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Natural synthesis of a DNA-binding protein from the C-terminal domain of DNA gyrase A in *Borrelia burgdorferi*

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We have identified a 34 kDa DNA-binding protein with an HU-like activity in the Lyme disease spirochete *Borrelia burgdorferi*. The 34 kDa protein is translated from an abundant transcript initiated within the gene encoding the A subunit of DNA gyrase. Translation of the 34 kDa protein starts at residue 499 of GyrA and proceeds in the same reading frame as full-length GyrA, resulting in an N-terminal-truncated protein. The 34 kDa GyrA C-terminal domain, although not homologous, substitutes for HU in the formation of the Type 1 complex in Mu transposition, and complements an HU-deficient strain of *Escherichia coli*. This is the first example of constitutive expression of two gene products in the same open reading frame from a single gene in a prokaryotic cellular system.

Keywords: *Borrelia burgdorferi*/DNA gyrase/HU/IHF/Mu

Introduction

The spirochete *Borrelia burgdorferi*, a causative agent of Lyme disease, is one of a few prokaryotes with a genome composed of linear as well as circular DNA molecules (Barbour and Garon, 1987; Hinnebusch and Tilly, 1993; Saint Girons *et al.*, 1994). With a linear chromosome and up to 17 circular and linear plasmids, the *B.burgdorferi* genome may be the most complex of those found in the prokaryotes (Fraser *et al.*, 1997). Whereas the telomeres of linear replicons of some prokaryotes such as *Streptomyces* and a number of phages contain covalently attached proteins, the linear replicons of *Borrelia* have covalently closed hairpin ends (Hinnebusch and Tilly, 1993). The only other prokaryotic linear replicon identified to date containing hairpin telomeres is the prophage of coliphage N15 (Svarchevsky and Rybchin, 1984; Hinnebusch and Tilly, 1993). The presence of these unusual hairpin-containing linear replicons in the *B.burgdorferi* genome suggests that novel proteins may be involved in maintenance, replication and compaction of these molecules.

The small DNA-binding proteins HU and integration host factor (IHF) function in the macromolecular metabolism of DNA to assemble higher-order nucleoprotein complexes in other bacteria. HU and IHF, first identified for their roles in bacteriophage metabolism, are abundant in *Escherichia coli*, with concentrations of 30 000 dimers and 8500–17 000 dimers per cell, respectively (Rouviére-Yaniv and Gros, 1975; Nash *et al.*, 1977; Ditto *et al.*, 1994). HU, which exists as a dimer encoded by *hupA* and *hupB*, binds DNA non-specifically, while IHF, a dimer encoded by *himA* and *hip* (*himD*), binds at specific sequences (Nash, 1996). Many bacteria encode separate HU and IHF proteins; however, some prokaryotes, such as *Bacillus subtilis*, synthesize a single protein that functions in both roles (Micka *et al.*, 1991). Hbb, an HU/IHF homolog in *B.burgdorferi*, was identified and shown to substitute for both HU and IHF using a genetic complementation assay in *E.coli* based on bacteriophage λ growth (Tilly *et al.*, 1996).

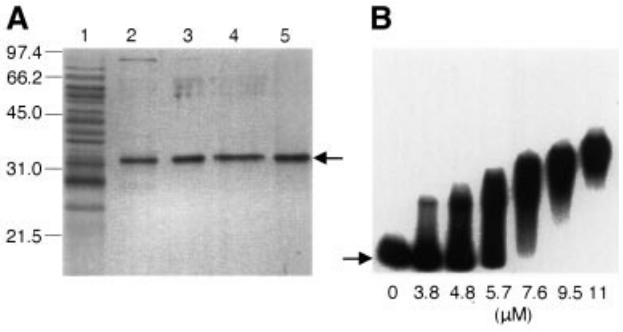
In addition to *hbb*, the recently completed sequence of the *B.burgdorferi* genome identified a limited complement of genes encoding proteins involved in DNA replication, repair and recombination, similar to the genomes of *Mycobacterium genitalium* and *Treponema pallidum* (Fraser *et al*., 1995, 1997, 1998). Included within this set of borrelial genes are single copies of *gyrA* and *gyrB*, encoding the A and B subunits of DNA gyrase (GyrA and GyrB), respectively (Huang, 1992; Fraser *et al.*, 1997). DNA gyrase, an A_2B_2 heterotetramer, is the only topoisomerase capable of introducing negative supercoiling into closed-circular DNA (Gellert *et al.*, 1976; Cozzarelli, 1980; Reece and Maxwell, 1991b; Wang, 1996). This reaction is dependent on the wrapping of DNA around the enzyme with positive writhe, which introduces negative writhe elsewhere in the molecule (Reece and Maxwell, 1991b). The domain of DNA gyrase that wraps DNA lies in the C-terminal third of GyrA (Reece and Maxwell, 1991a). Removal of this C-terminal domain abolishes the wrapping of DNA around the enzyme and eliminates the introduction of negative supercoiling, although not other enzymatic activities (Kampranis and Maxwell, 1996).

Pharmacological studies indicate the presence of a functional DNA gyrase activity in *B.burgdorferi* (Samuels and Garon, 1993). We report here that, in addition to synthesizing a functional DNA gyrase consisting of full-length GyrA and GyrB subunits, *B.burgdorferi* also synthesizes the C-terminal domain of GyrA as a distinct and abundant 34 kDa DNA-binding protein. This unique protein forms a higher-order nucleoprotein complex, substituting for HU in Mu transposition *in vitro*, and functions analogously to HU *in vivo* to support bacteriophage Mu growth in *E.coli*.

Results

Identification of the GyrA C-terminal domain

The 34 kDa GyrA C-terminal domain was identified in a biochemical screen for telomere-binding proteins in



C

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GyrA(a.a. 490-517)   YDEEVLKTSMSDLMQKENIVVMLTKKGF
                                || |||||||||||
N-terminal sequence              SDKMQKENIVVML·····
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Fig. 1. Identification of the GyrA C-terminal domain. (**A**) Purification of the 34 kDa GyrA C-terminal domain. Silver-stained 12.5% SDS–PAGE gel following the purification of the 34 kDa protein to apparent homogeneity. The clarified lysate was prepared from strain B31 (lane 1), and fractionated over heparin (lane 2), hydroxyapatite (lane 3), Mono S (lane 4) and phenyl Superose (lane 5) columns. Molecular mass standards shown in kDa. (**B**) EMSA. Increasing amounts of the 34 kDa protein incubated with an end-labeled DNA fragment. The arrow indicates migration of unbound DNA. (**C**) N-terminal sequence of the 34 kDa protein and alignment with GyrA. The N-terminal sequence is aligned with residues 490–517 of GyrA, predicted from the DNA sequence (BB0435; DDBJ/EMBL/GenBank accession No. AE001148) (Fraser *et al.*, 1997). Lines represent identity determined by sequencing; dots indicate predicted identity.

B.burgdorferi. A whole cell lysate was fractionated by heparin column chromatography, and fractions were screened for telomere-binding activity by electrophoretic mobility shift assays (EMSAs) using a sub-telomeric DNA fragment (Figure 1A and B). A DNA-binding activity localized to fractions containing a 34 kDa protein, which was further purified to apparent homogeneity (Figure 1A). N-terminal sequencing identified the 34 kDa protein as the GyrA C-terminal domain, encompassing residues 500–810 of the full-length GyrA protein (Figure 1C).

The large amount of the 34 kDa protein isolated, and the presence of a methionine codon, which could serve as a translational start codon, directly upstream of the N-terminal serine residue (Figure 1C) suggested that the protein was synthesized as a distinct protein independent of full-length GyrA. To determine whether the GyrA C-terminal domain was present in intact cells, or whether the protein arose as a result of proteolytic cleavage during purification, whole cell *B.burgdorferi* lysates were examined by immunoblotting analysis using a polyclonal antiserum to a recombinant *B.burgdorferi* GyrA C-terminal domain (Figure 2). The antiserum detected the 34 kDa GyrA C-terminal domain, and an ~91 kDa protein, predicted to be full-length GyrA. The 34 kDa protein was present in crude lysates at a 5- to 20-fold higher level than the 91 kDa protein. The cellular concentration of the GyrA C-terminal domain was estimated to be 34 000 molecules per cell based on regression analysis of a standard curve generated from immunoblots with known concentrations of the GyrA C-terminal domain (data not shown).

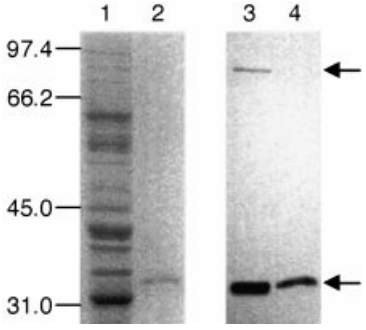

Fig. 2. Western analysis. Proteins were resolved by 7.5% SDS–PAGE. Lanes 1 and 3, whole cell *B.burgdorferi* lysate; lanes 2 and 4, purified GyrA C-terminal domain. Lanes 1 and 2 were stained with Coomassie Brilliant Blue. Lanes 3 and 4 were Western blotted and probed with anti-GyrA C-terminal domain antiserum. The arrows indicate the putative full-length 91 kDa GyrA (top) and the 34 kDa GyrA C-terminal domain (bottom). Molecular mass standards shown in kDa.

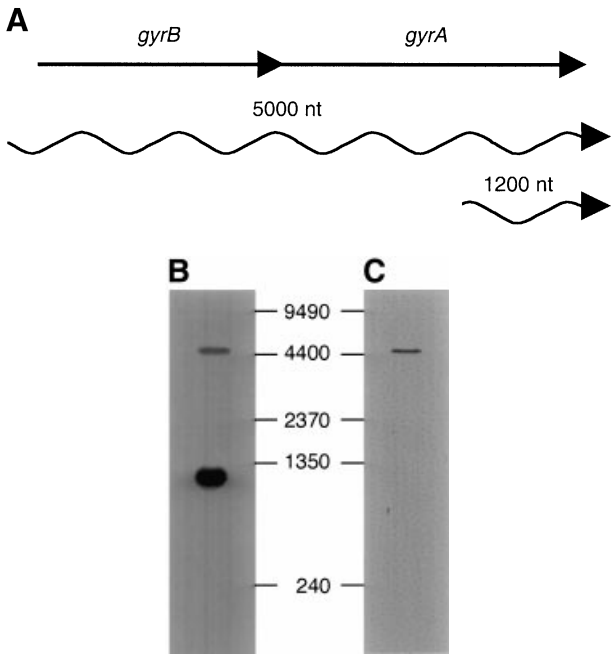

Fig. 3. Northern analysis. (**A**) Schematic of *gyrB* (2.0 kb) and *gyrA* (2.4 kb) genes located on the chromosome, the bicistronic transcript (5000 nucleotides) encoding both GyrB and GyrA proteins, and the monocistronic transcript (1200 nucleotides) encoding the 34 kDa protein (DNA shown as solid arrow, RNA as wavy arrow). (**B**) Northern blot of total cellular RNA probed with a PCR product specific for the 3′ end of *gyrA* encoding the 34 kDa GyrA C-terminus. (**C**) Northern blot of total cellular RNA probed with a PCR product specific for the *gyrB* coding sequence. Molecular size standards shown in nucleotides.

Analysis of the transcripts encoding the GyrA C-terminal domain

To determine whether the 34 kDa protein was expressed in *B.burgdorferi* as a novel gene product, we used Northern analysis to identify a transcript encoding the GyrA C-terminal domain. When using a probe specific for the 3′ end of *gyrA*, encoding the C-terminal 310 amino acid residues, two separate transcripts were detected: a 1200 nucleotide and a 5000 nucleotide transcript (Figure 3A and B). The 1200 nucleotide transcript is large enough to encode the GyrA C-terminal domain (310 amino

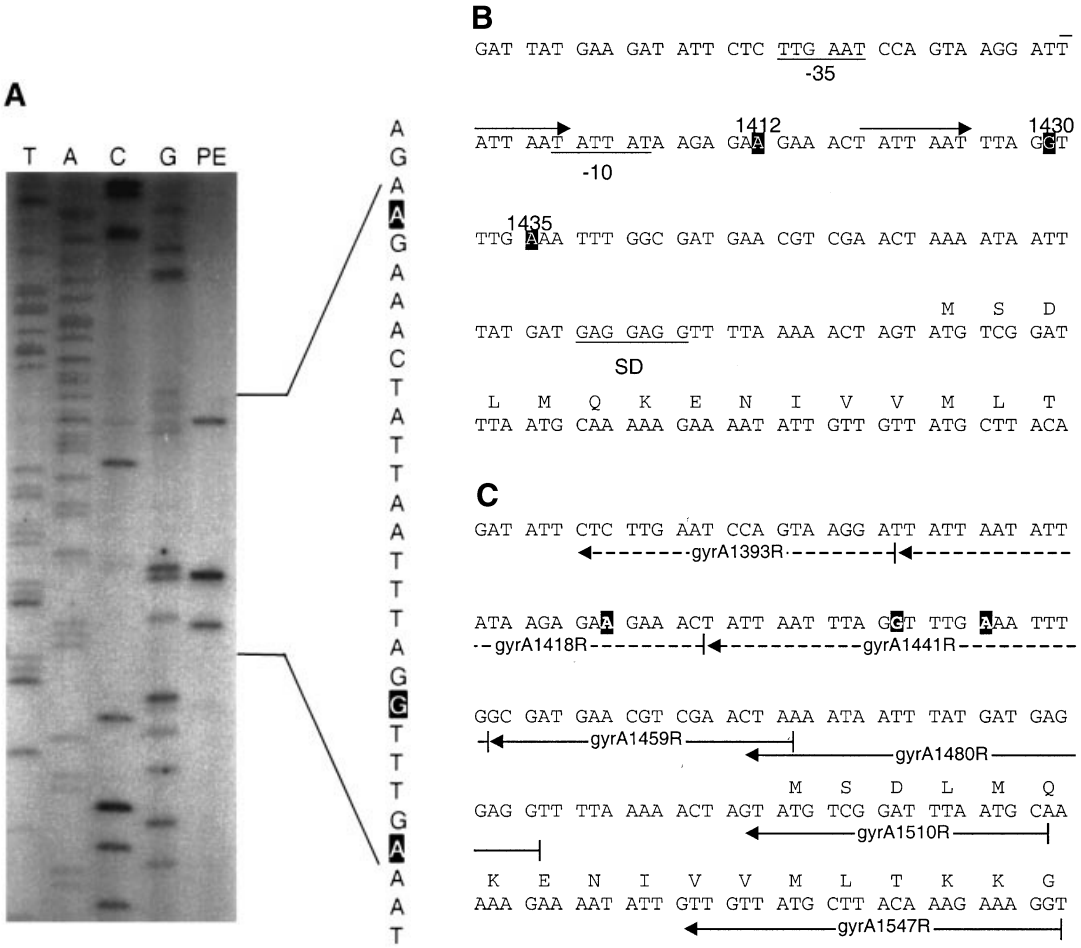

Fig. 4. Transcriptional start site identification. (**A**) Primer extension mapping of three RNA 5′ ends using primer gyrA1510R. Transcriptional start sites are indicated in inverted typeface. Two separate primers (gyrA1510R and gyrA1547R) identified the same three 5′ ends in three independent isolations of B31 RNA (data for gyrA1547R not shown). (**B**) Putative regulatory sequences. Transcriptional start sites are numbered and in inverted typeface. Possible –10, –35 and Shine–Dalgarno (SD) sequences are underlined. Two direct repeats are indicated with arrows. Amino acid residues of the GyrA C-terminal domain coding sequence are indicated above the appropriate codon. (**C**) Schematic representation of oligonucleotide probe hybridization. Nucleotides 1369–1548 of *gyrA* are shown. Arrows indicate oligonucleotides complementary to the sense strand. Solid arrows indicate oligonucleotides that hybridize with the 1200 nucleotide transcript, and dashed arrows indicate oligonucleotides that do not hybridize with the 1200 nucleotide transcript. Transcriptional start sites determined by primer extension are shown in inverted typeface. Amino acid residues of the GyrA C-terminal domain are indicated above the appropriate codon.

acids); however, it is not large enough to encode full-length GyrA (810 amino acids). A probe specific for the 5′ end of *gyrA*, encoding the N-terminal domain of GyrA, only hybridized with the 5000 nucleotide transcript (data not shown). We hypothesize that the larger transcript is a bicistronic message encoding both GyrB (634 amino acids) and GyrA. This is supported by the hybridization of a *gyrB*-specific probe to the 5000 nucleotide transcript (Figure 3C). As shown in Figure 3A, *gyrB* is located directly upstream of *gyrA*, with only 14 bp separating the two genes (Huang, 1992; Fraser *et al.*, 1997). The 1200 nucleotide transcript is expressed at a 4-fold higher level than the larger bicistronic message as assayed by quantifying the hybridization signals. We have also identified the GyrA C-terminal domain-specific transcript in a number of other species of *Borrelia* including *B.garinii*, *B.afzelii* and the relapsing fever spirochete *B.hermsii*, all of which contain predominantly linear genomes (data not shown).

Transcriptional start site analysis of the GyrA C-terminal domain-specific mRNA

Primer extension analysis was used to locate three putative transcriptional start sites for the 1200 nucleotide transcript located at nucleotides 1412, 1430 and 1435 of *gyrA* (Figure 4A). The presence of multiple transcriptional start sites is not uncommon in either *B.burgdorferi* genes or small DNA-binding protein genes from other prokaryotes (Kohno *et al.*, 1990; Marconi *et al.*, 1993; Tilly *et al.*, 1996). Although promoter sequences in *B.burgdorferi* have not been functionally defined, only the transcriptional start site at nucleotide 1412 has upstream sequences that resemble σ70 consensus promoters (Figure 4B). The absence of a consensus promoter sequence upstream of transcriptional start sites at nucleotides 1430 and 1435 is perhaps not unexpected, considering that the regions upstream of all three transcriptional start sites not only serve as regulatory sequences for transcription, but must maintain the open reading frame for full-length GyrA.

Two direct repeats of TATTAAT are also found in the promoter region (Figure 4B).

To confirm that the three sites identified were in fact 5′ ends of transcripts, we used a series of oligonucleotides flanking the three sites as probes in Northern hybridizations (Figure 4C). Five oligonucleotide probes complementary to regions downstream of the transcriptional start site at nucleotide 1435 all hybridized with both the 5000 nucleotide and 1200 nucleotide transcripts. However, two oligonucleotide probes complementary to regions upstream of transcriptional start sites at nucleotides 1430 and 1435 hybridized only with the 5000 nucleotide transcript. Oligonucleotide probe gyrA1441R, complementary to the region directly downstream of the transcriptional start site at nucleotide 1412, also failed to hybridize with the 1200 nucleotide transcript, suggesting that nucleotide 1412 is not used as often as the other two transcriptional start sites under standard culture conditions. These data are consistent with the primer extension data, which indicates that nucleotide 1430 serves as the major transcriptional start site (Figure 4A). The identified promoter region including all three transcriptional start sites for the 1200 nucleotide transcript functions in *E.coli* to regulate expression of a reporter gene (unpublished data) and to produce a functional GyrA C-terminal domain as described below (Figure 6).

Nucleoprotein complex formation

EMSAs were used to examine DNA-binding activity of the GyrA C-terminal domain. As the amount of protein added to the binding reaction was increased, the mobility of the DNA–protein complex was further retarded (Figure 1B). This indicates that multiple protein molecules are capable of binding to each DNA molecule. Similar results were obtained using the *ospAB* promoter sequence from the 50 kb linear plasmid, an *ospC* coding sequence from the 26 kb circular plasmid and synthetic telomere sequences from the 17 kb linear plasmid, suggesting that the DNA-binding activity of the protein is non-specific (data not shown).

Based on the abundance of the GyrA C-terminal domain and its non-specific DNA-binding activity, we hypothesized that the protein had an analogous function to the small DNA-binding protein HU of *E.coli*. An *in vitro* Mu transposition assay was used to test this hypothesis. The Type 1 complex is an early intermediate in the transposition reaction, and its formation is stringently dependent on the presence of HU (Craigie *et al.*, 1985). The GyrA C-terminal domain substituted for HU in the *in vitro* reaction, as assayed by the formation of the Type 1 complex in Mu transposition (Figure 5A). The protein was capable of promoting the donor-cleavage reaction over a wide range of concentrations (Figure 5B); however, IHF was required for the GyrA C-terminal domain to substitute for HU in the reaction (data not shown).

Complementation of an HU mutant

Genetic manipulation of *B.burgdorferi* is in the initial stages of development, which have been slowed by a complex undefined medium and the availability of only one selectable marker (Samuels *et al.*, 1994). Therefore, we investigated the *in vivo* activity of the GyrA C-terminal domain in a more experimentally tractable system. We

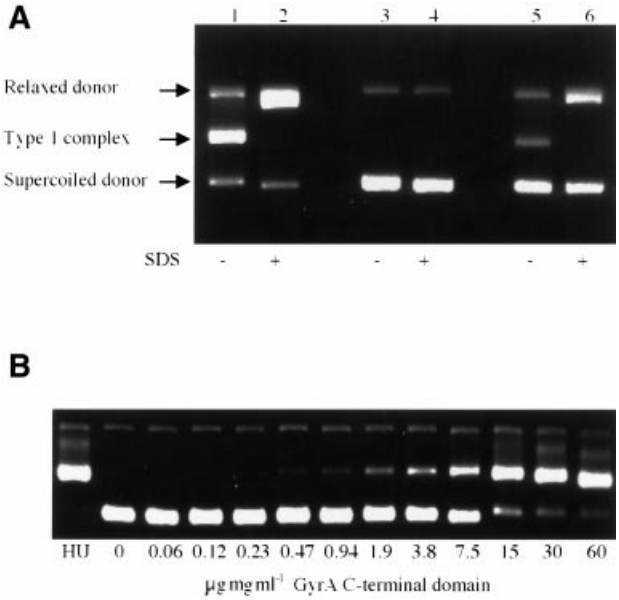

Fig. 5. Mu donor-cleavage reaction. (**A**) Substitution of the GyrA C-terminal domain for HU. The supercoiled donor plasmid is converted to the Type 1 complex in the presence of HU, Mu A and IHF. Mu A introduces two single-strand nicks into the supercoiled plasmid. The introduction of single-strand nicks does not result in a relaxed plasmid, due to the complex formed with Mu A, HU and IHF, which separates the Type 1 complex into two separate topological domains: relaxed and supercoiled. In the presence of SDS, the proteins dissociate from the Type 1 complex, resulting in a nicked open circular plasmid. Lanes 1 and 2, with HU; lanes 3 and 4, without HU; lanes 5 and 6, with GyrA C-terminal domain substituting for HU. (**B**) Titration of the GyrA C-terminal domain. Increasing amounts of the GyrA C-terminal domain were substituted for HU in the Mu donor-cleavage reaction at the indicated concentrations. Lane HU contains standard reaction components with HU at 7.5 μg/ml.

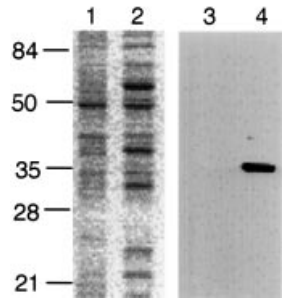

Fig. 6. Expression of the *B.burgdorferi* GyrA C-terminal domain in *E.coli*. Lanes 1 and 3, whole cell *E.coli* strain A5196 lysate; lanes 2 and 4, whole cell *E.coli* strain A5196 containing plasmid pTASK1. Lanes 1 and 2 were stained with Coomassie Brilliant Blue. Lanes 3 and 4 were examined by Western analysis. Molecular mass standards shown in kDa.

examined the ability of the gene encoding the GyrA C-terminal domain to complement a bacteriophage Mu plating deficiency in an HU-deficient strain of *E.coli* (A5196). HU, which is encoded by the *hupA* and *hupB* genes, is required for bacteriophage Mu to form plaques on *E.coli*. The GyrA C-terminal domain was expressed in *E.coli* A5196 from plasmid pTASK1 using the natural *B.burgdorferi* promoter within the *gyrA* gene. Synthesis of the GyrA C-terminal domain in *E.coli* was confirmed by Western analysis (Figure 6). *Escherichia coli* containing pTASK1 restored Mu growth as assayed by efficiency of

Table I. Bacteriophage Mu growth

Strain	plasmid	e.o.p.
N99	–	1
A5196 *hupA hupB*	–	$<4\times10^{-5}$
A5196 *hupA hupB*	pTASK1	1

A5196 relevant genotype: *hupA16*::*kan hupB11*::*cat* (Mendelson *et al.*, 1991); N99: isogenic parent strain. pTASK1: pCR2.1-TOPO with insert encoding *B.burgdorferi* GyrA C-terminal domain and endogenous promoter region.

plating (e.o.p.), which measured the ability of bacteriophage Mu to form plaques on the complemented *E.coli hupA hupB* mutant (Table I). Bacteriophage Mu readily formed plaques on wild-type *E.coli*, but e.o.p. was severely reduced in the *E.coli hupA hupB* mutant. The gene encoding the GyrA C-terminal domain complemented the *hupA hupB* mutant, restoring the Mu plating deficiency to wild-type levels.

Discussion

The GyrA C-terminal domain in *B.burgdorferi* is the first example of a bacterium naturally synthesizing a domain of DNA gyrase as a separate protein, distinct from the subunits found in the holoenzyme. The synthesis of the GyrA C-terminal domain is controlled by an expression system rarely found in a prokaryotic cellular genome: a smaller protein encoded on a separate transcript that is initiated within a full-length gene and translated in the identical reading frame as a larger protein. In *B.burgdorferi*, translation of the GyrA C-terminal domain is initiated at Met499 of GyrA, and transcription of the 1200 nucleotide mRNA encoding the protein is initiated from three separate start sites located within *gyrA*. The three start sites may be a means to regulate expression of the gene encoding the 34 kDa protein by using different start sites under different conditions. Based on the Northern and primer extension data, we predict that nucleotides 1430 and 1435, which are separated by one turn of the helix, are the two predominant start sites.

Expression of the gene encoding the 34 kDa protein may be complex considering that the promoter for the gene is located within *gyrA*, which is expressed at an ~4-fold lower level than the gene encoding the GyrA C-terminal domain. The GyrA C-terminal domain gene is also located within a gene (*gyrA*) that is regulated, in *E.coli*, by the level of DNA supercoiling (Menzel and Gellert, 1987). Adding to the complexity associated with transcription being initiated within another gene is the possibility that translation of the 34 kDa protein may be initiated from both the 1200 nucleotide transcript and the larger 5000 nucleotide bicistronic message.

As unexpected as the synthesis of this unique protein is the ability of the GyrA C-terminal domain to substitute for HU *in vitro* and complement an HU-deficient mutant of *E.coli in vivo*. Initial stages of Mu transposition both *in vitro* and *in vivo* require HU (Craigie *et al.*, 1985; Huisman *et al.*, 1989). The formation of the Type 1 complex necessitates the assembly of a multi-protein complex consisting of Mu A, HU and IHF on a supercoiled donor molecule (Chaconas *et al.*, 1996). We demonstrate here that the GyrA C-terminal domain from *B.burgdorferi* substitutes for HU in forming the Type 1 complex in Mu transposition. The eukaryotic protein HMG1 will also substitute efficiently for HU in this reaction (Lavoie and Chaconas, 1994). HU and HMG1, although from different domains of life, share similar activities both *in vitro* and *in vivo* (Bianchi, 1994). The efficient substitution of the *B.burgdorferi* GyrA C-terminal domain for HU in the Mu donor-cleavage reaction indicates a specific HU-like activity for the protein found only in the functionally related proteins HU and HMG1.

In *E.coli*, a 33 kDa protein consisting of the GyrA C-terminal domain can be generated by proteolytic cleavage of GyrA or by expression of a recombinant gene product (Reece and Maxwell, 1989, 1991a). The naturally occurring protein from *B.burgdorferi* and the homologous region from the *E.coli* GyrA are biochemically distinct, sharing only 24% identity at the amino acid level. The GyrA C-terminal domain from *E.coli* is acidic with a predicted isoelectric point (pI) of 4.0; in contrast, the naturally occurring 34 kDa protein from *B.burgdorferi* is basic with a predicted pI of 9.1. Like the GyrA C-terminal domain of *B.burgdorferi*, the 33 kDa protein from *E.coli* binds DNA non-specifically, with multiple protein molecules binding each DNA molecule (Reece and Maxwell, 1991a). Despite similar DNA-binding activities, the recombinant 33 kDa GyrA C-terminal domain from *E.coli* was not capable of substituting for HU under standard reaction conditions in the *in vitro* Mu donor-cleavage reaction (data not shown), indicating that the *B.burgdorferi* GyrA C-terminal domain possesses a unique HU-like function that is not merely a consequence of binding DNA non-specifically.

The GyrA C-terminal domain not only forms higher-order nucleoprotein complexes *in vitro*, but the gene encoding the GyrA C-terminal domain complements an *E.coli hupA hupB* mutant as measured by bacteriophage Mu growth. This indicates that the protein also functions in forming these assemblies *in vivo*. The gene previously identified as encoding the HU/IHF homolog in *B.burgdorferi*, *hbb*, is incapable of complementing the defect in Mu growth found in *hupA hupB E.coli* strains (Tilly *et al.*, 1996). However, *hbb* complements an HU/IHF-deficient mutant as assayed by restoring a bacteriophage λ packaging deficiency (Tilly *et al.*, 1996). Whereas λ growth requires either HU or IHF, Mu growth has a strict requirement for HU (Huisman *et al.*, 1989; Mendelson *et al.*, 1991). The previously identified HU/IHF homolog, Hbb, may be synthesized at much lower levels than the GyrA C-terminal domain, since extensive attempts to purify Hbb from *B.burgdorferi* have been unsuccessful (Tilly *et al.*, 1996). In addition, immunoblot analyses with an antibody against a synthetic peptide reveal little Hbb protein in cell lysates (S.W.Knight, C.Schwanke and D.S.Samuels, unpublished data). We report here that the GyrA C-terminal domain is an abundant non-specific DNA-binding protein in *B.burgdorferi* with an activity and a cellular concentration similar to HU. Taken together, these data suggest that Hbb, although it shares sequence homology with HU and IHF, may have a more limited role in DNA metabolism than homologs found in other prokaryotes, and that the GyrA C-terminal domain may provide the major HU-like activity in *B.burgdorferi*.

The GyrA C-terminal domain has not been identified as a naturally synthesized DNA-binding protein in any other prokaryote outside of the *Borrelia*. However, the activities of DNA gyrase and HU are linked. HU stimulates DNA gyrase decatenation activity *in vitro* (Marians, 1987) and HU suppressor mutations have been mapped to *gyrB* (Malik *et al.*, 1996). DNA gyrase and HU alter the superhelical path of the DNA helix using different mechanisms. DNA gyrase affects DNA topology by changing the linking number, and HU affects DNA writhe by bending or wrapping the helix. The *Borrelia* have apparently been able to join the supercoiling and bending activities of these two proteins more intimately by synthesizing the DNA gyrase domain partially responsible for wrapping DNA as a separate protein with an HU-like activity. We speculate that this unique protein evolved as an adaptation for the presence of linear hairpin-containing replicons. Alternatively, the unique expression of the GyrA C-terminal domain gene from *gyrA* may be a means for *Borrelia* to compensate for its small genome size of ~1.2 Mb by expressing domains of larger proteins as distinct functional products.

Materials and methods

GyrA C-terminal domain purification

A crude lysate of high passage *B.burgdorferi* strain B31 was prepared from an 18 l culture grown in BSK-H medium (Sigma) at 32°C. Nucleic acid was precipitated and the lysate was clarified as previously described (Margolis and Samuels, 1995). The clarified lysate was dialysed against 20 mM Tris–HCl pH 8 (the pH of Tris solutions was measured at 25°C), 0.5 mM EDTA, 10% glycerol (H buffer) and loaded onto a 5 ml Econo-Pac heparin cartridge (Bio-Rad). The column was eluted with a 100 ml linear gradient from 0–1 M NaCl in H buffer at 2 ml/min. Fractions containing the 34 kDa protein were dialysed against 10 mM sodium phosphate pH 6.8, in 10% glycerol and loaded onto a 2 ml CHT2-I hydroxyapatite column (Bio-Rad). The column was eluted with a 20 ml linear gradient from 10 to 500 mM sodium phosphate pH 6.8, in 10% glycerol, followed by a 10 ml linear gradient from 500 to 1000 mM sodium phosphate pH 6.8, in 10% glycerol at 1 ml/min. Fractions containing the 34 kDa protein were dialysed against 10 mM sodium phosphate pH 6.8, 0.5 mM EDTA, 10% glycerol (P buffer) and loaded onto a 1 ml Mono-S column (Pharmacia). The column was eluted with a 15 ml linear gradient from 0 to 0.5 M NaCl in P buffer, followed by a 5 ml linear gradient from 0.5 to 1 M NaCl in P buffer at 1 ml/min. Fractions containing the 34 kDa protein were pooled and mixed with an equal volume of 3.4 M $(NH_4)_2SO_4$, 100 mM sodium phosphate pH 7, 10% glycerol, 1 mM EDTA, and loaded onto a 1 ml phenyl Superose column (Pharmacia). The column was eluted with 15 ml of a 1.7–0 M $(NH_4)_2SO_4$ and 50–10 mM sodium phosphate pH 7, linear gradient in 10% glycerol, 1 mM EDTA at 0.5 ml/min. Fractions containing the 34 kDa protein were dialysed against 50 mM Tris–HCl pH 7.5, 0.1 M KCl, 0.1 mM EDTA, 5 mM DTT, 50% glycerol and stored at –20°C.

Electrophoretic mobility shift assay

A 255 bp PCR product amplified from the 17 kb linear plasmid beginning 23 bp from the left end was used as an EMSA substrate. The substrate was prepared as previously described (Margolis and Samuels, 1995) using the following primers kindly provided by K.Tilly: 10 pmol of TL16g (5′-AGACTAATAAAATAATGAATA-3′) end-labeled with [γ-^{32}P]dATP and 25 pmol of TL16h (5′-GTATTTTGACTCAAA-ACTTTA-3′). Approximately 2.1 µg of labeled DNA was incubated at 25°C for 30 min with the 34 kDa GyrA C-terminal domain in 10 mM HEPES pH 7.5, 90 mM KCl, 300 µg/ml BSA, 200 µg/ml poly(dI-dC), 10% glycerol. DNA–protein complexes were resolved by electrophoresis through a 4% polyacrylamide gel and visualized by autoradiography.

Immunoblots

Whole cell *B.burgdorferi* lysates were prepared by boiling cells in SDS–PAGE loading buffer (125 mM Tris–HCl pH 6.8, 4% SDS, 20% glycerol, 1.4 M 2-mercaptoethanol and 0.2% bromophenol blue). Samples were fractionated by 7.5% SDS–PAGE. For Western analysis, samples were transferred to a PVDF membrane and incubated with rabbit antiserum raised to a recombinant GyrA C-terminal domain, followed by goat antibody to rabbit immunoglobulin G conjugated with horseradish peroxidase. The size and intensity of bands were determined using a Gel Doc 1000 system with Multi Analyst software (Bio-Rad).

RNA analysis

Total RNA was isolated from cultures using TRIzol™ reagent as described by the manufacturer (Gibco-BRL). Total RNA (15 µg) was fractionated on 1.2% formaldehyde–agarose gels and transferred to nylon membranes. Hybridization with oligonucleotide probes (Marconi *et al.*, 1993) and with PCR probes (Brown, 1996) was performed as previously described. The intensity and size of bands were determined using an Image Acquisition and Analysis system (Ambis) and a Gel Doc 1000 system with Multi Analyst software (Bio-Rad), respectively.

Primer extension was used to map the RNA 5′ ends for the 1200 nucleotide transcript encoding the GyrA C-terminal domain. One picomole of end-labeled primer was incubated with 7 µg of total cellular RNA in 50 mM KCl, 10 mM Tris–HCl pH 8.3, 5 mM $MgCl_2$, 1 mM each dNTP and 20 U RNase inhibitor. Extension was performed at 42°C with 50 U of MuLV reverse transcriptase. Extension products were resolved on a 6% polyacrylamide sequencing gel, and sizes were determined by use of a DNA sequencing ladder. Two different primers were used in the primer extension assay: gyrA1510R (5′-GCATTAAATCCGACATAC-3′) and gyrA1547R (5′-CCTTTCTTTGTAAGCATAACAAC-3′).

Mu donor cleavage reaction

Mini-Mu donor plasmid pBL07, HU, IHF and Mu A were kindly provided by G.Chaconas. Type 1 *in vitro* reactions were performed as previously described with all reaction components at twice the standard concentrations (Naigamwalla and Chaconas, 1997). The GyrA C-terminal domain, when substituted for HU, was used at a concentration of 7.5 µg/ml or as indicated. After incubation at 30°C for 5 min, the reactions were divided into equal aliquots and 1% SDS was added to one aliquot. Reactions were resolved on 1% agarose gels in 1× TAE buffer, stained with ethidium bromide (0.5 µg/ml) for 30 min and destained in H_2O for 30 min.

Complementation

The portion of the *gyrA* gene encoding the GyrA C-terminal domain, including the promoter, was cloned into the plasmid vector pCR2.1-TOPO (Invitrogen). Primers gyrA1213F (5′-AAAGATGCAAGGGAGAG-GC-3′) and gyrA2433R+*Sph*I (5′-AGCATGCTTATTTAATAA-ATTTTGA-3′) were used to PCR amplify nucleotides 1213–2433 of *gyrA* from *B.burgdorferi* strain B31. The 1220 bp product was cloned into the vector resulting in plasmid pTASK1 and transformed into TOP10F′ cells according to the manufacturer's instructions (Invitrogen). Plasmid pTASK1 was isolated and transformed into competent *E.coli* strain A5196 (*hupA hupB*) (Mendelson *et al.*, 1991). Synthesis of the 34 kDa GyrA C-terminal domain in *E.coli* strain A5196 was confirmed by Western analysis as described above using 12.5% SDS–PAGE.

Induction of Mu *cts*62 lysogens (kindly provided by K.Drlica) and preparations of lysate were performed as previously described (Bukhari and Ljungquist, 1977). Mu titers were measured by plaque formation on N99 lawns at 37°C on 1% Luria–Bertani (LB) Ca Mg plates (LB supplemented with 1 mM $CaCl_2$, 2.5 mM $MgSO_4$) with 0.5% LB top agar. The e.o.p. was measured as the ratio of bacteriophage Mu titer on the mutant strain to the titer on the wild-type strain.

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