Borrelia burgdorferi ftsZ Plays a Role in Cell Division

Lydia Dubytska,1 Henry P. Godfrey, 2 and Felipe C. Cabello 1*

Departments of Microbiology and Immunology 1 and Pathology, 2 New York Medical College, Valhalla, New York 10595

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ftsZ is essential for cell division in many microorganisms. In Escherichia coli and Bacillus subtilis, FtsZ plays a role in ring formation at the leading edge of the cell division septum. An ftsZ homologue is present in the Borrelia burgdorferi genome (ftsZBbu). Its gene product (FtsZBbu) is strongly homologous to other bacterial FtsZ proteins, but its function has not been established. Because loss-of-function mutants of ftsZBbu might be lethal, the tetR/tetO system was adapted for regulated control of this gene in B. burgdorferi. Sixty-two nucleotides of an ftsZBbu antisense DNA sequence under the control of a tetracycline-responsive modified hybrid borrelial promoter were cloned into pKSS1. This construct was electroporated into a B. burgdorferi host strain carrying a chromosomally located tetR under the control of the B. burgdorferi flaB promoter. After induction by anhydrotetracycline, expression of antisense ftsZ RNA resulted in generation of filamentous B. burgdorferi that were unable to divide and grew more slowly than uninduced cells. To determine whether FtsZBbu could interfere with the function of E. coli FtsZ, ftsZBbu was amplified from chromosomal DNA and placed under the control of the tetracycline-regulated hybrid promoter. After introduction of the construct into E. coli and induction with anhydrotetracycline, overexpression of ftsZBbu generated a filamentous phenotype. This suggested interference of ftsZBbu with E. coli FtsZ function and confirmed the role of ftsZBbu in cell division. This is the first report of the generation of a B. burgdorferi conditional lethal mutant equivalent by tetracycline-controlled expression of antisense RNA.

Knowledge regarding cell division in bacteria has been primarily obtained using gram-positive and gram-negative bacteria as experimental models (8, 12, 41). Most bacterial cells grow in length with little change in cell diameter, until they reach a critical size that is generally twice their original length (14, 57). Cell division is then initiated in the middle diameter of the cell with the formation of a contractile ring comprised largely of FtsZ (4, 6, 14, 51, 57). The role of cell division genes is the first report of the generation of a B. burgdorferi conditional lethal mutant equivalent by tetracycline-controlled expression of antisense RNA.

High-temperature treatment of E. coli results in complete and immediate cessation of division and formation of filamentous cells that lack visible constriction points and the contractile ring (8, 14). FtsZ is also rate limiting for septum initiation. Moderate increases in its level results in a minicell phenotype because there is an increase in division frequency at the cell poles and the average cell resulting from these divisions are smaller, suggesting that septation mediated by FtsZ is occurring earlier in the cell cycle (56). High levels of FtsZ, on the other hand, completely inhibit division (34, 36). The ftsZ gene is also essential for cell division in gram-positive organisms. In Bacillus subtilis, ftsZ is required for both cell division and the formation of the sporulation septum (4), while in Streptomyces coelicolor, ftsZ is required for septation but not for viability (47).

The lack of information regarding mechanisms of cell division and morphogenesis in B. burgdorferi and other spirochetes (41) is due at least in part to a lack of molecular genetic tools relative to species such as E. coli and B. subtilis. Despite the recent development of a number of genetic methods for manipulating B. burgdorferi (11, 17, 45), cell division mutants of B. burgdorferi have not yet been isolated, nor is it known whether they are lethal (which would preclude their isolation) (15, 23, 40, 55). Isolation of conditionally lethal mutants in other bacteria has permitted isolation of mutants in genes that play an essential role in bacterial metabolism or are essential for bacterial survival (23, 24, 27, 50, 58). Isolation of conditional lethal mutants as a genetic tool in B. burgdorferi has been limited by the lack of knowledge regarding the nutritional requirements of this bacterium (10, 45). Furthermore, its slow growth and the inability to use solid replica plating makes rapid identification of mutant bacteria unable to grow in limiting media difficult (10).

A regulatory system based on the tet operon of the E. coli TnJ0 transposon (5, 19–21, 54) has been widely used for tight regulation of eukaryotic and prokaryotic gene expression (3, 5, 32, 50, 58) and permits analysis of conditionally lethal mutants (15, 26, 27, 29). In the absence of tetracycline or its nonantibiotic analogues such as anhydrotetracycline (A4C), the TetR repressor binds to the tetO operator that has been fused to the promoter of a target gene. Binding of TetR inhibits binding of RNA polymerase and transcription of the target gene. In the presence of tetracycline or its analogues, TetR conformation is altered so that it cannot bind to tetO, RNA polymerase can bind to the target gene promoter, and transcription occurs (5,
Gene expression in this system is tetracycline dose dependent, so that changes in its concentration permit variations in target gene expression with titration of the biological effects of a regulated phenotype (5, 54). Because the affinity of tetracycline and its derivatives is 1,000- to 100,000-fold greater for TetR than for the ribosome, induction of TetR-controlled gene expression occurs well before antibiotic-induced ribosomal inhibition (5).

The Tet system has been used to regulate expression of antisense RNA (asRNA) in bacteria and indirectly modulate gene expression so as to permit titration of gene expression and generate a functional equivalent of conditionally lethal mutants. This has been accomplished by blocking FtsZBbu synthesis by regulated gene expression of asRNA to ftsZBbu (26, 27, 58). These experiments suggest that FtsZBbu plays a role in cell division in B. burgdorferi and that ftsZBbu expression in particular and B. burgdorferi gene expression in general can be negatively controlled by the use of tetracycline-regulated expression of asRNA.

### Table 1. Oligonucleotides used in these studies

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**Materials and Methods**

**Bacterial strains, plasmids, and media.** *E. coli* DH5α was obtained from H. Bujard (32). *E. coli* TOP10 and pCR2.1-TOPO were purchased (TOPO TA cloning kit, Invitrogen, Carlsbad, Calif.). Low-passage infectious *B. burgdorferi* strain 297 was provided by M. V. Norgard (1). pKPS11 was provided by S. Samuels (17). *E. coli* cells were grown in Luria-Bertani (LB) broth (Gibco-BRL, Gaithersburg, MD). *B. burgdorferi* was grown in BSK-H medium (Sigma, St. Louis, Mo.) with 6% rabbit serum (Sigma).

**DNA manipulations.** DNA manipulations were performed by standard methods (46). All enzymes used in plasmid constructions were obtained from New England Biolabs, Beverly, MA. Total DNA was purified from cultures using the QIAquick gel extraction kit (QIAGEN, Valencia, Calif.).

**DNase I digestion.** DNase I digestion was performed with 5% DNase I (Roche, Mannheim, Germany) in 10 mM Tris/HCl, pH 7.5, and 10 mM MgCl2. DNA manipulation protocols were: denaturation for 2 min at 94°C for 1 cycle, followed by 3 cycles of 10°C for 10 s, 53°C for 10 s, and 72°C for 2 min, with a final extension at 68°C for 5 min.

**Oligonucleotide primers used in the present study were purchased (GenoSys**
Biotechnology, The Woodlands, Tex.). The structure of all constructs was confirmed by restriction enzyme analysis and by PCR amplifications with appropriate primers and DNA sequence analysis of amplicons. All primers used in the present study are listed in Table 1.

**Construction of plasmid for creation of B. burgdorferi 297 containing chromosomal tetR.**

tetR was amplified by PCR from *E. coli* DH5αZ chromosomal DNA using forward and reverse primers TetR1 and TetR2 and the *B. burgdorferi* B31 flaB promoter was amplified from total *B. burgdorferi* DNA with primers FlaB1 and FlaB2 (Table 1). These PCR products were digested with KpnI, ligated, and cloned into pCR2.1-TOPO (Invitrogen) to yield pCR2.1/tetR. This construct was electroporated into *E. coli* TOP10 (Invitrogen) and selected on ampicillin plates according to the manufacturer's instructions. The structure of tetR under the control of the flaB promoter was confirmed by DNA sequencing. tetR was inserted in the nonessential chromosomal luxS gene (BB0377) as follows (Fig. 1A). A PCR fragment containing tetR under the flaB promoter was then amplified from pCR2.1/tetR with primers TetR3 and TetR4 (Table 1). TetR3 was designed to contain a sequence homologous to the *aph(3′)-IIIa* kanamycin resistance gene from *Enterococcus faecalis* (53). TetR4 was designed to contain a sequence homologous to the *B. burgdorferi* luxS gene. A second PCR fragment containing *aph(3′)-IIIa* under the control of its own promoter was amplified from pAT112 (53) by using primers KanR1 and KanR2 (Table 1). KanR1 was designed to contain a sequence homologous to *B. burgdorferi* luxS, KanR2 was designed to contain a sequence homologous to tetR. A third PCR fragment containing approximately 900 bp of the 3′ end of *B. burgdorferi* metK (BB0376) and the 5′ region of luxS was amplified from total DNA *B. burgdorferi* 297 with primers LuxS1 and LuxS2; LuxS2 was designed to contain a sequence homologous to tetR. A fourth PCR fragment containing the 3′ end of luxS and approximately 1,050 bp of 5′ sequence of the BB0378 gene was amplified from *B. burgdorferi* strain 297 with primers LuxS3 and LuxS4 (Table 1); LuxS3 was designed to contain a sequence homologous to *aph(3′)-IIIa*. All DNA fragments were purified with a QIAquick gel extraction kit, mixed together, and fused by long PCR (48) using the primers LuxS1 and LuxS4. To minimize polar effects, tetR and the kanamycin resistance marker *aph(3′)-IIIa* were inserted in the opposite orientation to that of luxS. The resulting PCR product was cloned into pCR2.1-TOPO. The recombinant plasmid was electroporated into *E. coli* TOP10, and electroporants were selected on LB agar plates containing ampicillin and kanamycin according to the manufacturer's instructions. Plasmid DNA from ampicillin-kanamycin-resistant clones was purified. The presence of the insertion was detected by PCR with the primers LuxS5 and LuxS6, and its sequence was confirmed by restriction and sequence analysis.

**FIG. 1.** (A) Production of *B. burgdorferi* 297 containing chromosomally located tetR by allelic exchange of a construct containing tetR from Tn10 of *E. coli* and the kanamycin resistance gene *aph(3′)-IIIa* from *Enterococcus faecalis* into the central region of the *B. burgdorferi* luxS gene (BB0377). (B) Production of plasmids used these studies. Sequences: 1, *P* tetl hybrid promoter containing tetO sequences from Tn10 of *E. coli* inserted into *B. burgdorferi* bmpA promoter sequence; 2, antisense ftsZ (asftsZ) corresponding to nucleotides 1 to 62 of *B. burgdorferi* ftsZ ORF. Diagram 3 illustrated the construction of pLD6 for regulated expression of asftsZ RNA in *B. burgdorferi*. (C) Construction of pLD7 from pCR2.1-TOPO for regulated expression of ftsZsse in *E. coli*. See Materials and Methods for details of the constructions.
Construction of plasmids for ATc-regulated expression. To create a hybrid promoter (Ptetl) that could interact with TetR and function in B. burgdorferi, nucleotides 190 to 35 bp upstream from the B. burgdorferi B31 bmpA starting codon were amplified from B. burgdorferi total DNA by PCR using forward and reverse primers Ptetl1 and Ptetl2 and purified (Table 1). A second amplicon containing the Tn10 tetO sequences fused to bmpA on either side of the bmpA promoter sequence was generated by PCR of B. burgdorferi total DNA using forward and reverse primers Ptetl3 and Ptetl4 and purified. Ptetl3 (Table 1) contained tetO DNA sequences (wavy underlines) on either side of the /H1100235 promoter. Ptetl4 (Table 1) contained ATG fused to nucleotides 120 to 139 of bmpA (double underline). These amplicons were fused by using long PCR (48) to generate the hybrid promoter Ptetl (Fig. 1B). This construct was purified, and its structure was confirmed by DNA sequencing. To create pLD6 (Fig. 1B), a plasmid containing DNA sequences coding for asftsZBbuRNA, primers Ptet1 and...
AtsZ were used in long PCR (48) to generate an ampiclon with Perl fused to asfsZ (Fig. 1B). Primer AtsZ (Table 1) contained a KmPl site (underlined), a universal terminator sequence (boldface), asfsZ/ftsZ DNA sequences, and a sequence complementary to the Perl promoter (italicized). The resulting PCR product was cloned into pCR2.1-TOPO and subsequently electroporated into E. coli TOP10. Plasmid DNA from electroporants selected with ampicillin on LB agar plates was purified, and the DNA fragment containing Perl fused to asfsZ was excised and subcloned into the KmPl and Psfl sites of pkFSS1 (Fig. 1B). To create pLD7 (Fig. 1C), DNA containing the complete ftsZ/ftsZ gene was amplified from B. burgdorferi genomic DNA and fused with a DNA segment containing Perl by long PCR (48) using primers FtsZ1 and FtsZ2 and purified. Primer FtsZ1 (Table 1) contained a DNA sequence complementary to Perl (italics). Primer FtsZ2 (Table 1) contained a KmPl site (underlined). This ampiclon was fused to Perl using long PCR (48) and Tet1 and FtsZ2 as forward and reverse primers. The resulting ampiclon was cloned into pCR2.1-TOPO, transformed into E. coli DH5αZ, and selected on LB agar plates with 50 μg of ampicillin/ml (Fig. 1C). Structures of all plasmid constructs were confirmed by PCR amplification and DNA sequence analysis.

Electroporation of B. burgdorferi. B. burgdorferi 297 was grown to mid-log phase (1 × 10^7 to 2 × 10^7 cells/ml) and electroporated with 10 to 30 μg of recombinant plasmid DNA. After overnight recovery, the electroporated cells were diluted and 1 × 10^8 to 2 × 10^8 cells in 200 μl of complete BSK-H medium containing 400 μg of kanamycin/ml for selection of clones of B. burgdorferi containing tetL and 400 μg of kanamycin/ml and 50 μg of streptomycin/ml for selection of clones containing 297/tetR and pLD6 were distributed in each well of 96 microwell plates (Corning, Inc., Corning, N.Y.). After 14 to 21 days, B. burgdorferi showing growth in microwells were cultured in 1 ml of complete BSK-H medium with appropriate antibiotics. DNA was extracted from these cultures by using High Pure PCR template preparation kit (Roche Diagnostics Corp., Indianapolis, Ind.). DNA of kanamycin-resistant colonies of B. burgdorferi 297/tetR was analyzed by PCR for the presence of the insertion of tetL and promoter-kanamycin gene fusion using primers LuxS5 and LuxS6 to confirm it was the result of a double crossover. The fusion of asfsZ/ftsZ with the Perl promoter was confirmed by sequence analysis using Perl.

Detection of TetR by immunoblotting. Total proteins of B. burgdorferi 297/tetR were extracted from 1 × 10^7 to 2 × 10^7 cells by lysing them in Laemmli buffer. Lysate proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by silver staining or immunoblotting (49) with rabbit anti-E. coli TetR polyclonal antibody (a generous gift from Kai Schönig). Immunoblots were developed with alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G (Jackson ImmunoResearch, West Grove, Pa.) and enhanced chemiluminescence technology according to the manufacturer’s instructions (ECL Western blotting kit, GE Healthcare Amersham Biosciences, Piscataway, N.J.), and read by using a Storm 860 PhosphorImager and ImageQuant software (Molecular Dynamics, Sunnyvale, Calif.).

Real-time quantitative reverse transcription-PCR (RT-PCR) analysis. Total RNA was prepared from B. burgdorferi 297 by using TRIzol (Invitrogen) according to the manufacturer’s instructions. All RNA samples were treated with DNase I (Promega Corp., Madison, Wis.) to remove genomic DNA. RNA-free mRNA (50 to 100 ng in a volume of 20 μl) was reverse transcribed by using a reverse transcription system kit (Promega) according to the manufacturer’s instructions. Reverse-transcribed samples were denatured for 5 min at 95°C and stored at −20°C until use. ftsZ mRNA and flaB mRNA were quantified by using an ABI Prism 7500HT sequence detection system (Applied Biosystems, Foster City, Calif.). TaqMan reactions were performed in a volume of 25 μl using the TaqMan Universal PCR Master Mix (Applied Biosystems). TaqMan probes and forward and reverse primers were designed with Primer Express 2 software (Applied Biosystems). For ftsZ quantitation, FtsZ3 and FtsZ4 were used as forward and reverse primers and TET/TAMRA was used as the probe (Table 1). For flaB quantitation, FlaB3 and FlaB4 were used as forward and reverse primers and fTAM/FAM/TAMRA was used as the probe (Table 1). PCR was performed under the following conditions: 50°C for 2 min, followed by 95°C for 10 min, and then 45 cycles of 95°C for 15 s and 60°C for 1 min. The threshold cycle (Ct) for ftsZ and flaB mRNA was calculated by Sequence Detection software (Applied Biosystems); levels of ftsZ mRNA relative to flaB reference mRNA were calculated as described previously (43). PCR amplification was confirmed by measurement of amplicon sizes of ftsZ and flaB by agarose gel electrophoresis.

Microscopic analysis of cell length. Cultures of B. burgdorferi 297/tetR and derivatives containing pLD6 and pkFSS1 (10^7 cells/ml) were stained with acridine orange and examined by fluorescence microscopy without or with induction with 1.5 μg of ATc/ml. B. burgdorferi cells in three independent slides were examined (magnification, ×1,250), and the cells in 10 fields (ca. 100 cells per field) in each slide were counted and assessed for the presence of the filamentous phenotype. Spireochete lengths on photomicrographs of similar fields were also measured, and a stage micrometer was used to convert these lengths to μm. A similar approach was used to analyze E. coli DH5αZ and E. coli DH5αZ(pLD7).

Statistical analysis. The effect of addition of ATc to B. burgdorferi 297 and derivatives in culture from three independent experiments was analyzed by a two-way analysis of variance with a repeated measures Bonferroni posttest. Significance levels were set at P < 0.05.

RESULTS

Comparison of ftsZ/ftsZ open reading frame (ORF) with other bacterial FtsZ proteins. B. burgdorferi ftsZ open reading frame (ORF) codes for a protein with a molecular mass of 42,971 Da. Its deduced amino acid sequence shows strong similarity to other bacterial FtsZ amino acid sequences particularly over the first 312 codons and has 50, 50, 46, and 46% identity with FtsZ proteins of Treponema pallidum, B. subtilis, Leptosira interrogans, and E. coli, respectively (Fig. 2). Within the highly conserved N terminus region, FtsZ_mmu has a Gly-rich block, G138MG GGTGTCG1420, identical to the characterized GTP-binding site of E. coli FtsZ (37), and a N222IDFADV229 consensus sequence that is part of a putative GTP-hydrolyzing domain (13, 39). The C-terminal moiety of the borrelial FtsZ homologue also contains a D385DDIDVPFLKR395 conserved region that is
thought to be important for interaction with FtsA and ZipA (33). The gene product of ftsZ_Bbu is thus highly homologous to its counterparts in other bacteria.

Construction of a B. burgdorferi 297 strain expressing TetR from a chromosomally inserted tetR gene. Construction of the tetR insertion is shown in Fig. 1 and is described in detail in Materials and Methods. Electroporation of pCR2.1-TOPO containing the luxS flanking regions, tetR, and aph(3')-IIIa into B. burgdorferi 297 yielded four kanamycin-resistant clones; results from one of these clones are shown in Fig. 3A. The tetR insertion into the luxS gene was confirmed by PCR analysis, which showed that longer amplicons were generated from clones with the inserted gene than from wild-type B. burgdorferi 297. Immunoblotting demonstrated the presence of TetR (Fig. 3B). One of the clones was used for all subsequent studies.

Development of filamentous phenotype in B. burgdorferi in response to ATc-regulated production of asftsZ RNA. Construction of pLD6 to mediate regulated synthesis of asftsZ RNA in B. burgdorferi 297/tetR is shown in Fig. 1 and is described in detail in Materials and Methods. pLD6 contained an asDNA fragment complementary to the initial 62 nucleotides of the ftsZ_Bbu coding strand under the control of the Ptet promoter, a hybrid promoter containing two tetO sequences fused with bmpA promoter sequences (Fig. 1B). To prevent readthrough from the asDNA, a termination signal was placed at the end of the antisense sequence. Twenty-four hours after expression of asftsZ_Bbu RNA was induced in mid-log-phase cultures of B. burgdorferi 297/tetR, clones containing pLD6 were selected by using appropriate antibiotics. Twenty-four hours after expression of asftsZ_Bbu RNA was induced in mid-log-phase cultures of B. burgdorferi 297/tetR(pLD6) by the addition of ATc, large numbers of abnormally elongated, filamentous spirochetes were visible under the microscope (Fig. 4C). We were unable to see any septations in these cells. No such cells were present in uninduced cultures of B. burgdorferi 297/tetR (pLD6) (Fig. 4B) or in control cultures of B. burgdorferi 297/tetR in the presence (Fig. 4A) or absence (not shown) of ATc.

ATc-induced B. burgdorferi 297/tetR (pLD6) (Fig. 4D) were significantly longer than uninduced B. burgdorferi 297/tetR (pLD6) or control B. burgdorferi 297/tetR in the absence or presence of ATc (P < 0.001). Although uninduced B. burgdorferi 297/tetR (pLD6) were slightly longer than uninduced or induced B. burgdorferi 297/tetR (compare Fig. 4C with Fig. 4A and B), this difference was not statistically significant (Fig. 4D).

**FIG. 4.** Production of filamentous B. burgdorferi by ATc-mediated induction of asftsZ RNA. (A) B. burgdorferi 297/tetR cultured for 24 h in the presence of 1.5 μg of ATc/ml (acridine orange staining; magnification, ×1,250). (B) B. burgdorferi 297/tetR (pLD6) cultured for 24 h in the absence of ATc (acridine orange staining; magnification, ×1,250). (C) B. burgdorferi 297/tetR (pLD6) cultured for 24 h in the presence of 1.5 μg of ATc/ml (acridine orange staining; magnification, ×1,250). (D) Mean (± the SE) spirochete length (in μm) in cultures of B. burgdorferi 297/tetR and 297/tetR (pLD6) cultured for 24 h in the absence (−) or presence (+) of 1.5 μg of ATc/ml. Spirochete lengths (n = 20) on photomicrographs (magnification, ×1,250) from three independent experiments were measured for each experimental condition; a stage micrometer was used to convert these lengths to μm. ***, P < 0.001. (E) Mean growth of B. burgdorferi 297/tetR (triangles) and 297/tetR (pLD6) (circles) in the absence (open symbols) or presence (closed symbols) of 1.5 μg of ATc/ml added at time zero (arrow). The results from three independent experiments have been combined. Error bars are hidden behind the symbols. See Materials and Methods for details.
By 24 h of induction, filamentous cells accounted for 78 ± 11% (mean ± standard error [SE]) of cells examined in cultures of *B. burgdorferi* 297/tetR (pLD6) and were significantly more frequent (*P* < 0.001) than in uninduced *B. burgdorferi* 297/tetR (pLD6) (25 ± 7%) or in control induced or uninduced *B. burgdorferi* 297/tetR cultures at this time (14 ± 3% and 19 ± 3%, respectively). At 48 h after ATc induction, the percentage of filamentous cells in ATc-induced cultures of *B. burgdorferi* 297/tetR (pLD6) had fallen to 48 ± 4% and remained at this level at 72 h. We were unable to detect formation of the division septum in any of these cells. These values were significantly less than at 24 h (*P* < 0.01) but still significantly higher than the percentages of filamentous spirochetes in uninduced cultures of *B. burgdorferi* 297/tetR (pLD6) (23 ± 2%) or in control induced or uninduced *B. burgdorferi* 297/tetR cultures (14 ± 1% and 13 ± 1%, respectively).

*B. burgdorferi* 297/tetR showed no significant differences in growth in the absence or presence of ATc (Fig. 4E). Growth of *B. burgdorferi* 297/tetR (pLD6) was significantly less (*P* < 0.001) than that of *B. burgdorferi* 297/tetR in the absence of 1.5 µg of ATc/ml (Fig. 4E). However, growth of this recombinant strain was even more inhibited in the presence of ATc (*P* < 0.001). This suggests that the growth and cell division of *B. burgdorferi* was slowed by the lack of FtsZ protein occasioned by induced *asftsZBbu*.

**Quantitation of *ftsZBbu* mRNA after induction of production of *asftsZBbu* mRNA.** The filamentous borrelial phenotype was most probably a result of the inability of these cells to divide because of the lack of FtsZBbu generated by the induced *asftsZBbu*. As a first step toward understanding the mechanism of action of induced *asftsZ* RNA, *ftsZBbu* mRNA was examined by RT-PCR and real-time RT-PCR. RT-PCR indicated that induction of *ftsZBbu* RNA by 1.5 µg of ATc/ml for 24 h was associated with a sharp decrease in *ftsZBbu* mRNA compared to levels in uninduced *B. burgdorferi*. Culture of *B. burgdorferi* 297/tetR in the presence of ATc had no obvious effect on production of *ftsZ* mRNA (Fig. 5A). These results were quantitatively confirmed by real-time RT-PCR. Inhibition of *ftsZ* mRNA by induced production of *asftsZ* in *B. burgdorferi* 297/tetR (pLD6) was highly significant after 6 h of induction with ATc (Fig. 5B), indicating that the induction of *asftsZBbu* is associated with decreased *ftsZBbu* mRNA levels in these bacteria.

**Expression of *B. burgdorferi* *ftsZ* in *E. coli*.** Overexpression of autologous *FtsZ* in *E. coli* generates a minicell phenotype, whereas high levels of expression of heterologous FtsZ result in a filamentous phenotype (6, 34, 36, 55). To determine whether *ftsZBbu* could be ectopically expressed in *E. coli* and whether its gene product would interfere with *E. coli* *FtsZ* function, pLD7, containing *ftsZBbu* under the control of the *Ptetl* hybrid promoter (Fig. 1), was electroporated into *E. coli* DH5α. No bacteria carrying pLD7 (Fig. 1C) were obtained after electroporation of pLD7 into *E. coli* lacking expression of TetR (data not shown), but electroporation of pLD7 into *E. coli* DH5α expressing TetR (32) was successful. This could suggest that uncontrolled expression of *FtsZBbu* in *E. coli* was lethal. By 12 h after induction of synthesis of ectopic *FtsZBbu* in *E. coli* DH5α (pLD7) with 1.5 µg of ATc/ml, numerous extremely long filamentous cells were visible (Fig. 6C). No septations were visible in these *E. coli* cells. No filamentous cells were detected in control cultures of *E. coli* DH5α expressing TetR in the absence (not shown) or presence of ATc (Fig. 6A). Small numbers of filamentous cells were also present in *E. coli* DH5α (pLD7) in uninduced cultures (Fig. 6B), but they were much shorter than the filamentous cells seen in induced cultures (compare Fig. 6B and C), and their presence did not significantly increase mean bacterial length in these cultures (Fig. 6D). Both shorter and longer filamentous cells contained denser areas consistent with contractile rings (Fig. 6C and D). These experiments indicate that ectopically produced *FtsZBbu* can interfere with the function of the endogenous *FtsZ* protein in *E. coli*. Producing TetR in the absence (not shown) or presence of ATc was lethal. By 12 h after induction of synthesis of ectopic *FtsZBbu* in *E. coli* DH5α (pLD7) with 1.5 µg of ATc/ml, numerous extremely long filamentous cells were visible (Fig. 6C). No septations were visible in these *E. coli* cells. No filamentous cells were detected in control cultures of *E. coli* DH5α expressing TetR in the absence (not shown) or presence of ATc (Fig. 6A). Small numbers of filamentous cells were also present in *E. coli* DH5α (pLD7) in uninduced cultures (Fig. 6B), but they were much shorter than the filamentous cells seen in induced cultures (compare Fig. 6B and C), and their presence did not significantly increase mean bacterial length in these cultures (Fig. 6D). Both shorter and longer filamentous cells contained denser areas consistent with contractile rings (Fig. 6C and D). These experiments indicate that ectopically produced *FtsZBbu* can interfere with the function of the *E. coli* *FtsZ* protein in *E. coli*.

**DISCUSSION**

Although the deduced *ftsZBbu* gene product is highly homologous to other bacterial *FtsZ* proteins, the activity of *ftsZBbu* and *FtsZBbu* in cell division has not been previously examined. By combining tetracycline-regulated control of gene expression, a technique developed for eukaryotic cells but rarely used...
in bacteria (3), with antisense technology (29, 31, 35), it was possible to study the functionality of ftsZ_{Bbu} and confirm its activity in cell division. Blocking of FtsZ Bbu production by ATc-mediated induction of asftsZ_{Bbu} RNA resulted in decreased ftsZ_{Bbu} mRNA, a filamentous phenotype, and slow growth. Furthermore, regulated ectopic expression of ftsZ_{Bbu} in E. coli resulted in the appearance of filamentous E. coli as a result of functional protein interference of FtsZ Bbu with cell division proteins of E. coli, thus providing additional confirmation for the functionality of ftsZ_{Bbu} in cell division.

It was not unexpected that the ATc-mediated downregulation of ftsZ_{Bbu} expression resulted in a filamentous phenotype and slow growth in B. burgdorferi, since in the absence of FtsZ in other bacteria, the Z ring is not formed and there is a failure to assemble the complete divisome needed for cell division (34, 40, 55, 59). The observed low levels of ftsZ mRNA under the conditions of ATc-induced asRNA provided confirmation that the inhibition of ftsZ_{Bbu} expression mediated by induced asftsZ was specific for production of FtsZ_{Bbu}. Attempts to detect the presence of B. burgdorferi FtsZ protein in wild-type B. burgdorferi using cross-reacting anti-E. coli FtsZ rabbit antibodies in immunoblots of B. burgdorferi lysate proteins were unsuccessful (data not shown). This failure might be due to a lack of complete cross-reactivity of this antibody for the B. burgdorferi and E. coli FtsZ proteins or to the low levels of FtsZ_{Bbu} present under the conditions of growth.

The functional role of the B. burgdorferi FtsZ homologue and its involvement in B. burgdorferi cell division was also indirectly confirmed by our inability to obtain transformants with pLD7 in E. coli not expressing TetR. (pLD7 contained FtsZ_{Bbu} under the control of the TetR-susceptible P_{tetl} hybrid borrelial promoter). In contrast, transformants with pLD7 were easily obtained in the E. coli strain that expressed TetR. In this connection, it should be mentioned that many B. burgdorferi promoters are recognized by the E. coli protein synthesis machinery (11, 17, 49). These results suggested that constitutive expression of B. burgdorferi FtsZ was lethal for E. coli, as has been shown to be the case with other heterologously expressed bacterial FtsZ proteins (14). This hypothesis was confirmed by the appearance of filamentous E. coli after induction of expression of FtsZ_{Bbu} in E. coli (pLD7), indicating inhibition of E. coli cell division by FtsZ_{Bbu} (59). These findings indicate that FtsZ_{Bbu} is a cell division protein with function similar to its homologues in other bacteria and thus able to block cell division when expressed in a heterologous background (30, 34, 36, 38, 47, 55, 59). This functional identity of the B. burgdorferi ftsZ gene and FtsZ protein with other bac-

FIG. 6. Production of filamentous E. coli by ATc-mediated induction of ectopic ftsZ_{Bbu}. (A) E. coli DH5αZ cultured for 24 h in the presence of 1.5 pg of ATc/ml (acridine orange staining; magnification, ×1,250). (B) E. coli DH5αZ (pLD7) cultured for 24 h in the absence of ATc (acridine orange staining; magnification, ×1,250). (C) E. coli DH5αZ (pLD7) cultured for 24 h in the presence of 1.5 pg of ATc/ml (acridine orange staining; magnification, ×1,250). (D) Mean (± the SE) bacterial length (in μm) in cultures of E. coli DH5αZ and E. coli DH5αZ(pLD7) cultured for 24 h in the absence (−) or presence (+) of 1.5 pg of ATc/ml. Bacterial lengths (n = 20) on photomicrographs (magnification, ×1,250) from three independent experiments were measured for each experimental condition; a stage micrometer was used to convert these lengths to μm. ***, P < 0.001. See Materials and Methods for details.
terial homologues is fully consistent with the amino acid sequence and domain identity it shares with them (30, 36, 59).

*B. burgdorferi* is a genetically intractable organism. Its genome is unstable. There are no natural systems of horizontal gene transfer in spirochetes and, consequently, no simple and efficient method for introducing DNA into this bacterium. Its nutritional requirements are still incompletely known so that simple and defined media for its culture are lacking, and it grows slowly in the complex media that are available for its culture. Molecular genetic study of the *B. burgdorferi* genome has been hampered by the dearth of genetic tools to isolate and complement mutants and to manipulate gene expression although many strides have been made to remedy this in recent years (11, 45). It has therefore been difficult to isolate conditional lethal mutants in *B. burgdorferi*, a type of mutant that has been crucial for the development of genetic systems and physiological studies in other bacteria (5, 23, 26, 32, 35, 45, 50, 58, 59).

Many advances to manipulate the genome of *B. burgdorferi* have been the result of adaptation of genetic systems developed in other bacteria to this pathogen (11, 45). The present study of the function of ftsZ*Bbu* was only possible because we were able to adapt two genetic tools developed for the study of other bacteria, namely, *tetR*-regulated control of gene expression and asRNA technology (3, 27, 32, 35, 58). In adapting this system to *B. burgdorferi*, the *tetR* gene was placed under the control of the constitutive *B. burgdorferi* flAB promoter, and this DNA construct was inserted in the chromosome in *luxS*, a gene not required for *B. burgdorferi* growth and infectiousness (7); the DNA segment encoding antisense ftsZ RNA under the hybrid *Ptet* TetR-responsive promoter was located extrachromosomally in a plasmid (17). The chromosomal location of the *tetR* gene under a constitutive promoter is preferable to a plasmid location. Variations in gene dosage as result of variations in copy number are minimized in the chromosomal location, thus ensuring that the synthesis of TetR will remain constant throughout the cell division cycle (32, 44). By permitting regulated expression of *ftsZ* asRNA this system has for the first time generated the functional equivalent of a conditionally lethal mutant in *B. burgdorferi*.

*B. burgdorferi* cells containing both components of the Tet system regulated the expression of *ftsZ* asRNA and FtsZ in an ATc-dependent manner as measured by levels of *ftsZ* mRNA (Fig. 5). The reduced levels of FtsZ were associated with inhibition of cell division demonstrated by the emergence of *B. burgdorferi* cells with the filamentous phenotype and slow growth (Fig. 4). However, TetR repressor levels encoded from the *B. burgdorferi* chromosome were not totally able to suppress expression of *ftsZ* asRNA from the *Ptet* hybrid promoter since uninduced cultures of *B. burgdorferi* 297/letR (pLD6) contained cells that were slightly longer (Fig. 4B and D) and grew significantly more slowly (Fig. 4E) than the *B. burgdorferi* 297/letR controls. We do not believe that filamentous cells divide after the blocking of cell division, and we have some preliminary evidence suggesting that they in fact are dead (data not shown). This escape of repression by TetR was evident in *E. coli* pLD7, where small numbers of filamentous cells were visible in the absence of ATc induction (Fig. 6B). It might be a result of titration of the TetR repressor by an increased number of the extrachromosomally located molecules of *Ptet* generated by variations in the copy number of the pKFSS1 plasmid (2). However, we have previously demonstrated that pKFSS1 has a copy number of one relative to the *B. burgdorferi* chromosome (9), suggesting that other mechanisms may play a role in the imbalance generated between numbers of TetR molecules and *Ptet* promoters (28). These might include changes in pKFSS1 supercoiling, which might decrease the access of the repressor to a region in the plasmid where *Ptet* is located (2). Titration of the levels of TetR by excessive amounts of *Ptet* molecules is a problem that could be readily corrected by placing an increased number of copies of the *tetR* gene in the chromosome and by putting its transcription under the control of a stronger *B. burgdorferi* constitutive promoter. Alternatively, this imbalance could be potentially corrected by placing both the *tetR* repressor gene and the putative gene controlled by the *Ptet* promoter in the chromosome (15).

In summary, this is the first report to show the feasibility of the regulated control of gene expression in *B. burgdorferi* and the usefulness of asRNA for generating the physiologic equivalent of conditionally lethal mutants in this pathogen. These experiments suggest that FtsZ*Bbu* plays a role in cell division in *B. burgdorferi* and that *ftsZ*Bbu expression in particular, and gene expression in general, can be negatively controlled in this species by the use of tetracycline-regulated expression of asRNA.

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REFERENCES


RETRACTION

*Borrelia burgdorferi ftsZ* Plays a Role in Cell Division

Lydia Dubytska, Henry P. Godfrey, and Felipe C. Cabello

Departments of Microbiology and Immunology and Pathology, New York Medical College, Valhalla, New York 10595

Volume 188, no. 5, p. 1969–1978, 2006: The primer sequence of AftsZ in Table 1 will result in a DNA product that encodes a missense RNA rather than an antisense *ftsZ* RNA as stated in the paper. This critical error negates one of the major conclusions of the article, namely that the expressed antisense *ftsZ* RNA specifically inhibited levels of *ftsZ* mRNA and resulted in *Borrelia burgdorferi* cells with a filamentous phenotype. We are now exploring whether the induction of this missense *ftsZ* RNA results in specific abolition of FtsZ production or whether the filamentous phenotype is the result of nonspecific phenomena.

We have also discovered that the control panels in Fig. 4A and B and 6A contain incorrect photomicrographs. The correct photomicrographs exist and are available at http://www.nymc.edu/fcabello/RetractionJBact. The conclusions based on the experiments are not affected by these errors.