Cloning and Characterization of a *Rhizobium meliloti* Homolog of the *Escherichia coli* Cell Division Gene *ftsZ*

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Received 29 May 1991/Accepted 17 July 1991

The ftsZ gene is essential for initiation of cell division in Escherichia coli and Bacillus subtilis. To begin our studies of division arrest during differentiation of Rhizobium meliloti bacteroids, we isolated a R. meliloti ftsZ homolog, $ftsZ_{Rm}$. Degenerate primers directed towards a conserved region of ftsZ were used to amplify a segment of R. meliloti DNA by polymerase chain reaction, and the product of this reaction was then used to isolate positive clones from a bacteriophage library. The DNA sequence of an open reading frame containing the region of homology indicated that the R. meliloti FtsZ protein (FtsZ_{Rm}) is 50% homologous to the known E. coli and B. subtilis FtsZ proteins, but at 590 amino acids (63 kDa), it is predicted to be nearly 50% larger. Strong expression of an approximately 70-kDa labeled protein in a coupled in vitro transcription-translation system supports this prediction. The additional 200 amino acids appear to fall in a single internal domain highly enriched for proline and glutamine residues. When we regulated R. meliloti ftsZ (ftsZ_{Rm}) expression on a high-copy-number plasmid in E. coli with P_{tac} and lacl^a, cells were smaller than normal in the presence of low FtsZ_{Rm} levels (with no isopropyl- β -p-thiogalactopyranoside [IPTG]) and filamentous when FtsZ_{Rm} was overproduced (with IPTG). These results suggest that low levels of FtsZ_{Rm} stimulate E. coli cell division, while high levels may be inhibitory.

During development of nitrogen-fixing nodules in alfalfa, Rhizobium meliloti bacteria are released from infection threads into root cortical cells and surrounded by a plantderived peribacteroid membrane, and then they begin differentiating into bacteroids capable of fixing atmospheric nitrogen (19). Two important physiological changes that occur during differentiation are the cessation of cell division and the arrest of DNA replication after a few rounds (39). Mature bacteroids of R. meliloti have an increased nucleic acid content and a more rounded shape and are larger than their free-living predecessors (29). Occasional branched forms are also seen. Morphological changes during development in nonsymbiotic microbial systems such as Bacillus subtilis or Caulobacter crescentus have been well studied (20, 27), but the genes and factors involved in the morphological aspects of Rhizobium differentiation have not been defined. One potential way of addressing the mechanism of division arrest and bacteroid morphogenesis is to understand the genes and factors required for cell division control in Rhizobium cells. Since cell cycle mutants of R. meliloti bacteroids have not yet been isolated, our initial strategy involves isolating homologs of key Escherichia coli cell cycle genes that might be developmentally regulated in R. meliloti.

In *E. coli, ftsZ* is a key gene required for normal cell division (for reviews, see references 8, 15, and 21). FtsZ acts early in the cell division process, since heat induction of *ftsZ* temperature-sensitive mutant cells results in complete and immediate cessation of division (10), and the filamentous cells thus formed have no visible constriction points (4). FtsZ also appears to be rate limiting for septum initiation: moderately increasing the level of FtsZ results in a minicell phenotype because of an increase in division frequency at the cell poles, and the average cell becomes smaller, sug-

gesting that septation is occurring earlier in the cell cycle (42). Very high levels of FtsZ, however, completely inhibit division (5, 42). FtsZ protein appears to be a target of other division factors: the products of the *minCD* locus, which appear to suppress divisions at the cell poles, act to antagonize the FtsZ effect (5). Also, SulA, which is induced during DNA damage, inhibits the effect of FtsZ, resulting in cell filamentation (21).

The ftsZ gene is conserved in other eubacteria, as demonstrated by Southern blot and Western blot (immunoblot) analyses (7). The only other characterized ftsZ homolog, from *B. subtilis*, encodes a protein that is 50% identical to *E. coli* FtsZ (2) and is essential for synthesis of both vegetative and sporulation septa (3). However, expression of *B. subtilis* ftsZ in *E. coli* inhibits host cell division (2). In this paper, we report the isolation and initial characterization of a *R. meliloti* ftsZ homolog which shares sequence similarity with its *E. coli* and *B. subtilis* counterparts and affects *E. coli* cell division but codes for a significantly larger protein than its counterparts do.

MATERIALS AND METHODS

Media, chemicals, and enzymes. Ampicillin, kanamycin, and tetracycline were used in Luria broth (32) and plates as necessary at 50, 50, and 10 µg/ml, respectively. To detect inserts in pUC or pBluescript vectors, Luria broth plates with ampicillin were supplemented with a 20-mg/ml solution of 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside and IPTG (isopropyl- β -D-thiogalactopyranoside) in N,N-dimethylformamide to give a final concentration of 40 µg/ml. Restriction enzymes, T4 DNA ligase, and Klenow DNA polymerase were from New England BioLabs or Promega Biotec and were used according to the manufacturers' recommendations. Radiochemicals were from Amersham.

Bacterial strains, bacteriophages, and plasmids. All strains and plasmids used are listed in Table 1. JFL101 was obtained from J. Lutkenhaus.

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TABLE 1. Strains, phages, and plasmids used in this study

Strain, phage, or plasmid	Genotype or characteristics	Source or reference
R. meliloti		
RCR2011	Wild type	26
1021	Str ^r derivative of RCR2011	26
E. coli		
XL1-blue	recA1 endA1 gyrA96 thi hsdR17 supE44 relA1 lac (F' proAB lacPZΔM15, Tn10)	32
LE392	hsdR514 hsdM supE44 supF58 Δ(lacIZY)6 galK2 galT22 metB1 trpR55	32
pLK-17	lac mcrA mcrB hsdR gal supE	Stratagene
JFL101	ftsZ84 recA ilv deo ara(Am) lacZ125(Am) galU42(Am) trp(Am) tyrT supFA81(Ts)	J. Lutkenhaus
Bacteriophages		
λGE M- 11	λ replacement-cloning vector	Promega Biotec
λJC9	λGEM-11 containing 10- and 4-kb BamHI frag- ments from R. meliloti replacing central stuffer	This work
Plasmids		
pBluescript SK+	<i>lac</i> expression vector (Ap ^r) containing M13 replication origin	Stratagene
pUC118	pUC18 (Ap ^r) containing M13 replication origin	41
pUC119	Same as pUC118, with reversed polylinker	41
pJC8	220-bp cloned PCR prod- uct (<i>Eco</i> RI fragment)	This work
pJC05	3-kb XhoI fragment con- taining ftsZ-	This work
pJC06	2-kb BamHI-HindIII frag-	This work
pJC18	10-kb BamHI fragment	This work
рЈС062	0.22-kb deletion of C ter- minus of $ftsZ_{Rm}$	This work

PCR. Synthesis of a 221-bp polymerase chain reaction (PCR) product containing a segment of ftsZ from R. meliloti total DNA was accomplished with the following primers (Pu is a purine, Py is a pyrimidine, and N is G, A, T, or C): 5'CTTGAATTCAAPyACNGAPuGCNCAPuGC3' (to prime synthesis of the nontemplate strand) and 3'TACCCNCCNC CNTGNCCNTGCTTAAGTTC5' (template strand). Two segments of amino acid identity between the B. subtilis and E. coli FtsZ proteins that were spaced sufficiently far apart within the gene to give an easily detectable PCR product and that could yield relatively nondegenerate primer sequences, particularly at the 3' ends, were chosen. At the third position in a codon, maximum degeneracy was introduced in order to prevent a complete mismatch. Annealing potential at the 3' end was maximized by using only the first two invariant positions of the C-terminal amino acid codon. To facilitate cloning of the PCR product, we engineered the 5' termini to contain both an EcoRI restriction site and a CTT terminal trinucleotide to make the site more accessible to the EcoRI enzyme.

PCR reactions were performed on an Ericomp programmable thermal reactor in a 0.1-ml reaction mixture containing 10 mM Tris (pH 8.3), 1.5 mM MgCl₂, 50 mM KCl, 0.1 mg of gelatin per ml, deoxynucleoside triphosphates at 0.2 mM, primers at 1 μ M, 0.1 to 1 μ g of template DNA, and 3 U of *Thermus aquaticus* DNA polymerase (Amplitaq; Perkin-Elmer/Cetus). Each of the 30 cycles of amplification consisted of denaturation for 1 min at 95°C, annealing for 1 min at 55°C, and polymerization for 2 min at 74°C. PCR products were then separated by electrophoresis on a 2% agarose gel in Tris-borate-EDTA buffer at 250 V, visualized by ethidium bromide staining and UV light, excised, eluted, and purified by extraction with phenol-chloroform and precipitation with ethanol.

Preparation and manipulation of DNA. Total genomic DNA from *R. meliloti* 1021 was prepared as described previously (26). Minipreparations of plasmid DNA from *E. coli* were done by alkaline extraction (32). Large-scale plasmid preparations for use as templates in S-30 reactions were isolated from CsCl gradients as described elsewhere (32). Minipreparations of phage λ DNA were made from high-titer liquid lysates essentially as described elsewhere (25). DNA fragments were purified either by elution from agarose gels by freeze-squeeze (40) or directly from lowmelting-point GTG agarose (SeaKem) as described elsewhere (32).

Construction and screening of the phage library. A library of *R. meliloti* 1021 DNA, constructed by D. Bramhill, was made by ligating total DNA partially digested with *Bam*HI into *Bam*HI-cleaved arms of λ GEM-11 according to the instructions given by Promega Biotec. The phage were plated on *E. coli* LE392 or pLK-17 to give single plaques and transferred to GeneScreen nylon membranes (New England Nuclear); phage DNA was fixed to the filters as described before (22). The filters were screened with the insert from pJC8. After positives were purified and rescreened, phage DNA from λ JC9 was isolated, digested, blotted, and probed with the insert from pJC8 to map the gene.

Plasmid constructions. Plasmid pJC8 was made by ligating EcoRI-cleaved pUC119 to the gel-purified 221-bp PCR product which had been cleaved with EcoRI. Plasmids pJC18 and pJC05 were made by cloning the λ JC9 10-kb BamHI and 3-kb XhoI fragments that contained homology to the PCR product into BamHI- and XhoI-cleaved pUC119, respectively. In plasmid pJC05, the orientation of ftsZ is opposite to that of the vector lac promoter (P_{lac}) . Plasmid pJC06 was constructed by cleaving pJC05 with BamHI (in the pUC119 polylinker) and HindIII (downstream of ftsZ) and cloning it into pBluescript cleaved with BamHI and HindIII, placing ftsZ under the control of P_{lac} . For DNA sequencing, nested deletions from the polylinkers of pJC05 and pJC06 were made by using exonuclease III and S1 nuclease (Bethesda Research Laboratories) as previously described (32). Some parts of the sequence that were not covered by exonuclease deletions were subcloned into either pUC118 or pUC119 for sequencing. Plasmid pJC062 is an exonuclease III deletion derivative of pJC06, in which 395 nucleotides of R. meliloti DNA was deleted from the HindIII site.

DNA sequencing. Single-stranded DNA for sequencing was prepared from plasmids derived from pUC118, pUC119, or pBluescript SK+ by using the M13K07 helper phage as described elsewhere (31). DNA sequencing using the dideoxynucleotide chain termination method (33) was carried out with the Sequenase 2.0 kit (U.S. Biochemicals) using either the -40 primer from the kit (pJC05 derivatives) or the T7





FIG. 1. Ethidium bromide-stained gel showing PCR amplification of a segment of *R. meliloti ftsZ*. A 2% agarose gel was loaded with λ DNA digested with *BglI* (lane M), half of a PCR reaction mixture with *R. meliloti* DNA template (lane Rm), and half of a PCR reaction mixture with *E. coli* DNA template (lane Ec). The arrow denotes the position of the 210-bp marker.

promoter primer from Promega Biotec (pJC06 derivatives). Both dGTP and dITP mixes were used.

In vitro protein synthesis. Coupled transcription-translation reactions contained S-30 extracts made from *R. meliloti* RCR2011 and 1 μ g of the appropriate CsCl-banded plasmid DNA template and were performed as described previously (11). Proteins labeled with [³⁵S]methionine were diluted in sample buffer, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (17), and visualized by autoradiography using Kodak XAR-5 film.

DNA sequence analysis. DNA and protein sequence comparisons, characterizations, and mapping were done with the University of Wisconsin Genetics Computer Group programs (9). Data base homology searches were done with FASTA and TFASTA (18). Multiple sequence alignments were accomplished with TULLA (38).

Microscopic techniques. Bacterial cells grown in Luria broth with ampicillin were placed directly on glass slides without fixation and viewed under a Nikon light microscope with Nomarski optics. Photographs were taken with a Nikon FE 35-mm camera and Kodak Ektachrome (160 ASA) film.

RESULTS

Cloning the ftsZ_{Rm} gene. Since the DNA sequence homoldgy between the E. coli and B. subtilis ftsZ genes is only 50% (2) and since Rhizobium DNA has a much higher GC content, we did not expect to easily detect the R. meliloti homolog by Southern hybridization using an internal segment of the E. coli ftsZ gene as a probe. However, there are many short segments of amino acid identity between the E. coli and B. subtilis FtsZ proteins. We reasoned that a PCR approach using primers to these regions would successfully amplify internal R. meliloti-specific sequences that could be used as a 100% homologous probe to screen a genomic library. Therefore, primers corresponding to two relatively nondegenerate hexamer amino acid segments near the N terminus which would give rise to a predicted 221-bp PCR product were made (see Materials and Methods). After amplification of R. meliloti 1021 genomic DNA with these primers, a 221-bp product, which was by far the predominant species, was observed (Fig. 1). This product was cloned into pUC119 to make pJC8.

The DNA sequence of the cloned PCR product (pJC8) revealed a partial reading frame coding for 68 amino acids with 57% amino acid identity to *E. coli* FtsZ (FtsZ_{Ec}) and 62% identity to *B. subtilis* FtsZ (FtsZ_{Bs}), indicating that a portion of the *R. meliloti ftsZ* gene had been successfully

amplified. The GC-rich DNA sequence of the pJC8 insert was only 57 and 63% homologous to the corresponding regions in $ftsZ_{\rm Ec}$ and $ftsZ_{\rm Bs}$, respectively, suggesting that a hybridization approach would have been difficult. The DNA sequence of the amplified fragment also revealed closely spaced *PstI* and *NsiI* sites that would subsequently prove useful in locating ftsZ on a λ clone and determining its orientation.

As expected, the amplified region hybridized much more strongly to R. meliloti DNA than to E. coli DNA on a Southern blot; consequently, it was used to screen a λ library of R. meliloti DNA. Several positive clones were obtained; one, λ JC9 (Fig. 2), which contained a 10-kb BamHI insert which hybridized to the probe, was chosen for further study. Southern blot analysis of restriction enzymedigested R. meliloti genomic DNA and purified λ JC9 DNA indicated that the entire ftsZ gene was probably contained within the BamHI insert, based on the predicted position of the PCR product within the coding region and the mapped location of this PCR product within the BamHI fragment (Fig. 2). A 3-kb XhoI fragment likely containing the entire $ftsZ_{Rm}$ gene was subcloned (pJC05); it and another 2-kb BamHI-HindIII subclone with the insert in the opposite orientation (pJC06) were used for DNA sequencing (Fig. 2).

DNA and deduced protein sequence analysis of ftsZ. We obtained the nucleotide sequence of the 2-kb BamHI-HindIII fragment on both DNA strands by using the strategy diagrammed in Fig. 2. The sequence (Fig. 3) revealed a single long open reading frame coding for an acidic 590amino-acid protein, predicted to be 63.0 kDa with an isoelectric point of 4.91. This $ftsZ_{Rm}$ reading frame uses a high percentage of preferred *R. meliloti* codons throughout its entire length (1) and is preceded by a sequence highly homologous to the Shine-Dalgarno sequence important for ribosome binding (Fig. 3, underlined), suggesting that it is translated efficiently (36). An inverted repeat resembling a stable rho-independent terminator structure (30) lies 130 to 150 bp downstream from the stop codon, having a calculated ΔG of approximately -17 kcal (1 cal = 4.184 J) (12) (Fig. 3).

The $FtsZ_{Rm}$ protein is 45 to 50% identical to both the B. subtilis and the E. coli FtsZ proteins; a three-way protein alignment is shown in Fig. 4. A pairwise comparison of the three proteins gives comparable levels of relatedness. Strikingly, however, the predicted 590-amino-acid R. meliloti FtsZ protein is more than 50% larger than $FtsZ_{Ec}$ (383 residues) and $FtsZ_{Bs}$ (382 residues). In this alignment, the extra approximately 200 amino acids in $FtsZ_{Rm}$ are present in a single nonhomologous domain near the C terminus (Fig. 4). All three proteins have similar amino acid compositions; however, $FtsZ_{Rm}$ contains 8.1% proline residues versus 3.92% in FtsZ_{Ec} and 3.40% in FtsZ_{Bs} (Table 2). The nonhomologous domain in $FtsZ_{Rm}$ is especially enriched in proline and glutamine residues, which together account for over 30% of the total amino acids in this region (Table 2). In fact, when 202 amino acids from this region are removed from the $FtsZ_{Rm}$ sequence, the size, charge, and composition of this deleted protein (Table 2) become very similar to those of $FtsZ_{Ec}$ and $FtsZ_{Bs}$; the only exception is the presence in $FtsZ_{Rm}$ of three cysteine residues, while the other FtsZproteins lack cysteines.

We searched the data base for proteins with potential homology to $FtsZ_{Rm}$ but found no proteins other than $FtsZ_{Ec}$ and $FtsZ_{Bs}$ with significant sequence similarity. However, when the 200-amino-acid nonhomologous domain of $FtsZ_{Rm}$ was used to search the data base, among the proteins receiving the highest similarity scores were several wheat



FIG. 2. Restriction nuclease map and sequencing strategy for the *R. meliloti ftsZ* gene. Narrow open boxes denote λ GEM-11 DNA arms; filled boxes denote cloned *R. meliloti* DNA inserted into the *Bam*HI site. These include the 3-kb *Xhol* fragment and the 10-kb *Bam*HI fragment that hybridize to the *ftsZ* PCR product probe (lightly and densely hatched boxes, respectively) and another *Bam*HI insert that does not hybridize to the *ftsZ* probe (stippled box). Thick boxes denote portions of plasmid DNA flanking the inserted *Rhizobium* DNA. These include DNA from pUC119 and pBluescript SK + vectors (open boxes for pJC05 and pJC06, respectively), the *ftsZ* gene open reading frame (light stippled boxes), the segment of *ftsZ* and the vector *lac* promoters (P_{*lac*}) are indicated by arrows above the boxes. The sequencing strategy is indicated at the bottom; the arrows represent 5'-to-3' sequencing runs with single-stranded DNA generated by exonuclease III deletions of pJC05 (rightward arrows) or pJC06 (leftward arrows) or by subcloning into pUC119 or pBluescript SK +. The right end of the open arrow represents the extent of the deletion in pJC062. Restriction endonuclease cleavage sites are represented by vertical tick marks: B, *Bam*HI; H, *Hind*III; N, *Nsi*I; P, *Pst*I; Sa, *Sal*I; Sp, *Sph*I; Ss, *Sst*I; X, *Xho*I.

high-molecular-weight glutenins, which contain extensive proline-glutamine tracts that resemble the Pro-Gln motifs found in $FtsZ_{Rm}$.

In view of the possibility that the 200-amino-acid domain had originated by an insertion event or had been removed in *E. coli* and *B. subtilis* by a splicing event, perhaps analogous to σ^{k} processing in *B. subtilis* (37), we searched for potential splice sites and inverted repeats flanking the domain. A 7-base inverted repeat (TCGTCGC; Fig. 3) was found; the left repeat was located 11 nucleotides upstream from the start of the nonhomologous domain, while the right repeat was located at the C-terminal boundary of the domain. However, neither this sequence nor any potential splicing sequences were found in the other homologs, so the significance of the inverted repeat is unclear.

Detection of FtsZ protein. Previous observations by Corton et al. (7) support the prediction that $FtsZ_{Rm}$ protein is much larger than its homologs: a protein from *Agrobacterium tumefaciens*, a close relative of *R. meliloti* (43), was shown to migrate at a significantly higher molecular mass than *E. coli* FtsZ on a Western blot probed with anti-FtsZ antiserum (7). To further test the idea that $FtsZ_{Rm}$ is much larger than its *E. coli* and *B. subtilis* counterparts, we expressed [³⁵S] methionine-labeled $FtsZ_{Rm}$ in a *R. meliloti*-derived cell-free transcription-translation system (S-30 extract). When the full-length *ftsZ*_{Rm} gene was used as template, a tight triplet of bands migrating with an apparent molecular mass of approximately 70 kDa was strongly expressed (Fig. 5). The degree of expression was independent of whether *ftsZ*_{Rm} was in the same orientation as P_{lac} (pJC06) or in the opposite orientation (pJC05). This apparent size is 7 kDa larger than the predicted 63 kDa, which may be a result of aberrant migration, as has been observed with other acidic proteins such as bacterial σ factors (14). To demonstrate that this triplet represents $FtsZ_{Rm}$, a template was used (pJC062) that lacks the last 72 amino acids of $FtsZ_{Rm}$ and has a reading frame terminating 2 amino acids into the pUC119 polylinker, resulting in a 520-amino-acid protein predicted to be approximately 8 kDa smaller than the wild-type $FtsZ_{Rm}$ (Fig. 2). A triplet migrating with an apparent molecular mass of approximately 62 kDa was indeed observed (Fig. 5), strongly suggesting that the triplet represents FtsZ_{Rm}. The fact that the truncated species also migrates unusually slowly suggests that the features that cause aberrant migration are still intact in the truncated species; this idea is supported by the estimated isoelectric point of the truncated protein of 4.74, which is similar to that of the full-length protein.

To provide additional support for the existence of an unusually large *R. meliloti* FtsZ protein, we carried out Western analysis of $FtsZ_{Rm}$ synthesized from plasmids in *E. coli* with anti-FtsZ_{Ec} serum (7). In addition to the 40-kDa $FtsZ_{Ec}$ band, a band at approximately 70 to 80 kDa was visible on a blot containing protein from strain XL1-Blue (*lacI*^q) carrying plasmid-borne *ftsZ*_{Rm} under P_{*lac*} control (pJC06) and became at least 10 times more intense with protein from the same strain after induction with 1 mM IPTG over 24 h (results not shown). Multiple bands were not observed; this lack could be due either to the presence of

1	XDaI TCTAGATCGGTCGGCATATCGCCGGACGAGACAGGTGTTCGAGTTTGAAGGAATTGGCCG	60
61	GCTCGGCAAGGCGGCCAGGGAAAG <u>AGGAACAGGACATGGCCATCAACTTGCAGAAGC</u> M A I N L O K P	120
121	CGGACATTACCGAGCTGAAGCCGCGTATCACGGTCTTCGGCGTCGGCGGCGGCGGCGGCGGCGGCGGCGG	180
181	$\label{eq:constraint} \begin{array}{c} **** \\ \texttt{ACGCCGTCAACAACAATGATCACCGCCGGGCTCCAGGGCGTCGATTTCGTCGTCGCCCAACA} \\ \texttt{A} \ V \ N \ M \ \texttt{M} \ \texttt{I} \ \texttt{T} \ \texttt{A} \ \texttt{G} \ \texttt{L} \ \texttt{Q} \ \texttt{G} \ \texttt{V} \ \texttt{D} \ \texttt{F} \ \texttt{V} \ \texttt{V} \ \texttt{A} \ \texttt{N} \ \texttt{T} \end{array}$	240
241	CGGATGCCAGGCACTCACCATGACCAAGGCCGAGCGGATCATCCAGATGGGTGTTGCCG D A O A L T M T K A E R I I O M G V A V	300
301	TCACCGAAGGTCTTGGTGCCGGCTGGAGCCGCCGCCGCCGCCGCCGCGGAGAAT T E G L G A G S Q P E V G R A A A E E C	360
361	* GCATCGACGAGATCATCGATCACCTGCAGGGCACGCATATGTGCTTCGTCACCGCCGGCA I D E I I D H L Q G T H M C F V T A G M	420
421	TOGOCGOCCACCGOCACGOGTOCTOCTCCCATCCTCCCCAGCCTOCCCCCAACAAGG G G G T G T G A A P I V A Q A A R N K G	480
481	GTATCCTCACCGTCGGCGTCGTCACCAAGCCCTTCCATTTCGAAGGCGGACGCCGCATGC I L T V G V V T K P F H F E G G R R M R	540
541	GGATCGCCGACCAGGGTATTTCCGATCTTCAGAAGTCGGTCG	600
601	CGAACCAGAACCTCTTCCGCATCGCCAATGACAAGACGACCTTCGCGGACGCCTTCGCCA N Q N L F R I A N D K T T F A D A F A M	660
661	TGGCCGACCAGGTTCTTTATTCGGGCGTCGCCTGCATCACCGACCTCATGGTCAAGGAAG A D Q V L Y S G V A C I T D L M V K E G	720
721	GCCTCATCAACCTCGACTTCGCCGACGTCCGTTCGGTGATGGGCGCGCGC	780
781	TGATGGGTACGGGCGAAGCCTCCGGCGAGGGCCGCGAATGGCCGCGGAAGCCGCGA M G T G E A S G E G R A M A A A E A A I	840
841	TCGCCAACCCGCTGCTCGACGAAACCTCGATGAAGGGCGCTCAGGGTCTGCTCATCTCCA A N P L L D E T S M K G A Q G L L I S I	900
901	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	960
961	AGGAGGTCGATCCGGACGCCAACATCATTCTCGGCGGACCTTCGACGAAGAGCTCGAAG E V D P D A N I I L G A T F D E E L E G	1020
1021	GCCTCATTCGGGTTTCCGTCGTCGCCACCGCATCGACCGCACGGCGCGGAGGTGGCCG L I R V S V V A T G I D R T A A E V A G	1080
1081	GCCGCTCCGCCGACTTTCGTCCGCTGGTAGCGCCGAAGCCGATCGTCCGCCGCCGCCG R S A D F R P V A P K P I V R P S A A V	1140
1141	$\begin{array}{cccc} TTCCGGCTCAGCCGCAGCCGCAGCCGCAGCCGCGCGCGCG$	1200
1201	AGCAGCCGCTCCAGCAGCAGAATGTCGACCACATCGCGCTCGCCATTCGCGAGGCCGAAA Q P L Q Q Q N V D H I A L A I R E A E M	1260
1261	TGGAGCGCGAGCTCGACATCGCTGCGCGCGCGCGAGCGCGCGC	1320
1321	AGCCCCACCTCCAGGAAGAGGCCCTTCCGTCCGCAGAGCAAGCTCTTCGCCGGCGTCGCTC P H L Q E E A F R P Q S K L F A G V A P	1380
1381	CGACGGAGGCCGCACCGGTCATGCGGCCGGGCGCAGCCGGCCCGGGCCGGGCCGAGATGC T E A A P V M R P A Q P A P R P V E M Q	1440
1441	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	1500
1501	TOCGGCAGCAGCCGAGCCGGTACGCATGCCGAAGGTCGAGGACTTTCCGCCGGTCGTGA R Q Q A E P V R M P K V E D F P P V V K	1560
1561	AGGCAGAAATGGATTACCGGACGCAGCCGGCGCCTGCGGATCAGGAAGAAGCGCGGGCCGA A E M D Y R T Q P A P A H Q E E R G P M	1620
1621	TGGGACTCCTGAACGGATCACCAGCTCGCTCGGCTGCGTGCAACGGGAAGGGGAATG G L L N R I T S S L G L R E R E A T N V	1680
1681	TCTCGTCCGACATGACCGCAGCGCGCGCCGCCGCCGCCGCCGCCGCCGCCGCC	1740
1741	CGCCGGAAGCCAGCTCTATGCGCCGCGTCGCGGCCAGCTCGACGATCACGGTCGCGCTG P E A S L Y A P R R G Q L D D H G R A A	1800
1801	CACCGCAGATGCGGTCGCATGAAGACGATCAGCTCGAAATTCCGGCGTTCCTGCGCCGCC P Q M R S H E D D Q L E I P A F L R R Q	1860
1861	AGTCGAGCTGATACCTACGGCGCCGTGCGCTCGATCGGACGCGCAATGTCGCCGTAGTAC S $_{\rm S}$	1920
1921	TTTCAGTCGCCGCAGTTTTTCCTGAGCCGGCTCCGGTTCAGGGAAACATGCGGAGGCTGT	1980
1981	TCCTTCACCTTGCCGAGGCGCCCGGGCGCCATTTTTCGATTTTTTACGCGAAA	2040
2041	HINGIII TCAGAAGCTT 2050	

only one immunoreactive species or to the inability of this method to resolve the triplet bands. Although the antibody did not appear to be specific enough to detect a 70- to 80-kDa band above background from R. meliloti 1021 total protein, the IPTG-inducible 70- to 80-kDa band in E. coli is consistent with the size observed in vitro.

Effects of $FtsZ_{Rm}$ expression on E. coli growth and cell division. In order to determine whether the cloned $ftsZ_{Rm}$ exhibited biological activity, we assayed the effects of the cloned gene on E. coli cells. The presence of $ftsZ_{Rm}$ on a high-copy-number pUC derivative (pJC05) in strain XL1-Blue was not detrimental to E. coli growth or viability (data not shown), and cell morphology appeared to be similar to that of XL1-Blue cells containing pBluescript vector (Fig. 6a). E. coli XL1-Blue cells containing $lacI^{q}$ and $ftsZ_{Rm}$ under the control of P_{lac} (pJC06) had severalfold-lower levels of FtsZ than those containing pJC05, as assayed by Western blot (results not shown). Microscopic analysis revealed that the average length of these cells was much less than that of cells containing pJC05 or pBluescript vector (Fig. 6b), suggesting that low-level expression of $FtsZ_{Rm}$ results in hyperdivision. However, induction of these cells carrying pJC06 with 1 mM IPTG led to cell filamentation (Fig. 6d) and at least a 10-fold decrease in CFU after 24 h (data not shown), suggesting that the overproduced $FtsZ_{Rm}$ was directly or indirectly inhibiting the E. coli cell division system. We reasoned that intermediate levels of expression might result in a phenotype between the small-cell and filamentous phenotypes. This idea was supported by the appearance of mostly normal-length cells at IPTG concentrations between 1 and 10 µM, suggesting that an equilibrium had been reached between division stimulation and inhibition (Fig. 6c). Neither pJC06 nor pJC05 was able to complement the ftsZ84(Ts) mutation in JFL101, which results in filamentation at high temperature (data not shown); however, JFL101 does not have the $lacI^{q}$ allele and thus might allow a significantly higher level of $ftsZ_{Rm}$ expression than the XL1-Blue derivatives used in the procedures described above.

DISCUSSION

In *E. coli*, the ftsZ gene is essential for initiation of the division septum, the first known step in the separation of two bacterial cells. As a first step in our effort to understand the mechanism of division arrest during bacteroid differentiation, we have isolated a *R. meliloti* ftsZ homolog by using PCR to obtain a completely homologous probe internal to the gene. The high degree of amino acid identity shared between the *E. coli* and *B. subtilis* proteins facilitated the PCR approach, which was simple and rapid and allowed identification of definite ftsZ sequence homology before a library was searched. The sequences of only two ftsZ genes were known prior to this work. The PCR was successful since the amino acids in the region of the primers are

FIG. 3. Nucleotide and predicted protein sequences of the R. *meliloti ftsZ* gene. The sequence of the nontemplate strand is shown from an XbaI site 92 bases within the pJC05-pJC06 insert from the XhoI site to the HindIII site; the sequence is numbered starting with

the 5'-terminal base in the XbaI recognition sequence. The amino acid sequence is shown in single-letter code, with each letter below the first base of its corresponding codon; the period denotes a termination codon. The underlined sequence is the predicted ribosome-binding site. The inverted arrows represent inverted repeats which are part of a predicted rho-independent terminator stem. Asterisks above the sequence denote the bases to which PCR primers were directed. Solid lines above the sequence mark the positions of the TCGTCGC inverted repeats.

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FIG. 4. Amino acid sequence alignment of FtsZ proteins from *E. coli* (FtsZ_{Ec}), *R. meliloti* (FtsZ_{Rm}), and *B. subtilis* (FtsZ_{Bs}), indicated by Ec, Rm, and Bs, respectively. Identical amino acids in FtsZ_{Rm} and at least one of the others at a given position are marked with vertical dashes. The numbering system is relative to the FtsZ_{Rm} sequence.

TABLE 2. Properties and composition of known FtsZ proteins and $FtsZ_{Rm}$ domains^a

Property	FtsZ _{Rm}	FtsZ _{Ec}	FtsZ _{Bs}	FtsZ _{Rm} -ext ^b	FtsZ _{Rm} -int ^c
Mol wt	62,974	40,297	40,355	40,895	22,097
Net charge	-18	-18	-10	-17	-1
pI	4.91	4.54	4.84	4.61	6.82
No. of resi-	590	383	382	388	202
dues					
Mol% of ^d :					
Ala	13.9	12.3	11.5	13.4	14.9
Gly	7.8	11.0	10.0	10.6	2.5
Cys	0.5	0.0	0.0	0.8	0.0
Ile	5.4	5.7	8.2	7.0	2.5
Leu	6.4	7.6	7.1	7.2	5.0
Val	8.0	9.9	8.1	7.0	9.9
Met	4.1	4.2	2.9	4.4	3.5
Phe	2.7	3.4	2.4	3.1	2.0
Tyr	0.5	0.5	0.5	0.5	0.5
Тгр	0.0	0.0	0.0	0.0	0.0
Pro	8.1	3.9	3.4	3.6	16.8
Gln	7.8	3.7	5.2	4.6	13.9
Asn	2.5	4.4	6.3	3.4	1.0
Arg	7.0	4.7	4.2	6.4	7.9
Lys	2.2	4.4	6.0	2.3	2.0
Glu	7.0	6.8	7.3	6.4	7.9
Asp	5.3	7.1	5.5	6.7	2.5
His	1.4	1.0	0.5	1.3	1.5
Ser	4.1	3.1	4.7	4.6	3.0
Thr	5.4	6.3	6.3	6.7	3.0

^a Values in this table were obtained from the PEPTIDESORT program.

^b Compilation of amino acids 1 to 321 and 524 to 590 of $FtsZ_{Rm}$.

^c Compilation of amino acids 322 to 523 of $FtsZ_{Rm}$. ^d Values rounded to the nearest 10th.

completely conserved in all three bacteria, and the degeneracies within the primer sequences allowed for the changes at the DNA level.

The $FtsZ_{Rm}$ protein has over 200 amino acids more than



FIG. 5. Synthesis of $FtsZ_{Rm}$ in vitro. Shown is an autoradiograph of a sodium dodecyl sulfate-polyacrylamide gel loaded with [³⁵S]methionine-labeled protein from a *R. meliloti* S-30 extract primed with the following DNA templates: pBluescript SK+ (lane 1), no DNA (lane 2), pJC05 (lane 3), pJC06 (lane 4), and pJC062 (lane 5). The positions of ¹⁴C-labeled protein standards are indicated at the right (in kilodaltons).

its homologs, and they appear to be in a single nonhomologous domain. The weak amino acid sequence similarity to wheat prolamin storage proteins is due to the predominance of proline and glutamine repeats in the glutenins. It has been proposed that these repeats form a stacked β -sheet secondary structure which could account for the mechanical flexibility of these glutenous proteins (35). However, Chou-Fasman secondary structure predictions for this region of FtsZ_{Rm} do not predict an obvious β structure (24), leaving the possible role of this region unclear at present. The 7-bp inverted repeats that flank the region hint at the possibility of an insertion event giving rise to this domain, but the domain is not homologous to any insertion elements in the data base, so the evolutionary origins of this sequence remain uncertain.

When the 200 extra amino acids of $FtsZ_{Rm}$ are not included in protein comparisons, $FtsZ_{Rm}$ is highly similar to its homologs. One exception is that $FtsZ_{Rm}$ contains cysteine residues completely lacking in the other two proteins which could be involved in disulfide cross bridges. Interestingly, the C-terminal 65 amino acids of $FtsZ_{Ec}$ and $FtsZ_{Bs}$ contain unusually high percentages of proline and glutamine residues (respectively 13.8 and 10.8% for $FtsZ_{Ec}$ and 12.3 and 9.2% for $FtsZ_{Bs}$) relative to the 3 to 5% compositions for the proteins as a whole (Table 2), suggesting that this domain may have a structural motif which is greatly expanded in the glutamine- and proline-rich nonhomologous domain of $FtsZ_{Rm}$.

Efficient expression of the $ftsZ_{Rm}$ gene in the R. meliloti S-30 extract in either orientation with respect to P_{lac} is consistent with the predicted efficient codon usage and good ribosome-binding site and suggests that a promoter for $ftsZ_{Rm}$ lies within the upstream 190 bp present in both pJC05 and pJC06. It is not clear why multiple protein bands were seen. There are no alternative translation initiation sites (ATG or GTG) in the sequence upstream of the ftsZ open reading frame, making multiple starts unlikely. Differential C termini are very unlikely, since the same pattern of bands was observed with the protein truncated at its C terminus (24). Degradation, specific processing, or modifications are still open possibilities and can be tested. Western analysis with an antibody specific for $FtsZ_{Rm}$ is the best way to investigate whether this phenomenon is seen with proteins synthesized in vivo. A considerable number of smaller bands had been observed on Western blots with FtsZ proteins from bacteria closely related to E. coli (7), and an analogous pattern, including the triplet band seen in vitro, could be detectable with a specific antibody. The slower mobility of FtsZ_{Rm} relative to its predicted molecular weight could be due to its low pI, as is the case with σ factors, or to an unusual structure or folding pattern. It is notable that an unusual structure is postulated to be the cause of aberrantly slow migration of the high-molecular-weight wheat glutenins (13), which, as already noted, have sequence compositions similar to that of the nonhomologous domain of $FtsZ_{Rm}$.

With the R. meliloti ftsZ gene now cloned, the next step is to determine its possible role in septum initiation in Rhizobium species. Several of our findings argue that this gene is equivalent to the known E. coli ftsZ. First, expression of the gene is predicted to be efficient, and we observed strong in vitro expression. Second, $ftsZ_{Rm}$ maps physically to the large chromosome (24), which is the location of most of the housekeeping functions. Third, a reading frame homologous to ftsA, another essential cell division gene located immediately upstream from ftsZ in E. coli and B. subtilis, similarly lies just upstream from ftsZ in R. meliloti (23). Finally, an



FIG. 6. E. coli cells (XL1-Blue) containing pBluescript vector and 1 mM IPTG (a), pJC06 with no IPTG (b), pJC06 and 10 μ M IPTG (c), and pJC06 with 1 mM IPTG (d). Magnification, \times 530.

IPTG dependence phenotype for $ftsZ_{Rm}$, which seems to reflect complementation at low levels and division inhibition at high levels, both consistent with a functional ftsZ gene, has been demonstrated in E. coli. However, the situation in Rhizobium species may be complicated. For example, several lines of evidence suggest that a second copy of $ftsZ_{Rm}$ may exist. First, weak but discrete secondary bands were observed on Southern blots containing R. meliloti genomic DNA probed with the cloned PCR product (6). Second, there are precedents in R. meliloti for duplication of genes: there exist multiple homologs of genes involved in nodulation, such as the *nodD* family (16) and the duplicate *nodPQ* locus (34); in addition, there exist two copies of a chaperonin 60 (cpn60) gene, which is homologous to the essential groEL gene of E. coli (28). Third, in addition to the large crossreactive band on the Western blot of Agrobacterium proteins reported by Corton et al. (7), there is an equally strong band slightly smaller than 40 kDa, suggesting the presence of a

second FtsZ product. Experiments addressing whether the $ftsZ_{Rm}$ gene is essential in *R. meliloti*, what effect its expression has on cell division, and whether a second $ftsZ_{Rm}$ gene exists are in progress.

ACKNOWLEDGMENTS

We thank J. Lutkenhaus for strain JFL101 and for the anti-FtsZ serum, D. Bramhill for the *R. meliloti* genomic library, and T. Egelhoff, M. Yelton, and R. Fisher for the S-30 extracts. We thank our entire group for useful discussions, and we particularly acknowledge R. Fisher, J. Ogawa, and J. Schwedock for critically reading the manuscript.

This research was supported by USDA grant 88-37262-3978. W.M. was additionally supported by an NSF Postdoctoral Fellowship in Plant Biology.

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