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Interactions between Heterologous FtsA and FtsZ Proteins at the FtsZ Ring

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FtsZ and FtsA are essential for cell division in *Escherichia coli* and colocalize to the septal ring. One approach to determine what regions of FtsA and FtsZ are important for their interaction is to identify in vivo interactions between FtsA and FtsZ from different species. As a first step, the *ftsA* genes of *Rhizobium meliloti* and *Agrobacterium tumefaciens* were isolated and characterized. In addition, an FtsZ homolog that shared the unusual C-terminal extension of *R. meliloti* FtsZ1 was found in *A. tumefaciens*. In order to visualize their localization in cells, we tagged these proteins with green fluorescent protein (GFP). When *R. meliloti* FtsZ1-GFP or *A. tumefaciens* FtsZ-GFP was expressed at low levels in *E. coli*, they specifically localized only to the *E. coli* FtsZ ring, possibly by coassembly. When *A. tumefaciens* FtsA-GFP or *R. meliloti* FtsA-GFP was expressed in *E. coli*, they failed to localize detectably to the *E. coli* FtsZ ring. However, when *R. meliloti* FtsZ1 was coexpressed with them, fluorescence localized to a band at the midcell division site, strongly suggesting that FtsA from either *A. tumefaciens* or *R. meliloti* can bind directly to its cognate FtsZ. As expected, GFP-tagged FtsZ1 and FtsA from either *R. meliloti* or *A. tumefaciens* localized to the division site in *A. tumefaciens* cells. Therefore, the 61 amino acid changes between *A. tumefaciens* FtsA and *R. meliloti* FtsA do not prevent their direct interaction with FtsZ1 from either species, suggesting that those residues are not essential for protein-protein contacts. Moreover, the failure of the two non-*E. coli* FtsA derivatives to interact strongly with *E. coli* FtsZ in this in vivo system unless their cognate FtsZ was also present suggests that FtsA-FtsZ interactions have coevolved and that the residues which differ between the *E. coli* proteins and those of the two other species may be important for specific interactions.

The *ftsA* and *ftsZ* genes are essential for *Escherichia coli* cell division (12, 35). FtsA protein associates with the cell membrane (30) and the division septum (35), and genetic evidence suggests that it interacts with other cell division proteins, such as FtsI, FtsN, FtsQ, and FtsZ (14, 16). The first known step in septation is the assembly of a ring consisting of FtsZ protein at the future division site (6). FtsZ, present in archaea as well as eubacteria (3, 28, 39), is a GTPase with a GTP-binding domain that resembles that of tubulin (15, 29, 31). As with tubulin, the C termini of FtsZ proteins are highly variable, exemplified best by the two distinct *R. meliloti* FtsZ homologs (26, 27). Also by analogy with tubulin, the C termini are not essential for FtsZ polymerization, suggesting that the N-terminal portions of heterologous FtsZ proteins can coassemble (25).

Recently, it has been shown that FtsA colocalizes to the FtsZ septal ring and other FtsZ cytoskeletal structures in *E. coli* cells (2, 25). FtsA has significant sequence similarity to the ATPase superfamily, which includes actin and hsp70 chaperones (7). The function of FtsA, including its putative ATPase activity (33), is not yet understood. Studies with *ftsZ* and *ftsA* conditional mutants suggest that FtsA acts later than does FtsZ (1, 20, 35). FtsA levels in the cell have been estimated at 50 to 200 molecules, about 100-fold lower than FtsZ levels (6, 38). This high FtsZ/FtsA ratio appears to be essential for normal cell division, since perturbations in the level of either protein inhibit the process (13, 18, 37). Although *ftsA* and *ftsZ* are cotranscribed, promoters for *ftsZ* transcription lie in the upstream *ftsA* gene (40), resulting in more *ftsZ* transcription than *ftsA* transcription. Therefore, there is ample genetic and cytological evidence that *E. coli* FtsZ and FtsA interact either directly or indirectly.

In this paper, we report the cloning and sequencing of *ftsA* gene homologs of *Rhizobium meliloti* and *Agrobacterium tumefaciens*, two closely related members of the *Rhizobiaceae* family which have intimate and complex relationships with plants. In addition, we characterize an *A. tumefaciens ftsZ* homolog. These species are unusual in that their cells branch in response to cell cycle blockage (24) and that at least *R. meliloti* has two *ftsZ* homologs (27). The high degree of relatedness between the two species is advantageous for addressing questions of cross talk between different FtsA and FtsZ proteins. Here, we describe the development of a novel in vivo interaction system that uses green fluorescent protein (GFP) tags to directly visualize structures formed by FtsZ and FtsA. Using this system, we confirm our previous suggestion (25) that FtsZ proteins from different species can coassemble at the FtsZ ring. Furthermore, we provide evidence that both *R. meliloti* and *A. tumefaciens* FtsA proteins can localize to the *A. tumefaciens* FtsZ ring. However, we show that FtsA-GFP fusions from these two species cannot interact significantly with the FtsZ ring of *E. coli* unless one of their FtsZ proteins is colocalized there. This result suggests that FtsA contacts FtsZ directly and specifically and that interspecies FtsA-FtsZ interactions can occur as long as the sequence divergence between the homologs is not too great.

MATERIALS AND METHODS

Bacterial strains and plasmids. All strains and plasmids used in this study are listed in Table 1.

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TABLE 1. Strains and plasmids used in this work

Bacterial strain or plasmid	Relevant characteristics	Source and/or reference
Strains		
E. coli JM105	F′ *traD36 lacI*^{q} Δ(*lacZ*)M15 *proA*$^+$*B*$^+$/*thi rpsL endA sbcB15 hsdR4* Δ(*lac-proAB*)	Laboratory stock
A. tumefaciens A136	Strain C58 cured of Ti plasmid	Laboratory stock
R. meliloti MB501	Restriction-defective derivative of Rm1021	Laboratory stock
Plasmids		
pBC SK(+)	Cam^r, cloning vector^r	Stratagene
pBluescript SK(+)	Carb^r, cloning vector	Stratagene
pJC18	Carb^r, *R. meliloti ftsA-ftsZ1* inserted at *Bam*HI site of pBluescript SK(+)	26
pWM240	Carb^r, *A. tumefaciens ftsA* and 5′ end of *ftsZ1*, inserted at *Pst*I site of pBluescript SK(+)	This work
pRW16	Carb^r, 3′ end of *A. tumefaciens ftsZ1* cloned between *Pst*I and *Eco*RV sites of pBluescript SK(+)	This work
pWM261	Carb^r, *A. tumefaciens ftsA-ftsZ1*, cloned between *Bam*HI and *Hin*dIII sites of pBluescript SK(+)	This work
pSW213	Tet^r, broad-host-range IncP plasmid containing *lac* promoter, pIC-20R polylinker, and *lacI*^{q}	8
pWM176	Tet^r, pSW213 containing the *tac* promoter	27
pMK4	Tet^r, pWM176 containing $ftsZ_{Ec}$ under *tac* promoter control	25
pWM189	Tet^r, pWM176 containing $ftsZ1_{Rm}$ under *tac* promoter control	27
pGBC	Cam^r, GFP cloned between *Xba*I and *Bam*HI sites in pBC SK(+)	This work
pGBS	Carb^r, GFP cloned between *Xba*I and *Bam*HI sites in pBluescript SK(+)	This work
pAEG1	Cam^r, $ftsA_{Ec}$ cloned between *Sac*I and *Xba*I sites in pGBC	This work
pAAG1	Cam^r, $ftsA_{At}$ at *Sac*I and *Xba*I sites in pGBC	This work
pARG1	Cam^r, $ftsA_{Rm}$ cloned between *Not*I and *Xba*I sites in pGBC	This work
pZEG1	Cam^r, $ftsZ_{Ec}$ cloned between *Sac*I and *Xba*I sites and *lacI*^{q} cloned at *Eco*RI site in pGBC	pZG 25
pZ1RG1	Cam^r, $ftsZ1_{Rm}$ cloned between *Ecl*136II and *Xba*I sites and *lacI*^{q} cloned at *Eco*RI site in pGBC	This work
pZ1RG2	Tet^r, *Fse*I-*Kpn*I fragment from pZ1RG1 cloned between *Fse*I and *Kpn*I sites in pWM189	This work
pAEG2	Carb^r, $ftsA_{Ec}$ cloned between *Sac*I and *Xba*I sites in pGBS	pAG 25
pAAG2	Carb^r, $ftsA_{At}$ cloned between *Sac*I and *Xba*I sites in pGBS	This work
pARG2	Carb^r, $ftsA_{Rm}$ cloned between *Not*I and *Xba*I sites in pGBS	This work
pZEG2	Carb^r, $ftsZ_{Ec}$ cloned between *Sac*I and *Xba*I sites in pGBS	pZG 25
pZ1AG1	Carb^r, $ftsZ1_{At}$ cloned between *Sac*I and *Xba*I sites and *lacI*^{q} cloned at *Eco*RI site in pGBS	This work
pZ1AG2	Carb^r, $ftsZ1_{At}$ cloned between *Sac*I and *Xba*I sites in pGBS	This work
pAEG3	Carb^r Tet^r, pAEG2 ligated at *Pst*I site to pSW213	This work
pAAG3	Carb^r Tet^r, pAAG2 ligated at *Kpn*I site to pSW213	This work
pARG3	Carb^r Tet^r, pARG2 ligated at *Kpn*I site to pSW213	This work
pZEG3	Carb^r Tet^r, pZEG2 ligated at *Pst*I site to pSW213	This work
pZ1RG3	Carb^r Tet^r, pZ1RG2 ligated at *Kpn*I site to pSW213	This work
pZ1AG3	Carb^r Tet^r, pZ1AG2 ligated at *Kpn* site to pSW213	This work

Growth conditions. All *E. coli* and *R. meliloti* strains were growth in Luria-Bertani broth (10 g of tryptone per liter, 5 g of yeast extract per liter, 5 g of NaCl per liter). *A. tumefaciens* strains were grown in MG/L medium (5). Plasmids were maintained in these strains by the addition of 10 μg of tetracycline per ml, 50 μg of carbenicillin per ml (*A. tumefaciens* only), 50 μg of ampicillin per ml (*E. coli* only), or 25 μg of chloramphenicol per ml. Isopropyl-β-D-thiogalactopyranoside was added at a concentration of 40 μM to 1 mM to cells in early logarithmic phase (optical density at 600 nm, 0.05 to 0.1) to induce the genes downstream of the *tac* or *lac* promoter on plasmids. Inductions were done for 1 to 10 h, depending on the resulting strength of expression. Inductions were done for as short a period as possible to reduce the chances of toxic or other abnormal effects of the expressed proteins.

Chemicals and enzymes. Most restriction enzymes, T4 DNA ligase, and *Taq* polymerase were purchased from Promega. Pfu polymerase (Stratagene) was used for some PCRs. The remainder of restriction enzymes and Vent polymerase, used in conjunction with *Taq* polymerase for PCR, were purchased from New England Biolabs. Oligonucleotide primers for PCR were purchased from GIBCO/BRL.

Construction of expression plasmids. Plasmid and genomic DNA isolation, restriction digestion, ligation, transformation, Southern blotting, and library screening were performed essentially as described previously (32). To clone *R. meliloti ftsA*, a 10-kb *Bam*HI fragment from λ JC9 phage that hybridized to the *ftsZ* gene (26) was cloned into pUC119 (36) to yield pJC18. The region of pJC18 upstream of *ftsZ* corresponding to a 1.3-kb *Bam*HI-*Xba*I fragment was subcloned and sequenced.

To clone *A. tumefaciens ftsA* and *ftsZ1*, *A. tumefaciens* genomic DNA was used as the template for PCR amplification of a 0.2-kb fragment containing a highly conserved segment of *ftsZ*. The primers were the same as those used for PCR amplification of a conserved segment of *R. meliloti ftsZ1* (26). The PCR product was sequenced and found to have several sequence differences from that of *R. meliloti ftsZ1*. It was then used to probe a library made from 1- to 5-kb *Pst*I fragments from *A. tumefaciens* genomic DNA cloned into pBluescript SK(+). One positive clone containing a 2.5-kb *Pst*I fragment was chosen for further analysis. Upon sequencing this clone (pWM240), it was determined that it contained all of *ftsA* but only the N-terminal portion of *ftsZ1*. The remainder of the *ftsZ* gene was obtained by inverse PCR. First, an *Eco*RV site 0.5 kb upstream of the *Pst*I site within *ftsZ1* was identified. In order to obtain genomic sequence information downstream of *Pst*I, *A. tumefaciens* genomic DNA was cleaved with *Eco*RV and self-ligated. The circular *Eco*RV fragment containing the remainder of *ftsZ* was then PCR amplified with two divergently oriented primers. The resulting 2-kb PCR product was cleaved with *Pst*I and *Eco*RV and cloned into the *Pst*I and *Eco*RV sites of pBluescript SK(+) to make pRW16. The DNA sequence from the *Pst*I site confirmed that this fragment was contiguous to the *Pst*I fragment previously cloned. The entire *ftsZ1* gene was reconstructed by ligating the *Pst*I-*Hin*dIII fragment of pRW16 with the *Bgl*II-*Pst*I fragment of pWM240 to make pWM261, which was used as the source for *A. tumefaciens* FtsZ-GFP fusions.

All fusions to the M2 mutant version of GFP (10) were made to the C termini of FtsA and FtsZ. For expression in *E. coli*, pGBC was used as the cloning vector. This plasmid contains GFP inserted between the *Xba*I and *Pst*I sites of the pBC SK(+) polylinker so that GFP is in frame with the TCT triplet in the *Xba*I site and under control of the vector *lac* promoter. The general strategy used for making all fusions was to amplify the gene of interest by PCR and then ligate it to that *Xba*I site. Therefore, the downstream primers were all engineered with an *Xba*I site placed immediately after the last sense codon of the gene and in frame with the TCT triplet in the *Xba*I site of the vector. For fusing *ftsA* homologs to

GFP, the upstream primer was engineered to contain *Sac*I (*A. tumefaciens*) or *Not*I (*R. meliloti*) and was designed to fuse the N terminus of the vector *lacZ* gene in the SK polylinker with the first codon of *ftsA*. This strategy was used to circumvent potential translation problems associated with translational coupling to *ftsQ*. On the other hand, the upstream primers for amplification and cloning of *ftsZ* homologs carried the native *ftsZ* ribosome binding sites. The upstream primer for *R. meliloti ftsZ1* contained an *Eco*RV site; the PCR product was cleaved with *Eco*RV and *Xba*I and ligated to pGBC cut with *Ecl*136II (which leaves a blunt end at the upstream site for ligation with the *Eco*RV-cleaved end of the PCR product) and *Xba*I. The upstream primer for *A. tumefaciens ftsZ1* was engineered with a *Sac*I site so that the PCR product could be cloned into pGBC by using *Sac*I and *Xba*I.

For expression in *A. tumefaciens* and *R. meliloti*, the fusions were transferred to an IncP plasmid capable of replicating in *Rhizobiaceae.* For *A. tumefaciens*, this was done in two steps. First, fusions made in pBC SK(+) were transferred to pBluescript SK(+) because resistance to carbenicillin is a superior way to select for transformants. These plasmids were linearized by cleavage with *Kpn*I (*A. tumefaciens* and *R. meliloti* FtsA-GFP and FtsZ1-GFP) or *Pst*I (*E. coli* FtsA-GFP and FtsZ-GFP) and then ligated to *Kpn*I- or *Pst*I-linearized pSW213, the IncP plasmid used. The ligations were electroporated into *A. tumefaciens* A136, selecting for Carb^r and Tet^r. For expression in *R. meliloti*, inserts containing fusions in pBC SK(+) were directly cloned into the polylinkers of IncP plasmids pSW213 and pWM189 so that they would be transcribed from the *lac* or *tac* promoter and electroporated into MB501 by selecting for Tet^r.

DNA sequencing and analysis. Plasmid clones constructed as described above were sequenced from the insert ends with T7 or T3 polymerase primers or with custom oligonucleotide primers. Subsequent sequencing templates included partial deletions of the initial templates. All double-stranded DNA templates were prepared with a Prep-a Gene kit (Bio-Rad) and sequenced on an Applied Biosystems automated DNA sequencer at the Microbiology and Molecular Genetics Core Facility, University of Texas—Houston. Most DNA sequence data was obtained from both strands. The DNA and predicted protein sequences were analyzed with University of Wisconsin Genetics Computer Group software (17). Database searches for similarities with other proteins were performed with BLASTP and TFASTA. The FtsA protein alignment was assembled for publication with the SeqVu 1.0.1 program (Garvan Institute) and MacDraw Pro.

Microscopic techniques. To view cells, 5 to 10 μl of cells in broth were pipetted onto a glass slide and flattened with a small cover glass. Cells were viewed with an Olympus BX-60 phase-contrast microscope equipped with an oil-immersion objective (×100 magnification), 100-W mercury lamp, and a standard fluorescein isothiocyanate filter set for GFP. Images were captured with an Optronics DEI-750 three-chip color charged-coupled device camera. Images were digitized with a Scion LG3 framegrabber board and manipulated with Adobe Photoshop 3.0. A Tektronix Phaser 440 was used to make printouts of all images.

Nucleotide sequence accession numbers. The nucleotide sequence data reported here have been submitted to the GenBank database under accession nos. AF024659 and AF024660.

RESULTS AND DISCUSSION

Identification of *ftsA* homologs in *R. meliloti* and *A. tumefaciens*. The organization of the *ftsA-ftsZ* region of *R. meliloti* and *A. tumefaciens* is similar to that of *E. coli* and *Bacillus subtilis*, with an intergenic region of 60 bp for *E. coli*, 173 bp for *B. subtilis*, 93 bp for *R. meliloti* (Fig. 1), and 92 bp for *A. tumefaciens* (Fig. 2). We did not detect any significant sequence similarity between the *R. meliloti* or *A. tumefaciens* intergenic region and the others. The open reading frame corresponding to *R. meliloti ftsA* ($ftsA_{Rm}$) encodes a protein of 442 residues (Fig. 1), which would yield a 47-kDa product. The *A. tumefaciens ftsA* gene ($ftsA_{At}$) encodes a protein of 443 residues (Fig. 2). As in *E. coli*, both *ftsA* genes appear to be translationally coupled to *ftsQ* since the *ftsQ* stop and *ftsA* start codons overlap (Fig. 1 and 2). In addition, both *ftsA* genes are preceded by a potential ribosome binding site sequence. The codons for both genes are strongly biased toward the host codon preference.

It has previously been established that a second, unlinked copy of *ftsZ* (*ftsZ2*) exists in *R. meliloti* (9). Sequencing over 500 bp upstream of *ftsZ2* did not reveal any homology to *ftsA* (data not shown). When potential open reading frames were translated and used to search translated databases with TFASTA, no significant matches were detected. In addition, only one copy of *ftsA* was observed in hybridization experiments with digested *R. meliloti* genomic DNA (data not shown). Thus, it is likely that the *R. meliloti ftsA* we report here is the only copy of the gene in *R. meliloti*. Although the native expression of *ftsZ2* and *ftsA* has yet to be demonstrated, the intriguing possibility that two different FtsZ proteins interact with a single FtsA protein in *R. meliloti* cells exists.

$FtsA_{Rm}$ and $FtsA_{At}$ are similar to other known FtsA proteins and show the expected sequence homologies with members of the ATPase superfamily, such as actin, hsp70, and

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CGCACTCAAGGCGAGGGAGAAGATGCTGAAAGCCCAGGAGAAGCGGATATGAGTTTGTTC      60
 A  L  K  A  R  E  K  M  L  K  A  Q  E  K  R  I  *(ftsQ STOP)
                                  (ftsA START) M  S  L  F
BamHI
GGATCCGCCAATTTCGGCCTTCCGCGTCTGAAGCCCTTGCCCTCGAAGCGGTCGCATGTC      120
G  S  A  N  F  G  L  P  R  L  K  P  L  P  S  K  R  S  H  V

GTGTCGGTGCTCGATATCGGCTCGACCAAGGTGGTATGCATGATCGGCCGCCTGACGCCG      180
V  S  V  L  D  I  G  S  T  K  V  V  C  M  I  G  R  L  T  P

CGTGCCGAGAGCCAGATCCTGCCCGGGCGCACGCACAGCATCGAGGTCATCGGCATCGGT      240
R  A  E  S  Q  I  L  P  G  R  T  H  S  I  E  V  I  G  I  G

CACCAGAAGTCGCGGGGCGTCAAGAATGGCGTCATCGCCGATCTCGATGCGGTCGAAAGC      300
H  Q  K  S  R  G  V  K  N  G  V  I  A  D  L  D  A  V  E  S

GTCGTCCGGCTTGCGGTCGATGCAGCCGAGCGCATGGCGGGACTGACGATCGACAGCCTC      360
V  V  R  L  A  V  D  A  A  E  R  M  A  G  L  T  I  D  S  L

ATCGTGAATGTTTCAGCCGGCCGCCTGCAGAGCGACGTCTATACCGCGACGATCGATCTC      420
I  V  N  V  S  A  G  R  L  Q  S  D  V  Y  T  A  T  I  D  L

GGCGGGCAGGAGGTCGAGGCGAACGATCTGAAGAAGGTACTCGCTGCCGCGGGGCACCAG      480
G  G  Q  E  V  E  A  N  D  L  K  K  V  L  A  A  A  G  H  Q

TCGCTGCGCACCGACCGCGCCATCCTGCACTCTCTGCCGACCGGCTTCTCGCTGGACGGC      540
S  L  R  T  D  R  A  I  L  H  S  L  P  T  G  F  S  L  D  G

GAGCGCGGCATCCGCGACCCGCTGGCGATGTTCGGTGACGTTCTCGGCGTCGACATGCAT      600
E  R  G  I  R  D  P  L  A  M  F  G  D  V  L  G  V  D  M  H

GTGCTGACGGCGGAGCGGCCCGCACTGAAGAACCTCGAGCTCTGCGTCAATCGCGCTCAC      660
V  L  T  A  E  R  P  A  L  K  N  L  E  L  C  V  N  R  A  H

CTCTCGGTCGAGGGCATGGTCGCGACGCCTTATGCCAGCGGGCTTGCGGCACTCGTGGAT      720
L  S  V  E  G  M  V  A  T  P  Y  A  S  G  L  A  A  L  V  D

GACGAGGTCGAGCTCGGATGTGCGGCCATCGACATGGGTGGCGGTACGACGACGATCTCG      780
D  E  V  E  L  G  C  A  A  I  D  M  G  G  G  T  T  T  I  S

GTTTTCGCCGAAGGCAAGCTCGTCCATGCGGACGCCGTCGGCCTTGGCGGCCACCACGTC      840
V  F  A  E  G  K  L  V  H  A  D  A  V  G  L  G  G  H  H  V

ACGACCGATCTGGCGCGCGGCCTGTCCACCCGCATCGAGGATGCCGAGCGTCTGAAGGTC      900
T  T  D  L  A  R  G  L  S  T  R  I  E  D  A  E  R  L  K  V

GTGCACGGTTCGGCGCTTCCGAACAGCGCGGACGAGCGCGATATCATTTCGGTTCCGCCG      960
V  H  G  S  A  L  P  N  S  A  D  E  R  D  I  I  S  V  P  P

ATCGGCGAAGACGACCGCGACCAGCCGACGCACGTGCCGCGCGCTCTGGTCTCGCGCATC     1020
I  G  E  D  D  R  D  Q  P  T  H  V  P  R  A  L  V  S  R  I

GTTCGCGCTCGCATCGAAGAGACGCTGGAACTCATACGCGACCGCATCCAGCGGTCCGGT     1080
V  R  A  R  I  E  E  T  L  E  L  I  R  D  R  I  Q  R  S  G

TTCAGCCCGATCGTCGGCAAGCGCATCGTCTTGACGGGGGGAGCCAGCCAGCTGACCGGT     1140
F  S  P  I  V  G  K  R  I  V  L  T  G  G  A  S  Q  L  T  G

CTGCCGGAAGCGGCGCGGCGCATTCTTGCGCGCAATGTCCGCATCGGCCGCCCGCTCGGC     1200
L  P  E  A  A  R  R  I  L  A  R  N  V  R  I  G  R  P  L  G

GTATCCGGCCTGCCGGCCGCTGCCAAGGGCCCGGCCTTTTCCACGGCCGTCGGGCTGATG     1260
V  S  G  L  P  A  A  A  K  G  P  A  F  S  T  A  V  G  L  M

ATCTACCCTCAGGTCGCCGACCTCGAGACACATGCCGCCGGCAGCGGCATGTTCTCCACC     1320
I  Y  P  Q  V  A  D  L  E  T  H  A  A  G  S  G  M  F  S  T

CTCGGCGGCAACAGCCGTTTCGCCCGGATGGGGCAATGGCTGAAAGAAAGTTTCTAGATC     1380
L  G  G  N  S  R  F  A  R  M  G  Q  W  L  K  E  S  F  *

GGTCGGCATATCGCCGGACGAGACAGGTGTTCGAGTTTGAAGGAATTGGCCGGCTCGGCA     1440

AGGCGGCCAGGGAAAGAGAAGGAACAGGACATGGCCATCAACTTGCAGAAGCCGGACATT     1500
                    (ftsZ) M  A  I  N  L  Q  K  P  D  I
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FIG. 1. Nucleotide and predicted amino acid sequences of *R. meliloti ftsA*. Asterisks denote stop codons; the 3′ end of *ftsQ* and the 5′ end of *ftsZ1* are also shown. The potential ribosome binding site for *ftsA* is underlined. The sequence in pJC18 is shown from the *Bam*HI site to the right. The sequence to the left of the *Bam*HI site was obtained from a contiguous clone.

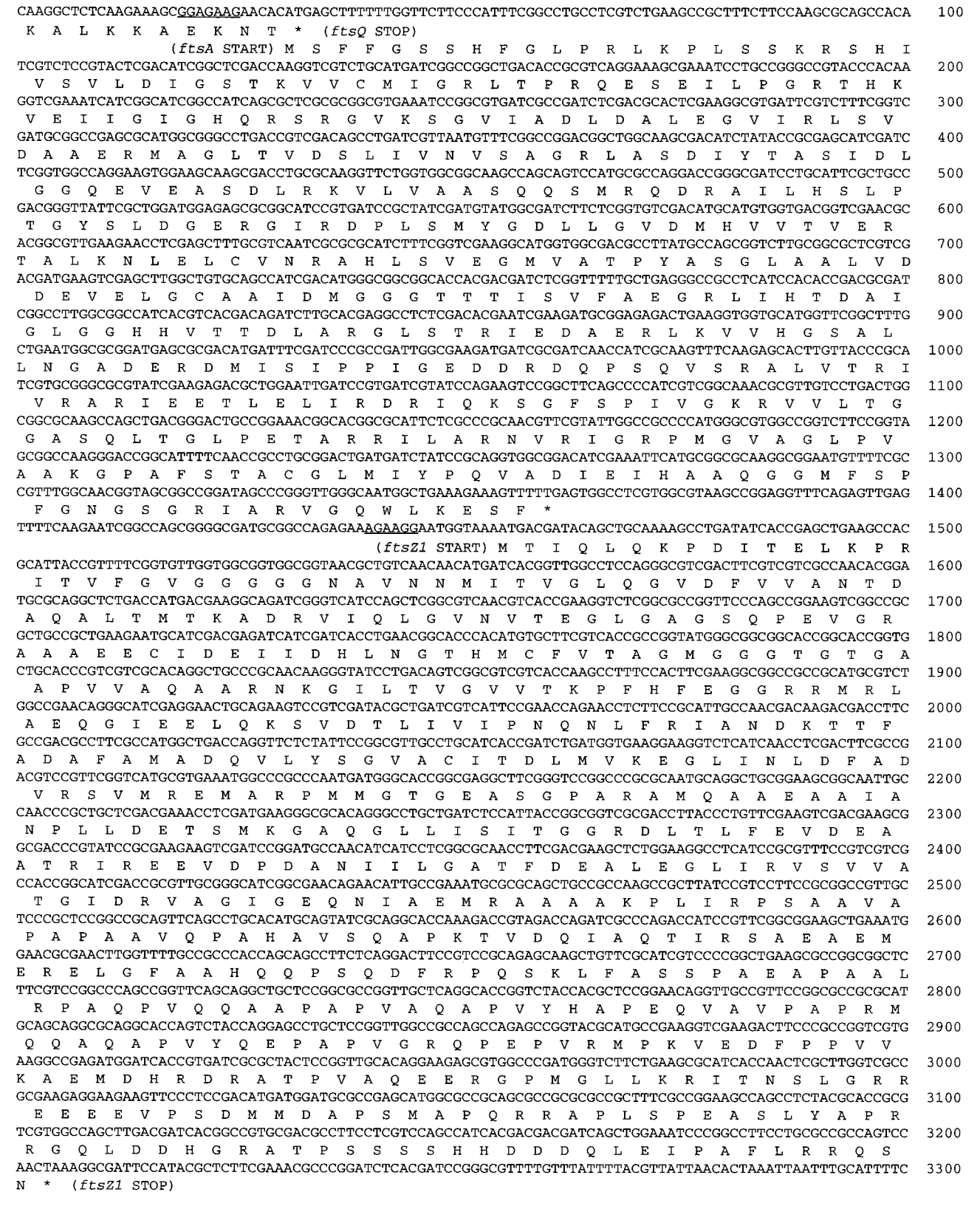

FIG. 2. Nucleotide and predicted amino acid sequences of the *A. tumefaciens ftsA* and *ftsZ1* genes. The potential ribosome binding sites for *ftsA* and *ftsZ1* are underlined. Asterisks denote stop codons.

MreB (7, 23). When several known FtsA proteins were aligned and compared, the region of highest sequence identity corresponded to residues 353 to 358 (IVLTGG) of $FtsA_{Rm}$ (Fig. 3). This is one of the two regions highly similar to MreB (19) and corresponds to the adenosine binding pocket in the crystal structures of actin, hsp70 (DnaK), and hexokinase. However, $FtsA_{Rm}$ and $FtsA_{At}$ also differ somewhat from other known FtsA proteins. They contain additional residues in three segments of the N terminus, corresponding to residues 1 to 15, 45 to 54, and 310 to 312. Perhaps these regions are involved in specific interactions, for example, with FtsZ1. In addition, the portion of *B. subtilis* FtsA ($FtsA_{Bs}$) carboxy-terminal from the connect 2 domain has virtually no sequence similarity to the others, whereas $FtsA_{Rm}$ and *E. coli* FtsA ($FtsA_{Ec}$) have significant similarity at their carboxy termini. The 31% identity between $FtsA_{Rm}$ and $FtsA_{Ec}$, 33% identity between $FtsA_{Rm}$ and $FtsA_{Bs}$, and 35% identity between $FtsA_{Ec}$ and $FtsA_{Bs}$ imply that all three proteins are comparably related in tests of pairwise combinations. This finding is consistent with the comparable relatedness of FtsZ proteins from the same three species (28).

An *ftsZ* homolog from *A. tumefaciens* encodes a protein highly similar to *R. meliloti* FtsZ1. Immediately downstream from $ftsA_{At}$ is a homolog of *ftsZ*, which encodes a predicted protein of 583 amino acids. Because it contains a large C-terminal extension similar but not identical to that of $FtsZ1_{Rm}$, we have designated the protein product $FtsZ1_{At}$ (Fig. 2). The $FtsZ1_{At}$ and $FtsZ1_{Rm}$ proteins are 81% identical overall but are 94% identical over the first 320 residues that comprise the conserved N-terminal portion of all FtsZ proteins (28) (Fig. 4). As is true with all FtsZ homologs, the greatest degree of divergence between $FtsZ1_{Rm}$ and $FtsZ1_{At}$ occurs in the first part of the C terminus, defined here as residues 320 to 460 of $FtsZ1_{Rm}$. This divergence is manifested by a number of amino acid substitutions as well as two deletions of $FtsZ1_{At}$ (relative to $FtsZ1_{Rm}$) and two deletions of $FtsZ1_{Rm}$ (relative to $FtsZ1_{At}$ (Fig. 4). These regions are rich in both glutamine and proline residues; as suggested previously for $FtsZ1_{Rm}$ (26), this may be a spacer domain that links the conserved N terminus with the conserved residues at the extreme C terminus. Interestingly, cell division proteins ZipA and FtsK also each contain an extensive proline- and glutamine-rich domain that may serve as a rigid spacer (22). The significant divergence of the FtsZ C termini in these closely related species further supports the idea that the C terminus is highly variable and may have a species-specific function.

Immunoblot studies of FtsZ proteins from diverse species originally suggested that two FtsZ homologs existed in *A. tumefaciens* (11). This data, in addition to our discovery of two homologs in *R. meliloti*, make it likely that *A. tumefaciens* indeed harbors another *ftsZ* gene. With the same PCR primers used for the isolation of $FtsZ1_{At}$, we isolated a PCR product that had homology to *ftsZ* but was significantly different from $ftsZ1_{At}$ (data not shown). We suggest that this represents a segment of a second *ftsZ* homolog in *A. tumefaciens*.

FtsZ proteins from two significantly different species can colocalize to the FtsZ ring. To determine whether $FtsZ1_{At}$ and $FtsZ1_{Rm}$ could coassemble in vivo, we first needed to confirm that the FtsZ1 proteins of *R. meliloti* and *A. tumefaciens* could localize to division sites of their respective hosts. We fused GFP to the C termini of the FtsZ1 proteins in a manner similar to the strategy previously reported (25). The fusions were initially cloned into high-copy-number plasmids, pBC SK(+) and pBluescript SK(+), that also contained $lacI^q$ to keep expression low. They were cloned into IncP plasmids, pWM176 and pSW213, and introduced into *A. tumefaciens* or *R. meliloti* to make *ftsZ1*/*ftsZ1*-GFP merodiploids. The localization of GFP fluorescence in these cells was then assayed by fluorescence microscopy.

As expected, $FtsZ1_{Rm}$-GFP localized to the midcell division site of *R. meliloti* (Fig. 5A) and $FtsZ1_{At}$-GFP localized to the cell midpoint of *A. tumefaciens* (Fig. 5B and 6A). Some cells exhibited a band at their midpoint with no visible constriction, whereas others had a dot located within a visible constriction (Fig. 5A and B) (data not shown). In addition, $FtsZ1_{Rm}$-GFP was able to localize exclusively to the midpoint of *A. tumefaciens* cells (Fig. 6B). Occasional branched cells that contained fluorescent dots or polymers were observed for both *A. tumefaciens* (Fig. 5C and D) and *R. meliloti* (data not shown). Such cells probably expressed too much fusion protein, because deliberate overexpression of FtsZ-GFP or untagged FtsZ in these species also usually induces branching (24). This branching phenotype is analogous to filamentation in *E. coli* and correlates with cell division inhibition in these species (24).

These results demonstrated that FtsZ1-GFP fusions from these species could localize to the cell division site, presumably because they were able to coassemble with the resident untagged FtsZ. Therefore, we can conclude that the GFP fusion technique to localize cell division proteins is feasible in species other than *E. coli*. Cells with FtsZ localized at their midpoint were observed at various stages of septation, suggesting that in these species FtsZ is also recruited early at the division site and that the presumptive FtsZ ring narrows along with midcell constriction. Finally, the ability of $FtsZ1_{Rm}$-GFP and $FtsZ1_{At}$-GFP to localize to the division site of either *A. tumefaciens* or *R. meliloti* suggests that FtsZ proteins from related but different species can readily assemble into heteropolymers. The fact that most of the cells which exhibited midcell fluorescence were normal in appearance also indicates that some of these heteropolymers may have been fully functional for septation.

Previously, we showed that a derivative of $FtsZ1_{Rm}$ with 206 C-terminal amino acids deleted could form polymers in *E. coli* cells when it was tagged with GFP. The fusion proteins also occasionally exhibited fluorescence at the midcell division site in the same cells (25). We speculated that this phenomenon was due to two simultaneous events, coassembly between $FtsZ1_{Rm}$ and *E. coli* FtsZ and assembly of $FtsZ1_{Rm}$ alone into an axial polymer that extended from pole to pole. To address this question further, we constructed a full-length $FtsZ1_{Rm}$-GFP fusion and expressed it in *E. coli* cells at different levels. When it was expressed at high levels, $FtsZ1_{Rm}$-GFP inhibited *E. coli* cell division and formed long polymers that were similar to the axial polymers previously observed (data not shown). However, when $lacI^q$ was cloned into the plasmid so that levels of the fusion protein were lower, $FtsZ1_{Rm}$-GFP did not form these polymers. Instead, $FtsZ1_{Rm}$-GFP clearly colocalized with *E. coli* FtsZ at the division site in nearly all *E. coli* cells examined (Fig. 6E), a pattern similar to that observed with $FtsZ_{Ec}$-GFP (Fig. 6F). These cells were normal in size, indicating that the mixed FtsZ ring might be functional. Likewise, $FtsZ1_{At}$-GFP also gave rise to fluorescent bands corresponding to FtsZ rings in *E. coli* (Fig. 6D), as well as long polymers in filaments containing higher, inhibitory levels of fusion protein (data not shown).

In a converse experiment, $FtsZ_{Ec}$-GFP was expressed in *A. tumefaciens* cells. The results were similar in general to those with $FtsZ1_{Rm}$-GFP and $FtsZ1_{At}$-GFP expression in *E. coli*. Whereas some cells exhibited fluorescent polymers that had no apparent localization (data not shown), many cells had a fluorescent band at midcell (Fig. 6C). Because axial fluorescence was strong and midcell fluorescence was weak, we surmised that unlocalized polymerization occurred when the $FtsZ_{Ec}$-

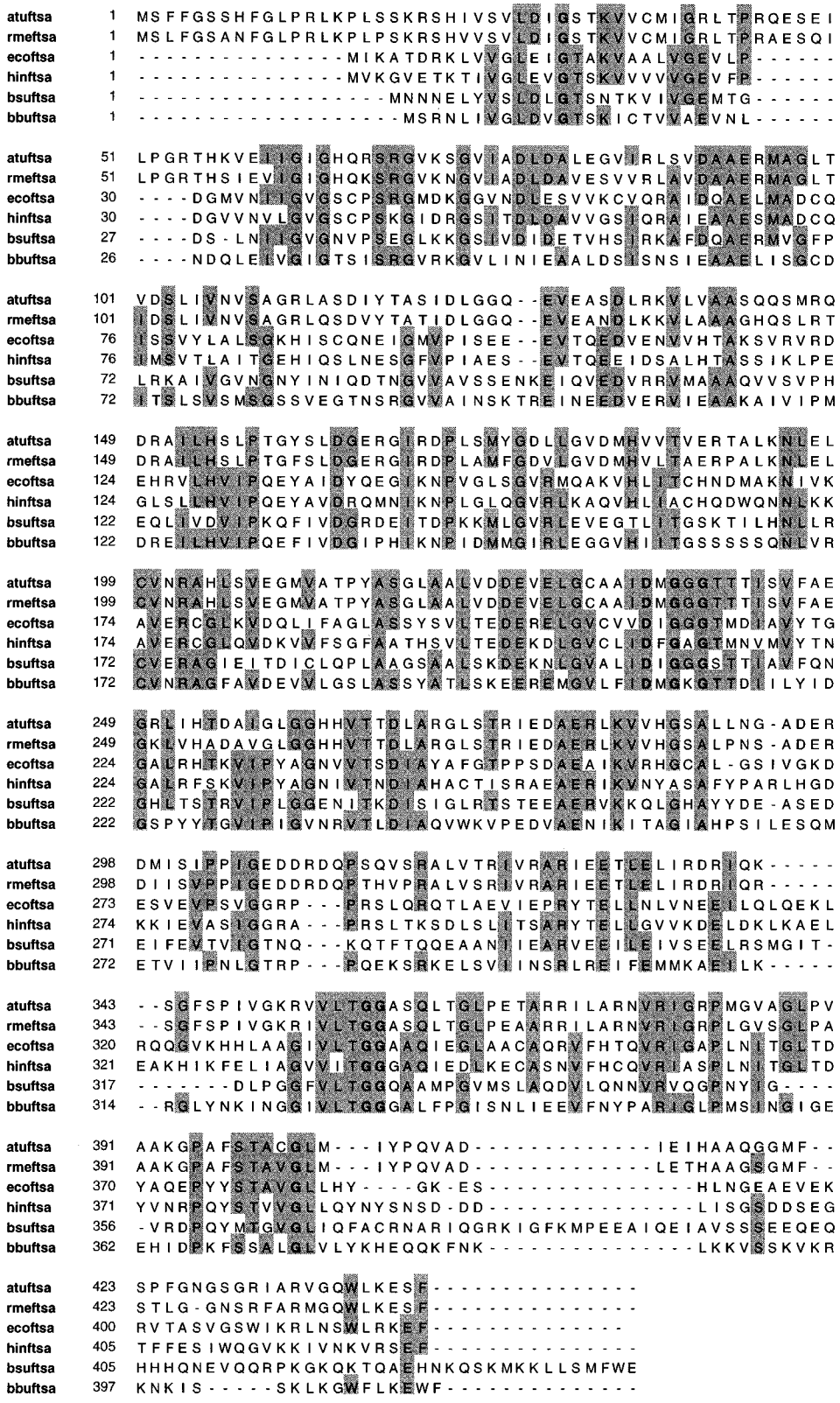

FIG. 3. Alignment of FtsA proteins of *B. subtilis* (bsuftsa), *A. tumefaciens* (atuftsa), *R. meliloti* (rmeftsa), *Haemophilus influenzae* (hinftsa), *Borrelia borgderferi* (bbuftsa), and *E. coli* (ecoftsa). The residue numbers for FtsA proteins are indicated at the beginning of each line. Residues identical among at least four of the six FtsA proteins are shaded. Residues invariant among the ATPase family (7) are shown in boldface. Dashes indicate gaps.

```
Rm   1 MAINLQKPDITELKPRITVFGVGGGGGNAVNNMITAGLQGVDFVVANTDA 50
       | | ||||||||||||||||||||||||||||||||| ||||||||||||||
At   1 MTIQLQKPDITELKPRITVFGVGGGGGNAVNNMITVGLQGVDFVVANTDA 50

Rm  51 QALTMTKAERIIQMGVAVTEGLGAGSQPEVGRAAAEECIDEIIDHLQGTH 100
       |||||||| | || || ||||||||||||||||||||||||||||||| |||
At  51 QALTMTKADRVIQLGVNVTEGLGAGSQPEVGRAAAEECIDEIIDHLNGTH 100

Rm 101 MCFVTAGMGGGTGTGAAPIVAQAARNKGILTVGVVTKPFHFEGGRRMRIA 150
       |||||||||||||||||| |||||||||||||||||||||||||||||| |
At 101 MCFVTAGMGGGTGTGAAPVVAQAARNKGILTVGVVTKPFHFEGGRRMRLA 150

Rm 151 DQGISDLQKSVDTLIVIPNQNLFRIANDKTTFADAFAMADQVLYSGVACI 200
         |||  ||||||||||||||||||||||||||||||||||||||||||||
At 151 EQGIEELQKSVDTLIVIPNQNLFRIANDKTTFADAFAMADQVLYSGVACI 200

Rm 201 TDLMVKEGLINLDFADVRSVMREMGRAMMGTGEASGEGRAMAAAEAAIAN 250
       |||||||||||||||||||||| | ||||||||| ||| ||||||||
At 201 TDLMVKEGLINLDFADVRSVMREMARPMMGTGEASGPARAMQAAEAAIAN 250

Rm 251 PLLDETSMKGAQGLLISITGGRDLTLFEVDEAATRIREEVDPDANIILGA 300
       |||||||||||||||||||||||||||||||||||||||||||||||||||
At 251 PLLDETSMKGAQGLLISITGGRDLTLFEVDEAATRIREEVDPDANIILGA 300

Rm 301 TFDEELEGLIRVSVVATGIDRTAAEVAGRSADFRPVAPKPIVRPSAAVPA 350
       |||| |||||||||||||| |       |  |  | ||  ||||||
At 301 TFDEALEGLIRVSVVATGIDRVAGIGEQNIAEMRAAAAKPLIRPSAAVAP 350

Rm 351 QPQPTVSLQPVPQPQPVQQPLQQQNVDHIALAIR..EAEMERELDIAARA 398
         |       | |            || ||  ||  |||||||  ||
At 351 APAAVQPAHAVSQA.........PKTVDQIAQTIRSAEAEMERELGFAAHQ 392

Rm 399 QVAAPAPQPQPHLQEEAFRPQSKLFAGVAPTEAAPVMRPAQP........ 440
       |                 |||||||||    | ||    |||||
At 393 Q............PSQDFRPQSKLFAS.SPAEAPAALRPAQPVQQAAPAP 429

Rm 441 ........APRPVEMQAPVQPQMQAQPVQQEPTQVVRQQAEPVRMPKVED 482
                ||  |   ||   | || || |||  | | | ||||||||||
At 430 VAQAPVYHAPEQVAVPAPRMQQAQA.PVYQEPAPVGR.QPEPVRMPKVED 477

Rm 483 FPPVVKAEMDYRTQPAPAHQEERGPMGLLNRITSSLGLRREREATNVSSDM 532
       ||||||||||| |    |  |||||||||| ||| ||| || |   | |||
At 478 FPPVVKAEMDHRDRATPVAQEERGPMGLLKRITNSLGRREEE..EVPSDM 525

Rm 533 TAAAPSAASQQRRPLSPEASLYAPRRGQLDDHGRAAPQMRS.HEDDQLEI 581
          ||| | | | |||||||||||||||||||||||| |    | | ||||||
At 526 .MDAPSMAPQRRAPLSPEASLYAPRRGQLDDHGRATPSSSSHHDDDQLEI 574

Rm 582 PAFLRRQSS 590
       |||||||||
At 575 PAFLRRQSN 583
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FIG. 4. Alignment of *R. meliloti* FtsZ1 (Rm) and *A. tumefaciens* FtsZ (At) amino acid sequences. Vertical lines indicate identities. Dots indicate gaps.

GFP concentration was too high to support coassembly with the native *A. tumefaciens* FtsZ ring. In support of this idea, higher expression of the fusion protein correlated with an increase in unlocalized polymers and a dramatic increase in cell branching (data not shown). Therefore, it is likely that these FtsZ-GFP polymers interfere with normal cell division.

This result confirms our hypothesis that an FtsZ protein from one species can localize to the division site of a cell from a significantly different species. One possible mechanism is that the foreign and native FtsZ proteins coassemble to make a mixed FtsZ ring (as long as levels of the foreign protein are below a certain concentration). We speculate that when the foreign FtsZ exceeds a specific concentration relative to that of the native FtsZ, it can no longer intercalate and instead forms a homopolymeric structure visible as the axial polymer. We cannot rule out the possibility that the axial polymeric structure is an abnormal result of the GFP tag, although the ability of overproduced wild-type *E. coli* FtsZ to form long spirals in *E. coli* cells, as visualized by decorating them with $FtsA_{Ec}$-GFP (25), makes this possibility less likely. A more detailed understanding of the mechanism of coassembly and changes in polymerization state awaits further structural studies of the FtsZ ring.

Interspecies interaction between *R. meliloti* and *A. tumefaciens* FtsA and FtsZ. The evidence that *E. coli* FtsA and FtsZ interact in *E. coli* cells, combined with the high sequence similarities between FtsZ and FtsA proteins of *R. meliloti* and *A. tumefaciens*, suggested that interspecies interaction between these proteins could occur. This idea was tested by individually fusing GFP to each protein, which allowed us to distinguish between proteins of different species that localized to the FtsZ ring. The FtsA-GFP fusions, along with a *lacI*^{q} cassette, were cloned into plasmids and then introduced into *R. meliloti* and *A. tumefaciens* as was done with the FtsZ-GFP fusions.

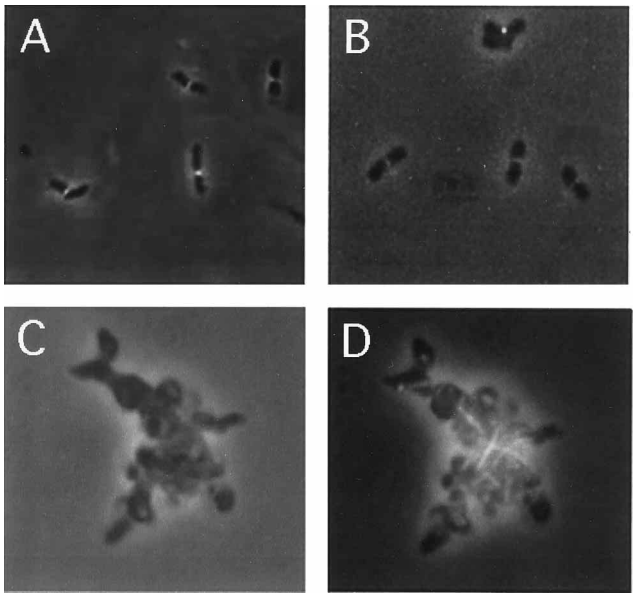

FIG. 5. Localization of GFP-tagged FtsZ proteins from *R. meliloti* and *A. tumefaciens* to the FtsZ ring of either species. (A) pZRG3 ($FtsZ1_{Rm}$-GFP) in *R. meliloti* MB501 (composite image); (B) pZAG3 ($FtsZ1_{At}$-GFP) in *A. tumefaciens* A136; (C and D) single cells of A136 containing $FtsZ1_{Rm}$-GFP by phase-contrast microscopy and fluorescence, respectively. Note cell branching and expansion to many times the volume of a normal cell, presumably because of fusion protein overexpression.

In the first demonstration of FtsA localization in a species other than *E. coli*, we found that $FtsA_{At}$-GFP fluorescence in *A. tumefaciens* localized to midcell as either a band or a dot corresponding to the midcell constriction (Fig. 7A and data not shown). These results suggest that FtsA localizes to the FtsZ structure at various stages during the septation process in these

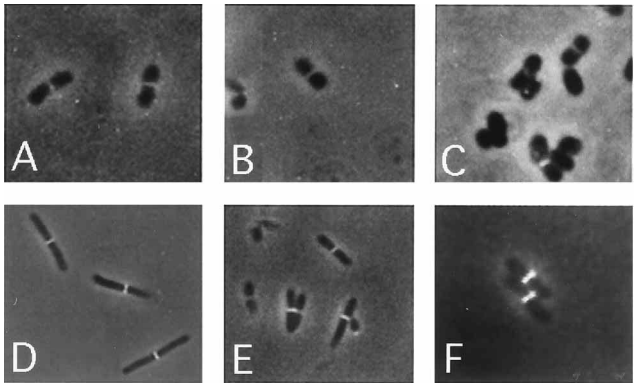

FIG. 6. Interspecies interaction of *R. meliloti*, *A. tumefaciens*, and *E. coli* FtsZ proteins in vivo. Shown are *A. tumefaciens* A136 (A to C) and *E. coli* JM105 (D to F) with the following plasmids: pZ1AG3 ($FtsZ1_{At}$-GFP) (A and D), pZ1RG3 ($FtsZ1_{Rm}$-GFP) (B and E), and pZEG3 ($FtsZ_{Ec}$-GFP) (C and F).

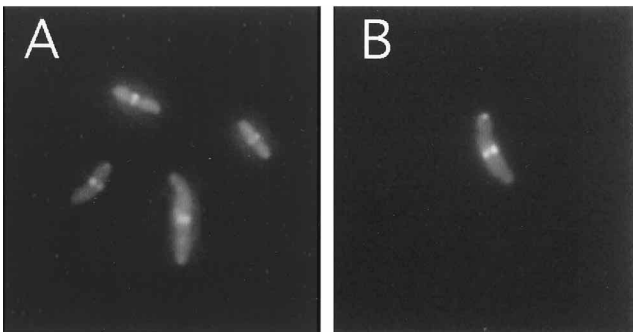

FIG. 7. Localization of GFP-tagged FtsA derivatives in *A. tumefaciens*. Shown are fluorescence micrographs of *A. tumefaciens* A136 containing pAAG3 ($FtsA_{At}$-GFP) (A) and pARG3 ($FtsA_{Rm}$-GFP) (B). Panel A is a composite image.

two species of *Rhizobiaceae*. Dots were also often seen at cell poles and were probably not inclusion bodies, as judged by phase-contrast microscopy (data not shown). These dots could be either a true reflection of FtsA localization, for example, persistence at the new pole after cell division, or an abnormal effect of overexpression of the GFP tag. Occasionally, highly branched cells that contained fluorescent dots or filaments were observed (data not shown); these were presumably cells in which the fusion was overexpressed and thus specifically inhibited cell division by analogy with FtsZ (24).

We then tested whether $FtsA_{Rm}$-GFP could localize to midcell in *A. tumefaciens*. The fluorescence localization and cell morphology were essentially the same as those with $FtsA_{At}$-GFP. This result demonstrated that the FtsA-GFP from one species could localize to the midcell constriction in the other species (Fig. 7B).

To independently confirm this interspecies interaction between the FtsA and FtsZ proteins of *A. tumefaciens* and *R. meliloti*, we used *E. coli* in a novel in vivo assay. This assay took advantage of the ability of $FtsZ1_{Rm}$ to colocalize at the *E. coli* FtsZ ring, as well as the larger cell size of and better control of gene expression in *E. coli*. When $FtsA_{Rm}$-GFP or $FtsA_{At}$-GFP was expressed in *E. coli* by itself, cells displayed uniformly green fluorescence (data not shown). This indicated that there was no detectable interaction between $FtsA_{Rm}$-GFP or $FtsA_{At}$-GFP with $FtsZ_{Ec}$. However, a fluorescent band was present at the midcell division site of a significant proportion of cells when $FtsZ1_{Rm}$ was coexpressed with $FtsA_{Rm}$-GFP in *E. coli*, with $FtsA_{Rm}$-GFP on a high-copy-number plasmid under *lac* promoter control and $FtsZ1_{Rm}$ on a low-copy-number IncP plasmid under *tac* promoter control (Fig. 8C). These bands, which presumably represented FtsZ ring structures, provided further evidence for specific interactions between $FtsA_{Rm}$ and $FtsZ1_{Rm}$ and between $FtsZ1_{Rm}$ and $FtsZ_{Ec}$. When $FtsA_{At}$-GFP was coexpressed with $FtsZ1_{Rm}$, it also displayed fluorescence at the FtsZ ring, as predicted from its interaction with $FtsZ1_{Rm}$ in *R. meliloti* (Fig. 8F). In contrast, when either $FtsA_{At}$-GFP or $FtsA_{Rm}$-GFP was expressed in cells containing only the IncP vector, no localization was detected (Fig. 8A and D). When $FtsZ_{Ec}$ was substituted for $FtsZ1_{Rm}$ in the IncP vector (pKM4), no clear localization was observed, except for occasionally diffuse bands or inclusion bodies that appeared to be localized randomly (Fig. 8B and E). These cells were more filamentous than were cells without pKM4, probably because of $FtsZ_{Ec}$ overproduction. Interestingly, whereas overproduction of $FtsA_{Ec}$ or $FtsA_{Ec}$-GFP routinely inhibited cell division of *E. coli* (25), overproduction of $FtsA_{Rm}$-GFP or $FtsA_{At}$-GFP without concomitant FtsZ overproduction caused little to no change in cell length. This lack of cell division inhibition is consistent with the failure of these non-*E. coli* FtsA proteins to interact significantly with *E. coli* FtsZ and potentially interfere with septation.

Conclusions and implications. Recent evidence has suggested that *E. coli* FtsA and FtsZ interact within *E. coli* cells, even when FtsZ is not in the ring conformation (2, 25). However, those studies were not able to confirm whether the FtsZ-FtsA interaction was direct or indirect. In this work, we have studied the interactions between FtsA and FtsZ proteins from different species in order to gain insight into their interaction specificity. Since many amino acid changes have already taken place among the *E. coli*, *R. meliloti*, and *A. tumefaciens* proteins, we were able to use them as natural variants to test

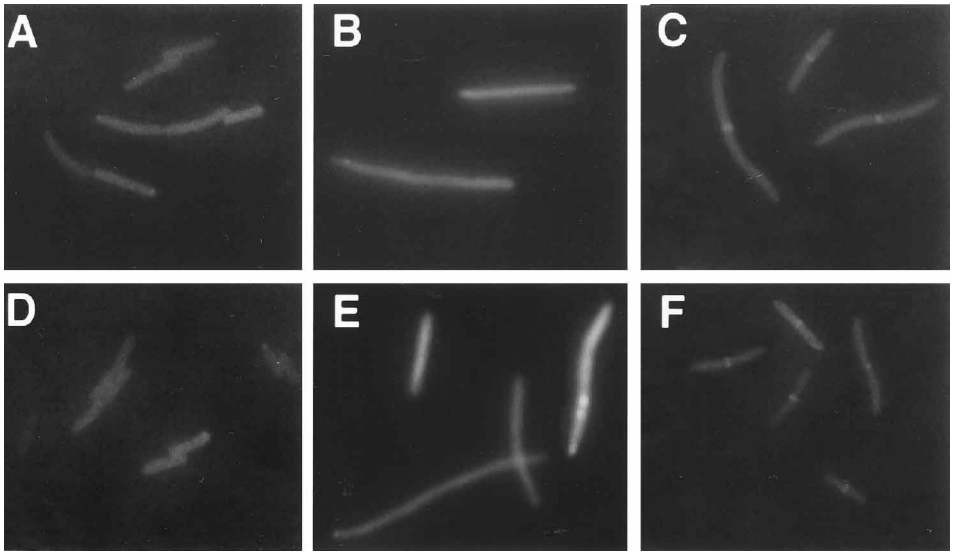

FIG. 8. Interspecies interaction of *R. meliloti* or *A. tumefaciens* FtsA and FtsZ at the *E. coli* FtsZ ring. Shown are fluorescence micrographs of *E. coli* JM105 containing pARG2 ($FtsA_{Rm}$-GFP) and pWM176 (IncP vector) (A), pARG1 ($FtsA_{Rm}$-GFP) and pMK4 ($FtsZ_{Ec}$) (B), pARG2 and pWM189 ($FtsZ1_{Rm}$) (C), pAAG2 ($FtsA_{At}$-GFP) and pWM176 (D), pAAG1 ($FtsA_{At}$-GFP) and pKM4 (E), and pAAG2 and pWM189 (F).

specificity. The GFP tagging technique is advantageous because it allows both the marking of only one protein and the testing of its ability to interact with others in vivo at the FtsZ ring. We used this strategy in a novel in vivo protein-protein interaction assay with *E. coli* to demonstrate that FtsA and FtsZ probably interact directly.

Our results suggest that FtsA and FtsZ proteins have coevolved, because FtsA proteins from species as distant as *E. coli* and *R. meliloti* do not appear to be able to substitute for one another. This idea is consistent with the failure of *B. subtilis ftsA* to complement an *E. coli ftsA* conditional mutant (4), although the reported toxicity of *B. subtilis ftsA* expression in *E. coli* (4) suggests that interaction between *B. subtilis* FtsA and *E. coli* FtsZ is possible. However, we found that FtsA-FtsZ interspecies interactions do occur when the species are sufficiently closely related. Since GFP fusions of either $FtsA_{Rm}$ or $FtsA_{At}$ can localize to the FtsZ ring of *A. tumefaciens* and both $FtsA_{Rm}$-GFP and $FtsA_{At}$-GFP can detectably localize to the FtsZ ring of *E. coli* only when either $FtsZ1_{Rm}$ or $FtsZ1_{At}$ is present there, $FtsA_{At}$ must be able to interact with $FtsZ1_{Rm}$ and $FtsA_{Rm}$ must be able to interact with $FtsZ1_{At}$. Moreover, the successful interaction in *E. coli* suggests that FtsZ-FtsA cognate pairs do not require other species-specific factors for their recognition, strongly suggesting that their interaction is direct. We can conclude that sequence drift between the *A. tumefaciens* and *R. meliloti* homologs of FtsA and FtsZ has not prevented their functional interaction. More specifically, positions corresponding to the 63 amino acids that differ between $FtsA_{Rm}$ and $FtsA_{At}$, particularly the 24 positions judged to have nonconserved changes, may not be critical for the ability of FtsA to bind to FtsZ (Fig. 3). Although there is a clustering of these changes at the C terminus, the otherwise even distribution of changes throughout the proteins is consistent with the idea that there may be no single domain of FtsA involved in its interaction with FtsZ (25). Further dissection of the various domains of FtsA by constructing mutants and interspecies chimeras may provide insight into how FtsA contacts FtsZ.

Interactions among FtsZ proteins from different species appear to be less stringent than are interactions among heterologous FtsA-FtsZ pairs. FtsZ homologs from *R. meliloti* and *A. tumefaciens* clearly colocalized to a very high proportion of *E. coli* FtsZ rings, even though the FtsZ homologs from *E. coli* and these two species of *Rhizobiaceae* have only about 50% amino acid identity. FtsZ-FtsA interaction may also be more sensitive to the FtsZ polymeric structure; for example, not every *E. coli* cell containing $FtsZ1_{Rm}$ and $FtsA_{Rm}$-GFP exhibited localization of fluorescence to the mixed FtsZ ring. Until more is known about the structure of the FtsZ ring, it is difficult to speculate on the mechanism of heterologous FtsZ coassembly. At low levels of expression of a foreign FtsZ or FtsZ-GFP in *E. coli*, cell division appears to be fairly normal, indicating that the FtsZ ring can tolerate the presence of different FtsZs and function properly. As our results suggest, even when it is colocalized with the native FtsZ, the foreign FtsZ retains its ability to contact its own FtsA. Perhaps the two FtsZ proteins can form a uniformly mixed protofilament bundle or sheet (21), not unlike the mixture possibly formed between $FtsZ_{Ec}$ and $FtsZ_{Ec}$-GFP that is fully functional for cell division (34). It also will be interesting to determine whether more distantly related FtsZ proteins, such as those from gram-positive bacteria and archaea, are able to colocalize to the *E. coli* FtsZ ring. The failure of some, but not others, to colocalize may provide insights into FtsZ structure and function that are not as easily attainable by studying the *E. coli* protein alone.

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