Borrelia burgdorferi *ftsZ* Plays a Role in Cell Division

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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 (*ftsZ*_{Bbu}). Its gene product (FtsZ_{Bbu}) is strongly homologous to other bacterial FtsZ proteins, but its function has not been established. Because loss-of-function mutants of *ftsZ*_{Bbu} might be lethal, the *tetR/tetO* system was adapted for regulated control of this gene in *B. burgdorferi*. Sixty-two nucleotides of an *ftsZ*_{Bbu} antisense DNA sequence under the control of a tetracycline-responsive modified hybrid borrelial promoter were cloned into pKFSS1. 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 FtsZ_{Bbu} could interfere with the function of *E. coli* FtsZ, *ftsZ*_{Bbu} 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 *ftsZ*_{Bbu} generated a filamentous phenotype. This suggested interference of *ftsZ*_{Bbu} with *E. coli* FtsZ function and confirmed the role of *ftsZ*_{Bbu} 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 has not been directly explored in spirochetes (41). However, null mutations in the *recA* gene in *Leptospira* spp. and the *cfpA* gene in *Treponema* spp. apparently interfered with cell division as judged by morphological alterations (25, 52). The *Borrelia burgdorferi* genome contains a homologue of *ftsZ* (*ftsZ*_{Bbu}) (18) and the *B. burgdorferi* protein (FtsZ_{Bbu}) encoded by *ftsZ*_{Bbu} is highly homologous to FtsZ proteins of other bacteria (4, 6, 8, 16, 22, 39, 57, 59), but the physiologic roles of *ftsZ*_{Bbu} and FtsZ_{Bbu} in cell division is unknown.

FtsZ is essential for cell division and acts early in this process in *Escherichia coli* (12, 14, 33). High-temperature treatment of *ftsZ* temperature-sensitive mutant cells in *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 organ-

isms. 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* Tn10 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 (ATc), 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,

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TABLE 1. Oligonucleotides used in these studies

Name	Gene	Sequence (5'→3')
AftsZ	<i>asftsZ</i>	TCGGGGTACCCCGTTAATTAATTAACCTTAGTTTAGAAGAACATACCGATAGTTAGTATAA TATTAGAAAAGTAATTGCGAAGGAGGTAAACTATTTCCCTTTACAAA ^a
FlaB1	<i>flaB</i>	CGGGGTACCCATATCATTCTCCATGATAAAATTTAA ^b
FlaB2		ACGCGGATCCGCTAAAATGCTAGGAGATTGTCT
FlaB3		AGCTCCCTCACCAGAGAAAAGA
FlaB4		GCAGCCTGCAAAAATTAACACA
6FAM/TAMRA	<i>ftsZ</i>	ATTTACAGCAATAGCTTCATCTTGGTTTGCTCCA
FtsZ1		TTGTTTGTAAAGGGGAAATAGTTTATGTGATTATAATGTGATTTTGTATATTTAAAG ^a
FtsZ2		CGCGGGTACCCCGCGCCATACCAGCAGTAATAAACACCATATCGG ^b
FtsZ3		CAACAACGTGAACGTAAATTTTCATCTTCA
FtsZ4		ATATGGCAAGGGCGAAAACA
TET/TAMRA	<i>kanR</i>	TATCCCCATAATCTCTTCAAGTTCAAGCAATGAAA
KanR1		AGTTTGGACCGAAAAAATAGTATCTAGGTACTAAAACAATTC ^c
KanR2		TGTGAAAGTGGGTCTTAAACCTTATAGCTTCTAAATTCTA ^d
LuxS1	<i>luxS</i>	GATCCAATGCCCTTGGAGCAGGGGATCAGGGAATAATATTTGGATATGCCTGCGATGAAA
LuxS2		GAATTGTTTGTAGTACCTAGATACTATTTTTTCGGTCCAAACT ^e
LuxS3		ACAATCTCCTAGCATTTTGTAGCTATTTTGGCCCTATGGGAT ^d
LuxS4		AGAACTTTTTATTCTTTTTTGTTTTTATGCTTAATGCATTTAGAATTTATTCAGGAATT
LuxS5		TGGATATGCCTGCGATGAAACAAAAAATTTTTTACCTGCT
LuxS6		CTTAATGCATTTAGAATTTATTCAGGAATTCCTAGCTATC
Ptet11	<i>bmpA</i>	GCTCTAGAGCGCCCTCAAAATGTTATTACTTCAATAATTAAGGAT ^f
Ptet12	<i>bmpA</i>	ATCGGGATCTTTAACAATTTCTA ^f
Ptet13		TAGAAATTGTTAAGGATCCCGATTCCCTATCAGTGATAGAGATTGATCCCTATCAGTGAT AGAGAAATAAAATAATAAGTGGAGAAATTATTGTTCTG ^g
Ptet14	<i>bmpA</i>	AATATAGCTTTGTTTGTAAAGGGGAAATAGTTTATGTCCTTTAATGAGATGCTTTAA ^h
TetR1	<i>tetR</i>	ACGCGGGTACCCATGATGTCTAGATTAGATAAAAGTA ^b
TetR2		CTAACTGCAGTTAAGACCCACTTTACATTTAAGTTGT
TetR3		TAGAATTTACAAGCTATAAGGTTTAAAGACCCACTTTCACA ^c
TetR4		ATCCCATAGGGCCAAAATAGCTAAAATGCTAGGAGATTGT ^c

^a KpnI site (underlined), universal terminator (boldface), and homology for *Ptet1* hybrid promoter (italic).

^b KpnI site (underlined); ATG (boldface).

^c Homology for *luxS* (italic).

^d Homology for *tetR* (italic).

^e Homology for *aph(3')-IIIa* (italic).

^f XbaI site (underlined) and homology for *bmpA* (double underline).

^g *tetO* (wavy underline) and -35 and -10 *bmpA* regions (boldface).

^h Homology for *bmpA* (double underline) and ATG (boldface).

19–21). 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 genes 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 (26, 27, 29, 31, 35). This has permitted study of the in vitro and in vivo expression of multiple genes (including virulence genes) in *S. aureus*, *Clostridium difficile*, and mycobacteria (15, 26, 27, 58). Antisense RNA molecules also play a role in the natural regulation of gene expression of many bacteria, including *Borrelia* spp. (42). To date, neither Tet-regulated systems nor asRNA has been used to manipulate gene expression in *Borrelia*.

We have now applied the regulated control of gene expression by ATc, an approach used by others to obtain tightly regulated control of gene expression in bacteria (5, 15), to isolate the physiological equivalent of conditional lethal mu-

tants. This has been accomplished by blocking FtsZ_{Bbu} synthesis by regulated gene expression of asRNA to *ftsZ*_{Bbu} (26, 27, 58). These experiments suggest that FtsZ_{Bbu} plays a role in cell division in *B. burgdorferi* and that *ftsZ*_{Bbu} expression in particular and *B. burgdorferi* gene expression in general can be negatively controlled by the use of tetracycline-regulated expression of asRNA.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. *E. coli* DH5αZ 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). pKFSS1 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 High Pure PCR Template Preparation kit (Roche, Mannheim, Germany), DNA restriction fragment isolation and PCR product purification was done using QIAquick gel extraction kit (QIAGEN, Valencia, Calif.); all methods were done according to the manufacturers' instructions. PCR amplification parameters were: denaturation for 2 min at 94°C for 1 cycle, followed by 38 cycles of 94°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

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, *PtetI* 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 *ftsZ*_{Bbu} in *E. coli*. See Materials and Methods for details of the constructions.

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) (7) 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 the 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.

<i>B. burgdorferi</i>	MEEALMKDYNMIDSHTRRFSTTNPTILKVIAGGGGSSNAVNRMI EYGV RDVEFIVANTD	60
<i>T. pallidum</i>	----MMNIELAPSGE--EFTLSPTFTVIKVIAGGGGSSNAVNRMMSCGLQCVEFIAANTD	52
<i>L. interrogans</i>	----MFDNDMSVSN-----AIKVVGVGGGGGNAVQHMC-EEVSDVEFFALNTD	45
<i>B. subtilis</i>	----MLEFETN-----IDGLASIKVIGVGGGGGNAVNRMIENEVQGV EYIAVNTD	46
<i>E. coli</i>	----MFEP-MELTND-----AVIKVIGVGGGGGNAVEHMRERIEGV EYFAVNTD	45
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<i>B. burgdorferi</i>	LQALQTSIAPIKIALGAKV TAGLGAGGKPEIGQAAAEEDIDVIRNHL SGADMVFITAGMG	120
<i>T. pallidum</i>	VQALSYSTAPKKLAIGTKVTRGLGAGDPEIG EKAAMEDAEAIASALQGANMVFITAGMG	112
<i>L. interrogans</i>	GQALSKSKVQNILQIGTNLT KGLGAGANPEIGKRAATEDRAKIEQLLEGADMVFITAGMG	105
<i>B. subtilis</i>	AQALNLSKAEVKMQIGAKLTRGLGAGANPEV GKKAEEESKEQIEEALKGADMVFVITAGMG	106
<i>E. coli</i>	AQALRKTAVGQTIQIGSGITKGLGAGANPEVGRNA AEDRDALRAALEGADMVFIAAGMG	105
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<i>B. burgdorferi</i>	GGTGTGAAPVIAQVAKELGILTVGVVTKPFPFEGPKKLRLAEQGINNLRKSVDTLIIIPN	180
<i>T. pallidum</i>	GGTGTGAAPVIAKIAREL GALTAVVTKPFRFEGRAKMMLAERGIEKLRTS DTVIVIPN	172
<i>L. interrogans</i>	GGTGTGGAAPVVAEVAKEMGILTVAVVTKPFPFEGPRRMKAAEQGIDELTKHVD SIIITVFN	165
<i>B. subtilis</i>	GGTGTGAAPVIAQIAKDLGALTGVGVTRPFTFEGRKRQLQAAGGISAMKEAVDTLIVIPN	166
<i>E. coli</i>	GGTGTGAAPVVAEVAKDLGILTVAVVTKPFPFEGKKRMAFAEQGITELSKHVD SLITIPN	165
	***** * * * *	
<i>B. burgdorferi</i>	QKLLTVVDKRTTIKDAFKRADDVLRMGVQGIAGLII EHGEVNIDFADVKSIMQGGQDALM	240
<i>T. pallidum</i>	QNLLSVVDKRCPIKETYL VADDLLRKSQVQISDLITLPGEVNLD FMDVKNTMEGQGYALI	232
<i>L. interrogans</i>	EKLLSVLGKGASLIDAFNAANDVLGNVKGVS ELITKPLINVD FADVRVMTNMGLAMM	225
<i>B. subtilis</i>	DRILEIVDKNTPMLEAFREADNVLRQGVQGISDLIATPGLINLDFADVKTIMSNKGSALM	226
<i>E. coli</i>	DKLLKVLGRGISLLDAFGAANDVLKGA VQGIAGLITRPGLMNVDFADVRTVMSEMGYAMM	225
	* * * * *	
<i>B. burgdorferi</i>	GIGYGKGENRAVDAATS AISNPLLEEVRIEGSKGLLVNVTGGDDFSLLELEEIMGIITVS	300
<i>T. pallidum</i>	GVGEGEGENRAVDAATAA INNPLLEETRIEGATRLLVAVRGSENLSMGEVDGVM SVVAKT	292
<i>L. interrogans</i>	GMGEASGENRAREAAEA AIISSPILLEDINLDGAKGVIVNITAGMDMSIGEFEEVGEVIRSF	285
<i>B. subtilis</i>	GIGIATGENRAAEAAKKA ISSPILLEA-IDGAQGVLMNITGGTNLSLYEVQEAADIVASA	285
<i>E. coli</i>	GSGVASGEDRAEEAAEMA ISSPILLEDIDLSGARGVLVNITAGFDLRLDFEFETVGN TIRAF	285
	* * * * *	
<i>B. burgdorferi</i>	VDDEATVIYGHAINSNLEDEIYVTVVATG-----FASKKQKEISSTPENN--T	346
<i>T. pallidum</i>	IDPDAAIIHGTSIDASMQRDRVTVIATGVPQASIS IAGDTHSSQIKTSSYGAVSTGVY	352
<i>L. interrogans</i>	ISDEAIVIAGTVIDPDMSDSMKVTVVVTG-----IEKVAMKRGFGVEKTS---	330
<i>B. subtilis</i>	SDQDVNMIFGVSVINENL KDEIVTVIATG-----FIEQEKDVTKPQRPS---	329
<i>E. coli</i>	ASDNATVVI GTSLDPMNDEL RVTTVATG-----IGMDKRPEITLV TNKQ---	330
	* * * * *	
<i>B. burgdorferi</i>	LSSKEFDTLMSGNQ-NAP--SGSYEQDSSFAAKSKNVNYF-----DDDI DVPTFLR	395
<i>T. pallidum</i>	ISSDEWNRAKSSKQPNLPGLATRNSAVQETRM EKNGVKGHTFGVPLPSVNEDI DEPTFLR	412
<i>L. interrogans</i>	--SLQQSASSFSNKTSAP--FLRKETE VVTGASNAPKTD S-----DDVNKSDI PSFLR	379
<i>B. subtilis</i>	-----LNQSIKTHNQSV P---KRDAKREEPQQQNTVSRHTS-----QPADDTL DIPTFLR	376
<i>E. coli</i>	--VQQPVMDRYQQHGMAP--LTQE QKPVAKVVNDNAPQTA-----KEPDYI DIPAF LR	379
	* * * * *	
<i>B. burgdorferi</i>	NLNKKSSDD	404
<i>T. pallidum</i>	NRNKGL---	418
<i>L. interrogans</i>	RR-----	381
<i>B. subtilis</i>	NRNKR G---	382
<i>E. coli</i>	KQAD-----	383

FIG. 2. Sequence comparison of FtsZ proteins of *B. burgdorferi* and selected other bacteria. Putative GTP-binding domains (*B. burgdorferi* FtsZ residues 118 to 128), GTP-hydrolyzing domains (*B. burgdorferi* FtsZ residues 214 to 232), and FtsA binding domains (*B. burgdorferi* residues FtsZ 389 to 395) are boxed. Identical residues between proteins indicated by an asterisk.

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 *PbmpA* on either side of the *bmpA* –35 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 the –35 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 *asfZ*_{Bbu} RNA, primers Ptetl1 and

AftsZ were used in long PCR (48) to generate an amplicon with *PtetI* fused to *asftsZ* (Fig. 1B). Primer AftsZ (Table 1) contained a *KpnI* site (underlined), a universal terminator sequence (boldface), *asftsZ_{Bbu}* DNA sequences, and a sequence complementary to the *PtetI* 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 *PtetI* fused to *asftsZ_{Bbu}* was excised and subcloned into the *KpnI* and *PstI* sites of pKFSS1 (Fig. 1B). To create pLD7 (Fig. 1C), DNA containing the complete *ftsZ_{Bbu}* gene was amplified from *B. burgdorferi* genomic DNA and fused with a DNA segment containing *PtetI* by long PCR (48) using primers FtsZ1 and FtsZ2 and purified. Primer FtsZ1 (Table 1) contained a DNA sequence complementary to *PtetI* (italics). Primer FtsZ2 (Table 1) contained a *KpnI* site (underlined). This amplicon was fused to *PtetI* using long PCR (48) and Tet1 and FtsZ2 as forward and reverse primers. The resulting amplicon was cloned into pCR2.1-TOPO, transformed into *E. coli* DH5 α Z and selected on LB agar plates with 50 μ g 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 μ g of recombinant plasmid DNA. After overnight recovery, the electroporated cells were diluted and 1×10^6 to 2×10^6 cells in 200 μ l of complete BSK-H medium containing 400 μ g of kanamycin/ml for selection of clones of *B. burgdorferi* containing *tetR* and 400 μ g of kanamycin/ml and 50 μ g 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 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 *tetR-flaB* promoter-kanamycin gene fusion using primers LuxS5 and LuxS6 to confirm it was the result of a double crossover. The fusion of *asftsZ_{Bbu}* with the *PtetI* promoter was confirmed by sequence analysis using *PtetI*.

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 Schöning). Immunoblots were developed with alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G (Jackson ImmunoResearch, West Grove, Pa.) and enhanced chemifluorescence technology according to the manufacturer's instructions (ECF 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. DNA-free mRNA (50 to 100 ng in a volume of 20 μ l) 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 7900HT sequence detection system (Applied Biosystems, Foster City, Calif.). TaqMan reactions were performed in a volume of 25 μ l 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 6FAM/TAMRA was used as the probe (Table 1). PCRs were 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 (C_T) 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.

Growth of *B. burgdorferi* 297 and its derivatives. Cultures were inoculated at a final concentration of 10^5 cell/ml in 3 ml of complete BSK-H medium containing 6% rabbit serum, incubated at 34°C in 1% CO₂ in the absence or presence of inducer to final concentrations of 10^8 cells/ml. Growth of *B. burgdorferi* cells was assessed by counting cells stained with acridine orange under a fluorescence microscope (49).

FIG. 3. Analysis of *B. burgdorferi* 297/*tetR*. (A) PCR analysis shows a larger amplicon in *B. burgdorferi* 297/*tetR* (lane 3) than in wild-type (lane 2) or in *B. burgdorferi* clone not containing *tetR* insertion (lane 4). Lane 1 shows PCR amplicon from pCR2.1-TOPO containing the DNA fragment used for *tetR* insertion by allelic exchange (see Fig. 1). (B) Silver stain and immunoblot of sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel. Immunoblot with rabbit anti-TetR shows TetR immunoreactivity in lysates from *B. burgdorferi* 297/*tetR* clone containing chromosomal *tetR* insertion (lane 1) and in *E. coli* (lane 3) but not in wild-type *B. burgdorferi* 297 (lane 2).

Microscopic analyses of cell length. Cultures of *B. burgdorferi* 297/*tetR* and derivatives containing pLD6 and pKFSS1 (10^6 cells/ml) were stained with acridine orange and examined by fluorescence microscopy without or with induction with 1.5 μ g of ATc/ml. *B. burgdorferi* cells in three independent slides were examined (magnification, $\times 1,250$), 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. Spirochete lengths on photomicrographs of similar fields were also measured, and a stage micrometer was used to convert these lengths to μ m. A similar approach was used to analyze *E. coli* DH5 α Z and *E. coli* DH5 α Z(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_{Bbu}* gene product and 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*, *Leptospira interrogans*, and *E. coli*, respectively (Fig. 2). Within the highly conserved N terminus region, FtsZ_{Bbu} has a Gly-rich block, G₁₁₈MG GGTGTG₁₂₆, identical to the characterized GTP-binding site of *E. coli* FtsZ (37), and a N₂₂₃IDFADV₂₂₉ 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 D₃₈₅DDIDVPTFLR₃₉₅ conserved region that is

FIG. 4. Production of filamentous *B. burgdorferi* by ATc-mediated induction of *asfZ* RNA. (A) *B. burgdorferi* 297/*tetR* cultured for 24 h in the presence of 1.5 μ g of ATc/ml (acridine orange staining; magnification, $\times 1,250$). (B) *B. burgdorferi* 297/*tetR* (pLD6) cultured for 24 h in the absence of ATc (acridine orange staining; magnification, $\times 1,250$). (C) *B. burgdorferi* 297/*tetR* (pLD6) cultured for 24 h in the presence of 1.5 μ g of ATc/ml (acridine orange staining; magnification, $\times 1,250$). (D) Mean ($\pm$ the SE) spirochete length (in μ m) in cultures of *B. burgdorferi* 297/*tetR* and 297/*tetR* (pLD6) cultured for 24 h in the absence (–) or presence (+) of 1.5 μ g of ATc/ml. Spirochete lengths ($n = 20$) on photomicrographs (magnification, $\times 1,250$) from three independent experiments were measured for each experimental condition; a stage micrometer was used to convert these lengths to μ m. ***, $P < 0.001$. (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 μ g 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.

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 *asfZ* RNA. Construction of pLD6 to mediate regulated synthesis of *asfZ* 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. After electroporation of pLD6 into *B. burgdorferi* 297/*tetR*, clones containing pLD6 were selected by using appropriate antibiotics. Twenty-four hours after expression of *asfZ*_{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).

By 24 h of induction, filamentous cells accounted for $78 \pm 11\%$ (mean $\pm$ 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) ($25 \pm 7\%$) or in control induced or uninduced *B. burgdorferi* 297/*tetR* cultures at this time ($14 \pm 3\%$ and $19 \pm 3\%$, 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 $48 \pm 4\%$ 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) ($23 \pm 2\%$) or in control induced or uninduced *B. burgdorferi* 297/*tetR* cultures ($14 \pm 1\%$ and $13 \pm 1\%$, 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 *asftsZ*_{Bbu}.

Quantitation of *ftsZ*_{Bbu} mRNA after induction of production of *asftsZ*_{Bbu} RNA. The filamentous borrelial phenotype was most probably a result of the inability of these cells to divide because of the lack of FtsZ_{Bbu} generated by the induced *asftsZ*_{Bbu}. As a first step toward understanding the mechanism of action of induced *asftsZ* RNA, *ftsZ*_{Bbu} mRNA was examined by RT-PCR and real-time RT-PCR. RT-PCR indicated that induction of *asftsZ*_{Bbu} RNA by 1.5 μg of ATc/ml for 24 h was associated with a sharp decrease in *ftsZ*_{Bbu} 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 *asftsZ*_{Bbu} is associated with decreased *ftsZ*_{Bbu} 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 *ftsZ*_{Bbu} could be ectopically expressed in *E. coli* and whether its gene product would interfere with *E. coli* FtsZ function, pLD7, containing *ftsZ*_{Bbu} under the control of the *Ptet* hybrid promoter (Fig. 1), was electroporated into *E. coli* DH5 α . 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* DH5 α Z expressing TetR (32) was successful. This could suggest that uncontrolled expression of FtsZ_{Bbu} in *E. coli* was lethal. By 12 h after induction of synthesis of ectopic FtsZ_{Bbu} in *E. coli* DH5 α Z (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* DH5 α Z pro-

FIG. 5. ATc-induced production of *asRNA* in *B. burgdorferi* 297/*tetR* (pLD6) is associated with decreased *ftsZ* mRNA. A. RT-PCR analysis of *ftsZ* and *flaB* mRNA from *B. burgdorferi* 297/*tetR* and *B. burgdorferi* 297/*tetR* (pLD6) cultured for 24 h in the absence (–) and presence (+) of 1.5 μg of ATc/ml. (B) Relative expression of *ftsZ* mRNA (*ftsZ*/*flaB* ratio) by real-time RT-PCR in *B. burgdorferi* 297/*tetR* (●) and *B. burgdorferi* 297/*tetR* (pLD6) (○) in the presence or absence of 1.5 μg of ATc/ml added at time zero (arrow). There is significantly less *ftsZ* mRNA present in *B. burgdorferi* 297/*tetR* (pLD6) than in *B. burgdorferi* 297/*tetR* after induction of *asftsZ* by ATc ($P < 0.001$, two-way analysis of variance). ***, $P < 0.001$.

ducing TetR in the absence (not shown) or presence of ATc (Fig. 6A). Small numbers of filamentous cells were also present in *E. coli* DH5 α Z (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 FtsZ_{Bbu} can interfere with the function of the *E. coli* FtsZ protein in *E. coli*.

DISCUSSION

Although the deduced *ftsZ*_{Bbu} gene product is highly homologous to other bacterial FtsZ proteins, the activity of *ftsZ*_{Bbu} and FtsZ_{Bbu} 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

FIG. 6. Production of filamentous *E. coli* by ATc-mediated induction of ectopic *ftsZ_{Bbu}*. (A) *E. coli* DH5 α Z cultured for 24 h in the presence of 1.5 μ g of ATc/ml (acridine orange staining; magnification, $\times 1,250$). (B) *E. coli* DH5 α Z (pLD7) cultured for 24 h in the absence of ATc (acridine orange staining; magnification, $\times 1,250$). (C) *E. coli* DH5 α Z (pLD7) cultured for 24 h in the presence of 1.5 μ g of ATc/ml (acridine orange staining; magnification, $\times 1,250$). (D) Mean ($\pm$ the SE) bacterial length (in μ m) in cultures of *E. coli* DH5 α Z and *E. coli* DH5 α Z(pLD7) cultured for 24 h in the absence (–) or presence (+) of 1.5 μ g of ATc/ml. Bacterial lengths ($n = 20$) on photomicrographs (magnification, $\times 1,250$) from three independent experiments were measured for each experimental condition; a stage micrometer was used to convert these lengths to μ m. ***, $P < 0.001$. See Materials and Methods for details.

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 *PtetI* 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-

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, 27, 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, 31, 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 *Ptetl* 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 *Ptetl* hybrid promoter since uninduced cultures of *B. burgdorferi* 297/*tetR* (pLD6) contained cells that were slightly longer (Fig. 4B and D) and grew significantly more slowly (Fig. 4E) than the *B. burgdorferi* 297/*tetR* 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 *Ptetl*

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 *Ptetl* promoters (28). These might include changes in pKFSS1 supercoiling, which might decrease the access of the repressor to a region in the plasmid where *Ptetl* is located (2). Titration of the levels of TetR by excessive amounts of *Ptetl* 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 *Ptetl* 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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