Characterization of the *ftsZ* Gene from *Mycoplasma pulmonis*, an Organism Lacking a Cell Wall

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The *ftsZ* gene is required for cell division in *Escherichia coli* and *Bacillus subtilis*. In these organisms, FtsZ is located in a ring at the leading edge of the septum. This ring is thought to be responsible for invagination of the septum, either causing invagination of the cytoplasmic membrane or activating septum-specific peptidoglycan biosynthesis. In this paper, we report that the cell division gene *ftsZ* is present in two mycoplasma species, *Mycoplasma pulmonis* and *Acholeplasma laidlawii*, which are eubacterial organisms lacking a cell wall. Sequencing of the *ftsZ* homolog from *M. pulmonis* revealed that it was highly homologous to other known FtsZ proteins. The *M. pulmonis* *ftsZ* gene was overexpressed, and the purified *M. pulmonis* FtsZ bound GTP. Using antisera raised against this purified protein, we could demonstrate that it was expressed in *M. pulmonis*. Expression of the *M. pulmonis* *ftsZ* gene in *E. coli* inhibited cell division, leading to filamentation, which could be suppressed by increasing expression of the *E. coli* *ftsZ* gene. The implications of these results for the role of *ftsZ* in cell division are discussed.

The *ftsZ* gene is essential for cell division in both the gram-negative bacterium *Escherichia coli* and the gram-positive bacterium *Bacillus subtilis* (3, 10). In *B. subtilis*, *ftsZ* is also required for formation of the sporulation septum (10). In the gram-positive, filamentous bacterium *Streptomyces coelicolor*, the *ftsZ* gene is not essential for viability; however, it is required for septation (19). The mycelia of an *ftsZ* null mutant lack septa, and the mutant is unable to sporulate, which is a process requiring the massive synthesis of septa. The requirement of *ftsZ* for cell division in these three diverse bacteria suggests that it may be utilized by all bacteria. Indeed, *ftsZ* has been found in a wide variety of eubacteria (9).

In both *E. coli* and *B. subtilis* FtsZ undergoes a dynamic localization during the cell cycle (4, 25). In nondividing cells, FtsZ is in the cytoplasm, whereas in dividing cells it is located at the leading edge of the septum in a pattern designated the FtsZ ring. This finding led to speculation that FtsZ may be a cytoskeletal protein involved in septation (4, 16). The abnormal septal morphology of some *ftsZ* mutants suggests that the FtsZ ring functions as a guide for the invagination of the septum (5). In this capacity, its immediate function could be either to initiate septal specific peptidoglycan biosynthesis or to bring about invagination of the cytoplasmic membrane.

Both the *E. coli* and *B. subtilis* FtsZ proteins have been purified and have been shown to bind and hydrolyze GTP (13, 20, 24, 25). In addition, these proteins have been found to form filaments in vitro in the presence of GTP (7, 21). These results support the proposal that FtsZ functions as a cytoskeletal element.

To further explore the function of *ftsZ* in cell division, we set out to see if *ftsZ* is present in bacteria that lack a cell wall. If present, it would suggest that the immediate function of the FtsZ ring is to mediate invagination of the cytoplasmic membrane rather than to activate septum-specific peptidoglycan biosynthesis. We find that at least two mycoplasma species, *Mycoplasma pulmonis* and *Acholeplasma laidlawii*, which lack

cell wall peptidoglycan, contain an *ftsZ* gene that is homologous to that present in gram-negative and gram-positive bacteria. We show that *ftsZ* is expressed in *M. pulmonis* and that expression of this *ftsZ* in *E. coli* inhibits division. In addition, we show that the *M. pulmonis* FtsZ binds GTP.

MATERIALS AND METHODS

Bacterial strains, plasmids, and phage. The *E. coli* K-12 strains W3110 and UT481 were used as routine cloning hosts. SF100 (*ompT*) (1) was used as a host for expressing the *M. pulmonis* *ftsZ* gene. Bacteria were grown in L broth supplemented with the appropriate antibiotics (ampicillin at 100 µg/ml and spectinomycin at 50 µg/ml). Morphological studies were carried out with plasmids in W3110. MCZ84 and MCZ26 are *E. coli* K-12 derivatives that carry *ftsZ84*(Ts) and *ftsZ26*(Ts), respectively (12). Two mycoplasma species, *M. pulmonis* and *A. laidlawii*, were used in this study. A genomic library of *M. pulmonis* was constructed in λZAP, which was obtained from Stratagene.

The plasmids used in this study are listed in Table 1. pCX62 contains the *M. pulmonis* *ftsZ* gene and was obtained by excision of a positive clone following screening of a λZAP library with the PCR fragment. It contains a genomic 3.8-kb *HindIII* fragment in the vector pBK-CMV. pCX64 was obtained by deleting 1.3 kb of DNA upstream of the *EcoRI* site in pCX62. pCX65 contains the insert from pCX64 cloned into the expression vector pJF118 so that the *ftsZ* gene can be expressed from *ptac*. pJF118 is a pBR322-based replicon. pCX66 was obtained by cloning an *RsaI*-*HindIII* fragment from pCX64 into pJF118, resulting in the *ftsZ* gene cloned downstream of the *ptac* promoter. It has less DNA between the start of the *ftsZ* gene and *ptac* than pCX65. pCX67 contains the *ftsZ* gene with the ribosome-binding site (RBS) changed by PCR to match that of the *B. subtilis* *ftsZ* gene. pBS58 contains the *E. coli* *ftsQ*, *ftsA*, and *ftsZ* genes cloned into pGB2, which is a pSC101-based replicon.

Amplification of *M. pulmonis* DNA. *M. pulmonis* and *A. laidlawii* DNAs were kindly provided by K. Dybvig (University of Alabama at Birmingham). The primers that were utilized corresponded to two highly conserved regions of the FtsZ protein with the sequences GGGTGTG and NLDFADV. The sequences of these primers, which incorporated the codon usage preference of *M. pulmonis*, were 5'-AGAGGATCCGG(AT)GG(AT)GG(AT)AC(AT)GG(TAC)AC(ATC)GG and 5'-GATGAATTCAC(GA)TC(AT)GCG(GA)AA(GA)TC(TA)A(GA)(GA)TT. PCRs were performed with *Taq* polymerase in a COY Tempcycler II for 35 cycles with each cycle consisting of 1 min at 94°C, 1 min at 45°C, and 2 min at 72°C. The amplified DNA was digested with *Bam*HI and *Eco*RI (sites contained within the primers) and was cloned into the corresponding sites of pUC19, yielding plasmid pCX60. This *Bam*HI-*Eco*RI fragment was subsequently cloned into pGB2 (8) to yield pCX61. This plasmid lacks homology to λZAP, allowing it to be used for probing libraries.

Cloning of the *ftsZ* gene from *M. pulmonis*. To clone the *ftsZ* gene from *M. pulmonis*, a library of chromosomal DNA was constructed in λZAP (Stratagene). *HindIII* fragments ranging in size from 2.5 to 6 kb were purified and ligated into λZAP digested with *HindIII* and treated with calf intestinal phosphatase. The ligated mixture was packaged in vitro with Gigapack II packaging extracts from

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TABLE 1. Bacterial plasmids used

Plasmid	Relevant characteristics	Reference or source
pBK-CMV	Vector contained in λZAP	Stratagene
pGB2	pSC101-derived vector	8
pJF118EH	Expression vector	15
pCX60	pUC19 with 321-bp PCR fragment	This work
pCX61	pGB2 with 321-bp PCR fragment	This work
pCX62	Phagemid containing 3.8-kb <i>Hind</i> III fragment	This work
pCX64	pBK-CMV with 2.5-kb <i>Eco</i> RI- <i>Hind</i> III fragment	This work
pCX65	pJF118 with the 2.5-kb <i>Eco</i> RI- <i>Hind</i> III fragment	This work
pCX66	pJF118 with the 2.2-kb <i>Rsa</i> I- <i>Hind</i> III fragment	This work
pCX67	pJF118 with the <i>M. pulmonis</i> <i>ftsZ</i> gene obtained by PCR and containing a modified RBS	This work
pBS58	Contains the <i>ftsQ</i> , <i>ftsA</i> , and <i>ftsZ</i> genes of <i>E. coli</i>	5

Stratagene. This size-selected library was screened with nick-translated pCX61 as a probe. Three positive plaques were selected for further study.

DNA sequencing. Double-stranded plasmid DNA was used for sequencing. A set of nested deletions of the insert of pCX64 was generated by using an Erase-a-Base kit obtained from Promega. Sequencing was performed with T7 Sequenase II from U.S. Biochemicals. Additional primers were synthesized and used to sequence any gaps remaining after sequencing of the nested set of deletions and to sequence the complementary strand.

Photomicroscopy. Cells were fixed with 2% glutaraldehyde for 30 min, washed with saline, resuspended in saline, and mounted on microscope slides. The cells were photographed with a phase-contrast microscope.

Overexpression and purification of FtsZ. The *ftsZ* gene from *M. pulmonis* was cloned into the expression vector pJF118 in an attempt to overexpress *ftsZ*. Cells containing pCX65 did not express *ftsZ*, whereas cells containing pCX66 did express some *ftsZ*. However, for the purposes of purification, we wished to increase this expression. Therefore, the RBS and sequence extending to the initiation codon were modified by PCR to match the sequence present in the *B. subtilis* *ftsZ* gene. The primers used were 5'-ACTGGATCCAGGAGGATTTAGCATGTCGGATTGATA and 5'-TCAAAGCTTTGATCTCTTGAAGATCA, with pCX64 as a template. The resultant PCR fragment was cloned into pJF118EH (15) to yield pCX67. Cells containing this plasmid produced an abundant band at 41 kDa, corresponding to the *ftsZ* gene product. To obtain FtsZ for generating antiserum, SF100(pCX67) was grown to log phase and was induced by the addition of 2 mM isopropyl- β -D-thiogalactopyranoside (IPTG) for 1 h. Cells were harvested by centrifugation and were broken by being passed through a French press. The lysate was clarified by sequential centrifugation, first at 10,000 rpm for 10 min (Beckman J2-21) and then at 40,000 rpm for 60 min (Beckman L8-55, Ti80 rotor). The second supernatant was separated on sodium dodecyl sulfate (SDS)-12.5% polyacrylamide gel electrophoresis (PAGE) gels, and FtsZ was electroeluted from the gel. A 500- μ g amount of this protein was sent to Cocalico Biologicals, Inc., for generation of antiserum in rabbits.

Nucleotide sequence accession number. The sequence data for the *M. pulmonis* *ftsZ* gene and protein have been assigned GenBank accession number MPU34931.

RESULTS

In an initial attempt to determine if wall-less bacteria contain FtsZ, lysates from several different species of mycoplasma were analyzed by Western blotting (immunoblotting) with antisera raised against the *E. coli* and *B. subtilis* FtsZ proteins. Although these antisera detect FtsZ in a wide range of eubacterial species, none of the mycoplasma lysates examined appeared positive. In a separate approach to try to clone the *ftsZ* gene, we used a PCR approach which has been used successfully to clone the *ftsZ* gene from several bacterial species (18). Several degenerate primers, biased for A and T in the third position, were synthesized corresponding to the most conserved regions of the protein. One pair of primers, corresponding to the conserved regions GGGTGTG and N(L/V)DFAD, yielded the expected-size PCR product with chromosomal DNAs from *M. pulmonis* and *A. laidlawii*. These amplified products were cloned and sequenced. Comparison of the deduced amino acid sequences of these fragments revealed significant similarity with other bacterial FtsZ proteins. This re-

sult, along with the high A+T contents of the PCR fragments, suggested that we had cloned a portion of the *ftsZ* gene from these organisms.

Cloning the *ftsZ* gene from *M. pulmonis*. Southern analysis with the cloned PCR fragment as a probe revealed that the *ftsZ* gene in *M. pulmonis* was present on a 3.8-kb *Hind*III fragment. Therefore, a genomic library containing *Hind*III restriction fragments in the range of 2.5 to 6 kb was constructed in λ ZAP. Plaques from the resultant library were screened by hybridization with pCX61, a pGB2 derivative containing the PCR fragment (Table 1), and three positive clones were selected. Restriction mapping of the plasmids obtained by *in vivo* excision of the λ ZAP clones revealed that they all contained the same 3.8-kb *Hind*III fragment, although one contained an additional 1.7-kb fragment. Interestingly, none of these plasmids affected cell morphology, suggesting that the *ftsZ* gene was not efficiently expressed. This conclusion stems from the observation that expression in *E. coli* of an *ftsZ* gene from other distantly related bacteria, such as *B. subtilis*, results in inhibition of cell division (2). Further hybridizations indicated that the *ftsZ* gene must be near the center of the 3.8-kb *Hind*III fragment and that the entire gene should be located on an internal 2.5-kb *Eco*RI-*Hind*III fragment (Fig. 1).

Sequence of the *ftsZ* gene. The 2.5-kb *EcoRI-HindIII* fragment was subcloned to give pCX64 and was used for DNA sequence analysis (Fig. 1). Analysis of the sequence revealed one large open reading frame preceded by a recognizable RBS.

[illegible]

FIG. 1. Nucleotide sequence and deduced amino acid sequence of the *M. pulmonis* *ftsZ* gene. The nucleotide sequence of the *ftsZ* region from *M. pulmonis*, along with the deduced amino acid sequence of the open reading frame, is presented. The *ftsZ* gene begins at bp 391 to 393 and ends with a termination codon at 1561 to 1563. The gene for methionyl-tRNA synthetase begins at bp 1727 to 1729 and runs to the end of the fragment. Putative RBSs are underlined, and relevant restriction sites are indicated.

FIG. 2. Comparison of *M. pulmonis* FtsZ with other FtsZ proteins. The FtsZ proteins were aligned by using Megalign (DNASTar). The FtsZ proteins include *M. pulmonis* (Mp), *B. subtilis* (Bs) (2), *E. coli* (27), and the portion of the *Acholeplasma* FtsZ corresponding to the PCR fragment obtained in this study.

AGGAG, six nucleotides upstream of the start codon. Analysis of the deduced amino acid sequence confirmed that this open reading frame corresponds to the *ftsZ* gene and confirmed that the entire gene had been cloned. The *ftsZ* gene begins at position 391 and consists of 390 codons encoding a protein with a molecular mass of 41.1 kDa. This is similar in size to the *E. coli* and *B. subtilis* FtsZ proteins. For the first 312 codons, the deduced amino acid sequence shows strong similarity with other FtsZ sequences, having 55% identity with *B. subtilis* FtsZ and 45% identity with *E. coli* FtsZ (Fig. 2). For the carboxy-terminal 70 to 80 residues, the FtsZ proteins display little similarity, and the extreme carboxy tail, which is highly conserved between the *E. coli* and *B. subtilis* FtsZ proteins, is not as well conserved in *M. pulmonis* FtsZ. In addition to the *ftsZ* open reading frame, a portion of another in the same orientation as that of *ftsZ* is present in our sequenced DNA. This second open reading frame begins 163 nucleotides downstream of *ftsZ* and encodes the N terminus of methionyl tRNA-synthetase. This synthetase is 48% identical to the *B. subtilis* synthetase and 25% identical to the *E. coli* synthetase for the first 282 residues. A portion of an open reading frame is also present upstream of *ftsZ*; however, the deduced product has no homology to proteins in the databases.

The nucleotide composition of this sequenced fragment is 71% A+T. This high A+T content is what is expected for this *Mycoplasma* species. The codon usage is also highly biased, with A or T usually found in the third position. For example, of 33 glycine codons, 30 have A or T in the third position. This expected bias was used in the design of the primers for PCR.

Expression of *ftsZ*. As noted above, pCX62 did not affect cell morphology, suggesting that *ftsZ* was not being expressed even though the plasmid contained more than 1 kb of DNA upstream of *ftsZ*. To try to express *ftsZ*, the *EcoRI*-*HindIII* fragment that was sequenced was cloned in the expression vector pJF118EH (15) downstream of the *tac* promoter. Full induction of the *tac* promoter by addition of 1 mM IPTG did not affect cell morphology of W3110(pCX65), and no obvious inducible band was seen by SDS-PAGE. The lack of apparent expression could be due to sequences upstream of *ftsZ* pre-

venting transcription reaching *ftsZ* or to a nonfunctional RBS. To try to differentiate between these possibilities, an *RsaI*-*HindIII* fragment, which lacks the first 327 bp of upstream DNA, was subcloned into the expression vector pJF118EH to give pCX66. W3110 containing pCX66 displayed an IPTG-sensitive phenotype. Addition of IPTG to a culture of W3110 (pCX66) resulted in inhibition of cell division and formation of long filaments (Fig. 3). Examination of lysates prepared 1 and 2 h after IPTG addition revealed that a band at the expected molecular mass, 41 kDa, was induced (data not shown).

To determine if the *M. pulmonis* *ftsZ* gene could complement an *ftsZ* mutant, pCX66 was introduced into strains carrying temperature-sensitive mutations in the *ftsZ* gene (MCZ84 and MCZ26). The resultant strains were streaked on plates containing a broad range of IPTG concentrations, and the plates were incubated at both 30 and 42°C. At 30°C, growth was inhibited at IPTG concentrations of 0.1 mM or greater. No growth was observed at 42°C even at intermediate IPTG concentrations (0.01 to 0.1 mM), indicating that the *M. pulmonis* *ftsZ* gene was unable to complement the *E. coli* mutations.

The filamentous phenotype and reduction in colony formation seen at IPTG concentrations of 0.1 mM or greater (Table 2) suggest that the *M. pulmonis* *ftsZ* gene interferes with the resident *E. coli* *ftsZ* gene. To test this, we examined IPTG sensitivity after introducing plasmid pBS58 carrying the *E. coli* *ftsZ* gene (17). This plasmid elevates the level of the *E. coli* FtsZ protein by three- to fourfold. pBS58 suppressed the inhibition of division (Fig. 4) and conferred resistance to IPTG (Table 2), indicating that the *M. pulmonis* protein interferes with *E. coli* FtsZ.

Purification of FtsZ and GTP binding. To purify FtsZ, we set out to improve expression. Expression of *Mycoplasma* genes in *E. coli* can be hindered because of differences in codon usage. *Mycoplasma* species use the UGA codon for tryptophan and use the arginine codons AGG and AGA, which are rarely used in highly expressed genes in *E. coli*. FtsZ lacks tryptophan, and increasing the gene dosage of an *arg* tRNA did not improve expression. Therefore, we used PCR to obtain a *M. pulmonis* *ftsZ* gene with the RBS and sequence extending to the initiation codon matching those of the *B. subtilis* *ftsZ* gene, which is efficiently expressed in *E. coli*. Plasmid pCX67, containing this modified gene, yielded high-level expression of the *M. pulmonis* *ftsZ* gene (Fig. 4). However, the FtsZ protein was unstable during purification, with proteolysis removing about 4 to 5 kDa. From in vitro experiments with trypsin and other proteases and either the *E. coli* or *B. subtilis* FtsZ proteins, we know that the C-terminal tail is very sensitive to proteolysis, resulting in a truncation of similar size (26). Use of an *E. coli* strain lacking the OmpT protease, SF100, slowed but did not eliminate the proteolysis occurring during purification. To obtain unprocessed FtsZ to use for initial studies and to raise antisera, FtsZ was purified by SDS-PAGE. To test this FtsZ for GTP binding, it was electrophoresed with bovine serum albumin (BSA) and *E. coli* FtsZ. The SDS-PAGE gel was transferred to a nitrocellulose filter and was blotted with [α -³²P]GTP. Autoradiography demonstrated that *M. pulmonis* FtsZ bound GTP as well as *E. coli* FtsZ (Fig. 4).

FtsZ in *M. pulmonis*. Since the antiserum to the *E. coli* FtsZ did not cross-react with the *M. pulmonis* FtsZ, an antiserum against this FtsZ was raised. The Western blot results are shown in Fig. 5. The antiserum to *M. pulmonis* FtsZ detected a single band with a molecular weight of 40,000 in the *M. pulmonis* lysate, demonstrating that the *ftsZ* gene is expressed during normal growth in this organism.

FIG. 3. Expression of the *M. pulmonis ftsZ* in *E. coli* blocks cell division. W3110 containing either the vector pJF118EH (A and B) or the *M. pulmonis ftsZ* plasmid pCX66 (C and D) was grown in Luria-Bertani medium containing ampicillin. Cells were photographed before (A and C) or 2 h after (B and D) the addition of IPTG to 0.5 mM.

DISCUSSION

The *ftsZ* gene has been shown to be essential for cell division in several bacteria that contain cell wall peptidoglycan (3, 10). In the present study, we demonstrate that at least two mycoplasma species which lack cell wall peptidoglycan contain an *ftsZ* gene that is homologous to other bacterial *ftsZ* genes. This gene is expressed in *M. pulmonis*, although we are unable at present to determine if this gene is essential for cell division in this organism. The purified protein has the ability to bind GTP and interferes with the *E. coli* division machinery in a manner much like that of other heterologous FtsZ proteins. These results demonstrate that *ftsZ* is present in organisms that lack peptidoglycan and suggest, along with other results (3, 19, 26), that FtsZ is a component required of all prokaryotic cell division events.

In bacteria in which it has been examined, FtsZ is localized at the leading edge of the septum in a pattern designated the

FtsZ ring (4, 25). It has been suggested that the FtsZ ring is a cytoskeletal element that functions to bring about the invagination of the septum (4, 16). In this role, it could function to cause invagination of the cytoplasmic membrane, which could act as a template for septal peptidoglycan biosynthesis, or it could directly activate septum-specific peptidoglycan biosynthesis, causing invagination of the cytoplasmic membrane. The

TABLE 2. The lethality of *M. pulmonis ftsZ* expression in *E. coli* is suppressed by plasmid pBS58

Strain	CFU at the following IPTG concn (mM) ^a :			
	0	0.05	0.1	0.25
W3110(pJF118EH)(pGB2)	275	294	308	316
W3110(pJF118EH)(pBS58)	192	178	179	191
W3110(pCX66)(pGB2)	191	72	0.047	0.023
W3110(pCX66)(pBS58)	184	190	177	154

^a CFU were determined by plating fresh overnight cultures on Luria-Bertani medium containing ampicillin (100 µg/ml) and IPTG at the concentrations indicated.

FIG. 4. Purification of *M. pulmonis* FtsZ and [α -³²P]GTP binding. (A) W3110(pCX67) was grown in Luria-Bertani medium containing ampicillin. Samples were taken before (lane 2) or 2 h after (lane 3) the addition of IPTG to 1 mM. Purified FtsZ was obtained by electroelution from preparative SDS-PAGE of a cell lysate clarified by high-speed centrifugation (lane 4). Lane 1 contains molecular weight markers (from the top, phosphorylase *b*, 97.4 kDa; serum albumin, 66 kDa; ovalalbumin, 45 kDa; carbonic anhydrase, 31 kDa; trypsin inhibitor, 21.5 kDa; and lysozyme, 14.4 kDa). (B and C) Samples of *E. coli* FtsZ (lane 1), BSA (lane 2), and *M. pulmonis* FtsZ (lane 3) were electrophoresed on two separate gels. One was stained with Coomassie blue (B), and the other was blotted to nitrocellulose which was incubated with [α -³²P]GTP, washed, and autoradiographed (C).

FIG. 5. *ftsZ* is expressed in *M. pulmonis*. A lysate of *M. pulmonis* was separated on an SDS-PAGE gel, transferred to nitrocellulose, and blotted with antibodies to *M. pulmonis* FtsZ. Lanes: 1 and 3, molecular weight markers (as in Fig. 4); 2 and 4, *M. pulmonis* lysate. Lanes 1 and 2 were stained with Coomassie blue, and lanes 3 and 4 are from immunoblotting.

findings, reported here, that FtsZ is present in bacteria that lack peptidoglycan and that this FtsZ behaves as a typical FtsZ argue that the primary role of FtsZ is to cause invagination of the cytoplasmic membrane. Presumably, bacteria that have peptidoglycan have evolved additional genes to couple peptidoglycan biosynthesis to the FtsZ ring.

The initial genomic clone of *M. pulmonis ftsZ* was not efficiently expressed in *E. coli*. This lack of expression was inferred from the absence of any morphological alterations in cells carrying the corresponding plasmid. This lack of expression could be due to the lack of promoters on the 3.8-kb insert or to a lack of recognition of expression signals in *E. coli*. In trying to express the *M. pulmonis ftsZ* gene in *E. coli*, we found that removal of a 327-bp fragment upstream of *ftsZ* allowed an exogenous promoter to boost *ftsZ* expression, suggesting that this segment contains a transcriptional terminator and that the *M. pulmonis* RBS was functional in *E. coli*. Nonetheless, the expression level was further augmented by changing the RBS to match that of the *B. subtilis ftsZ* gene, which is highly expressed in *E. coli*.

The *M. pulmonis* FtsZ protein is similar to the other FtsZ proteins that have been sequenced. These FtsZ proteins can be divided into two domains. The N-terminal domain consists of about 300 residues and is highly conserved, whereas the second domain is variable in both length and composition, with only a short segment near the carboxy end showing any similarity. From other work, we know that truncating the *E. coli* protein at residue 320 does not affect GTPase activity in vitro, although the protein loses the ability to complement an *ftsZ* mutation in vivo (22). Thus, the variable carboxy segment is essential. Previously, it was noted that this region from *E. coli*, *B. subtilis*, and *Rhizobium meliloti* was rich in proline and glutamine (18). This is not the case for the *M. pulmonis* FtsZ protein, since the carboxy-terminal segment contains only three prolines and three glutamines. The most notable feature of this variable segment in the *M. pulmonis* FtsZ protein is the high frequency of charged residues (39%), compared with 19% for the conserved region. This relatively high frequency of charged residues is seen in the other FtsZ proteins, although to a lesser extent.

Although the *M. pulmonis* FtsZ protein is highly conserved, we found that it did not complement *E. coli ftsZ* mutations. Since overexpression of the *E. coli ftsZ* gene can also block division, we were careful to examine a broad range of inducer

concentrations; however, we were still unable to observe complementation. Instead, expression of the *M. pulmonis ftsZ* gene blocked cell division and led to filamentation. This cannot simply be too much total FtsZ, since an increase in *E. coli* FtsZ suppressed the negative effect of the heterologous FtsZ. This dominant negative phenotype is also seen with the expression of other heterologous *ftsZ* genes in *E. coli* (2, 18) and argues that *M. pulmonis* FtsZ is able to interact with the division machinery and jam it.

After this work was submitted, we became aware of two mycoplasma genome projects. The *Mycoplasma capricolum* project reported a short segment of an *ftsZ* gene (6), and the project on *Mycoplasma genitalium* found a complete coding sequence for an *ftsZ* gene (14). Comparison of reported FtsZ sequences with Megalign (PAM250 matrix) revealed that the FtsZ protein from *M. pulmonis* is most closely related to those of gram-positive bacteria, whereas the *M. genitalium* FtsZ protein is quite distinct from other FtsZ proteins (26). Interestingly, of the *fts* genes in *E. coli* that are believed to be involved in bacterial cell division, only *ftsZ* was found in the *M. genitalium* genome project. Other *fts* genes, e.g., *ftsQ*, *ftsA*, *ftsL*, and *ftsN*, could act to couple septal peptidoglycan biosynthesis to the FtsZ ring.

The strategy that we used to clone the *ftsZ* gene from *M. pulmonis* relied on using degenerate primers biased for the codon usage of this organism and corresponding to two conserved regions of FtsZ. This strategy has been used to clone *ftsZ* genes from several other eubacteria, including *R. meliloti* (18) and *Streptomyces coelicolor* (19). The regions that we chose included GGGTGTG, which is involved in GTP binding and hydrolysis (13, 20, 24), and NLDFAD, which is possibly also involved in GTP hydrolysis (11). Using degenerate primers corresponding to these regions, we have been able to isolate the gene from an *Archaeobacterium* species, *Haloferax volcanii*, as well (26). In addition, Osteryoung and Vierling (23) have identified an *ftsZ* gene in the expressed tag database of the plant *Arabidopsis thaliana*. Initial characterization of this gene indicates that it encodes a protein destined for the chloroplast. Taken together, these results suggest that all prokaryotic organisms and the eukaryotic organelles derived from them utilize FtsZ to carry out cell division.

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