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Borrelia burgdorferi EbfC defines a newly-identified, widespread family of bacterial DNA-binding proteins

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ABSTRACT

The Lyme disease spirochete, Borrelia burgdorferi, encodes a novel type of DNA-binding protein named EbfC. Orthologs of EbfC are encoded by a wide range of bacterial species, so characterization of the borrelial protein has implications that span the eubacterial kingdom. The present work defines the DNA sequence required for high-affinity binding by EbfC to be the 4 bp broken palindrome GTnAC, where ‘n’ can be any nucleotide. Two high-affinity EbfC-binding sites are located immediately 5’ of B. burgdorferi erp transcriptional promoters, and binding of EbfC was found to alter the conformation of erp promoter DNA. Consensus EbfC-binding sites are abundantly distributed throughout the B. burgdorferi genome, occurring approximately once every 1 kb. These and other features of EbfC suggest that this small protein and its orthologs may represent a distinctive type of bacterial nucleoid-associated protein. EbfC was shown to bind DNA as a homodimer, and site-directed mutagenesis studies indicated that EbfC and its orthologs appear to bind DNA via a novel α-helical ‘tweezer’-like structure.

INTRODUCTION

The Lyme disease spirochete, Borrelia burgdorferi, is maintained in nature through cycles of alternating infections of vertebrate hosts and tick vectors. To facilitate infection of those distinctive types of animals, and to enable efficient transmission between hosts and vectors, B. burgdorferi controls production of a large number of bacterial proteins. Among these are the Erp proteins, a polymorphic family of surface-exposed, outer membrane lipoproteins that are expressed throughout mammalian infection but largely repressed during tick colonization (1). Known functions of Erp family members include binding of the host serum components complement factor H and plasminogen, and adherence to the extracellular matrix protein laminin (2–10). All Lyme disease spirochetes contain multiple variants of related prophages, known as cp32 elements, which replicate episomally as 32 kb circular plasmids (11–14). Each cp32 contains a mono- or bicistronic-erp locus. The B. burgdorferi type strain, B31, is known to harbor 10 different cp32 family members, and encodes 13 distinct Erp proteins (1,15). Despite the often extensive sequence variation found among erp open reading frames, all erp loci share a unifying feature in possessing highly conserved sequences immediately 5′ of the open reading frames. Within this DNA sequence is the transcriptional promoter and two

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operator sites that bind cytoplasmic proteins (16). Transcriptional fusion studies identified that the operator region closest to the transcriptional promoter, Operator 2, is involved in transcriptional repression (16). Members of our laboratories recently discovered that a chromosomally encoded protein of *B. burgdorferi*, named EbfC, specifically binds two sites within *erp* Operator 2 (17).

Many other species of bacteria encode orthologs of EbfC, suggesting that this protein performs a function(s) conserved throughout the kingdom Eubacteria. These homologous proteins have been variously classed as ‘domain of unknown function’ 149, Pfam 2575, COG-0718 and YbaB (18,19). The 3D structures of the *Escherichia coli* and *Haemophilus influenzae* orthologs have been determined (18) and (http://www.rcsb.org/pdb/explore.do?structureId=1PUG). Both proteins crystallized as dimers, consisting of a central β-folded dimer-interface region and protruding α-helices at either end, forming a distinctive structure that has been described as having a ‘tweezer-like’ shape (18). The present studies extended characterization of the *B. burgdorferi* EbfC protein, demonstrating that this small protein both specifically and nonspecifically binds DNA, can bind DNA independently of context and alters DNA conformation. Site-directed mutagenesis approaches were also employed to investigate the mode of interaction between EbfC and DNA.

**MATERIALS AND METHODS**

**Site-directed mutagenesis**

Site-directed mutagenesis of both the *erpAB* Operator 2 and *ebfC* were performed by sequence overlap extension PCR mutagenesis (20). Primers used in the PCR reactions are listed on Table 1. All mutagenized plasmids were sequenced on both strands to verify the desired mutations.

**Recombinant EbfC**

Proteins were produced from either p462M5 (17) or mutant derivatives thereof (Table 1), using *E. coli* Rosetta (DE3) (pLysS) (Novagen). Following induction by addition of 1 mM isopropylthio-β-galactopyranoside, bacteria were harvested by centrifugation and lysed by sonication in 20 mM NaPO₄, 0.5 M NaCl, 30 mM imidazole, pH 7.4. Lysates were cleared by centrifugation and injected onto 5 ml HisTrap-HP columns using an AKTA-FPLC with UPC-900 UV absorbance monitor and Fra920 fraction collector (GE Healthcare).

Columns were subjected to the lysis buffer containing a linear gradient of imidazole ranging from 30 mM to 750 mM. Fractions (1 ml each) were collected, and aliquots subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) with Coomassie brilliant blue staining, to assess protein purity. Proteins were concentrated using YM-30 Centric columns (Amicon), then dialyzed against 50 mM Tris–HCl, 1 mM phenylmethanesulfonyl fluoride, 1 mM dithiorthiol (DTT), 10% glycerol, pH 7.5.

Recombinant protein concentrations were determined by bicinchoninic acid (BCA) protein assays (Pierce). Aliquots were snap frozen in liquid nitrogen, then stored at −80°C. Frozen protein preparations were thawed on ice immediately before use.

**Electrophoretic mobility shift assays**

Sequences of biotinylated DNAs used to define optimal EbfC-binding activities by electrophoretic mobility shift assays (EMSA) are illustrated in Figure 1. DNAs were produced by PCR amplification using appropriate primers, templates and LA Taq polymerase (Takara). Amplification consisted of 40 cycles of 94°C for 1 min, 55°C for 1 min and 68°C for 1 min. Reaction products were separated by agarose gel electrophoresis and visualized by staining with ethidium bromide. DNAs were extracted into nuclease-free water using Wizard SV gel purification system (Promega). DNA concentrations

| Construct | Primer name | Sequence (from 5’ to 3’) |
|-----------|-------------|--------------------------|
| K16A      | K16A-1      | TCT AGC GTT GCG AAT AAT ATT GAC AAT ATT AAA AAG G |
| K16A-2    |             | AAT ATT ATT CGC AAC GCT AGA CAT ATT TTT CAA AAA ATC TAA CCG |
| D20A      | D20A-1      | AAT ATT ATT GCC AAT ATT AAA AAG GAA ATT TCT AAA ATT ACG |
| D20A-2    |             | TTT AAT ATT GGC AAT ATT ATT CTT AAC ATG A |
| K23A      | K23A-1      | GAC AAT ATT GCA AAG GAA ATT TCT TCA AAA ATT ACG |
| K23A-2    |             | AAT TTC CTT TGC AAT ATT GTC AAT ATT ATT CTT AAC G |
| N77A      | N77A-1      | TCT GCT TTA GAT GCT GTC TCT AAG GTT AAA G |
| N77A-2    |             | GAC AGC ATC AGC TAA AGC AGA TTT AAT CAT TTG |
| D78A      | D78A-1      | GCT TTA AAT GCT GCT TCT AAG GTT AAA GAA G |
| D78A-2    |             | AGA GAC AGC ATG TAA AGA AGA TTT AAT CAT TTG |
| K82A      | K82A-1      | GCT GTC TCT GCG GTT AAA GAA GAG ATA AAA TTA AAA ACC ATG G |
| K82A-2    |             | TTC TTT AAC CGC AGA GAC GAC AGC ATC ATT TAA AGC AG |
| K84A      | K84A-1      | TCT AAG GTT GCA GAA GAG ATA AAA TTA AAA ACC ATG |
| K84A-2    |             | TAT CTC TTC TGC AAC ATC AGA GAC AGC ATC TTA TAA |
| E85A      | E85A-1      | AAG GTT AAA GCA GAG ATA AAA TTA AAA ACC ATG G |
| E85A-2    |             | TTT TAT CTC TGC TTA AAC ATC AGA GAC AGC ATC |
| K88A      | K88A-1      | GAA GAG ATA GCA TAA AAA ACC ATG GAA GTT CTT CC |
| K88A-2    |             | GGT TTT TAA TGC TAT CTC TTC TTT AAC ATG AG |

aAll PCR used p462M5 as template (17).
were determined spectrophotometrically by measuring absorbance at 260 nm, with an absorbance of 1.0 = 50 μg/ml DNA, then each diluted to a final concentration of 1 nM (10× stock).

EMSAs were performed using 100 pM biotin-labeled double-stranded DNA probes as previously described (16,17), with binding conditions of 50 mM Tris–HCl, 1 mM DTT, 8 μl/ml Protease inhibitor (Sigma, St. Louis, MO), 2 μl/ml Phosphatase Inhibitor Cocktail II (Sigma), 50 μg/ml bovine serum albumin, 10% glycerol, pH = 7.5 at room temperature. Binding reactions were allowed to proceed for 20 min at room temperature, before being subjected to electrophoresis through 6% DNA Retardation Gels (Invitrogen) for a total of 9000 V-min. Gels were transferred to nylon membranes, cross-linked by UV light and biotinylated DNAs were detected by Chemiluminescent Nucleic Acid Detection Modules (Pierce) and Kodak Biomax film.

Dissociation constant determination

Dissociation constants (K_d) for EbfC–DNA interactions in the absence of poly-dI-dC were determined by analyses of EMSA gel images (21). Exposed films were scanned in 8 bit depth at 1200 dpi resolution, analyzed using Image J 1.37v (22) and the ratios of bound:free DNA in each lane were determined. These values were transferred into Microsoft Excel for graphing and analyses. The quantity of DNA in each band or total lane was expressed as concentration of the known input DNA (23,24).

Binding was analyzed according to a model in which n molecules of protein (P) bind DNA (D):

\[ nP + D \rightleftharpoons P_nD \]

with the equilibrium constant:

\[ K_d = \frac{[P]^n[D]}{[P_nD]} \]  

The K_d for the titration of DNA with EbfC were determined by graphing the ratio of bound to free DNA for each reaction. These ratios were graphed in relation to known concentration of free protein [P]. Since the ratios of free protein to DNA were extremely high, the binding of protein to DNA did not appreciatively change concentration of free protein (21). The K_d is therefore the concentration of EbfC where the ratio of bound to free DNA = 1.

Rearranging Equation (2) and taking natural logs gives:

\[ \ln \frac{[D]}{[P_nD]} = -n \ln [P] + \ln K_d \]

Thus, for EbfC, a graph of ln ([D]/[EbfC_nD]) as a function of ln [EbfC] will have a slope equal to the negative value of the stoichiometry, −n, and an x-intercept at which \[ K_d = n \ln [EbfC] \].

For EMSAs with two DNA probes, the ratio of bound to free DNA cannot be determined for each specific DNA probe, because it is impossible to determine which DNA species is responsible for any single protein DNA complex. In these cases, the calculated binding activities were the ratios of protein concentrations at which each free-DNA species disappeared by 50%.

DNA-bending analyses

Five 150 bp DNA fragments, overlapping stepwise by 30 bp increments over a total of 250 bp, were produced...
by PCR, using the cloned \textit{erpAB} insert of pBLS434a as template (13). The upstream oligonucleotide primer for each DNA fragment was modified with a biotin moiety at the 5' end. Natural curvature of the DNAs was analyzed by electrophoresis through 15% polyacrylamide gels and staining with ethidium bromide. EMSAs using purified recombinant EbfC were performed as described above. Predictions of natural DNA-bending were calculated using bend.it, with default parameters (http://hydra.icgeb.trieste.it/dna/bend_it.html) (25).

**Size fractionation chromatography**

The abilities of the various recombinant EbfC preparations to form multimeric structures were analyzed by size fractionation chromatography as previously described (17). Proteins were individually loaded onto a Superdex 75 10/300 column (GE Healthcare) and separated using a Waters 600 pump and controller equipped with a Waters 996 UV/Vis detector.

**EbfC model**

The \textit{B. burgdorferi} EbfC was modeled based on the solved structure of the orthologous protein YbaB of \textit{H. influenzae} (PDB 1j8b) (18). The sequences of EbfC and YbaB were aligned with BlastP (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Amino acid side-chain substitutions were made at positions of different sequences with the O-program (26). The alignment showed that compared with YbaB, EbfC contains an additional amino acid residue (Leu63) inserted into the loop before the C-terminal z-helix. This loop (residues 61–65) was modified to contain Leu63 so that its side chain would be in close proximity to Phe59 and Ala67 to form hydrophobic interactions. The resulting EbfC model was minimized with the CNS program (27). The figure of the EbfC model was generated with Molscript (28) and Raster3D (29,30).

**Analysis of \textit{ebfC} sequences of Lyme disease \textit{Borrelia} spp.**

The \textit{ebfC} loci of Lyme disease borreliae were amplified by PCR using oligonucleotides \textit{BFC-18} (5'-CATCTGCT TAAACATACATAAA-3') and \textit{EBFC-19} (5'-GACAA TGGTCTTTATTATAAGGTG-3'), which are specific for sequences bordering the \textit{B. burgdorferi} strain B31 \textit{ebfC} open reading frame on the 5' and 3' sides, respectively. Bacteria analyzed in this work were \textit{B. burgdorferi} (\textit{sensu stricto}) strains B31, N40, 297 and Sh-2-82, \textit{Borrelia afzelii} strains PKo and VS461, \textit{Borrelia garinii} strain Ip90 and \textit{Borrelia bissettii} strains 25015 and DN127.

**RESULTS**

**Sequence-specific binding of DNA by EbfC**

Our earlier EMSA studies found that competitor DNAs containing the broken palindrome TGT\textsuperscript{\textbeta}/ACA competed for EbfC binding to \textit{erp} promoters (\textit{PerpAB}), while DNAs that instead contained 5'-CAC\textsuperscript{\textbeta}/ACA-3' did not compete (17). To more precisely define the sequence requirements for high-affinity interactions between EbfC and DNA, site-directed mutagenesis was employed to create a series of variants in the Operator 2 region of the strain B31 \textit{erpAB} promoter (\textit{PerpAB}).

Initially, mutants of \textit{PerpAB} were produced in which either site I, site II or both sites I and II were changed to 5'-CACACAACA-3', all in the context of otherwise normal \textit{PerpAB} DNA sequences (Figure 1). EMSA using a probe with the wild-type \textit{PerpAB} sequence, b-WT, yielded two observable DNA–EbfC complexes, with the slower mobility (upper) complex having a higher affinity interaction (Figure 2A). Probes that contain only one TGT\textsuperscript{\textbeta}/ACA palindrome (b-C1 and b-C2) exhibited the same two EbfC–DNA complexes, but at decreased relative levels (Figure 3A). The signal strengths of the slower mobility complexes for EbfC binding to either probes b-C1 or b-C2 were approximately one half the value for b-WT. There were no apparent differences in EbfC binding to DNA probes that contained only wild type site I or site II, indicating relatively equal affinities for both sites. However, the mutation of both TGT\textsuperscript{\textbeta}/ACA palindromes in probe b-C1/2 dramatically reduced EbfC binding (Figure 2A). These results confirm that EbfC preferentially binds the
The EbFC binds DNA as a homodimer

The \( K_d \) for EbFC binding to Perp DNA was determined by titrating increasing concentrations of recombinant EbFC to labeled DNA probe b-WT, followed by EMSA analysis of protein-DNA complex formation (Figure 4A). The protein:DNA ratios in all reactions were extremely high, such that binding of protein to DNA would not significantly affect the concentration of free protein. As described in the following paragraph, EbFC binds DNA as a homodimer. The calculated \( K_d \) for the reaction 2 EbFC + b-WT \( \leftrightarrow \) 2 EbFC/b-WT is 388 ± 108 nM.

Serial dilution was also performed to confirm that result and to determine binding stoichiometry (Figure 4B). This method allows estimation of the complex stoichiometry \( n \) and the \( K_d \) (32). These analyses yielded a binding stoichiometry of \( n = 2.0 \) EbFC monomers per DNA molecule, indicating that EbFC binds DNA as a dimer (Figure 4C). DNA-binding analyses of the \( E. coli \) and \( H. influenzae \) EbFC orthologs yielded that same result (Cooley, A. E., et al., submitted for publication). These data are also consistent with the observed formation of \( B. burgdorferi \) EbFC dimers in solution (17) and crystallization of the \( E. coli \) and \( H. influenzae \) orthologs as homodimers (18) and (http://www.rcsb.org/pdb/explore.do?structureId=1PUG). By this technique, the calculated \( K_d \) for dimeric recombinant EbFC binding to Perp Operator 2 DNA was 313 nM, consistent with the results obtained from our other analyses described above.

DNA-binding by EbFC is context independent

All of the probes used in the above studies contained EbFC binding site(s) in the context of PerpAB (Figure 1).
The effects of surrounding DNA sequences on EbfC binding were also examined through use of constructs created by insertion of short DNA sequences into the TA-cloning site of plasmid pCR2.1. Labeled probes were produced from those constructs by PCR using the vector’s M13F and M13R priming sites, and consisted of the DNA insert flanked by pCR2.1 DNA. That DNA is relatively rich in G + C content as compared with the high A + T content of PerpAB and the rest of the B. burgdorferi genome [www.invitrogen.com/content/sfs/vectors/pcr2_1topo_map.pdf and (15,33)]. Inclusion of a 29 bp sequence of PerpAB that contained two GTnAC palindromes yielded strong EbfC binding (Figure 5B, lanes 1–3 [b-SRK-A]). As a control, the same sequence in which both EbfC-binding sites were changed to 5’-ACAC-3’ did not form the specific EbfC–DNA complex 2 (Figure 5B, lanes 4–6 [b-SRK-B]). A single EbfC-binding site on either a 13 or 7 bp insert yielded production of the specific EbfC–DNA complex 2 (Figure 5B, lanes 7–9 [b-SRK-C] and 10–12 [b-SRK-D], respectively). Thus, we conclude that EbfC is able to bind GTnAC sequences regardless of the composition of surrounding DNA, and is therefore likely to bind any consensus sequence within the B. burgdorferi genome.

**Binding of EbfC affects DNA bending**

DNA-binding proteins often change the conformation of targeted DNA. To examine whether or not EbfC-binding alters DNA conformation, its effects upon erpAB 5’ DNA were examined.

Five overlapping 150 bp fragments of PerpAB were produced, spanning a 250 bp region of erpAB centered on the two high-affinity EbfC-binding sites (Figure 6A). Modeling analyses of this 250 bp sequence predicted an extended region of DNA curvature that includes the promoter −35 and −10 regions (Figure 6A). Polyacrylamide electrophoresis of the five 150 bp fragments confirmed that prediction, probes 4 and 5, which contain the predicted bent region toward their centers, migrated more slowly than did probe 1, which does not contain the predicted bent region (Figure 6B and C). This is the first reported occurrence of naturally bent DNA in a spirochete, and its juxtaposition with PerpAB suggests that the bend may impact upon levels of erp transcription.

The effect of EbfC on the innate bend in PerpAB was next examined by EMSA. EbfC complexes with DNA probes 2, 3 and 4 all migrated at the same rates, while free probe 4 was retarded relative to probes 2 and 3, as above (Figure 6D). Thus, binding of EbfC to erp Operator 2 alters the conformation of PerpAB DNA.

The ‘tweezer’ domain of EbfC is vital for DNA binding

The EbfC ortholog of H. influenzae KW20 Rd (YbaB, HI_0442) shares 27.5% identity and 59.3% similarity with B. burgdorferi EbfC (Figure 7A). As noted in the Introduction section, both the H. influenzae and E. coli EbfC orthologs have been crystallized, with both forming nearly identical structures with a ‘tweezer’-like appearance (18). The predicted gap between the ‘tweezer’ arms of EbfC ranges between 15 and 22 Å, comparable with the
diameter of B-DNA, suggesting that the protruding $\alpha$-helices could form the DNA-binding domain (18,34).

To address that hypothesis, site directed mutagenesis of

$B. burgdorferi$ EbfC was performed to individually replace nine of the amino acids in those $\alpha$-helices (Figure 7A and B). Each recombinant protein was expressed and purified two separate times, to guard against preparation artifacts. None of the mutated variants of EbfC was able to detectably bind $P_{erpaB}$, even when tested at protein concentrations 6.5-fold greater than the wild-type EbfC (Figure 7C).

Size exclusion chromatography demonstrated that wild-type EbfC and the K16A, D20A, K23A, D78A, K82A, K84A, E85A and K88A mutants all preferentially formed dimers in solution (data not shown) (17). The N77A mutant protein did not form higher order structures, indicating that this residue, which is predicted to be located adjacent to the $\beta$-folded region of EbfC, plays a role in dimerization. These results indicate that the dimerization and DNA binding functions of EbfC are distinct, with the DNA-binding function residing in the $\alpha$-helical ‘tweezer’ domains.

EbfC also forms tetramers and octamers in solution (17). The K16A, D20A, K23A, D78A, K82A, K84A, E85A and K88A mutants all retained ability to form those higher ordered multimers (data not shown), indicating that all aspects of EbfC multimerization are independent of its ability to bind DNA.

EbfC is conserved among Lyme disease spirochetes

The bacteria that cause Lyme disease have been divided into several genospecies, including $B. burgdorferi$ (sensu stricto) and $B. afzelii$, $B. garinii$ and $B. bissetti$, each of which is associated with different symptoms in humans and degrees of infectivity (35–37). To ascertain the degrees of EbfC sequence conservation among Lyme disease spirochetes, $ebfC$ loci were sequenced from several strains of distinct borrelial genospecies, isolated from across North America and Eurasia. All of the examined bacteria encode an identical EbfC protein, suggestive of constraints upon sequence variation and indicating that the results obtained from the present work on the EbfC protein of $B. burgdorferi$ strain B31 are applicable to all Lyme disease bacteria.
A previous study from our laboratories discovered that \emph{B. burgdorferi} EbfC is a DNA-binding protein. Orthologs of EbfC are encoded by a large number of other bacterial species, suggesting a kingdom-wide, conserved function for this protein. Analyses of crystallized EbfC orthologs of \emph{H. influenzae} and \emph{E. coli} revealed a unique, ‘tweezer’ like structure. The present studies indicated that the extending \alpha-helices that comprise the ‘tweezer’ arms are involved in binding DNA, a feature that suggests the EbfC family to constitute a new type of DNA-binding protein. EbfC bound to DNA as a dimer, consistent with crystallographic data from the \emph{H. influenzae} and \emph{E. coli} orthologs.

The present studies revealed that \emph{B. burgdorferi} EbfC preferentially binds DNA containing the sequence GT\textsuperscript{n}AC, where \(n\) = any nucleotide. This sequence occurs 833 times on the major chromosome of \emph{B. burgdorferi} strain B31, a frequency of one site every 1093 base pairs. The \emph{B. burgdorferi} EbfC can also bind DNA which lacks a GT\textsuperscript{n}AC sequence. These features suggest that EbfC is not a gene-specific regulatory protein, as originally hypothesized (16,17). Instead, this protein’s simple and abundant binding sequence, abilities to bind DNA nonspecifically and to alter DNA conformation, plus its small size, are characteristics shared with bacterial nucleoid-associated proteins (38,39). EbfC forms tetramers and octamers in solution, and mutations that prevent DNA-binding did not disrupt ability to form those higher ordered structures. Thus the multimerization and DNA-binding activities of EbfC are distinct, raising the possibility that EbfC might also multimerize when bound to DNA and thereby serve as a bridge to condense DNA. The genome of \emph{B. burgdorferi} is the most complex bacterial genome yet to be analyzed. The sequenced genome of the type strain, B31, consists of a linear major chromosome and more than 20 other linear and circular smaller chromosomes/plasmids/episomal prophages (15,33,40). The mechanisms by which this bacterium facilitates maintenance, partitioning and compaction of this complex genome are largely unknown (41). The well studied \emph{E. coli} compacts its chromosome through DNA supercoiling and interactions with small DNA-binding proteins, the nucleoid-associated or ‘histone-like’ proteins (38,39,42,43). To date, 12 major nucleoid-associated proteins have been identified in \emph{E. coli} (38,39,42). In addition to functioning in chromatin organization and compaction, nucleoid-associated proteins often play roles in a variety of processes, including stress response, chromosome segregation, and transcription regulation. Therefore, it is likely that EbfC contributes to the unique genomic organization and dynamic behavior of \emph{B. burgdorferi}, which is adapted for its specialized lifestyle in the ticks and subsequent transmission to vertebrates by tick bites.
of other DNA processes, such as modulation of transcription, transposition, recombination, repair and DNA replication (38,44–46). The combination of structural and regulatory roles suggests that the global genome functions of the nucleoid are tightly linked to its organizational ability (47,48). Prior to this work, only two B. burgdorferi proteins have been identified which exhibit activities resembling those of nucleoid-associated proteins. The B. burgdorferi Hbb protein, which resembles E. coli HU and integration host factor (IHF), can bind and bend DNA, and is capable of complementing some functions of an E. coli HU/IHF mutant (49–51). A second known protein, Gac, is produced from independent synthesis of the carboxy-terminal domain of GyrA, and can complement an E. coli HU mutant to promote Mu DNA transposition (52). The roles of those two putative nucleoid-associated proteins in maintaining B. burgdorferi DNA have not yet been defined. The B. burgdorferi genome does not contain any recognizable homologs of known bacterial nucleoid-associated proteins such as H-NS or Fis (33). Thus, the current picture is that B. burgdorferi compacts its segmented genome, which consists largely of linear DNAs, using only Hbb, Gac and, we hypothesize, EbfC.

The B. burgdorferi ebfC is located immediately 3′ of dnaX, and the two genes are transcriptionally linked [our unpublished results and (17,33)]. Thus, EbfC protein levels are likely linked to those of the dnaX products, the γ and τ subunits of DNA polymerase III. If additional analyses confirm that EbfC is in fact a nucleoid-associated protein, then the connection between DNA replication and DNA conformation afforded by the dnaX-ebfC locus may prove central to the vitality of B. burgdorferi. Consistent with that hypothesis, repeated attempts by our laboratories and others to inactivate B. burgdorferi ebfC have been unsuccessful (P. Stewart, personal communication), suggesting that EbfC may be an essential borrelial protein.

Almost all components of the B. burgdorferi segmented genome contain the same frequency of EbfC consensus-binding sites, with a site approximately every 630–650 bp. The cp32 family members are distinctive in having an EbfC-binding sequence every 630–650 bp. The erp Operator 2 sites are unique in being the only locations in B. burgdorferi where two GTnAC sites are found in close proximity. The present studies demonstrated that EbfC exhibits a greater affinity for DNA containing two GTnAC sites than for DNA with only one site. Many bacterial nucleoid-associated proteins influence gene transcription levels as consequences of the locations of preferred binding sites relative to promoters (43,44,53,54). Noting that ebfC transcription is linked to that of DNA polymerase III subunits, and erp genes are maximally expressed during periods of rapid bacterial division, we hypothesize that cellular EbfC levels could serve as a signal of bacterial replication rates to influence expression of Erp lipoproteins. High-affinity EbfC-binding sequences are also scattered throughout the B. burgdorferi genome. Although some sites have been identified in close proximity to known transcriptional promoters (55,56), the effects of EbfC upon those genes remain to be investigated.

In conclusion, B. burgdorferi EbfC possesses both sequence-specific and sequence-independent DNA binding activities. Genes orthologous to ebfC are found in the genomes of a broad range of bacterial species, and the orthologs of E. coli and H. influenzae are also DNA-binding proteins (Cooley,A.E. et al., submitted for publication). Thus, characterization of B. burgdorferi EbfC impacts upon the entire kingdom Eubacteria. It also appears that EbfC family members interact with DNA through a unique fold, and represent a novel type of DNA-binding protein.

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