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Multiple Interaction Domains in FtsL, a Protein Component of the Widely Conserved Bacterial FtsLBQ Cell Division Complex▽†

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A bioinformatic analysis of nearly 400 genomes indicates that the overwhelming majority of bacteria possess homologs of the *Escherichia coli* proteins FtsL, FtsB, and FtsQ, three proteins essential for cell division in that bacterium. These three bitopic membrane proteins form a subcomplex *in vivo*, independent of the other cell division proteins. Here we analyze the domains of *E. coli* FtsL that are involved in the interaction with other cell division proteins and important for the assembly of the divisome. We show that FtsL, as we have found previously with FtsB, packs an enormous amount of information in its sequence for interactions with proteins upstream and downstream in the assembly pathway. Given their size, it is likely that the sole function of the complex of these two proteins is to act as a scaffold for divisome assembly.

The division of an *Escherichia coli* cell into two daughter cells requires a complex of proteins, the divisome, to coordinate the constriction of the three layers of the Gram-negative cell envelope. In *E. coli*, there are 10 proteins known to be essential for cell division; in the absence of any one of these proteins, cells continue to elongate and to replicate and segregate their chromosomes but fail to divide (29). Numerous additional nonessential proteins have been identified that localize to midcell and assist in cell division (7–9, 20, 25, 34, 56, 59).

A localization dependency pathway has been determined for the 10 essential division proteins (FtsZ→FtsA/ZipA→FtsK→FtsQ→FtsL/FtsB→FtsW→FtsI→FtsN), suggesting that the divisome assembles in a hierarchical manner (29). Based on this pathway, a given protein depends on the presence of all upstream proteins (to the left) for its localization and that protein is then required for the localization of the downstream division proteins (to the right). While the localization dependency pathway of cell division proteins suggests that a sequence of interactions is necessary for divisome formation, recent work using a variety of techniques reveals that a more complex web of interactions among these proteins is necessary for a functionally stable complex (6, 10, 19, 23, 24, 30–32, 40). While numerous interactions have been identified between division proteins, further work is needed to define which domains are involved and which interactions are necessary for assembly of the divisome.

One subcomplex of the divisome, composed of the bitopic membrane proteins FtsB, FtsL, and FtsQ, appears to be the bridge between the predominantly cytoplasmic cell division proteins and the predominantly periplasmic cell division proteins (10). FtsB, FtsL, and FtsQ share a similar topology: short amino-terminal cytoplasmic domains and larger carboxy-terminal periplasmic domains. This tripartite complex can be divided further into a subcomplex of FtsB and FtsL, which forms in the absence of FtsQ and interacts with the downstream division proteins FtsW and FtsI in the absence of FtsQ (30). The presence of an FtsB/FtsL/FtsQ subcomplex appears to be evolutionarily conserved, as there is evidence that the homologs of FtsB, FtsL, and FtsQ in the Gram-positive bacteria *Bacillus subtilis* and *Streptococcus pneumoniae* also assemble into complexes (18, 52, 55).

The assembly of the FtsB/FtsL/FtsQ complex is important for the stabilization and localization of one or more of its component proteins in both *E. coli* and *B. subtilis* (11, 16, 18, 33). In *E. coli*, FtsB and FtsL are codependent for their stabilization and for localization to midcell, while FtsQ does not require either FtsB or FtsL for its stabilization or localization to midcell (11, 33). Both FtsL and FtsB require FtsQ for localization to midcell, and in the absence of FtsQ the levels of full-length FtsB are significantly reduced (11, 33). The observed reduction in full-length FtsB levels that occurs in the absence of FtsQ or FtsL results from the degradation of the FtsB C terminus (33). However, the C-terminally degraded FtsB generated upon depletion of FtsQ can still interact with and stabilize FtsL (33).

While a portion of the FtsB C terminus is dispensable for interaction with FtsL and for the recruitment of the downstream division proteins FtsW and FtsI, it is required for interaction with FtsQ (33). Correspondingly, the FtsQ C terminus also appears to be important for interaction with FtsB and FtsL (32, 61). The interaction between FtsB and FtsL appears to be mediated by the predicted coiled-coil motifs within the periplasmic domains of the two proteins, although only the membrane-proximal half of the FtsB coiled coil is necessary for interaction with FtsL (10, 32, 33). Additionally, the transmembrane domains of FtsB and FtsL are important for their interaction with each other, while the cytoplasmic domain of FtsL is

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TABLE 1. Strains and plasmids

Strain or plasmid	Relevant genetic marker(s) or feature(s)[a]	Source or reference
Strains		
MC4100	F$^-$ *araD139* Δ*lacU169 relA1 rpsL150 thi mot flb5301 deoC7 rbsR*	Laboratory collection
JOE309	MC4100 *araD*$^+$	14
NB811	JOE309 *ftsL*::Tn*phoAL81*Δ*IS50R*(Kan^r) Δ(λ*attL-lom*)::*bla araC* P_{BAD}-*ftsL*	10
MDG277	JOE309 Δ*ftsL*::*kan* Δ(λ*attL-lom*)::*bla araC* P_{BAD}-*ftsL*	33
MDG252	JOE309 Δ*ftsL*::*kan* Δ(λ*attL-lom*)::*bla lacI*^{q} pDSW204-*gfp-ftsQ*/pJMG197	This study
MDG253	JOE309 Δ*ftsL*::*kan* Δ(λ*attL-lom*)::*bla lacI*^{q} pDSW204-*gfp-ftsB*/pJMG197	This study
MDG254	JOE309 Δ*ftsL*::*kan* Δ(λ*attL-lom*)::*bla lacI*^{q} pDSW204-*gfp-ftsW*/pJMG197	This study
MDG255	JOE309 Δ*ftsL*::*kan* Δ(λ*attL-lom*)::*bla lacI*^{q} pDSW204-*gfp-ftsI*/pJMG197	This study
Plasmids		
pAM238	pSC101 ori Spec^r pLac	26
pAM-*ftsL*	pAM238-*ftsL*	N. Buddelmeijer
pBAD33	pACYC184 ori Cm^r pBAD promoter	36
pJMG197	pBAD33-*ftsL*	27
pNG162	pSC101 ori Spec^r *lacI*^{q} P_{204}	30
pMDG29	pNG162-$flag_3$-*ftsL*	This study
pMDG30	pNG162-$flag_3$-*ftsL* Met16start	This study
pMDG31	pNG162-$flag_3$-*ftsL* Asp30start	This study
pMDG32	pNG162-$flag_3$-*ftsL* Ala90stop	This study
pMDG33	pNG162-$flag_3$-*ftsL* Ile100stop	This study
pMDG34	pNG162-$flag_3$-*ftsL* Gln114stop	This study
pMDG35	pNG162-*zapA*-F_{cyto}-*ftsL*	This study
pMDG36	pNG162-*zapA*-F_{cyto}-*ftsL* Ile100stop	This study

[a] Spec^r, spectinomycin resistance; Cm^r, chloramphenicol resistance; Amp^r, ampicillin resistance; Km^r, kanamycin resistance.

not necessary for interaction with FtsB, which has only a short 3-amino-acid cytoplasmic domain (10, 33).

In this study, we focused on the interaction domains of FtsL. We find that, as with FtsB, the C terminus of FtsL is required for the interaction of FtsQ with the FtsB/FtsL subcomplex while the cytoplasmic domain of FtsL is involved in recruitment of the downstream division proteins. Finally, we provide a comprehensive analysis of the presence of FtsB, FtsL, and FtsQ homologs among bacteria and find that the proteins of this complex are likely more widely distributed among bacteria than was previously thought.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. The bacterial strains and plasmids used in the paper are described in Table 1. Strains were grown in NZY rich medium for all experiments except the wrinkled-colony screen, where cells were grown on M63 minimal medium (14, 50). Concentrations of antibiotics were as follows: ampicillin, 25 μg/ml (chromosome); chloramphenicol, 30 μg/ml (high-copy-number plasmid) or 10 μg/ml (low-copy-number plasmid); kanamycin, 40 μg/ml; spectinomycin, 100 μg/ml. Strains were supplemented with 0.2% L-arabinose or 0.2% D-glucose to induce or repress expression of pBAD-regulated *ftsL*.

Standard laboratory techniques were used for DNA cloning and analysis, PCR, electroporation, transformation, and P1 transduction (57). Individual bacterial strains and plasmids were constructed as described below. Enzymes for DNA manipulation were from New England Biolabs.

Strain construction. Strain MDG252 was constructed by transforming JOE309 with pJMG197 (pBAD33-*ftsL*) and selecting for growth on plates with chloramphenicol. The resulting transformants were transduced with a P1 lysate from WM2240 (DY329 Δ*ftsL*::*kan*/pWM1845) and selected for growth on plates with kanamycin (40 μg/ml) plus arabinose to generate strain MDG279 (JOE309 Δ*ftsL*::*kan*/pBAD33-*ftsL*) (24). Strain MDG279 was then transduced with a P1 lysate from JOE61 [MC4100 Δ(λ*attL-lom*)::*bla lacI*^{q} P_{DSW207}-*gfp-ftsQ*] (J. Chen, unpublished strain) and selected for growth on plates with low ampicillin (25 μg/ml).

Strain MDG253 was constructed by transducing MDG279 with a P1 lysate from NB805 and selecting for growth on plates with low ampicillin (25 μg/ml) (11).

Strain MDG254 was constructed by transducing MDG279 with a P1 lysate from NWG563 and selecting for growth on plates with low ampicillin (25 μg/ml) (30).

Strain MDG255 was constructed by transducing MDG279 with a P1 lysate from JOE63 [MC4100 Δ(λ*attL-lom*)::*bla lacI*^{q} P_{DSW207}-*gfp-ftsI*] (J. Chen, unpublished strain) and selecting for growth on plates with low ampicillin (25 μg/ml).

Plasmid construction. Oligonucleotide primers used for the construction of all plasmids are listed in Table S1 in the supplemental material.

Plasmids pMDG29, -32, and -33 were constructed by PCR amplification of the *ftsL* construct from pAM-*ftsL* using the oligonucleotides 5′ NcoI FtsL full for all reactions and pAM rev, 3′ XbaI FtsL A90stop, or 3′ XbaI FtsL I100stop, respectively. The resulting PCR products were digested with NcoI and XbaI and then ligated into a purified digest (NcoI and XbaI) of pMDG7 (pNG162-$flag_3$-*ftsB*), lacking *ftsB* (33).

Plasmids pMDG30 and -31 were constructed by PCR amplification of the *ftsL* construct from pMDG29 using the oligonucleotides pTrc Rev for both reactions and 5′ NcoI FtsL Met16start or 5′ NcoI FtsL D30start, respectively. The resulting PCR products were digested with NcoI and XbaI and then ligated into a purified digest (NcoI and XbaI) of pMDG7 (pNG162-$flag_3$-*ftsB*), lacking *ftsB*.

Plasmid pMDG34 was constructed by PCR amplification of the *ftsL* Gln114stop construct from pAM-*ftsL* Gln114stop using the oligonucleotides 5′ NcoI FtsL full and pAM rev. The resulting PCR products were digested with NcoI and XbaI and then ligated into a purified digest (NcoI and XbaI) of pMDG7 (pNG162-$flag_3$-*ftsB*), lacking *ftsB*.

Plasmids pMDG35 and -36 were constructed by PCR amplification of the *ftsL* construct from pMDG29 using the oligonucleotides gfp-L-5′EcoRI-2 for both reactions and pTrc Rev or 3′ XbaI FtsL I100stop, respectively. The resulting PCR products were digested with EcoRI and XbaI and then ligated into a purified digest (EcoRI and XbaI) of pMDG1, lacking *ftsB* (55). Plasmid pMDG1 was constructed in a similar manner as that described for pMDG3, except that in the final step the *lacI*^{q} P_{204}-*zapA*-*malF*$_{cyto}$-*ftsB* fragment was ligated into pBAD42, replacing *araC* P_{BAD} (33).

Depletion strain experiments. Strains were grown overnight at 37°C in 5 ml of NZ containing arabinose and appropriate antibiotics. The overnight cultures were diluted 1:100 into fresh medium with arabinose and appropriate antibiotics and were grown at 37°C to an optical density at 600 nm (OD_{600}) of ~0.3. A sample of the culture was washed once with NZ medium lacking sugar and diluted 1:100 into prewarmed NZ (37°C) with either arabinose or glucose plus the appropriate antibiotics and grown at 37°C. Under these conditions, depletion of complementing FtsL from strain MDG277 takes approximately 3 h.

SDS-PAGE and Western blot analysis. Whole-cell samples for Western blot analysis were prepared by precipitation of protein with trichloroacetic acid

(TCA). Samples were separated by Tris-glycine SDS-PAGE and transferred by semidry blot electrotransfer onto Immobilon-P polyvinylidene fluoride (PVDF) membranes (Millipore). The PVDF membranes were probed with anti-FtsL polyclonal antibodies (lab collection, 1:5,000 dilution), anti-FtsB polyclonal antibodies (lab collection, 1:5,000 dilution), anti-FtsQ polyclonal antibody (lab collection, 1:1,000 dilution), anti-FLAG monoclonal antibody (1:1,000, Sigma), or anti-beta-lactamase antibodies (lab collection). Secondary anti-mouse or anti-rabbit antibodies conjugated to horseradish peroxidase were used with the ECL Plus (Amersham) detection reagents for immunodetection.

Membrane preparation and coimmunoprecipitation experiments. Coimmunoprecipitation experiments were performed in cells depleted of complementing FtsL as described above but in 50-ml culture volumes. Membrane preparations for the coimmunoprecipitation experiments were performed as previously described (55). Protein concentrations for the *n*-dodecyl-β-D-maltopyranoside (DDM) solubilized membrane fractions were determined by bicinchoninic acid (BCA) assay (Pierce). Immunoprecipitation experiments were performed with 200 μg of membrane fractions in a volume of 400 μl incubated with 20 μl of anti-FLAG M2 affinity gel (Sigma-Aldrich) at 4°C with mixing for 2 h. Samples were then washed three times with buffer I plus 0.1% DDM and resuspended in 60 μl of sample buffer (New England Biolabs). The supernatant was conserved after the first spin for analysis of proteins that were not immunoprecipitated.

Premature targeting experiments. Strains were grown overnight at 30°C in 5 ml of NZ with arabinose and appropriate antibiotics and then diluted 1:100 into fresh medium containing arabinose and antibiotics and grown to an optical density at 600 nm (OD_{600}) of ~0.3. A sample of the culture was washed once with NZ medium lacking sugar and diluted 1:100 into 5 ml of prewarmed NZ (30°C) with glucose, including the appropriate antibiotics, and then grown at 30°C. Cells were depleted of the complementing FtsL for 210 min, at which time IPTG (isopropyl-β-D-thiogalactopyranoside) was added to the cultures at a final concentration of 10 μM for the final 30 min of growth.

Microscopy. After cultures were grown for the appropriate time, samples were fixed (15) and mounted onto 1% agarose cushions as previously described (62). Cells were examined using an Eclipse TE200-E inverted microscope (Nikon) with a 100× plan apo-differential interference contrast (apo-DIC) oil-immersion lens. The final processing of images for presentation was done using Adobe Photoshop.

Bioinformatic analysis. Homologs of FtsQ, FtsL, and FtsB were identified using the following hidden Markov models (HMM) obtained from Pfam (http://pfam.sanger.ac.uk): FtsB (PF04977), FtsL (PF04999), FtsQ (PF03799 and PF084878), and MraW (PF01795). With these HMMs, we searched a GenBank complete bacterial genome list of 374 bacterial genomes using HMMER 2.3.2 (http://hmmer.janelia.org). Those results that were above the significance cutoff from Pfam (ls.C file) were collected and analyzed further.

Additional FtsL homologs were identified using two methods. First, we built new HMMs in which the seed alignment was based on FtsL homologs from *Firmicutes* or from *Actinobacteria*. To generate these HMMs, we used BLASTP to identify FtsL homologs for the seed alignments, using as a query either the FtsL homolog from *B. subtilis* or that from *Streptomyces coelicolor* (2). Significant hits were collected and aligned using HMMER 2.3.2 to generate HMMs. These new HMMs for FtsL homologs were then used as described above.

The second method that we used to identify FtsL homologs was the gene synteny method described in Results. Using the results collected from the HMM for MraW, a custom Perl script determined the chromosomal orientation of the *mraW* homolog, identified the downstream open reading frames (ORFs), and determined if those ORFs had predicted transmembrane domains by using PHOBIUS (http://phobius.cbr.su.se) and coiled-coil domains by using COILS (http://www.ch.embnet.org/software/COILS_form.html) (39, 46). We collected these results and analyzed them for the criteria of FtsL homologs described in Results. As an additional confirmation of these putative FtsL homologs identified by synteny, we used these proteins to search for homologs (PSI-BLAST, http://www.ncbi.nlm.nih.gov/blast/Blast.cgi), since the FtsL homolog of related organisms should share homology (3).

In cases where we were unable to identify FtsQ homologs by using the HMMs as described above, we examined the ORFs upstream of *ftsA* and *ftsZ* and downstream of peptidoglycan synthesis genes, which are common sites for *ftsQ* homologs. ORFs at these chromosomal positions were analyzed by the transmembrane prediction program TMHMM 2.0 (http://www.cbs.dtu.dk/services/TMHMM/) to identify bitopic membrane proteins (41). Those bitopic membrane proteins were then used to search PSI-BLAST for homologs that might be identified as FtsQ.

In some cases FtsB homologs were identified in certain bacteria but not in their close relatives. Further analysis revealed that this could occur when the ORF encoding the FtsB homolog was not annotated in the genomic sequence. For the epsilonproteobacteria, no FtsB homologs were identified in a search of our list of epsilonproteobacteria. However, the Pfam database identified an FtsB homolog in the epsilonproteobacterium *Sulfurovum* sp. (strain NBC37-1). Using this FtsB homolog from *Sulfurovum* sp., we identified FtsB homologs in the remaining epsilonproteobacteria (PSI-BLAST). The FtsB homologs identified by this manner are predicted to have both transmembrane and coiled-coil domains and are all downstream of the enolase (*eno*) homolog, which tends to be a conserved position for *ftsB* homologs in numerous organisms. Figure 6 was made using the Interactive Tree of Life (iTOL) web server (http://itol.embl.de) (45).

RESULTS

The FtsL C terminus is necessary for interaction with FtsQ. We have taken a gene truncation approach to identify domains of FtsL that are important for interaction with partner proteins. We were led to take this approach by the finding of a particular mutation obtained in a hunt for *ftsL* mutations using the "wrinkled-colony" screen previously described for the identification of mutations in *ftsQ* (32). This screen yields partially functional mutations. While we obtained several mutations in the different domains of FtsL, the most informative one was an early truncation near the periplasmic C terminus of FtsL, at residue Gln114 out of 121 amino acids. This mutation was of particular interest given the importance of the FtsB C terminus for interaction with FtsQ (33). Additionally, the C terminus of FtsL appears to be important for its function, since FtsL was initially identified by alkaline phosphatase translation fusions to the C terminus of FtsL, at amino acids 81, 88, and 112, which inactivated its function (35). Based on these previous observations, we examined the role of the FtsL C terminus in complementation and for interaction with partner proteins.

To characterize the role of the FtsL C terminus, we cloned three C-terminal truncations of FtsL, at residues Ala90, Ile100, and Gln114, and full-length *ftsL* onto a low-copy-number plasmid (pNG162) with a FLAG epitope tag fused to the N-terminal domain. The FLAG epitope tag assisted in Western blot analysis and coimmunoprecipitation experiments. These C-terminal truncations do not alter the coiled-coil prediction for FtsL (COILS program, data not shown) (46). The constructs were expressed in strain MDG277, which has a pBAD-regulated complementing copy of *ftsL*. In all of the following experiments in this paper, *ftsB* and *ftsQ* are expressed from their native chromosomal loci.

Expression of FLAG-FtsL Gln114stop in cells depleted of complementing FtsL resulted, as did the untagged mutant, in a wrinkled-colony morphology, while expression of FLAG-FtsL gave a wild-type colony morphology (Fig. 1B and A). Microscopic examination of cells from the FLAG-FtsL Gln114-expressing colonies revealed a mix of wild-type-sized and filamentous cells, indicating a defect in cell division (Fig. 1D). In contrast to the Gln114stop, FLAG-FtsL Ala90stop or FLAG-FtsL Ile100stop did not restore any growth to cells depleted of complementing FtsL (data not shown and Table 2). Western blot analysis showed that the levels of FLAG-FtsL Gln114stop were similar to those of FLAG-FtsL and that the FtsB levels were also similar when either construct was expressed (Fig. 2, lane 6 versus lane 7). The levels of FLAG-FtsL Ala90stop and FLAG-FtsL Ile100stop were reduced compared to FLAG-FtsL, but increased expression of these truncations did not restore growth of cells depleted of complementing FtsL (Fig. 2, lanes 4 and 5 versus lane 7, and data not shown).

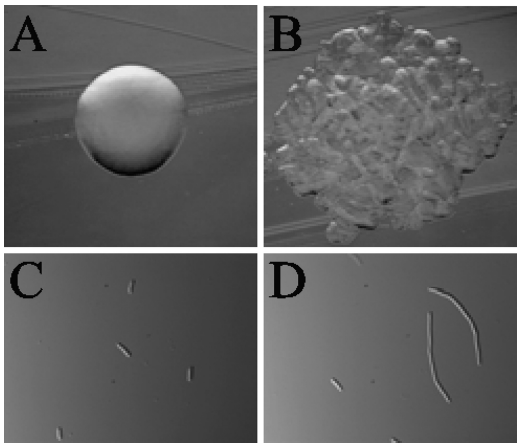

FIG. 1. Phenotype of an FtsL mutant identified in a wrinkled-colony screen. (A and B) Colonies formed on NZ medium by the strain MDG277 depleted of complementing FtsL and expressing either FLAG-FtsL from pMDG29 (A) or FLAG-FtsL Gln114stop from pMDG34 (B). (C and D) Differential interference contrast (DIC) micrographs of cells isolated from the colonies in panels A and B, respectively.

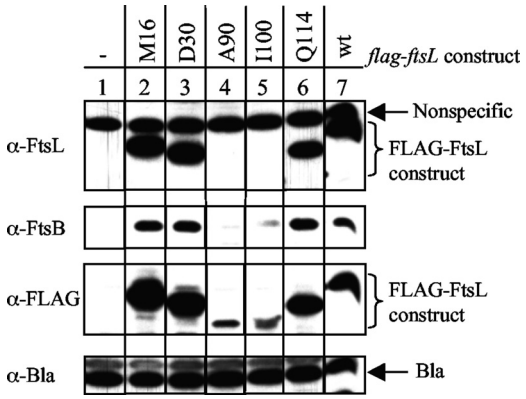

FIG. 2. Analysis of protein levels of N- and C-terminal truncations of FtsL. Western blot analysis of TCA-precipitated whole-cell samples that were grown in the presence of glucose to repress expression of the complementing FtsL. The FLAG-tagged FtsL constructs were induced with 20 μM IPTG. Samples were separated on 15% acrylamide gels. The α-Bla (anti-β-lactamase) blot is used as a loading control. An equivalent of 0.05 OD_{600} units was loaded per lane. The strain used was MDG277 (all lanes). The plasmids used were pNG162 (−, empty vector, lane 1), pMDG30 (M16, FLAG-FtsL Met16start, lane 2), pMDG31 (D30, FLAG-FtsL Asp30start, lane 3), pMDG32 (A90, FLAG-FtsL Ala90stop, lane 4), pMDG33 (I100, FLAG-FtsL Ile100stop, lane 5), pMDG34 (Q114, FLAG-FtsL Gln114stop, lane 6), and pMDG29 (wt, FLAG-FtsL, lane 6).

We next performed coimmunoprecipitation experiments to determine whether the defects in complementation by the C-terminal truncations of FtsL result from an inability to interact with FtsB and/or FtsQ. These experiments were done in cells depleted of complementing FtsL. All three FtsL truncations coimmunoprecipitated with FtsB (Fig. 3A, lane 6, and B, lanes 5 and 6). However, in contrast to FLAG-FtsL (Fig. 3A, lane 4), FLAG-FtsL A90stop and FLAG-FtsL Ile100stop failed to coimmunoprecipitate with FtsQ, while FLAG-FtsL Gln114stop showed a greatly diminished interaction with FtsQ (Fig. 3A, lane 6, and B, lanes 5 and 6). The levels of FtsQ in the whole-cell samples and coimmunoprecipitation supernatants indicate that the inability of the FtsL truncations to efficiently interact with FtsQ was not a result of the absence of FtsQ (Fig. 3A, lanes 3 and 9, and B, lanes 2, 3, 8, and 9). Taken together, these results suggest that a portion of the FtsL C-terminal periplasmic domain is required for the interaction with FtsQ but that the last 31 amino acids of FtsL are dispensable for interaction with FtsB.

TABLE 2. FtsL N- and C-terminal truncations

FtsL construct[a]	Complementation[b]	FtsB level[c]	FtsB interaction[d]	FtsQ interaction[d]	Downstream recruitment[e]
Wild type	+	+	+	+	+
Empty vector	−	−	NA[f]	NA	−
Met16start	+	+	ND[g]	ND	ND
Asp30start	Wrinkled	+	+	+	−
Ala90stop	−	−	+	−	ND
Ile100stop	−	−	+	−	+
Gln114stop	Wrinkled	+	+	+/−	ND

[a] FtsL constructs were generated by placing a stop codon or a start codon at the amino acid position listed. Full-length FtsL is 121 amino acids. The empty vector in these experiments is pNG162.

[b] FtsL constructs were tested for complementation by expression in a strain depleted of complementing FtsL (MDG277). +, wild-type colony formation; −, lack of colony formation; wrinkled, abnormal colony morphology that contains filamentous cells (Fig. 1B and D).

[c] FtsB levels were determined by Western blot analysis. +, presence of full-length FtsB; −, reduction of full-length FtsB and appearance of a degradation product of FtsB.

[d] The interaction of FtsL constructs with FtsB or FtsQ was determined by coimmunoprecipitation experiments as described in the text. +, interaction similar to FtsL wild type; −, no detectable interaction; +/−, greatly diminished interaction relative to the ability of FtsL wild type to interact with FtsQ.

[e] The recruitment of downstream division proteins was tested by the methods described in Results. In the case of FtsL Ile100stop, its ability to recruit FtsI was examined using the premature targeting system. In the case of FtsL Asp30start, the ability to recruit FtsW was examined.

[f] NA, not applicable.

[g] ND, not determined.

It should be noted that despite the interaction of both FLAG-FtsL Ala90stop and FLAG-FtsL Ile100stop with FtsB, we observed reduced levels of full-length FtsB and a truncation product of FtsB in whole-cell samples (Fig. 3A, lane 3, and B, lane 2). This reduction in full-length FtsB levels when the C-terminal truncations of FtsL were expressed probably results from the lack of interaction with FtsQ, which normally protects the FtsB C terminus from cleavage (33).

The FtsQ-interacting region of FtsL is dispensable for recruiting FtsI. We have shown that, while a portion of the FtsB C terminus is necessary for interaction with FtsQ, it is not required for recruitment of the downstream division proteins FtsW and FtsI (33). Therefore, we asked whether a portion of the FtsL C terminus that interacts with FtsQ is required for the recruitment of the downstream division proteins. To assess such interactions, we used a method that causes forced localization of FtsL Ile100stop to potential division sites, in the absence of upstream protein, and examined the recruitment of various Fts proteins fused to green fluorescent protein (GFP). This method, called premature targeting, has been used to study interactions among the division proteins independently of the recruitment pathway (30, 31). To promote this forced localization to midcell, we fused FtsL Ile100stop to ZapA, a small nonessential cytoplasmic protein that interacts directly

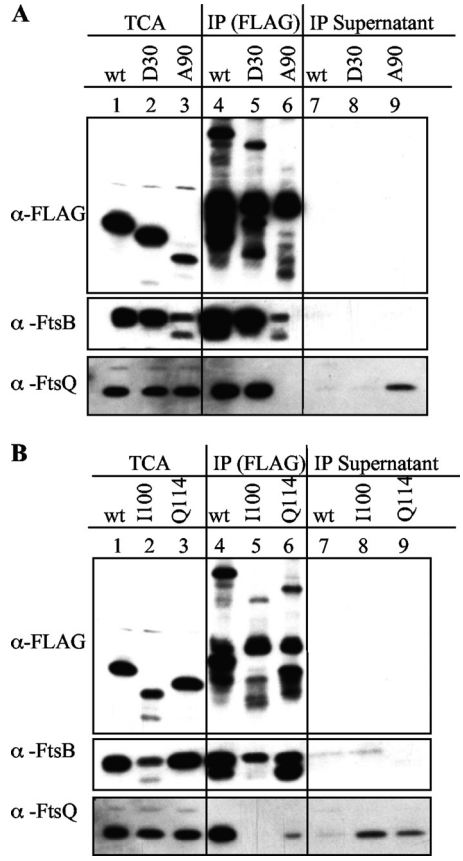

FIG. 3. Role of the FtsL N and C termini in interaction with FtsB and FtsQ. Cells were induced with 10 μM IPTG for all samples except those expressing FLAG-FtsL Ala90stop or FLAG-FtsL Ile100stop, which were induced with 60 μM IPTG. The whole-cell TCA-precipitated samples (lanes 1 to 3, TCA), the anti-FLAG antibody-immunoprecipitated samples [lanes 4 to 6, IP (FLAG)], and the supernatants after immunoprecipitation with anti-FLAG antibody (lanes 7 to 9, IP supernatant) were subjected to Western blot analysis. The slower-migrating bands at the top of the anti-FLAG blots (lanes 4 to 6) represent dimers of the FLAG-FtsL constructs previously described (11, 26). These dimers occur because of disulfide bond formation between cysteines of two FtsL monomers that occurs artifactually during sample preparation (J. M. Ghigo and N. Buddelmeijer, unpublished results). The bands in the lower part of the anti-FLAG blots likely are a mix of incompletely denatured FLAG-FtsL constructs and proteolysis of these constructs, which occurs during sample preparation. The faster-migrating bands seen in lanes 4 and 6 of the anti-FtsB blots for both panel A and panel B likely result from proteolysis of FtsB during sample preparation. Immunoprecipitation results similar to those presented in this figure were obtained when anti-FtsQ was used for protein immunoprecipitation (data not shown). Samples were separated on 15% acrylamide gels for the anti-FLAG and anti-FtsB blot assays and on 10% acrylamide gels for the anti-FtsQ blot assays. The strain used was MDG277 (all lanes). The plasmids used were pMDG29 (wt, FLAG-FtsL, lanes 1, 4, and 7 in both panel A and panel B), pMDG31 (D30, FLAG-FtsL Asp30start, lanes 2, 5, and 8 in panel A), pMDG32 (A90, FLAG-FtsL Ala90stop, lanes 3, 6, and 9 in panel A), pMDG33 (I100, FLAG-FtsL Ile100stop, lanes 2, 5, and 8 in panel B), and pMDG34 (Q114, FLAG-FtsL Gln114stop, lanes 3, 6, and 9 in panel B).

with FtsZ (31, 34, 38). The ZapA fusions were expressed from a low-copy-number vector, pMDG1, in strains depleted of complementing FtsL and expressing GFP fusions to FtsQ, FtsB, or FtsI. As a positive control for septal localization, we expressed GFP-FtsQ, since the localization of FtsQ does not depend on the presence of FtsL. In all samples tested, we observed septal localization bands of GFP-FtsQ (Fig. 4A, D, G, and J).

As a negative control, we expressed FLAG-FtsL Ile100stop without fusing it to ZapA and found that no septal localization bands were observed for GFP fusions to FtsB or FtsI (Fig. 4H and I). This lack of recruitment of FtsB and FtsI presumably results from the inability of FLAG-FtsL Ile100stop to interact with FtsQ, preventing localization of the truncated protein to potential division sites. However, when FtsL Ile100stop is fused to ZapA, thereby forcing its localization to potential division sites, we observed septal localization bands for both GFP-FtsB and GFP-FtsI (Fig. 4K and L) as occurred when a control ZapA fusion to wild-type FtsL was used (Fig. 4E and F). Western blot analysis showed that the levels of the GFP fusions and the FtsL Ile100stop constructs were similar in cells expressing ZapA-FtsL Ile100stop and in those expressing FLAG-FtsL Ile100stop (data not shown). These results indicate that the FtsQ-interacting region of FtsL is not necessary for the recruitment of the downstream division protein FtsI. While we did not directly examine the ability of ZapA-FtsL Ile100stop to recruit the protein immediately downstream, FtsW, previous work has shown that when FtsW or FtsI is present at division sites, the other protein is also present (30).

Despite restoring localization of FtsB and FtsI, presumably with FtsW, expression of a ZapA fusion to FtsL Ile100stop failed to restore complementation of the FtsL-depleted cells (Fig. 4J to L and data not shown). We suspect that this results from a previously observed inability to recruit FtsN to ZapA-formed division complexes (30, 31, 33).

The cytoplasmic domain of FtsL is involved in recruitment of the downstream division proteins. The role of the various domains of the bitopic Fts proteins, i.e., the cytoplasmic, transmembrane, and periplasmic domains, has been analyzed by swapping a given domain of an Fts protein with the analogous domain from a membrane protein not involved in cell division (37). Based on results from this method, it had been suggested that the cytoplasmic domain of FtsL is necessary for complementation of an FtsL depletion strain (37). A similar domain swap analysis also suggested that the cytoplasmic domain of FtsI was essential for its function (37). However, results from both deletions of the cytoplasmic domain and a bioinformatics analysis of the predicted transmembrane domain of FtsI suggest that the cytoplasmic domain of FtsI is in fact dispensable for its function (64). It was suggested that the previous results with the cytoplasmic swap construct of FtsI may have been misleading because the positioning of the transmembrane segment may have been altered in the membrane by the swap construct (64). Given the results with the FtsI cytoplasmic domain, we reexamined the role of the FtsL cytoplasmic domain using deletions.

To determine what role the cytoplasmic domain plays in FtsL function, we cloned two cytoplasmic truncations, at Met16 and Asp30, by starting translation at these positions and fusing the FLAG epitope tag to the N terminus of the truncations. Several transmembrane domain prediction programs predicted that the cytoplasmic truncations of FtsL leave substantial portions of the cytoplasmic domain intact, with the Asp30start truncations being 5 to 9 amino acids away from the

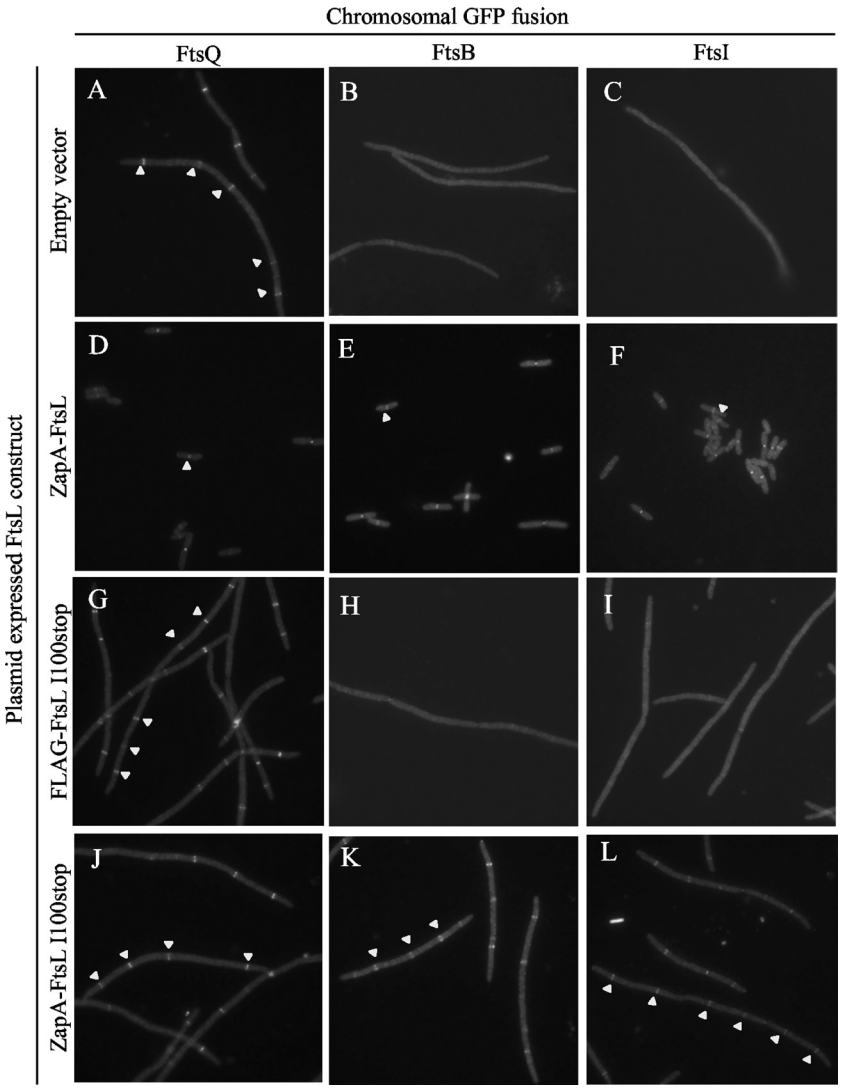

FIG. 4. The FtsL C terminus is dispensable for recruitment of the downstream division protein FtsI. Premature targeting analysis and microscopy of FtsL variants were performed in cells depleted of complementing FtsL as described in Materials and Methods. Shown are representative fluorescence micrographs from one of several fields of cells taken in a single experiment, with white arrowheads indicating the positions of septal bands. Note that expression of ZapA-FtsL complements the strain and results in wild-type-sized cells (D to F). The strains and plasmids used were GFP-FtsQ (MDG252 [A, D, G, and J]), GFP-FtsB (MDG253 [B, E, H, and K]), GFP-FtsI (MDG255 [C, F, I, and L]), empty vector (pNG162 [A to C]), ZapA-FtsL (pMDG35 [D to F]), FLAG-FtsL I100stop (pMDG33 [G to I]), and ZapA-FtsL I100stop (pMDG36 [J to L]).

first predicted amino acid in the transmembrane segment (data not shown). These truncations were cloned into a low-copy-number plasmid, pNG162, and transformed in the strain MDG277.

Expression of FLAG-FtsL Met16start complemented cells depleted of wild-type FtsL, forming wild-type-like colonies on plates and exhibiting wild-type cell size in liquid culture (Table 2). In contrast, expression of FLAG-FtsL Asp30start in cells depleted of wild-type FtsL resulted in wrinkled colonies on plates, and examination of cells from these wrinkled colonies revealed filamentous cells (Table 2). Western blot analysis shows that both FLAG-FtsL Met16start and FLAG-FtsL Asp30start are expressed well and stabilize FtsB (Fig. 2, lanes 2 and 3). Given the above results with the FtsL C-terminal truncations, the ability of FLAG-FtsL Asp30start to stabilize FtsB suggests both that it can interact with FtsB and that the resulting FtsB/FtsL complex is competent for interaction with FtsQ. Coimmunoprecipitation experiments indicate that FLAG-FtsL Asp30start does interact with FtsB and FtsQ (Fig. 3A, lane 5). Thus, the inability of FLAG-FtsL Asp30start to efficiently complement does not result from a disruption of interaction with FtsB and FtsQ. Rather, we suspected that the defect was due to a disruption of the interaction with the downstream division proteins, preventing recruitment of these proteins to potential division sites.

To determine if FLAG-FtsL Asp30start interacts with the downstream division proteins, we expressed the truncation construct in cells depleted of complementing FtsL and exam-

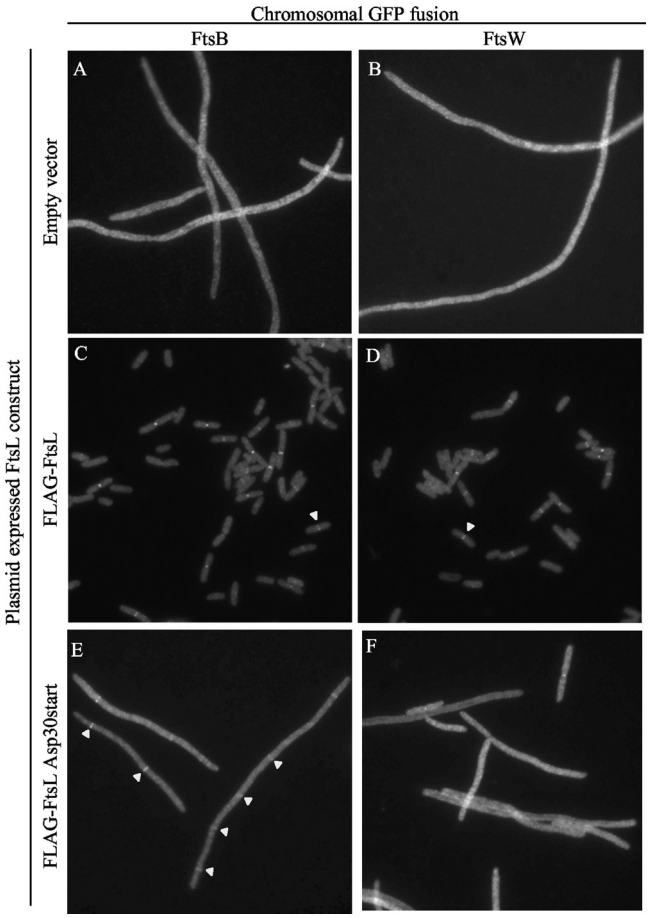

FIG. 5. The FtsL cytoplasmic domain is necessary for recruitment of the downstream division protein FtsW. Depletion of complementing FtsL was performed as described in Materials and Methods with 10 μM IPTG induction of the GFP fusions and FtsL constructs. Shown are representative fluorescence micrographs from one of three replicate experiments, with white arrowheads indicating the positions of septal bands. Note that expression of FLAG-FtsL complements the strain and results in wild-type-sized cells (C and D). The strains and plasmids used were GFP-FtsB (MDG253 [A, C, and E]), GFP-FtsW (MDG254 [B, D, and F]), empty vector (pNG162 [A and B]), FLAG-FtsL (pMDG29 [C and D]), and FLAG-FtsL Asp30start (pMDG31 [E and F]).

ined the recruitment of GFP-FtsB or GFP-FtsW fusions. When we expressed FLAG-FtsL Asp30start, we observed septal localization bands when GFP-FtsB was expressed (Fig. 5E). However, we did not observe septal localization signals when expressing GFP-FtsW with FLAG-FtsL Asp30start (Fig. 5F), while the control FLAG-FtsL construct did allow septal localization for both GFP fusions (Fig. 5C and D). Western blot analysis suggests that the absence of septal localization bands for GFP-FtsW in cells expressing FLAG-FtsL Asp30start did not result from low levels of the GFP fusions (data not shown). As expected, in empty-vector control experiments, there was no septal localization for GFP fusions to FtsB or FtsW (Fig. 5A and B). The results presented here suggest that the cytoplasmic domain of FtsL plays a role in the recruitment of the downstream division proteins.

Phylogenetic analysis of the conservation of FtsB, FtsL, and FtsQ. In *E. coli*, FtsB and FtsL can interact in the absence of FtsQ, and this FtsB/FtsL complex when prematurely targeted to the divisome can recruit downstream division proteins (30). This FtsQ-independent interaction between FtsL and FtsB is also observed for the *B. subtilis* homologs, as their homologs, $FtsL_B$ and DivIC, interact when expressed in *E. coli* in the absence of other *B. subtilis* proteins (18, 55). Furthermore, the finding that the FtsQ homologs of *B. subtilis* and *S. pneumoniae* are nonessential at low temperatures and on rich media, respectively, indicates that the homologs of FtsL and FtsB in these bacteria must be able to interact sufficiently in the absence of FtsQ to function in cell division (4, 44). These results raise the possibility that some bacteria that have homologs of FtsB and FtsL could lack an FtsQ homolog and still have a functional divisomal complex. This reasoning led us to examine a diverse set of bacteria for the presence of homologs of FtsB, FtsL, and FtsQ; the distribution of these proteins among bacteria has not been assessed in detail heretofore. In fact it was recently reported that the *Campylobacterales*, an order of the *Epsilonproteobacteria*, have FtsL and FtsB homologs but lack an FtsQ homolog (48).

To identify homologs of FtsB, FtsL, and FtsQ in a set of 374 bacteria, we used the FtsB, FtsL, and FtsQ hidden Markov models (HMMs) from the Pfam database (22). This approach allowed us to identify homologs of FtsB and FtsQ in many phylogenetically divergent bacteria. In contrast, the HMM for FtsL identified FtsL homologs only within the gamma-, beta-, and deltaproteobacteria and not in other bacteria known to have FtsL-like proteins, e.g., *B. subtilis*, *S. pneumoniae*, and *Streptomyces coelicolor*. It should be noted that the FtsL proteins from the Gram-positive bacteria have weak homology to *E. coli* FtsL and were designated FtsL based on their gene context (see below) and on a similar structure, i.e., a small membrane protein with a predicted coiled-coil motif (5, 17, 53). This lack of homology among FtsL proteins could explain the low number of bacteria previously identified as having FtsL homologs. Another factor limiting the identification of FtsLs is that a small number of FtsL homologs, only seven, were used to generate the FtsL HMM whereas the HMM for FtsB used 75 FtsB homologs.

We identified additional FtsL-like proteins based on the conserved position of *ftsL* downstream of *mraW* (63). The *mraW* gene encodes an *S*-adenosylmethionine-dependent methyltransferase with an unknown cellular function but which is nonetheless a highly conserved protein in bacteria (12). We identified homologs of MraW with the MraW HMM from the Pfam database and sought among the downstream open reading frames (ORFs) those that were predicted to be membrane proteins with a coiled-coil sequence. We designated those genes downstream of MraW homologs that matched the above criteria as encoding putative FtsL-like proteins, even though they were not necessarily homologs of canonical FtsLs. For the sake of clarity, we will refer to those proteins identified by this gene context, or synteny method, as FtsL homologs.

To further strengthen the inferences using the synteny method, we asked whether the 202 FtsL homologs that we identified using the HMMs for FtsL, as described in Materials and Methods, were the first ORFs downstream of *mraW* in their respective genomes, a position found with the previously

characterized FtsLs. All but one FtsL homolog identified in this way were the first ORF downstream from *mraW*. The one outlier, from *Thiomicrospira crunogena*, was the second gene downstream of *mraW*. Thus, the synteny method was able to identify likely FtsL homologs in over 99% of bacteria that were examined.

Based on our bioinformatic analysis of likely homologs of FtsB, FtsL, and FtsQ, we can divide bacteria into three groups: bacteria that have homologs of FtsB, FtsL, and FtsQ; bacteria that have no apparent homologs for FtsB, FtsL, and FtsQ; and finally, bacteria that have homologs of some but not all of the proteins in the FtsB/FtsL/FtsQ complex (Fig. 6; see Table S2 in the supplemental material). The largest group is made up of those in which we could identify homologs for all three proteins, a total of 295 bacteria out of 374. This group includes a morphologically and phylogenetically diverse set of bacteria that includes representatives from the *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chlorobi*, *Deinococcus-Thermus*, *Firmicutes*, *Fusobacteria*, *Proteobacteria* (excluding the *Epsilonproteobacteria*), and *Spirochaetes*.

In contrast, the smallest group is composed of bacteria that have no identifiable homologs for FtsB, FtsL, and FtsQ, a total of 22 bacteria. This group is mainly composed of bacteria known to lack a peptidoglycan cell wall, including the *Mollicutes* (*Mycoplasma*), *Dehalococcoidetes*, and the planctomycete *Rhodopirellula baltica* (28, 42, 49, 60). The remaining bacteria in our list that lack homologs of FtsB, FtsL, and FtsQ are two endosymbionts, *Baumannia cicadellinicola* and *Buchnera aphidicola* strain Cc, and the hyperthermophile *Thermotoga maritima*. It should also be noted that we were unable to identify homologs of FtsB, FtsL, and FtsQ in representatives of the *Archaea* (data not shown).

While a small number of bacteria lack homologs of all three proteins, we identified 57 bacteria that are missing homologs of one or two proteins within the FtsB/FtsL/FtsQ complex. We were unable to identify homologs for all three proteins in all representatives of the phylum *Cyanobacteria*, *Aquifex aeolicus*, *Deinococcus radiodurans*, *Deinococcus geothermalis*, and the remaining obligate intracellular bacteria (e.g., *Chlamydia* and *Chlamydophila* species, *Ehrlichia* species, and *Buchnera* species). Interestingly, while all of the epsilonproteobacteria and two actinobacteria, *Frankia alni* and *Frankia* sp. strain CcI3, have predicted homologs of FtsB and FtsL, we were unable to identify a homolog of FtsQ. For the obligate intracellular bacteria, we found that various combinations of FtsB, FtsL, and FtsQ occurred in these organisms. The lack of a complete FtsQ/FtsB/FtsL complex in these bacteria could reflect the gene loss and smaller genomes that occurred in the evolution of these bacteria from a free-living to an intracellular lifestyle (13). In the phylum *Cyanobacteria*, we found homologs for only FtsL and FtsQ; we could not identify homologs of FtsB.

Finally, in our search for FtsB homologs we found that some bacteria, specifically the *Bacillus cereus* group, composed of *B. cereus*, *B. thuringiensis*, and *B. anthracis*, which have predicted homologs of FtsB and FtsL, have an additional small membrane protein with a predicted coiled-coil motif that appears to be a second FtsB. Given that this extra FtsB protein has homology to the canonical FtsB homolog of *Bacillus* species and given its conservation within the *Bacillus cereus* group, this protein might play a role in some novel aspect of cell division for these bacteria.

DISCUSSION

Identification of interaction domains within FtsL. Results from a variety of methods suggest that the assembly of a stable divisome occurs through a broader set of protein interactions than is indicated by the linear dependency pathway. For example, overexpression of certain cell division proteins or expression of *ftsA* mutants can suppress defects in or absence of several cell division proteins (6, 23, 24). A model that includes a broader set of interactions among the divisomal proteins for assembly of the *E. coli* divisome also matches more closely assembly of the *B. subtilis* divisome, which does not occur by a linear pathway but appears to have more interdependency for septal localization (21).

In this paper, we identified a domain of the FtsL C terminus that is necessary for interaction with FtsQ but not with other division proteins. In combination with our studies that identified a region of the FtsB C terminus involved in interaction with FtsQ, this provides the first example of protein domains that are required for a cooperative interaction in the divisome; specifically, portions of both the FtsB and FtsL C termini are required for interaction with FtsQ (also with its C terminus). This cooperative interaction might be conserved in other bacteria, as indicated by results from the *in vitro* analysis of the *S. pneumoniae* homologs of FtsB, FtsL, and FtsQ, where formation of an FtsB/FtsL heterodimer is necessary for interaction with FtsQ (52). Additional structural investigations of this *S. pneumoniae* complex revealed that the homologs of FtsB and FtsL interact with FtsQ via their C-terminal domains (48).

To identify the domains of FtsL that are involved in interactions with other division proteins, we initially used a wrinkled-colony screen for mutants, the results of which led us to study the C terminus of FtsL. By generating a series of C-terminal truncations of FtsL, we found that a portion of the FtsL C terminus is necessary for interaction with FtsQ but not for interaction with FtsB or the downstream division proteins. We found that the truncation mutant FtsL Gln114stop, identified by the wrinkled-colony screen, weakly interacts with FtsQ as determined by coimmunoprecipitation experiments, indicating that the region of FtsL after Gln114 plays a role in this interaction. Further truncation of the FtsL C terminus, as in FtsL Ile100stop, completely disrupts the ability of FtsL to interact with FtsQ and results in an inability to complement an FtsL depletion strain. Taken together, these results suggest that the FtsL C terminus is involved in interaction with FtsQ; specifically, this region of FtsL must include amino acids that are N and C terminal to Gln114.

We also examined the role of the FtsL N-terminal cytoplasmic domain by generating truncations that are unlikely to affect the positioning of the transmembrane domain. We find that a portion of the FtsL cytoplasmic domain, between Met16 and Asp30, plays a role in the recruitment of the downstream division protein FtsW but is not necessary for interaction with FtsB and FtsQ. We also observed that overexpression of the FtsL Asp30start construct was partially dominant negative, presumably due to competition with the wild-type FtsL for interaction with FtsB and FtsQ (unpublished results). The

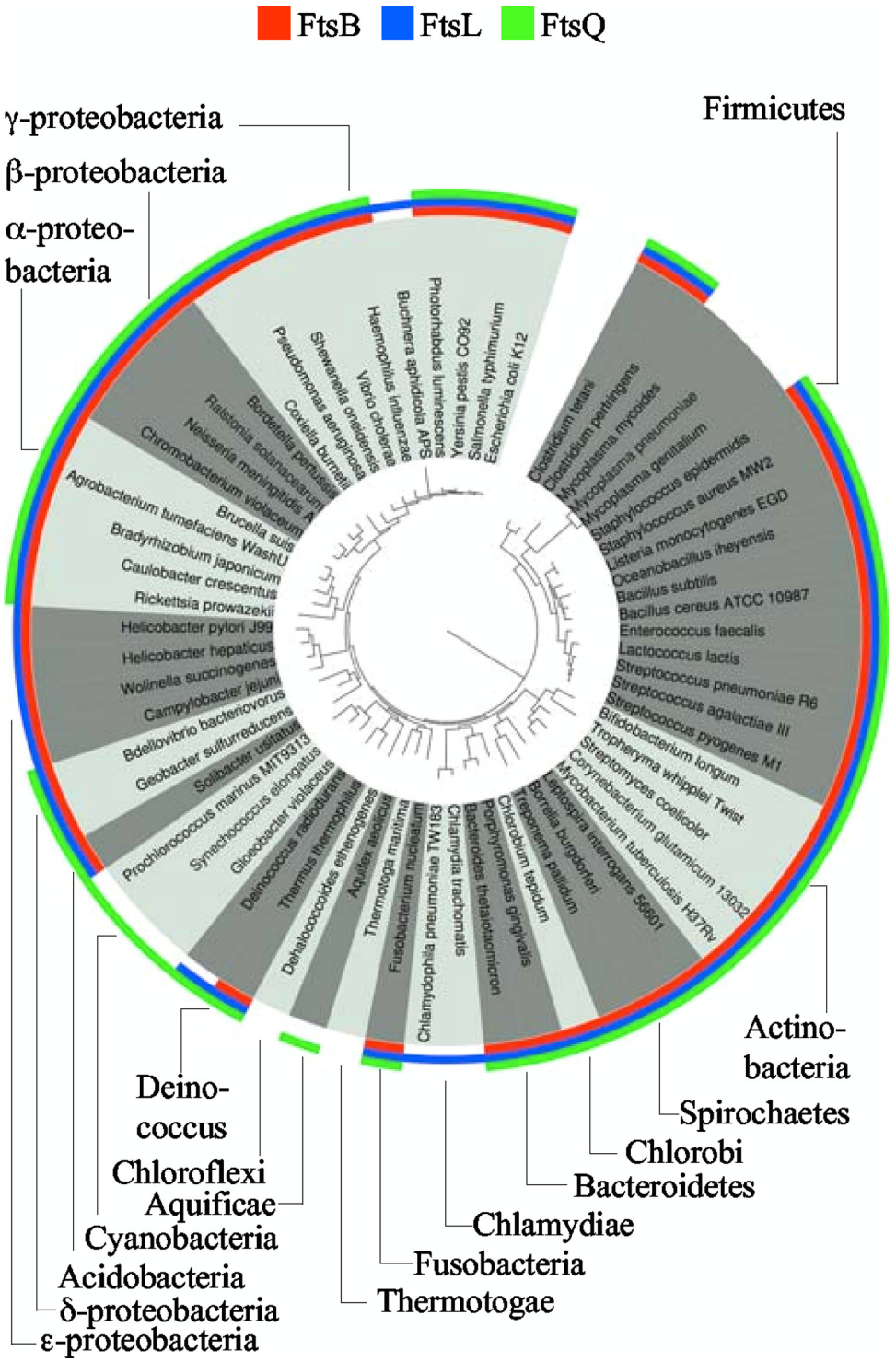

FIG. 6. Phylogenetic distribution of FtsQ, FtsL, and FtsB homologs. Organisms identified to have homologs of FtsQ (green line), FtsL (blue line), and FtsB (red line) are indicated on the phylogeny tree (based on 16S rRNA). Shown here is a subset of the 374 bacterial genomes examined, with representatives from every major phylum (see Table S2 in the supplemental material for a complete list).

FtsL Asp30start is likely defective in the recruitment of FtsW, given that FtsL Asp30start failed to recruit FtsW to potential division sites and that FtsW is the protein immediately downstream of FtsL in the dependency pathway. FtsW is a polytopic membrane protein with 10 transmembrane segments and has both N and C termini and several loops within the cytoplasm that could interact with the FtsL cytoplasmic domain (43).

Previous studies indicated that the transmembrane and

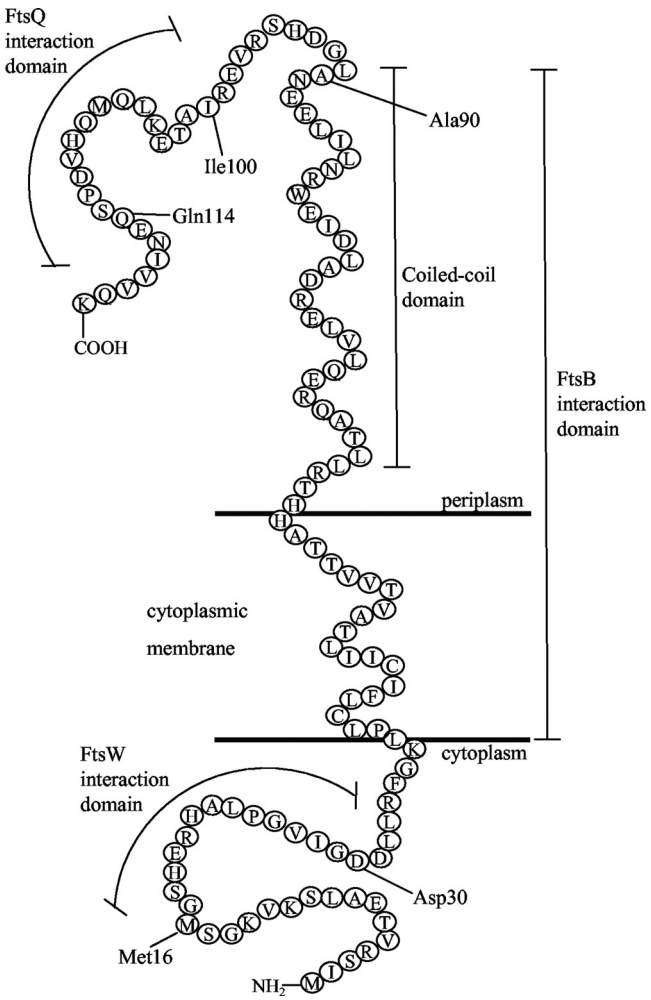

FIG. 7. A model for the interaction domains of FtsL. Shown is a schematic diagram of FtsL showing the positions of the cytoplasmic (residues 1 to 37), transmembrane (residues 38 to 57), and periplasmic (residues 58 to 121) domains. The positions of the C-terminal truncations and start sites for the N-terminal truncations are indicated. The positions of the FtsW interaction domain (residues 16 to 30), the FtsB interaction domain (residues 38 to 90), and the FtsQ interaction domain (residues 100 to 114) of FtsL are shown.

coiled-coil domains of FtsL are important for interaction with FtsB (10). Given that most of the cytoplasmic domain and C terminus of FtsL is dispensable for interaction with FtsB, our results suggest that the interaction between FtsB and FtsL requires only the transmembrane and coiled-coil motif of FtsL. In an analogous fashion, we have found that the transmembrane and coiled-coil motif of FtsB are necessary for interaction with FtsL (33).

Based on our results, in combination with previous work, we propose a model for assembly of the FtsB/FtsL/FtsQ complex and the additional interactions of components of this complex with the upstream and downstream division proteins (Fig. 7). Assembly of an FtsB/FtsL complex is mediated via their transmembrane and membrane-proximal periplasmic domains, which include their predicted coiled-coil domains. This FtsB/FtsL complex is competent for interaction with FtsQ and the downstream division proteins. The structure formed between the C termini of FtsB and FtsL could be the template for interaction with FtsQ; FtsQ may interact with amino acid sequences in both FtsB and FtsL or with sequences in only one of the components of this structure. Surface-exposed residues of the FtsQ C terminus have been shown to be required for interaction with FtsB and FtsL, while the periplasmic polypeptide transport-associated (POTRA) and transmembrane domains are important for localization of FtsQ to midcell (32, 58, 61). Recruitment by the FtsB/FtsL complex of the downstream division proteins minimally requires the cytoplasmic domain of FtsL, which presumably interacts with FtsW, the first known protein downstream of this complex.

Conservation of homologs of the FtsB/FtsL/FtsQ complex. We assessed the presence of FtsB, FtsL, and FtsQ in a set of phylogenetically distinct bacteria by looking for homologs of these three proteins. By using a more up-to-date bioinformatics approach, including synteny, we find that there is more conservation of these proteins than previously reported (47, 51, 63). The conservation of homologs of FtsB, FtsL, and FtsQ in a majority of the bacteria that we examined suggests that this complex is important for cell division in bacteria with diverse shapes, including rods, cocci, branched filaments, branched rod-shaped cells, spirals, and curved cells. Furthermore, homologs of these three proteins are present in bacteria that divide in a different manner from the prototypical binary fission of *E. coli*, such as by endospore formation (*B. subtilis*), fruiting body formation (*Myxococcus xanthus*), budding (*Hyphomonas neptunium*), and multiple fission (*Bdellovibrio bacteriovorus*). The conservation of FtsB/FtsL/FtsQ homologs in these diverse bacteria suggests that this subcomplex has evolved to cooperate with the divisomal proteins present in these bacteria, which could be homologs of FtsW and FtsI or of uncharacterized division proteins.

The absence of homologs of any of these three proteins from some bacteria, such as the *Mollicutes*, the *Dehalococcoidetes*, and the planctomycete *Rhodopirellula baltica*, may be related to their lack of a peptidoglycan cell wall. As a result, they may contain only a subset of the cell division proteins known to be present in the model organisms used for studying cell division, which have a different cell envelope structure than what is present in these bacteria. Most of the *Mollicutes* have an FtsZ homolog but lack many of the other cell division proteins (1). However, homologs of FtsQ were identified in two planctomycetes, and a homolog of FtsL is present in one of those as well, although it remains to be determined if these proteins are actually involved in cell division in these bacteria (54).

Since in some bacteria, e.g., *B. subtilis* and *S. pneumoniae*, the FtsB and FtsL homologs can function in the absence of an FtsQ homolog (4, 44), we looked for bacteria in which FtsB and FtsL were present but there was no FtsQ homolog. This was the case for members of the *Actinobacteria* (*Frankia*) and the epsilonproteobacteria, which have homologs of FtsB and FtsL but lack an identifiable homolog of FtsQ. However, it is possible that, in spite of our extensive analysis of these genomes, we were unable to identify an existing FtsQ homolog in them. Alternatively, these FtsQ-less bacteria could have another protein that replaces the function of FtsQ. We did find that immediately upstream of the *ftsA* and *ftsZ* homologs in the epsilonproteobacteria, a common position for *ftsQ* homologs, is a gene that encodes a putative membrane protein with a

predicted peptidyl-prolyl *cis-trans* isomerase domain (data not shown). Many of the FtsQ homologs that we identified have predicted POTRA domains, which are implicated in chaperone-like activity, raising the possibility that the putative chaperone activity of FtsQ is replaced by the peptidyl-prolyl isomerase in the epsilonproteobacteria.

We also found that in addition to homologs of FtsB and FtsL, members of the *Bacillus cereus* group, which includes the species *B. cereus*, *B. anthracis*, and *B. thuringiensis*, have an additional FtsB-like protein. This additional FtsB homolog could play a role in the binary fission of these bacteria, and/or it could be involved in the sporulation process.

Finally, the use of the synteny method allowed us to identify putative homologs of FtsL in a diverse set of bacteria. Even in the case of the characterized FtsL homologs from *E. coli* and *B. subtilis*, which share a similar domain structure, gene context, and interactions, these proteins do not share significant homology at the amino acid level. Just as the FtsL homologs from *E. coli* and *B. subtilis* lack significant homology, so do some of the FtsL homologs that we identified from the phylogenetically diverse set of bacteria. This divergence in sequence of FtsL homologs suggests that there is not a specific protein sequence for FtsL homologs but that instead the main determinant for FtsL homologs appears to be the presence of a membrane-bound protein with a coiled-coil domain, which can still interact with its partner proteins, FtsB and FtsQ.

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