most recently, FlaA from Serpulina hydysenteriae (46) has been shown by several criteria to be posttranslationally modified by glycosylation.

For over a decade, both ultrastructural analysis and biochemical isolation and characterization indicated that the PF filaments of B. burgdorferi differed from those of other spirochetes. These PFs were said to be comprised primarily of a 41-kDa FlaB protein (5, 6, 14, 16). However, we recently found a flaA homolog in B. burgdorferi which mapped in a flagellum/chemotaxis operon (fla/che) (20, 23). In addition, B. burgdorferi flaA was found to be expressed in growing cells, and the encoded protein reacted with an antisera directed to FlaA of T. pallidum (23).

In this study, we examined whether B. burgdorferi FlaA is associated with the PFs, and we also characterized FlaA in detail. Using a new procedure to isolate the PFs, we found that FlaA purified along with the PFs. These results, along with the analysis of a spontaneously occurring PF-deficient mutant, HB19Fla−, isolated by Sadziene et al. (63), indicate that FlaA is a PF protein. We also analyzed the transcription and translation of flaA in the wild type and HB19Fla−. The results indicate that expression of both flaB (63) and flaA is likely to
be controlled at the translational level. Finally, we present evidence that FlAA of *B. burgdorferi* is glycosylated and that it is antigenically similar to its counterparts from other spirochete species.

**MATERIALS AND METHODS**

**Bacterial strains.** The strains of *B. burgdorferi* senso stricto (3) include 212 (17), HB19 and HB19Fla– (64), and B31 (39). *B. burgdorferi* cells were grown in BSK-II medium without gelatin (4). *S. hyodysenteriae* B204 (61), *Treponema denticola* VS461 (62) and the PF-deficient *flg* mutant HLT1 were cultured as previously described (45, 62). Other spirochete cell pellets or lysates were from the following sources: *Borrelia afzelii* VS461, *Borrelia garinii* GV2, and *Borrelia hermsii* HS1 from T. Schwab (Rocky Mountain Laboratories, Hamilton, Mont.), *Treponema phagedenis* Kazan 5 and T. pallidum subsp. pallidum from R. Limberger and K. Wicher (Wadsworth Center for Laboratories and Research, Albany, N.Y.), and *Spirocheta aurantia* M4 from E. P. Greenberg (University of Iowa, Iowa City).

**DNA and RNA manipulation.** Unless noted, all procedures for genetic manipulations were carried out according to standard methods (2). The PCR was done with PrimeZyme (Biometra) DNA polymerase (24). The amplified PCR products were cloned into pGEM-T vector (Promega) for further manipulation. Reverse transcription (RT-PCR) was done with PrimeZyme (Biometra) DNA polymerase (24). The amplified PCR products were cloned into pGEM-T vector (Promega) for further manipulation. The reverse transcription (RT-PCR) was done with PrimeZyme (Biometra) DNA polymerase (24). The amplified PCR products were cloned into pGEM-T vector (Promega) for further manipulation.

**Purification of *T. pallidum* and *S. hyodysenteriae* polyclonal anti-FlaA antibodies.** Monoclonal antibodies to *T. pallidum* and *S. hyodysenteriae* polyvalent anti-Fla antibodies have been previously described and were obtained from S. Norris (University of Texas Medical School, Houston) and M. Jacques (University of California, Irvine). Western blot assays were performed as previously reported (23, 28). Primary antibodies were usually diluted from 1:1,000 to 1:5,000. Secondary antibodies were usually diluted 1:10,000 in a primary and predicted secondary structure similar to those of its counterparts from other bacteria (23, 29).

**RESULTS**

**Coisolation of the FlAA protein with the PFs.** We have previously shown that the *B. burgdorferi* 38-kDa FlAA homolog has a primary and predicted secondary structure similar to those of its counterparts from other bacteria (23, 29). Based on this information, we hypothesized that FlAA was closely associated with FlAB and resided on the PF filament. Accordingly, we isolated the PFs and tested whether FlAA also copurified during the purification procedure. To minimize the disruption of the FlAA from FlAB, we used a simple protocol that avoided harsh chemical treatment. *B. burgdorferi* cells were first treated with Triton X-100 to remove the outer membrane sheath (10). The PFs were then isolated by shearing followed by centrifugation (crude PF preparation). Further purification was achieved by
CsCl isopycnic gradient centrifugation (purified PF preparation). Electron microscopic examination of the purified PFs indicated that this preparation was relatively free of debris (Fig. 1). As shown by SDS-PAGE, both the 41- and 38-kDa proteins were the prominent protein species in both the crude and purified PFs (Fig. 2a, lanes 3 to 5). Several other proteins, of 60 kDa, 33 kDa, and 31 kDa, were detected in the crude PFs (Fig. 2a, lanes 3 and 4) but not the purified PF preparations (Fig. 2a, lane 5) and were thus likely contaminants. Immunoblotting with T. pallidum anti-FlaA confirmed that the 38-kDa protein was FlaA (Fig. 2b, lanes 2 to 4; Fig. 2c), and the 41-kDa protein corresponded to FlaB (Fig. 2c) PFs isolated from T. denticola were used as a positive control for FlaA (Fig. 2b, lane 1) (62). SDS-PAGE and Coomassie blue staining revealed that several proteins were released into the Triton X-100 supernatant fraction (not shown). However, immunoblot analysis revealed that although some FlaB partitioned into this fraction (Fig. 2c, lane 3, bottom), no FlaA was detected (Fig. 2c, lane 3, bottom). These results suggest that FlaA is not associated with the outer membranes and that it resided with the PFs. Because both FlaA and FlaB proteins were the major proteins detected following PF purification, they evidently are closely associated and comprise the PFs. FlaA and FlaB also copurified in crude PF preparations from strains B31 (Fig. 2a, lane 4) and HB19 (not shown), indicating that FlaA and FlaB associate with the PFs in other strains of B. burgdorferi. In cell lysates, and in both crude and purified PFs, the amount of FlaA was considerably less than that of FlaB (Fig. 2a).

**Peptide analysis of FlaA.** Although the protein which purified along with the PFs reacted with an anti-FlaA antiserum, we had no direct proof that the flaA gene (20, 23) encodes that protein. In our attempts to analyze this protein by N-terminal amino acid analysis, we found that it was blocked to Edman degradation. Accordingly, purified FlaA was analyzed by mass fingerprinting in a MALDI-TOF mass spectrometer after tryptic digestion. The mass-to-charge ratios (m/z) of the protonated molecular ions corresponding to six tryptic peptides (1,065.22, 1,188.28, 1,630.63, 1,644.60, 1,786.80, and 2,063.80 Da) were searched against those of all proteins in the NCBI database with masses of 30 to 40 kDa (58,035 entries). Allowance was made for up to one missed tryptic cleavage site per peptide. Five of the peptides matched values for the protein encoded by B. burgdorferi flaA (NCBI accession no. 2688608) to within 0.24 Da. The sixth peptide, that with an m/z of 2,063.80, matched to within 0.35 Da a peptide from the same protein with the condition that both methionine residues contained by the peptide would have become oxidized. The identification of the protein encoded by flaA was then confirmed by fragmenting the doubly or triply charged ions corresponding to all six of the peptides in a triple-quadrupole mass spectrometer and matching the fragments with those expected for the peptide sequences identified in the mass fingerprinting experiment. The results from five of the six peptides were in accord with the expected sequences. One peptide, however, did not match the sequence predicted for it. This was the peptide with an m/z of 2,063.80 Da, which was also the one that matched with lowest precision in the fingerprinting experiment. We therefore judge this one match to be spurious. Accordingly, we conclude that the protein which reacts to the specific FlaA antiserum is encoded by the flaA gene previously identified.

**Dissociation of FlaA from FlaB after treatment with Sarkosyl.** To explore why previous studies failed to detect FlaA associated with PFs, we repeated the purification procedure by using a method similar to one described by other investigators (6, 16). The major difference between these two methods is that the other method (6, 16) used Sarkosyl. We found that the use of Sarkosyl resulted in the release of most of the B. burgdorferi proteins (Fig. 3, lane 2). No FlaA was detected by Coomassie blue staining in the PF pellet after the first round of Sarkosyl lysis (Fig. 3a, lanes 3 and 5). We also tested for FlaA during PF purification by Western blot analysis. All of the FlaA

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**FIG. 1.** Electron microscopy of CsCl-purified PFs obtained by Triton X-100 extraction of B. burgdorferi from strain 212 negatively stained with uranyl acetate. Bar = 0.1 μm.

**FIG. 2.** Analysis of PFs by SDS-PAGE and immunoblotting. (a) Cell lysates and PF preparations were subjected to SDS-PAGE (12% [wt/vol] polyacrylamide gel) and stained with Coomassie brilliant blue. Approximately 50 μg of protein was loaded in each lane. Lanes: 1, protein markers; 2, B. burgdorferi 212 cell lysate; 3, crude PFs of 212; 4, crude PFs of B31; 5, purified PFs of 212. (b) Western blotting with antiserum to T. pallidum FlaA. Approximately 10 μg of protein was loaded per lane. Lanes: 1, purified T. denticola (Td) PFs (63); 2, B. burgdorferi 212 cell lysate; crude PFs of 212; 4, purified PFs of 212. (c) Western blotting using polyclonal T. pallidum FlaA and monoclonal H9724 to B. burgdorferi FlaB during purification of 212 PFs.
disassociated from the PFs and was released into the supernatant after the first round of treatment with Sarkosyl (Fig. 3b, lane 2); no FlaA was detected in the final PF preparation (Fig. 3b, lanes 3 to 7). These results suggest that FlaA disassociated from the PFs during purification using Sarkosyl. In other experiments, FlaA was found to be associated with the PFs after the first treatment with Sarkosyl, but it dissociated after subsequent rounds of treatment (26). The above results, along with the evidence that FlaA is considerably lower in concentration than FlaB, likely account for the failure to detect FlaA in previous B. burgdorferi PF preparations. Electron microscopic examination of the PFs prepared by both methods revealed no obvious differences in structure (data not shown).

**Defect in expression of FlaA in HB19Fla**— Sadziene et al. isolated a spontaneously occurring nonmotile PF-deficient mutant, HB19Fla— (63). This mutant synthesized the hook- basal body structures but did not synthesize the flagellar filaments. Western and Northern blot analysis indicated that although HB19Fla— failed to produce the FlaB protein, the encoding message was still produced at a level equivalent to the wild-type level. Accordingly, Sadziene et al. suggested that FlaB could be regulated on the translational level (63). HB19Fla— is thus likely to be deficient in the last stage of PF assembly, i.e., assembly of the PF filament after hook- basal body synthesis.

We hypothesized that like FlaB, FlaA is involved in the last stage of filament assembly. To test this hypothesis, we analyzed HB19Fla— with respect to FlaA synthesis by immunoblot analysis. SDS-PAGE and Coomassie blue staining revealed that FlaB was evident in the parental HB19 cell lysate but absent in HB19Fla— (Fig. 4a). Western blot analysis revealed that both FlaA and FlaB were also present in HB19 but absent in HB19Fla— (Fig. 4b). These results extend those of Sadziene et al. (63) and suggest that both FlaA and FlaB failed to be expressed in HB19Fla—.

We examined whether the lack of expression of FlaA is caused by a genetic defect in flaA or failure to be transcribed. The region containing flaA from HB19Fla— was amplified by using a pair of primers derived from the adjacent genes orfA and cheA (23) and then sequenced. Comparison of the HB19Fla— region to that of strain 212 revealed 100% identity at the nucleotide level (GenBank accession no. U62900). These results suggest that flaA is intact in HB19Fla—.

We used RT-PCR analysis to test if flaA is transcribed in HB19Fla— (23, 28). Three pairs of primers spanning the adjacent and central regions were used: orfA to flaA, internal regions of flaA, and flaA to cheA (20, 23). Three specific products of the predicted sizes were successfully amplified from the mRNAs of HB19 and HB19Fla— (22). By extracting the PCR products from the gel and reamplifying with internal primers, the bands of the predicted sizes were shown to be the desired specific amplified products (22). These results, together with those obtained by Western blot analysis, suggest that flaA is transcribed but not translated in HB19Fla—.

We compared the amount of flaA message in HB19Fla— to that in the wild type, using RNase protection assays. As a control, we also compared 16S rRNA messages in the two strains. There were no obvious differences between HB19 and HB19Fla— with either the flaA or 16S rRNA riboprobe (Fig. 5). The RNA protection was specific, as hybridization with yeast RNA was negative. We quantitatively compared the amounts of mRNA protected by the riboprobes with a PhosphorImager. Using four different amounts of total RNA in two different experiments, we found no significant difference in RNase protection between HB19 and HB19Fla— with either riboprobe. For example, we found that with 20 µg of total RNA and the flaA riboprobe, 11,069 cpm was protected in HB19 and 9,830 cpm was protected in HB19Fla—. These results indicate that HB19Fla— synthesized flaA message in an amount similar to the wild-type amount.
FlaA is a glycosylated protein. As already mentioned, we were unable to sequence the N-terminal region of the purified protein. For this reason, and because S. hyodysenteriae FlaA has been reported to be glycosylated (46), we tested if B. burgdorferi FlaA underwent a postranslational modification. We used three different methods to test for glycosylation. First, we used the purified native FlaA protein and the highly sensitive ECL glycoprotein detection system to test for glycosylation. As expected, the positive control serum transferrin reacted strongly (Fig. 6, lane 1). In addition, we found that B. burgdorferi FlaA also yielded a strong reaction, indicating that the native protein was glycosylated (Fig. 6, lane 2). As a negative control, the cytoplasmic motility protein recombinant FliI (28) failed to react (Fig. 6, lane 4), as did the recombinant FlaA containing a deletion of the N-terminal region (29) (Fig. 6, lane 3). Also as controls, we found that reactivity was sodium metaperiodate dependent, as omitting this reagent resulted in no reactivity of FlaA and serum transferrin (not shown). Second, we tested whether FlaA reacted with five different lectins: GNA, SNA, MAA, PNA, and DSA. Only SNA and GNA gave positive reactions (Fig. 7a). SNA binds to α-(2-6)-linked sialic acid, and GNA binds to terminal mannose residues. Recombinant FliI and FlaA were negative (not shown), whereas all positive controls reacted as expected (Fig. 7a, serum transferrin). Because reactivity could not be removed by pretreating proteins by boiling in 2% (wt/vol) SDS, 10 mM EDTA, or 8 M urea (not shown), the attached sugar residues are evidently covalently bound to FlaA. Finally, native and recombinant N-glycosidase F was found to remove the lectin reactivity as detected with SNA and GNA (Fig. 7b). Similar results were found when the positive control serum transferrin was treated with N-glycosidase F. These results taken together suggest that FlaA is a glycosylated protein that contains both sialic acid and mannose.

Epitopes of FlaA conserved among spirochete species. We have previously shown that anti-T. pallidum FlaA antibody reacted strongly with a 38-kDa protein of B. burgdorferi cell lysates (23). We investigated whether antibodies to FlaA proteins of other spirochetes reacted with B. burgdorferi FlaA. Monoclonal antibodies against S. aurantia and T. pallidum FlaA failed to react with B. burgdorferi FlaA. The polyclonal anti-S. hyodysenteriae FlaA antibody reacted positively with both T. pallidum and S. hyodysenteriae FlaA proteins in cell lysates, while the reactions with other species were inconclusive (data not shown). On the other hand, antibody specific to T. pallidum FlaA demonstrated strong reactivity to FlaA from many spirochete species as determined by Western blotting of whole-cell lysates (Fig. 8). The reactive proteins were identical in size to those reported in the literature for each FlaA of the species examined (8, 46, 54, 62). As a control, the T. denticola flgE mutant HLS1, which is deficient in PF synthesis (62), failed to react with the FlaA antiserum. We also tested several Borrelia species, including B. burgdorferi sensu stricto (strains 212, HB19, and B31), B. afzelii, B. garinii, and B. hermsii. All produced significant amounts of FlaA of approximately 38 kDa (Fig. 8b). B. hermsii reacted less intensely than the other spe-

![FIG. 6. Detection of glycosylated FlaA protein by periodate oxidation followed by hydrazine detection. The proteins were subjected to SDS-PAGE (12% (wt/vol) polyacrylamide gel) and tested by a glycoprotein detection system. Lanes: 1, serum transferrin (positive control); 2, purified native FlaA; 3, recombinant FlaA with N-terminal deletion (amino acids 1 to 26); 4, purified recombinant FliI.](http://jb.asm.org/)

![FIG. 7. Lectin binding to FlaA and sensitivity to N-glycosidase F treatment. Approximately 0.5 to 1.0 µg of protein was loaded in each lane and tested as described in Materials and Methods. (a) Lectin SNA reactivity. Lanes: 1, recombinant FlaA; 2, native FlaA; 3, recombinant FliI; 4, serum transferrin; 5, lectin GNA to native FlaA. (b) Individual proteins were incubated with recombinant N-glycosidase F as described in Materials and Methods and detected with lectin SNA after electrophoresis and blotting. Lanes: 1, FlaA after enzyme treatment; 2, FlaA before enzyme treatment; 3, serum transferrin after enzyme treatment; 4, serum transferrin before treatment.)](http://jb.asm.org/)

![FIG. 8. (a) Cross-reactivity of FlaA among the spirochetes. Cell lysates (approximately 10 µg of protein was loaded in each lane) from different species of spirochetes were studied by Western blotting with anti-T. pallidum FlaA antiserum. T. denticola flgE is a T. denticola flgE mutant. (b) Conservation of FlaA among Borrelia species, determined by Western blot analysis using anti-T. pallidum FlaA and cell lysates of various Borrelia species.)](http://jb.asm.org/)
cies. As expected, the control HB19Fla— failed to react. These results suggest that epitopes on FlaA are evolutionarily well conserved among the spirochetes, including many species of *Borrelia*.

**DISCUSSION**

*B. burgdorferi* has often been cited as being distinct from other spirochetes in having only one protein component comprising its flagellar filament (6, 14, 16, 21, 71). Two lines of experimental data have contributed to this concept. First, electron microscopy indicated that there was no obvious outer layer surrounding a filament core (5, 6, 14, 44). Second, in the analysis of purified PFs, only a single major protein band was observed with SDS-PAGE and two-dimensional gel electrophoresis (6, 16). Our recent finding of a flaA homolog in *B. burgdorferi* led us to reassess this notion (23). We found that both FlaA and FlaB copurified with the PFs in a gentle isolation procedure. In addition, the mutant which lacked PFs, HB19Fla—, also lacked both FlaA and FlaB. Thus, both biochemical and genetic findings strongly indicate that FlaA and FlaB comprise the PFs of *B. burgdorferi*. This experimental approach has been previously used to demonstrate that multiple protein species comprise the PFs of other spirochete species (48, 62).

Several reasons can account for FlaA not previously being detected as a PF protein. First, Sarkosyl was used to purify the PFs in two earlier studies (6, 16). Our results indicate that this ionic detergent completely released FlaA from the flagellar filament during purification (26). Second, FlaA is an acidic protein different from those of other *B. burgdorferi* strains. S. aurantia did not release FlaA from the PFs of *S. hyodysenteriae* during purification (26). Second, FlaA is an abundant protein in other spirochete species and is expressed at levels approximately equal to those of FlaB (7, 8, 42, 48, 54, 69). In contrast, the amount of *B. burgdorferi* FlaA was approximately 10% of the amount of FlaB, which can be easily missed on SDS-PAGE, especially since FlaA and FlaB have similar molecular weights. Finally, *B. burgdorferi* FlaA does not induce a strong immune response during infection, as its counterparts in other spirochetes are expressed in relation to other *B. burgdorferi* antigens such as FlaB during Lyme disease (29). Thus, FlaA was not selected as a potential diagnostic antigen for Lyme disease.

Several lines of evidence suggest that *B. burgdorferi* FlaA is similar to its counterparts from other spirochetes. *B. burgdorferi* FlaA has 54 to 59% amino acid sequence similarity to FlaA proteins of *T. pallidum*, *S. aurantia*, and *S. hyodysenteriae* (23). In addition, as with the FlaA proteins of these bacteria (9, 36, 43), *B. burgdorferi* has an N-terminal signal peptidase cleavage site involved in protein export. In fact, expression of intact *B. burgdorferi* and other spirochete FlaA proteins in *E. coli* results in cell death (29, 36, 57), and the N-terminal region is in part responsible for this lethality (29, 36). Western blot analysis reinforces this similarity of the FlaA proteins. Using an antiserum to *T. pallidum* FlaA, we found cross-reactivity in three strains of *B. burgdorferi*, along with *B. afzelii*, *B. garinii*, *B. hermsii*, *S. aurantia*, *S. hyodysenteriae*, and *T. denticola* (Fig. 8b). Because FlaA was found associated with the PFs, these results emphasize its evolutionary importance and likely role in spirochete motility.

It is not clear at this time whether *B. burgdorferi* FlaA is a flagellar outer sheath protein. Evidently, removal of FlaA during purification does not result in morphological dissociation of the PFs, as intact PFs are obtained by using Sarkosyl. Preliminary experiments using *T. pallidum* anti-FlaA were unsuccessful in localizing the protein by immunoelectron microscopy (26). Future experiments which involve immunoelectron microscopy and antisera specific to *B. burgdorferi* FlaA should allow us to determine its precise location. In the analysis of other spirochete PFs, the PF sheath tended to be associated with the region proximal to the basal body (13). Because FlaA is expressed in *B. burgdorferi* in a considerably smaller amount than in other spirochetes, perhaps it is found only in the area near the hook region.

Glycosylated proteins are relatively rare in bacteria. They have been associated with many archaeal flagellins, S-layer proteins, and pilin (37, 66). More recently, glycoproteins have also been associated with flagella of specific species of members of the domain *Bacteria* (18, 53). With respect to spirochete PFs, FlaA has been shown to be glycosylated in *S. hyodysenteriae* (46), and Brahamsha and Greenberg reported that one of the FlaB proteins may be glycosylated in *S. aurantia* (8). The precise role of the carbohydrate moieties on the flagellar and PF proteins is unclear. One suggestion is that the carbohydrate residues are necessary for flagellum assembly in specific species of *Archaea* (37). Alternatively, the suggestion has been made that sugar residues on the flagellar glycoproteins may promote rotation of the flagella without their becoming entangled. One known attribute of many glycoproteins is that the carbohydrate regions are involved in increasing hydration (72). Perhaps these residues on FlaA bind water and act as a lubricant to promote PF rotation within the periplasmic space. Future experiments, which will require a sophisticated gene inactivation system, will be necessary to determine their precise function.

The spontaneously occurring PF-deficient mutant HB19Fla— has yielded significant information with respect to *B. burgdorferi* virulence, motility, and PF synthesis (24, 30, 31, 63, 64). We realize that construction of site-directed mutations in *B. burgdorferi* motility genes would be the preferable means to analyze structure and function. However, a genetic transfer system for *B. burgdorferi* is in the very early stages of development (59); thus, we are presently constrained in experimental design relating to genetics. With this limitation in mind, HB19Fla— has been shown to be less invasive of cultured cells than the wild type (63), and it has been shown to serve as a live attenuated vaccine for mice (64). These results suggest that motility and/or PFs are likely to be an important virulence factor for *B. burgdorferi*. In addition, HB19Fla— has been essential in developing a model of *B. burgdorferi* motility and in demonstrating that PFs in part dictate the shape of the entire cell (30, 31). Our previous studies have shown that HB19Fla— still produced many motility and chemotaxis proteins (FlI, FlIG, FlIM, FlIN, FlHA, FlHB, and CheA) required for early flagellar assembly (22, 24). These results are consistent with the morphological data that HB19Fla— synthesizes the hook-based body region but not the filament structure (63).

Analysis of HB19Fla— suggests that flagellar gene expression in *B. burgdorferi* differs from that of other bacteria. Both flaA and flaB messages were synthesized in HB19Fla—, but the encoding proteins were not detected by Western blotting (Fig. 4 and 5) (63). These results are consistent with translational control of expression of both genes. A similar conclusion was reached with respect to flaB expression in *T. phagedenis* (49). More recently, a flaE mutant of *T. denticola* was found to synthesize flaB message but not FlaB protein (47). In *B. burg-
dorferi, flaA and flaB reside in different operons: flaB is in a monocistronic operon mapping at approximately 125 kb on the chromosome (11, 20, 65); flaA is the first of five chemotaxis and motility genes mapping on an operon at 722 kb on the chromosome (20, 23, 25). Evidently, HB19Fla−− has a genetic defect that blocks expression of both FlaA and FlaB in two separate operons. In addition, cheA is downstream of flaA, and CheA but not FlaA is expressed in HB19Fla−− (22). Apparently there is selective gene expression within the flaA operon. How translational control in B. burgdorferi is achieved is unclear. Perhaps antisense mRNA regulates flaA and flaB expression (19). Alternatively, RNA (58) or protein processing could be involved (32). In Salmonella typhimurium and other bacteria, the last stage of flagellum synthesis is tightly regulated on the transcriptional level (51, 52). Our results and those of others suggest that B. burgdorferi and other spirochetes adopted a different strategy to control PF filament synthesis.

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