Cloning and Characterization of *ftsN*, an Essential Cell Division Gene in *Escherichia coli* Isolated as a Multicopy Suppressor of *ftsA12*(Ts)

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A new cell division gene, *ftsN*, was identified in *Escherichia coli* as a multicopy suppressor of the *ftsA12*(Ts) mutation. Remarkably, multicopy *ftsN* suppressed *ftsI23*(Ts) and to a lesser extent *ftsQ1*(Ts); however, no suppression of the *ftsZ84*(Ts) mutation was observed. The suppression of *ftsA12*(Ts), *ftsI23*(Ts), and *ftsQ1*(Ts) suggests that FtsN may interact with these gene products during cell division. The *ftsN* gene was located at 88.5 min on the *E. coli* genetic map just downstream of the *cytR* gene. *ftsN* was essential for cell division, since expression of a conditional null allele led to filamentation and cell death. DNA sequence analysis of the *ftsN* gene revealed an open reading frame of 319 codons which would encode a protein of 35,725 Da. The predicted gene product had a hydrophobic sequence near its amino terminus similar to the noncleavable signal sequences found in several other Fts proteins. The presumed extracellular domain was unusual in that it was rich in glutamine residues. A 36-kDa protein that was localized to the membrane fraction was detected in minicells containing plasmids with the *ftsN* gene, confirming that FtsN was a membrane protein.

About a dozen genes that appear to be specifically required for cell division in *Escherichia coli* have been identified previously (6, 19). However, the lack of any exhaustive mutant hunts makes it likely that additional cell division genes will be isolated. Identification of new division genes and elucidation of the complex interplay among all of the division genes are essential to a full understanding of the mechanism of cell division in *E. coli*.

Of the cell division genes that have been identified, six are located in the 2-min region, and these are among the most extensively characterized. They include *ftsA*, *ftsI*, *ftsL* (*mraR*), *ftsQ*, *ftsW*, and *ftsZ*. Among these genes, the *ftsZ* gene plays a key role in cell division. Elevated levels of FtsZ induce minicell formation, whereas a small decrease in FtsZ results in inhibition of division, suggesting that FtsZ is limiting for cell division (13, 46). It has also been suggested previously that FtsZ is the target of the division inhibitors SulA and MinCD (4, 5, 24, 28). The FtsZ protein, which is present at approximately 20,000 molecules per cell, forms a dynamic ring structure at the division site during cell division (7). Largely on the basis of this behavior, it was suggested that FtsZ might function as a cytoskeletal protein to orchestrate the circumferential invagination of the cytoplasmic membrane and cell wall to form the septum. Recently, FtsZ was shown to be a GTP-binding protein with weak GTPase activity, raising the possibility that FtsZ might use GTP to form a structure and to modulate its dynamic localization (17, 29, 33). Consistent with this hypothesis, the specific activity of the FtsZ GTPase is dependent upon the FtsZ concentration, suggesting that FtsZ must oligomerize in order to express the GTPase activity (16, 17, 29, 45).

The products of the *ftsA*, *ftsI*, and *ftsQ* genes are thought to function after FtsZ in cell division (2, 19). Cell fractionation studies have demonstrated that the FtsA protein, present at about 200 molecules per cell, is peripherally

associated with the inner surface of the cytoplasmic membrane (32). Examination of the resumption of cell division in *ftsA3* filaments suggests that FtsA may be a component of the septum (40). This suggestion arises from the observation that division sites that were bypassed at the nonpermissive temperature could not be immediately utilized upon a return to permissive conditions. It is thought that the FtsA3 protein, which is irreversibly denatured, blocks the division site. Recent observations suggest that the ratio of FtsZ to FtsA is critical for cell division to occur, despite the dramatic difference in their levels (14, 18). This raises the possibility that FtsA may interact with FtsZ.

Three of the other gene products, FtsL, FtsQ, and PBP3 (*ftsI* gene product), are transmembrane proteins (9, 11, 23). They have similar topologies, with a noncleavable, N-terminal signal sequence acting to transport the main body of the protein to the periplasmic space and to tether the protein to the cytoplasmic membrane. It is well known that PBP3, the product of *ftsI*, is one of the four high-molecular-weight penicillin-binding proteins and that it is specifically required for septal peptidoglycan biosynthesis (30, 38). It has both transglycosylase and transpeptidase activities, and the latter can be specifically inhibited by certain β -lactams (38). An interaction between PBP3 and FtsA has been previously suggested on the basis of the ability of one allele of *ftsA* to affect the binding of a β -lactam to PBP3 (39). Although little is known about the action of the *ftsQ* gene product, our previous observations suggest that it functionally interacts with FtsZ, FtsA, and PBP3 (14). More recently, *ftsL* has been identified as an essential cell division gene that might have some role in coupling cell division and peptidoglycan physiology (23). Mutations in *ftsL* can result in either filamentation or cell lysis (41). Clearly, proteins with this transmembrane topology would be ideally situated to affect peptidoglycan physiology in response to intracellular signals.

Obviously, revealing and dissecting interactions among the above-mentioned cell division genes and other cell

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TABLE 1. Bacterial strains

Strain	Relevant marker(s)	Source or reference
JFL110	<i>ftsA12</i> (Ts) <i>recA</i>	28
BUG6	<i>ftsA6</i> (Ts)	34
TC27	<i>ftsA27</i> (Ts)	W. Donachie
TOE23	<i>ftsI23</i> (Ts)	2
TOE1	<i>ftsQ1</i> (Ts)	2
JFL101	<i>ftsZ84</i> (Ts) <i>recA</i>	27
JC10240	<i>srl::Tn10 recA56</i>	13
JKD41(pKD123)	W3110 <i>ftsN::kan</i> (pKD123)	This work
JKD42(pKD123)	Same as JKD41 but <i>srl::Tn10 recA56</i>	P1(JC10240) × JKD41(pKD123) selecting for Tet ^r and screening for temperature sensitivity
V355.594	<i>recD</i>	36
P678-54	<i>minB</i>	1
W3110	Prototroph	Laboratory collection
MCA12 ^a	<i>ftsA12</i> (Ts) <i>leu::Tn10</i>	P1 transduction
MCA27 ^a	<i>ftsA27</i> (Ts) <i>leu::Tn10</i>	P1 transduction
MCZ84 ^a	<i>ftsZ84</i> (Ts) <i>leu::Tn10</i>	P1 transduction
MCI23 ^a	<i>ftsI23</i> (Ts) <i>leu::Tn10</i>	P1 transduction
MCQ1 ^a	<i>ftsQ1</i> (Ts) <i>leu::Tn10</i>	P1 transduction

^a This strain was constructed by P1 transduction in two steps. First, *leu::Tn10* was introduced into the strain carrying the temperature-sensitive mutation by selecting for Tet resistance and screening for temperature sensitivity. P1 was then grown on this transductant and infected into MC4100, selecting for Tet resistance and again screening for temperature sensitivity.

division genes are crucial to understanding their exact roles in the cell division process. In this study, we sought to identify *ftsA*-interacting genes by isolating multicopy-dependent suppressors of the *ftsA12*(Ts) mutation. By this strategy, a previously undescribed gene, *ftsN*, was isolated. Gene disruption showed that *ftsN* is essential for cell division and viability. When present on a multicopy plasmid, *ftsN* also suppressed *ftsI23*(Ts) and *ftsQ1*(Ts), suggesting that it may interact with PBP3 and FtsQ as well as FtsA. It did not suppress *ftsZ84*(Ts). Interestingly, FtsN appears to have the same transmembrane topology as FtsQ, PBP3, and FtsL.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used are listed in Table 1.

Plasmids. The suppressor plasmids p2-5 and p1-11 and several derivatives are shown in Fig. 1. The suppressor plasmids p2-5 and p1-11 were obtained by selecting for multicopy suppression of *ftsA12*(Ts). Additional suppressor plasmids, p3-8 and p2-14, had inserts that overlapped the inserts in p2-5 and p1-11 (data not shown). The suppressor plasmids were selected from a library that contained chromosomal DNA partially digested with *TaqI* and *Sau3A* ligated into pBR322 double-digested with *ClaI* and *BamHI*. Derivatives of p2-5 containing the *kan* cassette in the suppressor gene were obtained as described later in the text. pKD1 is a pBR322 derivative containing the *kan* cassette from pUC4K. pKD123 contains the chromosomal insert from p1-11 cloned into a derivative of the temperature-sensitive replicon pEL3 (13) that carries Cm^r in place of Amp^r. pKD140 contains the 2.1-kb *EcoRV*-*SphI* fragment of p2-5 cloned into pBR322, and pKD138 contains this frag-

FIG. 1. Restriction maps of the plasmids. The thick segment of the line at the top indicates the overlapping region of the inserts in Kohara phage clones 538 and 539. The lines below the top line represent the inserts in each plasmid listed at the right. The size of each insert is given in parentheses. The triangle indicates the position of the *kan* insertion in p2-5K15 (B, *BamHI*). The open and shaded boxes indicate the ORFs for *cytR* and *ftsN*, respectively. The arrows indicate the direction of transcription.

ment cloned into the polylinker region of the expression plasmid pJF118EH (22).

DNA hybridization. DNA hybridization was used to locate the insert in p1-11 containing the *ftsN* gene on the *E. coli* genetic map. p1-11 was labeled with [α - 32 P]dCTP by the random primer method (35) using random hexamers purchased from Bethesda Research Laboratories. The Gene-Screen Plus filters that contained DNA from each clone of the phages of Kohara's lambda phage library of *E. coli* chromosomal DNA were prepared by Shambhavi Subbarao in our laboratory. The hybridization was carried out according to the instructions in the manual provided by NEN Research Products (DuPont).

Semirandom *kan* insertion mutagenesis. A detailed description of the semirandom *kan* insertion mutagenesis method will be published elsewhere. Briefly, to disrupt *ftsN* on p2-5, the plasmid was partially digested with *Hpa*II in the presence of ethidium bromide. The conditions for the digestion were first determined such that a majority of the plasmid molecules in the reaction mixture were linearized. Linear molecules were isolated from a low-melting-point agarose gel (35). The *kan* cassette plasmid pKD1, which was engineered to have the kanamycin (*kan*) resistance gene flanked by *Bst*BI sites, was digested with *Bst*BI, and the 1.4-kb fragment containing the *kan* resistance gene was isolated by using GeneClean. This fragment was mixed with the linear p2-5 molecules, ligated, and transformed into JFL110 [*ftsA12*(Ts)]. Transformants were selected on kanamycin plates at 30°C and screened for temperature sensitivity (i.e., loss of suppression). Plasmids were then isolated from these temperature-sensitive transformants and subjected to restriction analysis to locate the *kan* insertions.

Labeling plasmid-encoded proteins. The minicell strain P678-54 was transformed with different plasmids, and the minicells were isolated and labeled as described previously (12).

DNA sequencing. The 2.1-kb *Eco*RI-*Sph*I fragment presumably containing the *ftsN* gene was cloned from p2-5 into the *Hinc*II-*Sph*I site of M13mp19 and M13mp18. A set of nested deletions of the insert in M13mp19 was generated by using an ExoIII deletion kit purchased from Stratagene and following the accompanying instructions. Sequencing was done by the dideoxy method with Sequenase version II (U.S. Biochemical Corp.) according to the procedures provided by the manufacturer. To sequence the other strand, primers were made according to the sequence obtained from sequencing the complementary strand, and M13mp18 containing the insert was used as the template.

Cell fractionation. To localize the FtsN protein, the minicells containing p2-5 were labeled and fractionated into membrane and soluble fractions by the procedure described by Osborn et al. (31).

Photomicroscopy. Cells sampled for photomicroscopy were fixed in 10% formaldehyde. A thin layer of 1% agarose was made on a glass slide, and the fixed cells were spotted onto it. The cells were examined and photographed by phase-contrast microscopy.

Nucleotide sequence accession number. The sequence data for the *ftsN* gene and protein have been assigned the GenBank accession number L14281.

RESULTS

Isolation of a multicopy suppressor of *ftsA12*(Ts). To isolate multicopy suppressors of the *ftsA12*(Ts) mutation, a genomic library contained in a high-copy vector was introduced into

strain JFL110. Ampicillin-resistant transformants were selected at 37°C, a nonpermissive temperature for JFL110. Temperature-resistant transformants were restreaked onto ampicillin plates at 37°C to verify their temperature resistance. Plasmid DNA was isolated from transformants that showed better growth on plates at 37°C as judged by the size of the colonies and the morphology of the cells. Plasmid DNA from positive colonies was retransformed into JFL110 to confirm the suppression. Forty plasmids were thus obtained, and these were divided into six classes based upon the morphology of the transformants at 37°C and restriction analysis of the plasmid inserts. Class 1 contained inserts that encompassed the wild-type *ftsA* and neighboring *ftsZ* genes. Class 2 contained the *sdiA* gene (44), which has been shown to increase expression of the *ftsQ*, *ftsA*, and *ftsZ* genes when its dosage is increased. JFL110 carrying plasmids from these two classes produced short cells and minicells at 37°C. Classes 3 to 6 exhibited levels of suppression similar to one another but less efficient than those of classes 1 and 2 on the basis of the morphology of the suppressed cells. JFL110 containing these plasmids grew well at 37°C, but it still produced short filaments. Classes 3 to 6 were differentiated from one another only by the different sizes of the chromosomal inserts. Further restriction analysis of representatives (p1-11, p2-5, p2-14, and p3-18) of these four classes of plasmids revealed that they shared common restriction fragments, indicating that they may all contain the same suppressor gene. This was later confirmed by complementation of a null allele of the suppressor gene by these plasmids (see below). p1-11 and p2-5 had the least overlap, and they thus narrowed the location of the suppressor gene to a region of about 2 kb (Fig. 1). The 2.1-kb *Eco*RV-*Sph*I fragment that contained the overlapping region was subcloned from p2-5 into pBR322. The resultant plasmid, pKD140 (Fig. 1), suppressed *ftsA12*(Ts) as efficiently as p2-5.

Specificity of suppression. The suppression of *ftsA12*(Ts) by pKD140 was examined in more detail by quantitating the effect of pKD140 on the plating efficiencies of JFL110 at different temperatures. At 37°C, pKD140 completely suppressed *ftsA12*(Ts), since the plating efficiency was 1 (relative to the efficiency of plating at 30°C). At 42°C, the suppression was not complete, although the plating efficiency was increased more than 100-fold over that of the control containing pBR322 (data not shown). To determine the specificity of the suppression, pKD140 was introduced into an isogenic set of strains constructed in the MC4100 background. These strains carried either *ftsA* mutations, *ftsA12*(Ts) or *ftsA27*(Ts), or other *fts* mutations, *ftsZ84*(Ts), *ftsQ1*(Ts), or *ftsI23*(Ts). pKD140 suppressed *ftsA12*(Ts) in this background to a similar degree, but it had only a marginal effect on *ftsA27*(Ts) (Table 2). Surprisingly, pKD140 suppressed *ftsI23*(Ts) very well and also showed significant suppression of *ftsQ1*(Ts). However, pKD140 did not suppress *ftsZ84*(Ts) and actually had a negative effect on the plating efficiency. pKD140 did not suppress a conditional null allele of *ftsA* (15), suggesting that the residual activity of mutant FtsA proteins is necessary for the suppression (data not shown).

Mapping the suppressor gene. Since representative plasmids from classes 3 to 6 all contained an overlapping region and presumably the same suppressor gene, we chose one to locate the insert on the *E. coli* physical map. This was done by using Kohara's library (25), which consists of 481 ordered clones of lambda phages that contain overlapping fragments covering the entire *E. coli* chromosome. p1-11 was labeled and used to probe filters containing the DNA from each

TABLE 2. Suppression specificity of multicopy *ftsN*

Strain	Mutation	Presence of pKD140 ^a	Relative efficiency of plating at the following temp (°C) ^b	
			37	42
MCI23	<i>ftsI23</i>	—		2×10^{-8}
		+		1×10^{-2}
MCA12	<i>ftsA12</i>	—	1.2×10^{-5}	2×10^{-7}
		+	0.5	1×10^{-5}
MCA27	<i>ftsA27</i>	—		2×10^{-6}
		+		2×10^{-5}
MCQ1	<i>ftsQ1</i>	—		7×10^{-5}
		+		7×10^{-3}
MCZ84	<i>ftsZ84</i>	—		1×10^{-5}
		+		5×10^{-7}

^a Strains either contained pKD140 (+) or did not contain pKD140 but did contain pBR322 (—).

^b Plating efficiencies at these temperatures are relative to the plating efficiencies at 30°C.

lambda clone. Two clones, clones 538 and 539, showed a positive signal. These two phages carry overlapping chromosomal inserts from 88.5 min of the *E. coli* genetic map (Fig. 1). This mapping result was consistent with the restriction maps of the inserts and the chromosome covering this region (Fig. 1). Except for one of the *Pst*I sites which is missing in the insert contained in p1-11, the restriction maps of these inserts match that of the chromosome.

Interruption of the suppressor gene. The map position of the suppressor gene suggested that it might be a new gene, a suggestion which was later confirmed by sequencing. In addition, the gene was later found to be an essential cell division gene and was therefore designated *ftsN*. To determine whether *ftsN* was necessary for cell division, we decided to disrupt the chromosomal copy of *ftsN*. To do this, the *ftsN* gene on p2-5 was first disrupted by using a semi-

FIG. 2. Interruption of *ftsN*. p2-5 was linearized by digestion with *Aat*II and *Nde*I, and the digested plasmid DNA was transformed into V355.594 (*recD*) containing pKD123. Integration of *ftsN::kan* was obtained by selection for Kan^R. *ftsN::kan* was then transduced into W3110 containing pKD123 to give JKD41(pKD123).

FIG. 3. *ftsN* is required for cell division. Exponentially growing cells of JKD41(pKD123) were shifted from 30 to 42°C. Samples for photomicroscopy were taken before (1) or 4 h after (2) the shift.

random *kan* insertion method (see Materials and Methods). The semirandom *kan* insertion method was employed because of the lack of convenient restriction sites in the insert and the fact that we did not know the exact location of the *ftsN* gene. Three plasmids, p2-5K15, p2-5K39, and p2-5K48, were obtained by screening for *kan* insertions that abolished the ability of pKD140 to suppress *ftsA12*(Ts). These insertions were assumed to be within the *ftsN* gene. p2-5K15 was chosen to disrupt the chromosomal copy of *ftsN*.

The strategy used to disrupt the chromosomal *ftsN* gene is outlined in Fig. 2. Since it was likely that *ftsN* was essential for cell viability, the disruption was done in the presence of pKD123, which is temperature sensitive for replication and which carries the 3.6-kb chromosomal fragment from p1-11 (Fig. 1). p2-5K15 was linearized by digestion with *Nde*I and *Aat*II and was transformed into V355.394 (36) containing pKD123. Kanamycin-resistant transformants were selected at 30°C. Of 31 transformants obtained, 30 were determined to be temperature sensitive for growth. A P1 lysate was prepared from one of the temperature-sensitive transformants and was used to transduce the wild-type strain W3110 containing pKD123 to kanamycin resistance. The resultant strain, JKD41(pKD123), was temperature sensitive for growth and formed long filaments at 42°C (Fig. 3). These filaments contained virtually no constrictions; occasionally, one occurred at the midpoint of the filament. JKD41 (pKD123) had a plating efficiency of 2.5×10^{-6} at 42°C. The

FIG. 4. Identification of the FtsN protein. Minicells isolated from strain P678-54 containing various plasmids were labeled with [³⁵S]methionine. The labeled proteins were separated by SDS-PAGE and visualized by autoradiography. Samples in each lane are as indicated. The solid arrowhead points to the band corresponding to the FtsN protein, which has an estimated molecular mass of 36 kDa. The open arrowhead points to the LacI protein, whose molecular mass is known to be 38.6 kDa. Molecular mass standards (in kilodaltons) are on the left.

plating efficiency was completely rescued by p2-5, whereas it was unaffected by p2-5K15, confirming that the temperature sensitivity and the filamentation phenotypes of JKD41 (pKD123) were indeed a result of inactivation of the chromosomal *ftsN* gene. The other suppressor plasmids, p1-11, p2-14, and p3-18, also complemented JKD41(pKD123), confirming that they contained *ftsN* (data not shown). pGB2, a vector plasmid incompatible with pKD123, could not replace pKD123 at 30 or 37°C (data not shown), indicating that *ftsN* was also required for cell growth at a low temperature. Nucleoid staining of the filaments obtained from JKD41 (pKD123) grown at the nonpermissive temperature showed regularly distributed nucleoids (data not shown), suggesting that the lack of FtsN did not obviously affect DNA synthesis and nucleoid segregation. To determine whether the filamentation was due to induction of the SOS response, the *recA56* mutation was introduced into JKD41(pKD123). The resultant strain, JKD42(pKD123), was temperature sensitive and still filamented at 42°C, indicating that filamentation was not due to induction of the SOS response. We concluded that *ftsN* is an essential gene that is required for cell division.

Identification of the FtsN protein. Minicell labeling was used to specifically label the proteins encoded by p2-5, p2-5K15, pKD138, and pJF118EH. p2-5 contains a chromosomal fragment of about 6 kb including the *ftsN* gene (Fig. 1). p2-5K15 is the same as p2-5 except that it contains a *kan* insertion in the *ftsN* gene (Fig. 1). pKD138 has a 2.1-kb *EcoRV-SphI* fragment from p2-5 (Fig. 1) cloned downstream of the *tac* promoter in pJF118EH. If the *ftsN* gene in pKD138 is in the same orientation as the *tac* promoter, induction by isopropyl-β-D-thiogalactopyranoside (IPTG)

FIG. 5. Nucleotide sequence of *ftsN* gene and deduced amino acid sequence of gene product. The possible -35 and -10 regions are underlined. The 7-bp inverted repeats overlapping the putative promoter are indicated by arrows. The hydrophobic segment of 27 amino acids is boxed. The open triangle indicates the position of the *kan* insertion present in p2-5K15. Arrows within the ORF indicate the six-amino-acid and four-amino-acid segments that are repeated.

should increase its expression. The labeled minicell lysates were separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels, and the autoradiogram is shown in Fig. 4. A protein with a molecular mass of about 36 kDa was detected in minicells containing p2-5 or pKD138 but not in minicells containing p2-5K15 or the vector pJF118EH. The labeling of this protein in cells containing pKD138 was obviously increased in the presence of IPTG. We concluded that *ftsN* encodes a protein of about 36 kDa and is transcribed counterclockwise in the same direction as *cytR* (39), which must map very close to the *ftsN* gene.

Sequence analysis of *ftsN*. The 2.1-kb *EcoRV-SphI* fragment containing the *ftsN* gene was subcloned from p2-5 into M13 and sequenced on both strands. One large open reading frame (ORF) and the 3' end of another were found. The partial ORF corresponded to the 3' end of *cytR*, which has been cloned, sequenced, and mapped at 88.5 min, confirming our mapping results (42). The nucleotide and deduced amino acid sequences of the other ORF, located immediately downstream of *cytR* and in the same orientation, are presented in Fig. 5. This ORF, consisting of 319 codons, starts with a GTG codon and would encode a protein with a calculated molecular mass of 35,725 Da, consistent with the estimate of the size of FtsN by SDS-PAGE. The initiation codon lacks a good neighboring consensus ribosome-binding site (37). This fact along with the presence of the GTG initiation codon is consistent with the poor expression of this protein observed in minicells, in which it is expressed less efficiently than the LacI protein (Fig. 4). The computer program DNA Star was applied to screen the NBRF (release 34) and SwissProt (release 21) data bases for known or potential ORFs with deduced protein sequences showing homology to that of FtsN; no significant homologies were found. A putative promoter with a good match to the *E. coli*

FIG. 6. Hydropathy profile of the FtsN protein sequence.

consensus promoter sequence was found just upstream of the ORF. A 7-bp inverted repeat with a spacer sequence of 13 bp (GCCCCGCCN₁₃GGCGGGC) was found overlapping the putative promoter region for *ftsN* (indicated in Fig. 5). A search of GenBank for homology to this inverted sequence was performed, but no exact match was found. By sequencing the *Bam*HI-*Sph*I fragment and the *Bam*HI-*Eco*RV fragment from p2-5K15 (Fig. 1), the *kan* insertion in p2-5K15 was determined to be at the *Hpa*II site at position 312, 71 codons from the presumed 5' end of the *ftsN* gene (Fig. 5). The hydropathy profile of the deduced FtsN protein sequence was obtained with the sequence analysis program DNA Strider (Kyte Doolittle analysis [26]), and it shows a hydrophobic segment from amino acid 29 to 55 (Fig. 5 and 6). This feature of FtsN is also present in FtsQ, which has a hydrophobic segment from amino acid 25 to 49 (47). The hydrophobic segment of FtsQ has been previously shown to function as a membrane-spanning segment (23), and it is required for the membrane localization of FtsQ (20). It is likely that FtsN has a membrane topology similar to that of FtsQ, with its larger C-terminal part located in the periplasm, its smaller N-terminal part located in the cytoplasm, and the connecting hydrophobic segment spanning the membrane. Another noticeable feature of the FtsN sequence is that a stretch of amino acids running from positions 113 to 225 is unusually rich in glutamine; out of 113 amino acids, 36 are glutamine (32%). Also, the six-amino-acid sequence TPEQRQ is found twice near the middle of the protein, as is the four-amino-acid sequence QSKP (Fig. 5).

Subcellular localization of FtsN. The hydrophobicity analysis of the FtsN sequence reveals a hydrophobic segment near its N terminus, suggesting that FtsN is a transmembrane protein. To test whether FtsN was a membrane protein, minicells containing p2-5 were labeled with [³⁵S]methionine and fractionated into soluble (cytoplasm plus periplasm) and membrane fractions. Proteins in different cellular fractions were analyzed by SDS-PAGE and autoradiography. As shown in Fig. 7, FtsN was detected in the membrane fraction but not in the soluble fraction. This supports the suggestion that FtsN is a transmembrane protein that is presumably anchored to the cytoplasmic membrane through the hydrophobic segment near its N terminus.

Effect of overproduction of FtsN. To overexpress the FtsN protein and to determine the effect of an increased level of FtsN on cell growth and division, pKD138, which contains *ftsN* under the control of the *tac* promoter, was introduced into strain W3110. When the expression of *ftsN* was induced from this plasmid by the addition of IPTG, no obvious effect on cell growth or cell division was observed. We then added a consensus ribosome site, AGGAGG, 7 bp before the GTG

FIG. 7. Cellular localization of the FtsN protein. Minicells containing pKD2-5 were labeled with [³⁵S]methionine and fractionated. Proteins were separated by SDS-PAGE and visualized by autoradiography. Lanes: M, membrane; C, cytoplasm (plus periplasm); T, total cell lysate. The arrowhead points to the FtsN protein band. Molecular mass standards (in kilodaltons) are on the left.

initiation codon of the gene by polymerase chain reaction to further increase the expression of FtsN. The resultant plasmid was introduced into W3110. Upon induction by IPTG, the cells formed short filaments and were lysed. It was also noticeable that the cell ends were blunt compared with those of control cells. Coomassie blue staining of SDS-PAGE gels confirmed that the FtsN protein was effectively overexpressed, resulting in a readily detectable band in whole-cell lysates (15). We concluded that cell division is not very sensitive to the level of FtsN, although a very high level of the protein caused some degree of division inhibition and cell lysis.

DISCUSSION

By isolating multicopy suppressors of the *ftsA12*(Ts) mutation, a previously undescribed gene, *ftsN*, was identified and mapped at 88.5 min. Inactivation of the gene resulted in filamentation and cell death, indicating not only that it is an essential gene but that it is an essential cell division gene. Multicopy *ftsN* completely suppressed *ftsA12*(Ts) at 37°C but had only a marginal effect at 42°C and only a marginal effect on the *ftsA27*(Ts) mutation. Surprisingly, multicopy *ftsN* suppressed *ftsI23*(Ts) and, to a lesser extent, *ftsQ1*(Ts). In contrast, it did not suppress *ftsZ84*(Ts) but actually appeared to enhance the mutation, since the plating efficiency of a strain with this mutation was actually decreased.

The *ftsN* gene is located immediately downstream of the *cytR* gene. It is transcribed in the same direction as *cytR*, and no obvious transcription terminator was found between these two genes. It is possible that a promoter(s) upstream of *cytR* may contribute to expression of *ftsN*, but it is clearly not required for the suppression observed here, since DNA fragments containing the *ftsN* gene and only the 3' portion of the *cytR* gene suppressed both *ftsA12*(Ts) and *ftsI23*(Ts) when present on a multicopy plasmid. The initiation codon appears to be a GTG codon that lacks a recognizable

ribosome-binding site. This is consistent with the poor level of expression of *ftsN* observed in this study. Furthermore, adding a consensus ribosome-binding site 7 bp upstream of the GTG codon significantly enhanced expression of FtsN.

Several possibilities for the mechanism of the suppression of the *fts* mutations exist. One is that an elevated level of the FtsN protein might increase the expression of the *ftsA*, *ftsI*, and *ftsQ* genes. This possibility is considered unlikely, at least for suppression of the *ftsA* and *ftsQ* mutations. If FtsN regulates a promoter upstream of *ftsA* or *ftsQ* and increases the expression of either gene when its level is increased, increased expression of *ftsZ*, which lies immediately downstream of *ftsA*, would also occur. The *sdiA* gene which positively regulates the P2 promoter of *ftsQ* is able to suppress both *ftsZ84*(Ts) (44) and *ftsA12*(Ts) (this paper). However, multicopy *ftsN* does not suppress *ftsZ84*(Ts) but actually aggravates the defect in the mutant cells (Table 2). This argues that the expression of *ftsA* and *ftsQ* is not increased in the presence of multicopy *ftsN* and that *ftsN* exerts its suppression effect at a posttranscriptional level.

It has been shown previously that MreB negatively regulates *ftsI* expression (43). Therefore, it is possible that the suppression of *ftsI23*(Ts) by multicopy *ftsN* is through a negative effect on the function of MreB. Such an effect would be expected to induce the formation of spherical cells in wild-type strains, but cells containing *ftsN* on a multicopy plasmid had a normal rod shape (Fig. 3). Therefore, if the expression of *ftsI* is affected by an increased dosage of *ftsN*, it is by an *mreB*-independent mechanism. A similar argument may be made that the suppression is not through increasing PBP5 or PBP6, which have been shown to restore division of *ftsI23*(Ts) mutant cells but which induce spherical cells in *ftsI*⁺ strains (3).

Another possibility is that the FtsN protein functions in concert with FtsA, PBP3, and FtsQ in division. One of the hypothetical roles of FtsA in division is to mediate an interaction between FtsZ in the cytoplasm and the septum-specific peptidoglycan synthetic machinery in the periplasm. PBP3, the cell division-specific peptidoglycan synthetase encoded by *ftsI*, is one of the components of the peptidoglycan synthetic machinery probably interacting directly with *ftsA* (39). FtsN and FtsQ may be additional components. FtsQ has been proposed to be the mediator between cytoplasmic FtsZ and penicillin-insensitive peptidoglycan synthesis which is postulated to function in the periplasm in early septal peptidoglycan synthesis before PBP3 (30). However, morphological analysis of double mutants of *rodA* and either *ftsZ*, *ftsA*, *ftsQ*, or *ftsI* suggested that FtsQ, like FtsA and PBP3, was not required in the FtsZ-dependent early division event but that it acted later (2). Therefore, it is possible that following the early FtsZ event, FtsA, FtsQ, PBP3, and FtsN replace penicillin-insensitive peptidoglycan synthesis and complex with FtsZ to continue septal peptidoglycan synthesis. The recently identified FtsL (23, 41) may also participate in this complex.

Note that suppression of *fts* mutations by an increase in another *fts* gene product is not generally observed. For example, an increase in *ftsI* expression did not suppress mutations in *ftsA*, *ftsQ*, or *ftsZ* (21). Also, we have previously observed that an increase in *ftsQ* expression did not suppress *ftsA*, *ftsI*, or *ftsZ* mutations but was more detrimental to strains carrying these mutations than to a wild-type strain (14). Therefore, the multicopy *ftsN* suppression may be unique among the *fts* genes. Also, note that the multicopy *ftsN* suppression reported here includes suppression of an intracellularly located defect, FtsA12(Ts), and

extracellularly located defects, FtsI23(Ts) and FtsQ1(Ts). However, these proteins are all colocalized to the cytoplasmic membrane and could physically interact as suggested above. Therefore, it is possible that an increase in FtsN may help the functioning of the mutated gene products, perhaps by promoting the formation of a septal biosynthetic complex among gene products that are less efficient because of mutation.

Recently, we described alleles of *ftsZ* that affect cellular morphology through an altered geometry of the septum (8). This altered geometry of the septum coincided with an altered geometry of the FtsZ ring, raising the possibility that the FtsZ ring was directly affecting septal peptidoglycan biosynthesis. We also described alleles of *ftsZ* that result in cell lysis at the nonpermissive temperature, and the lysis appeared to be linked to the division site (8). These alleles might alter the interaction between FtsZ and the septum-specific peptidoglycan synthetic system consisting of PBP3, FtsA, FtsQ, FtsL, and FtsN.

Our results have identified a new gene whose product is similar to several other *fts* gene products. The gene products of *ftsQ*, *ftsL*, *ftsI*, and *ftsN* all appear to have a noncleavable signal sequence located near their amino termini which functions to transport the main body of the protein to the periplasmic space and anchor it to the cytoplasmic membrane. With FtsN, we have shown only that it is located in the membrane fraction and have inferred that its topology is similar to that of the others on the basis of a similar hydrophobicity profile. For each of the proteins, the amino-terminal sequence preceding the transmembrane signal sequence is longer than what is typical for a protein with a cleavable signal sequence, raising the possibility that it may have some function in either helping to maintain the topology of the protein or interacting with cytoplasmic components. In addition, each of these gene products is present at a relatively small number of molecules per cell (in the range of 20 to 50 molecules), and yet the cell division machinery is relatively insensitive to their overproduction (10, 11, 15, 23). Cell division is inhibited only when the levels of these gene products are increased more than 100-fold. This is in sharp contrast to FtsZ and the peripheral membrane protein, FtsA. A three- to fourfold increase in either of these proteins is inhibitory to cell division (14, 18). Thus, although the *ftsL*, *ftsQ*, *ftsI*, and *ftsN* gene products are essential for cell division to occur, the process is relatively insensitive to their levels.

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