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Domain-Swapping Analysis of FtsI, FtsL, and FtsQ, Bitopic Membrane Proteins Essential for Cell Division in *Escherichia coli*

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FtsI, FtsL, and FtsQ are three membrane proteins required for assembly of the division septum in the bacterium *Escherichia coli*. Cells lacking any of these three proteins form long, aseptate filaments that eventually lyse. FtsI, FtsL, and FtsQ are not homologous but have similar overall structures: a small cytoplasmic domain, a single membrane-spanning segment (MSS), and a large periplasmic domain that probably encodes the primary functional activities of these proteins. The periplasmic domain of FtsI catalyzes transpeptidation and is involved in the synthesis of septal peptidoglycan. The precise functions of FtsL and FtsQ are not known. To ask whether the cytoplasmic domain and MSS of each protein serve only as a membrane anchor or have instead a more sophisticated function, we have used molecular genetic techniques to swap these domains among the three Fts proteins and one membrane protein not involved in cell division, MalF. In the cases of FtsI and FtsL, replacement of the cytoplasmic domain and/or MSS resulted in the loss of the ability to support cell division. For FtsQ, MSS swaps supported cell division but cytoplasmic domain swaps did not. We discuss several potential interpretations of these results, including that the essential domains of FtsI, FtsL, and FtsQ have a role in regulating the localization and/or activity of these proteins to ensure that septum formation occurs at the right place in the cell and at the right time during the division cycle.

The formation of the cell septum in the bacterium *Escherichia coli* takes place at a precise place in the cell and at a precise time in the division cycle. A number of genes have been identified that appear to play essential roles in this process, including a cluster of *fts* (temperature-sensitive filamentation) genes located in the min-2 region of the *E. coli* chromosome. The *ftsZ* gene encodes an abundant cytoplasmic protein which forms a ring near the site of cell division prior to the formation of the cell septum (2). The *ftsA* gene encodes a peripheral membrane protein that may interact with FtsZ (9). Three genes in this cluster, *ftsI*, *ftsQ*, and *ftsL*, code for cytoplasmic membrane proteins with simple structures (4, 7, 18). Each of these three proteins has a similar topological organization: an amino-terminal hydrophilic cytoplasmic sequence, a single hydrophobic transmembrane segment, and a hydrophilic periplasmic domain (Fig. 1). Proteins with this topology have been termed bitopic membrane proteins. All three proteins are made in very small amounts, with estimates ranging from 25 to 150 molecules per cell (1, 7, 11, 18).

The *ftsI* gene codes for a penicillin-binding protein (PBP), PBP3, which exhibits a transpeptidase activity related to peptidoglycan synthesis (22). (We refer to this protein as FtsI throughout this paper.) This activity is encoded in the portion of the polypeptide chain which is localized to the periplasm. Mutations and antibiotics that inactivate FtsI prevent septum formation but do not interfere with cell elongation (38). The enzymatic activity of FtsI is thus presumed to have a role in peptidoglycan synthesis only during septum formation. FtsI is localized to the cell septum at later stages of cell growth (42).

While mutations in the *ftsL* and *ftsQ* genes have phenotypes very similar to those in the *ftsI* gene, very little information about the functions of these two Fts membrane proteins exists. The periplasmic domain of FtsL is composed of an amino acid sequence which has features of an α-helical leucine zipper (18). Preliminary mutagenesis data on this domain of FtsL are consistent with its functioning as a leucine zipper, although no direct evidence for this proposed role has been obtained (18a). No information about the possible function of any of the domains of FtsQ exists.

We are interested in studying the role of this set of membrane proteins in the cell division process. As part of these studies, we are seeking to determine the functions of the domains of each of the proteins that are located in the three different subcellular compartments—the cytoplasm, the membrane, and the periplasm. Because of their simple bitopic structure, these proteins can be readily dissected into their component parts. Studies of many membrane proteins involved in other cellular processes have pointed to a variety of different possible roles for their membrane-spanning segments (MSSs). Among these roles are (i) membrane anchors for the cytoplasmic and/or exported domain of a protein (reference 27 and results for FtsQ described below), (ii) domains for homodimerization of the protein (24), (iii) domains of interaction with MSS of other membrane proteins (3, 8), (iv) functional domains in proteins that act as channels or pores in the membrane (20), and (v) signals for subcellular localization, for instance to the Golgi compartments in eukaryotic cells (30, 32).

The cytoplasmic domains of bitopic membrane proteins have also been studied. In eukaryotic cells, sequence motifs in these domains sometimes act to localize proteins to specific subcellular compartments such as the endoplasmic reticulum or the lysosome (17, 31, 37). For bitopic membrane proteins that act as signalling molecules, the cytoplasmic domains can activate regulatory cascades or even directly regulate gene expression. In other cases, the sequences appear to act only as part of the anchoring portion of the protein, ensuring its proper topology and arrangement. In these cases, replacing the

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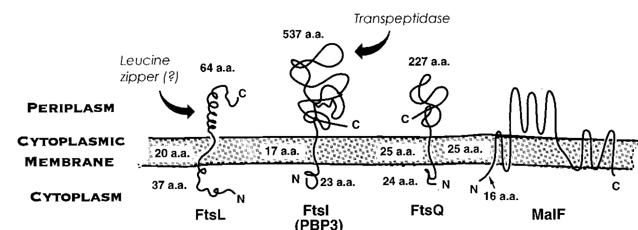

FIG. 1. Membrane topology of proteins used in this study (see introduction for details). From MalF, only the short amino-terminal cytoplasmic domain and the first MSS were used. These can be deleted without affecting the transport activity of MalF. a.a., amino acid.

domain with another unrelated but suitable sequence may result in a protein that is still functional (35).

In many cases, the exported portion of this class of membrane proteins encodes the primary functional activity. Examples are PBPs in *E. coli* (15) and a host of membrane receptors in eukaryotic cells.

To determine the roles of the various domains of the Fts membrane proteins, we have initiated an approach involving the exchange of domains between them. In addition, we have exchanged into these proteins a cytoplasmic and/or MSS domain of a protein involved in a process totally unrelated to cell division. For these latter constructs, we have chosen the membrane protein MalF, which is required for maltose transport in *E. coli* (14, 21). The simplified rationale behind this approach is as follows. Replacing any one domain of a protein with that of another and assaying the protein's function would indicate whether the sequence of that specific domain is required for the function of the protein. If any of the domains are required for subcellular localization to the cell septum, then those domains may be exchangeable among the Fts proteins; however, the MalF domain would not suffice for proper function. If any pair or if all three of the proteins form a complex via, e.g., their MSSs, then certain classes of exchanges may complement each other, even though a single exchange may eliminate the function.

According to this simple rationale, such studies could reveal the important domains of a protein and thus allow a focus for further genetic and biochemical analyses. We recognize (see Discussion) that the results of such an analysis will likely be more difficult to evaluate than this simple description would suggest. However, we believe, as illustrated by the results described below, that these findings open new avenues of research that are important both for understanding how the Fts proteins function and for understanding some important features of the organization of membrane proteins in general.

MATERIALS AND METHODS

Strains, plasmids, and media. The strains and plasmids used in this study are listed in Table 1. Rich and minimal media were NZY and M63, respectively (18, 19). Ampicillin was used at 200 μg/ml in rich medium and at 50 μg/ml in minimal medium. Kanamycin was used at 60 or 100 μg/ml in minimal medium and at 40 μg/ml in rich medium. Minimal medium contained thiamine (0.5 μg/ml) and 0.01% leucine and 0.005% isoleucine for complementation of temperature-sensitive (Ts) mutants, but not for transductions. L-Arabinose (0.2, 0.02, 0.002, and 0.0002%) or D-glucose (0.2%) was added to medium to modulate expression of *fts* alleles cloned under control of the P_{BAD} promoter (pBAD derivatives [19]). In addition, minimal medium always contained 0.2% glycerol, which served as the primary carbon source on plates containing low concentrations of arabinose or glucose.

Complementation. (i) Complementation of Ts mutants. Swap constructs and control plasmids were transformed into strains carrying Ts alleles of either *ftsI* or *ftsQ*, selecting for ampicillin resistance on NZY plates at 30°C. Isolates were tested for complementation by streaking onto NZY-ampicillin and M63-ampicillin plates with and without arabinose or glucose (to modulate expression of the

TABLE 1. Bacterial strains and plasmids used

Strain or plasmid	Relevant genetic marker(s) or feature(s)[a]	Source or reference
Strains		
KS272	F^- Δ*lacX74 galE galK thi rpsL* Δ*phoA* (*Pvu*II)	38a
LMG194	KS272 Δ*ara714 leu*::Tn*10*	19
LMG64	KS272 *ftsI23 leu*::Tn*10 recA*::*cat*	This study
MJC129	KS272 *ftsQ*(Ts) *recA*::*cat*	7a
MJC431	KS272 *ftsI*::Tn*phoA*I137ΔIS*50R* (Kan^r)/pLMG173 (Amp^r)	7a
LMG145	KS272 *ftsL*::Tn*phoA*L81ΔIS*50R* (Kan^r)/pLMG180 (Amp^r)	18
MJC295	KS272 *ftsQ*::Tn*phoA*80 (Kan^r)/ pLMG161 (Amp^r)	7a
Plasmids		
pBAD18	Arabinose regulation, Amp^r	19
pBAD18-Kan	Arabinose regulation, Kan^r	19
pLGM130	*malF*	This study
pLMG161	*ftsQ*	This study
pLMG173	*ftsI*	This study
pLMG180	*ftsL*	This study
pLD30	$I_{Eag}/I_{Msc}/I$	This study
pLD45	$L_{Eag}/L_{Msc}/L$	This study
pLD47	$F_{Eag}/F_{Msc}/F$	This study
pLD39	$I/I_{Msc}/I$	This study
pLD41	$I_{Eag}/I/I$	This study
pLD43	$F_{Eag}/F_{Msc}/I$	This study
pLD55	$I_{Eag}/I_{Stu}/I$	This study
pLD57	$F_{Eag}/I_{Msc}/I$	This study
pLD59	$L_{Eag}/I_{Msc}/I$	This study
pLD61	$Q_{Eag}/I_{Msc}/I$	This study
pLD63	$F_{Eag}/L_{Msc}/L$	This study
pLD65	$I_{Eag}/L_{Msc}/L$	This study
pLD67	$Q_{Eag}/L_{Msc}/L$	This study
pLD75	$I/I_{Stu}/I$	This study
pLD88	$I_{Eag}/I_{Msc}/L$	This study
pLD90	$F_{Eag}/F_{Msc}/L$	This study
pLD92	$Q_{Eag}/Q_{Msc}/L$	This study
pLD94	$L_{Eag}/F_{Msc}/L$	This study
pLD96	$L_{Eag}/Q_{Msc}/L$	This study
pLD98	$L_{Eag}/I_{Msc}/L$	This study
pLD100	$L_{Eag}/I_{Msc}/L + I_{Eag}/L_{Msc}/I$	This study
pLD102	$I_{Eag}/I_{Msc}/Q$	This study
pLD104	$F_{Eag}/F_{Msc}/Q$	This study
pLD106	$L_{Eag}/L_{Msc}/Q$	This study
pLD108	$Q_{Eag}/F_{Msc}/Q$	This study
pLD110	$Q_{Eag}/I_{Msc}/Q$	This study
pLD112	$Q_{Eag}/L_{Msc}/Q$	This study
pLD114	$Q_{Eag}/Q_{Msc}/I$	This study
pLD116	$L_{Eag}/L_{Msc}/I$	This study
pLD118	$I_{Eag}/F_{Msc}/I$	This study
pLD120	$I_{Eag}/Q_{Msc}/I$	This study
pLD122	$I_{Eag}/L_{Msc}/I$	This study
pLD134	$F_{Eag}/Q_{Msc}/Q$	This study
pLD135	$I_{Eag}/Q_{Msc}/Q$	This study
pLD136	$L_{Eag}/Q_{Msc}/Q$	This study
pLD137	$Q_{Eag}/Q_{Msc}/Q$	This study
pLD139 (Kan^r)	*ftsI*	This study
pLD140 (Kan^r)	$F_{Eag}/F_{Msc}/I$	This study
pLD141 (Kan^r)	$L_{Eag}/I_{Msc}/I$	This study
pLD142 (Kan^r)	$I_{Eag}/Q_{Msc}/I$	This study
pBS120	ss/I_{perip}	4
pLD138	ss/Q_{perip}	This study
pMAL-c2	MBP fusion vector	New England BioLabs
pDSW156	MBP-FtsI (perip)	This study
pLMG322	MBP-FtsL (perip)	This study
pLMG182	P_{BAD}-*ftsL-phoA* L81 fusion	This study

[a] ss, signal sequence; perip, periplasmic domain.

plasmid-borne gene being tested) (19), and growth was scored after 18 and 36 h at 42°C. Identical control plates were incubated at 30°C. Complementation for the *ftsI*(Ts) and *ftsQ*(Ts) mutants was done on plates containing 0.2% glucose, no sugar, and 0.002, 0.02, and 0.2% arabinose.

(ii) Complementation of null mutants by transduction. Swap constructs were tested for complementation by transduction essentially as previously described (7, 19). Briefly, derivatives of LMG194 (*ftsL*$^+$ *ftsI*$^+$ *ftsQ*$^+$ *leu*::Tn*10*) carrying plasmids encoding the swap alleles to be tested (or control plasmids) were transduced to kanamycin resistance and leucine prototrophy on M63 plates. The lysates were prepared on LMG145 (*ftsL*::Tn*phoA* Leu$^+$/pLMG180), MJC431 (*ftsI*::Tn*phoA* Leu$^+$/pLMG173), or MJC295 (*ftsQ*::Tn*phoA* Leu$^+$/pLMG161). The plates were incubated at 37°C and examined daily for at least 3 days, by which time complementing plasmids typically yielded 50 to 100 transductants per plate, while noncomplementing plasmids yielded fewer than 5 transductants per plate. Some of the transductants were made phage free and analyzed. Isolates obtained from complementing plasmids bred true and contained the plasmid originally present in the recipient, as expected, whereas isolates obtained from noncomplementing plasmids often gave rise to faster-growing variants upon restreaking and contained the *fts*$^+$ plasmid from the donor strain.

The degrees of complementation of *ftsL* and *ftsI* mutants by swap proteins with P1 transductions were tested on plates containing 0.2, 0.02, and 0.002% arabinose, no sugar, and 0.2% glucose. For *ftsQ*, 0.2 and 0.002% arabinose and 0.2% glucose were tested. Complementation was observed only at 0.2% arabinose for *ftsL* and *ftsQ*, but over a wide range of arabinose concentrations for *ftsI*.

Molecular biological procedures and oligonucleotides. Standard techniques were employed for cloning and analysis of DNA, PCR, electroporation, and transformation (36). Enzymes used to manipulate DNA were from New England BioLabs. DNA sequencing was performed for double-stranded plasmid templates either manually (Sequenase; U.S. Biochemicals) or with an automated sequencer (ABI).

The oligonucleotides (5′→3′) used to make the different constructions were as follows: 1, pBAD18-5′ (CTGTTTCTCCATACCCGTT); 2, pBAD18-3′alt (GG CTGAAAATCTTCTCT); 3, F_{Eag}3′ (CATCGGCC GGCGTCGCTTTGCCAC CAATG); 4, F_{Eag}5′ (GACGCCGGCCGATGGTCAGTGCTAGGTC); 5, F_{Msc} 3′ (TATTCCCCTT GGCCATACATTAAAACA); 6, F_{Msc}5′ (ATGTATGGC CAAGGGGAATACCTG); 7, I_{Eag}3′ (ACGGCCGCTGATAAA GTTGGCAT G); 8, I_{Eag}5′ (ATCAGCGGCCGTTTTGCGTTGTTATGC); 9, I_{Msc}3′ (ACCA CGCTACGTGGCCAAGCA CAAAAG); 10, I_{Msc}5′ (TGCTTGGCCACGTAG CGTGGTTACAA); 11, I_{Stu}3′ (ACGCTACGCGGAGGCCTCCCAGA AA AG); 12, I_{Stu}5′ (TGCTTGGCCTCCGCGTAGCGTGGTTACAA); 13, L_{Eag}3′ (ACGGCCGCTGATAAAGTTGGCATG); 14, L_{Eag}5′ (CGATTCGGCCGGC TGCCACTCTGCCTG); 15, L_{Msc}3′ (CGGTATGGTGGCCAGTGGTTACCA C); 16, L_{Msc}5′ (CCACTGGCCACCATACCCGTTTA); 17, Q_{Eag}3′ (ACGGCC GCCATTATTGCGGCGAG); 18, Q_{Eag}5′ (AATGGCGGCCGTCTGGCGGG GATCC); 19, Q_{Msc}3′ (TGCGCATCTTGGCCAAGGCCCAACACG); 20, Q_{Msc}5′ (GGGCCTTGGCCAAGATGCGCAACGCC); 21, FtsIΔ5′ (CGTTTA TCCTTATTTGAATTCTACGATATTTTC); 22, MBP-L_{per} (CATCATACCCG TTTACTGACC); and 23, phoAss3′ (TGATGGCCATTGGCTTTTGTCA CAGG).

Construction of wild-type genes under P_{BAD} control. Plasmids that express swap proteins starting from pLMG130 (*malF*), pLMG161 (*ftsQ*), pLMG173 (*ftsI*), and pLMG180 (*ftsL*) were constructed. These are derivatives of pBAD18 and express the genes indicated under the control of the *araBAD* promoter (19). pLMG130 was constructed by subcloning a 2.2-kbp *Eco*RI-*Hin*dIII fragment containing the wild-type *malF* gene and part of the *malG* gene from plasmid pDHB32 (6) into the same sites of pBAD18. To construct pLMG161, a 931-bp *Eco*RI-*Kpn*I (partial digest) fragment from plasmid pMJC103 (7) was cloned into the same sites of pBAD18. The *Eco*RI site is 13 bp upstream of the ribosome binding site (RBS) for *ftsQ*, and the second *Kpn*I site, at the end of the fragment, is 42 bp downstream of the stop codon. Construction of pLMG173 was done in two steps. First, a *Hpa*I-*Nru*I (partial digest) fragment encoding the *ftsI* gene from pZ26::Tn*phoA*L112 (7) was subcloned into the *Sma*I site of pBAD18. Both orientations of the insert were obtained, and a plasmid with an insert orientation that placed *ftsI* under P_{BAD} control was chosen. Second, by using oligonucleotide 21 and site-directed mutagenesis, an *Eco*RI site was introduced 9 bp upstream from the proposed RBS of *ftsI*. Then, the sequences upstream of this RBS were deleted by dropping out the DNA between the *Eco*RI site of the vector and the new *Eco*RI site. This placed *ftsI* immediately downstream of the P_{BAD} promoter and eliminated all non-*ftsI* sequences. Plasmid pLMG180, which contains a 412-bp fragment encoding *ftsL* under P_{BAD} control, has been described elsewhere (18). All four genes cloned into pBAD18 were contained between the *Eco*RI and *Hin*dIII sites of the vector and retained their native RBSs.

Construction of swaps. Versions of the pLMG plasmids described above, in which each gene contained a restriction site for *Eag*I and *Msc*I flanking the MSS, were constructed according to the following steps (here described for *ftsL*) (see Fig. 2).

(i) Introduction of an *Msc*I site at the 5′ end of the MSS. The cytoplasmic domain and MSS were amplified by PCR with primers 1 (5′ pBAD primer) and 15 with pLMG180 as the template. The 5′ pBAD primer aligns to vector sequences beyond the RBS of the gene and the *Eco*RI site of the vector. Primer 15 contains an *Msc*I site at its 5′ end and introduces this site near the 3′ end of the PCR product. The amplified DNA fragment was made blunt end with T4 DNA polymerase, digested with *Eco*RI, and then ligated into pBAD18 that had been digested with *Eco*RI and *Sma*I. The resulting plasmid was called pLD1.

(ii) Introduction of an *Eag*I site at the 3′ end of the MSS. The cytoplasmic domain was amplified by PCR with primers 1 (5′ pBAD primer) and 13 with pLMG180 as the template. Primer 13 introduced an *Eag*I site near the 3′ end of the PCR fragment. The amplified fragment was made blunt-end with T4 DNA polymerase, cut with *Eco*RI, and ligated into pBAD18 that had been digested with *Eco*RI and *Sma*I. The resulting plasmid was called pLD11.

(iii) Introduction of both the *Eag*I and the *Msc*I sites into the same fragment of *ftsL*. The MSS was PCR amplified with primer 14, which introduced an *Eag*I site at the 5′ end of the MSS, and primer 2 (3′ pBAD primer) with pLD1 as the template. The fragment obtained was digested with *Eag*I and *Sph*I and ligated into pLD11 that had been digested with the same enzymes. The resulting plasmid was named pLD16.

(iv) Construction of a complete *ftsL* gene containing both the *Eag*I and the *Msc*I restriction sites. The periplasmic domain of *ftsL* was amplified by PCR with primers 16 and 2 with pLMG180 as the template. Primer 16 introduced an *Msc*I site at the 5′ end of the fragment encoding the periplasmic domain. The fragment obtained was digested with *Msc*I and *Hin*dIII and ligated into pLD16 that had been cut with the same enzymes. The resulting plasmid was called pLD45, and its gene is referred to as $L_{EagI}/L_{MscI}/L$ (i.e., $L_{cyt}/L_{mss}/L_{perip}$) to indicate the presence of the restriction sites at the ends of the corresponding domains. $L_{EagI}/L_{MscI}/L$ was functional, as judged by complementation, as were $I_{EagI}/I_{MscI}/I$, $Q_{EagI}/Q_{MscI}/Q$, and $F_{EagI}/F_{MscI}/F$.

(v) Construction of genes with swapped domains. Chimeric *ftsL* genes were constructed by exchanging the fragments that correspond to the cytoplasmic and MSS domains. For instance, to construct MSS swaps for FtsL, the *Eag*I-*Msc*I fragment of pLD45, which encodes the FtsL MSS, was substituted by *Eag*I-*Msc*I fragments derived from *malF*, *ftsI*, and *ftsQ* to produce $L_{EagI}/F_{MscI}/L$, $L_{EagI}/I_{MscI}/L$, and $L_{EagI}/Q_{MscI}/L$. For cytoplasmic domain substitutions, fragments flanked by the *Eco*RI and *Eag*I sites were exchanged, and for double substitutions (cytoplasmic domain and MSS), *Eco*RI-*Msc*I fragments were exchanged.

The construction of the L/I/L plus I/L/I double-swap plasmid (pLD100) was achieved as follows. Plasmid pLD98 containing the L/I/L swap was digested with *Xba*I, blunt ended, and digested with *Hin*dIII. Both of these sites are at the ends of the hybrid L/I/L gene, with the *Xba*I/blunt site being closest. Then, an *Eco*RI/blunt-I/L/I-*Hin*dIII fragment from pLD122 was cloned into the treated pLD98 plasmid, placing the I/L/I gene (with its own RBS) just downstream of L/I/L.

Cloning *ftsI* swaps into pBAD18-Kan. Because β-lactamase destroys the substrate fluorescein hexanoic 6-aminopenicillanic acid (FLU-C_6-APA) used to test penicillin-binding activity, wild-type *ftsI* and three swap alleles were subcloned into a vector that confers Kan^r rather than Amp^r. This was accomplished by digesting pLMG173 (*ftsI*), pLD43 ($F_{EagI}/F_{MscI}/I$), pLD59 ($L_{EagI}/I_{MscI}/I$), and pLD120 ($I_{EagI}/Q_{MscI}/I$) with *Eco*RI and *Bsa*I, gel isolating the 2-kbp DNA fragment carrying the *ftsI* allele, and ligating this fragment into pBAD18-Kan (19) that had been cut with the same enzymes. The resulting plasmids were named pLD139 to 142.

Cloning of MBP-fusion proteins. (i) MBP-FtsI (periplasmic domain). Plasmid pMAL-c2, which allows for fusion of proteins to the carboxyl terminus of a signal sequenceless maltose-binding protein (MBP) derivative, was digested with *Eco*RI, made blunt ended by treatment with Klenow fragment in the presence of deoxynucleoside triphosphates, and cut with *Hin*dIII. This vector fragment was then ligated to the 1.7-kbp *Msc*I-*Hin*dIII fragment from pLD30 carrying the periplasmic domain of FtsI. The resulting plasmid was converted from Amp^r to Kan^r by digestion with *Dra*I, which cuts twice within the *bla* gene, and ligating in a 1.3-kbp fragment (*Bam*HI, then made blunt ended with Klenow fragment) conferring Kan^r from plasmid pUC4K (from Pharmacia Biotech). The resulting plasmid was named pDSW156.

(ii) MBP-FtsL (periplasmic domain). DNA encoding the periplasmic domain of *ftsL* was amplified from pLMG180 by PCR with primers 22 and 2. After treatment with T4 DNA polymerase and *Hin*dIII, the resulting 210-bp fragment was ligated into pMAL-c2 that had been digested with *Xmn*I and *Hin*dIII. Ampicillin-resistant clones were screened for expression of an MBP-FtsL fusion protein by Western blotting with antibody against FtsL. The resulting plasmid, pLMG322, produced a fusion with no polylinker-encoded amino acids between MBP and FtsL.

Antibodies. Antibodies against synthetic peptides derived from the sequence of FtsI (FtsI-1, MKAAAKTQKPKRQEEHC; FtsI-2, CEPDALTTGDKNEFV) or FtsL (FtsL-1, CREQLVLERDALDIE; FtsL-2, CMQHVDPSQENIVVQK) were raised in rabbits by ImmunoDynamics. FtsI-1 corresponds to the first 16 residues of the cytoplasmic domain, and FtsI-2 corresponds to the last 14 residues of the mature (28) periplasmic domain. FtsL-1 and FtsL-2 are derived from periplasmic domain; FtsL-1's sequence is close to the MSS, and FtsL-2 corresponds to the carboxyl terminus of FtsL. A strong reaction of the antibodies against FtsI was observed after 4 months of boostings, while for FtsL, 8 months were necessary. The antibody against FtsI was affinity purified (34) against the isolated periplasmic domain of FtsI. Antibody against FtsL was similarly purified against an MBP-FtsL (periplasmic domain) fusion protein expressed by pLMG322.

Western blotting. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose (36).

Immunodetection was performed with antipeptide antibodies against FtsI or FtsL and the ECL Western blotting system (Amersham). For quantitation, film was scanned with a computing densitometer (Molecular Dynamics).

To determine the steady-state levels of expression of FtsI swap proteins, derivatives of KS272 or LMG64 carrying appropriate plasmids were grown at 30°C in NZY-ampicillin to an optical density at 600 nm (OD_{600}) of ~0.5. Cultures were then diluted fivefold into 42°C NZY-ampicillin containing arabinose to induce expression of the plasmid-borne swap allele, if any. After 1 h, the OD_{600} had returned to about 0.5, and microscopic examination of LMG64 derivatives revealed extensive filamentation (except in the cases of pLMG173 and pLD30). One milliliter of cells was pelleted by centrifugation and resuspended in 100 μl of SDS-sample buffer per OD_{600} unit of 0.5. Samples were boiled for 5 min, and 10 μl was loaded onto a 10% polyacrylamide-SDS gel.

The steady-state levels of FtsL swap proteins were determined similarly, except that cultures were always grown at 37°C, and the swap constructs were detected in *ftsL*$^+$ cells only (KS272 and LMG194). Cells containing swaps or control plasmids (pLMG180, pLD45, and pBAD18) and cells containing the chromosomal insertion mutation complemented by pLMG180 were grown to mid-log phase in NZY-ampicillin (plus 0.2% arabinose in the case of the null mutant). Cells were harvested, resuspended in fresh medium with 0.2% arabinose, and diluted 10-fold into NZY-0.2% arabinose; a fraction of the culture of the null mutant was resuspended and grown in NZY-0.2% glucose. After 2.5 h, the null mutant had formed filaments in the presence of 0.2% glucose but showed normal cells and efficient complementation in the presence of arabinose. At this time, 1 ml of cells was pelleted by centrifugation and resuspended in 100 μl of SDS-sample buffer per OD_{600} of 0.5. Samples were boiled for 5 min, and 10 μl was loaded onto a 15% polyacrylamide-SDS gel.

Detection of FtsI with FLU-C_6-APA. FLU-C_6-APA was synthesized as described elsewhere (33), and its concentration in 50 mM sodium phosphate buffer (pH 7.0) was determined by using E_{494} = 68 mM^{-1} as the extinction coefficient.

The strains were grown overnight in NZY-kanamycin, inoculated 1:500 into fresh medium, and grown at 37°C to an OD_{600} of ~0.5. Expression of the plasmid-borne FtsI protein (if any) was then induced by adding arabinose to a final concentration of 0.002%, except for MBP-FtsI expressed from pDSW156, which was induced with 50 μM isopropyl-β-D-thiogalactopyranoside. One hour after induction, the cells were harvested by centrifugation and resuspended in spheroplasting buffer (50 mM Tris-HCl [pH 8.0], 5 mM Na_2EDTA, 500 mM sucrose). One milliliter of culture at an OD_{600} of 1.0 was resuspended in 50 μl; for cultures at other ODs, the volume of buffer was adjusted accordingly.

Spheroplasting buffer rendered the outer membrane permeant to FLU-C_6-APA. For routine assays, FLU-C_6-APA was added to a final concentration of 20 μM, and assay mixtures were incubated at 30°C for 30 min. Samples were withdrawn at various times, added to an equal volume of 2× Laemmli gel-loading buffer to quench the reaction, and boiled for 5 min. A 10-μl volume was loaded onto a 10% polyacrylamide-SDS gel, and proteins were separated by electrophoresis. Proteins labelled with FLU-C_6-APA were visualized and quantitated with a Molecular Dynamics FluorImager 575. A second gel was run to determine the level of FtsI by Western blotting. Samples for Western blotting were generally diluted by 10- to 100-fold.

For the experiment shown in Fig. 5, procedures were modified to allow lysis of the cells. Cells resuspended in spheroplasting buffer were converted to spheroplasts by incubation on ice in the presence of 0.2-mg/ml lysozyme for 20 min. FLU-C_6-APA was then added to a final concentration of 20 μM, and spheroplasts were incubated at 30°C for 20 min, at which time a sample was taken and added to 2× Laemmli gel-loading buffer to assess binding of FLU-C_6-APA to FtsI derivatives in intact spheroplasts. The remaining spheroplasts were then pelleted in a Microfuge, the buffer containing FLU-C_6-APA was discarded, and the spheroplasts were lysed by resuspension in 50 mM Tris-HCl (pH 8.0)–5 mM $MgCl_2$. After incubation for 5 min at 30°C in the presence of 1-μg/ml DNase I to reduce viscosity, an equal volume of 2× spheroplasting buffer was added, followed by FLU-C_6-APA to a final concentration of 20 μM. Samples were taken after 10 and 20 min of incubation at 30°C to assess binding of FLU-C_6-APA to FtsI derivatives in lysed spheroplasts.

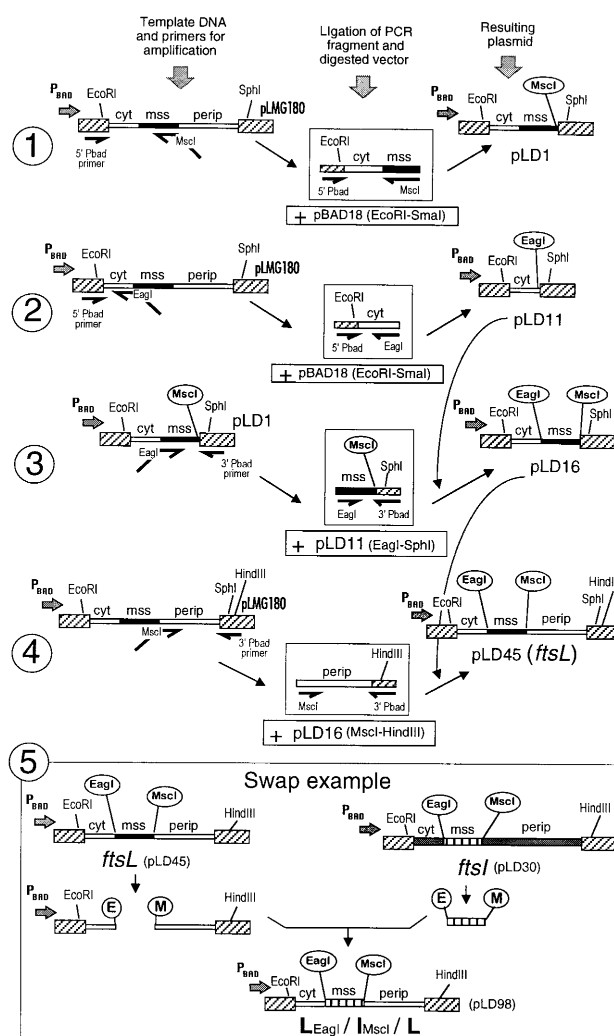

FIG. 2. Construction of plasmids encoding proteins with swapped domains. Numbers 1 through 5 correspond to the five steps described in Materials and Methods. cyt, cytoplasmic domain; mss, MSS; perip, periplasmic domain.

RESULTS

Assembling swap constructs. In planning to study Fts swap constructs, we considered it highly desirable to be able to control the levels of expression of the hybrid proteins in our analysis of their functionality. On the one hand, it seemed important to be able to examine the abilities of such constructs to complement *fts* mutants when they are expressed at lower levels that are at least somewhat comparable to wild-type levels. On the other hand, some constructs might exhibit only partial but significant functionality that would be observed only at high expression levels. Furthermore, since membrane proteins are often deleterious to the cell at high levels of expression, tight control of their expression could also be crucial for this analysis. Finally, dominant-negative effects of the swaps might be observed only at higher levels of expression. Therefore, all swap constructs were made starting with the *malF* and *fts* genes cloned into a plasmid containing the tightly regulated promoter for the arabinose operon. The plasmid that we used was pBAD18 as described by Guzman et al. (19).

We wished to obtain constructs in which one or two domains of each membrane protein were swapped with another. For each of the *fts* genes cloned into pBAD18, we introduced restriction sites at positions in the gene's DNA sequence corresponding to the amino-terminal and carboxy-terminal borders of the presumed transmembrane segment using PCR (Fig. 2). (It should be remembered at this point that, except for the very few membrane proteins for which three-dimensional structures exist, the definition of the exact borders of transmembrane segments is based only on sequence examination and the rules that have evolved for predicting these borders.) For *malF* (6, 14), a protein with eight MSSs, we introduced these restriction sites at the borders of the first MSSs. This MSS can be deleted without affecting the transport activity of MalF (12). In planning these constructs, we also attempted to

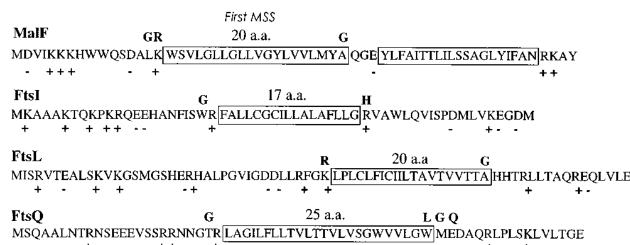

FIG. 3. Sequence of cytoplasmic domains and MSS of MalF, FtsI, FtsL, and FtsQ. MSSs are boxed. Boldface letters to the top of the protein sequences indicate amino acid (a.a.) changes resulting from introduction of *Eag*I and *Msc*I restriction sites before and after the MSS, respectively. Positively and negatively charged residues are also indicated. Only the first MSS of MalF was used in this study.

ensure that the charge distribution at each end of the MSS segment corresponded to that necessary to maintain the original topology of these proteins. That is, basic amino acids at the amino terminus of the MSS and acidic residues (or a net neutral charge) at its carboxy terminus are determinants of the appropriate orientation (5, 25, 39, 41). Relevant amino acid sequence changes encoded by the introduction of restriction sites into these genes are shown in Fig. 3.

After construction of the various swaps, we verified their structures by restriction mapping and by DNA sequencing of all gene segments made by PCR. We use a simple nomenclature to describe each swap construct. Each swap is designated by three letters, such that the first letter identifies the protein whose coding region corresponds to the amino terminus (the cytoplasmic domain) of the protein, the second corresponds to the protein from which the MSS was derived, and the third represents the protein from which the periplasmic domain was derived. Thus, QLL has the cytoplasmic domain of FtsQ and the MSS and periplasmic domains of FtsL.

Expression and topology of swap constructs. We tested our FtsI and FtsL constructs to ensure that each of them expressed its swap protein. For proteins containing the periplasmic domains of FtsI, we found that all constructs expressed substantial levels of the proteins as assayed by Western blots (Fig. 4). The amounts of protein varied according to the constructs. This variation was probably due to the differing efficiencies of the translation start signals for the particular protein from which the amino terminus of a swap derived. We note here that swap constructs in which the FtsQ amino-terminal domain preceded sequences from one of the other Fts proteins consistently expressed levels of hybrid protein lower than those seen with other swaps. These findings suggest that the FtsQ translation start signal, at least in these constructs, is weaker than those of FtsI and FtsL (Fig. 4). Previously presented results indicated that FtsQ has a weak translation start signal (26).

For FtsL, most of the constructs expressed protein detectable in Western blots with anti-FtsL antibody. However, we have never observed any product from QQL, QLL, or wild-type L in blots. For IIL and LLL, cross-reacting protein was seen in one of several blots that we performed. The anti-L antibody does not appear to be very effective, since it is also unable to reveal chromosomally expressed FtsL in blots. The failure to detect the constructs with a Q amino terminus is perhaps not surprising, since, as mentioned above, such constructs are expressed at lower levels in general. The failure to detect wild-type FtsL expressed from the plasmid is more surprising. However, since this plasmid effectively complements

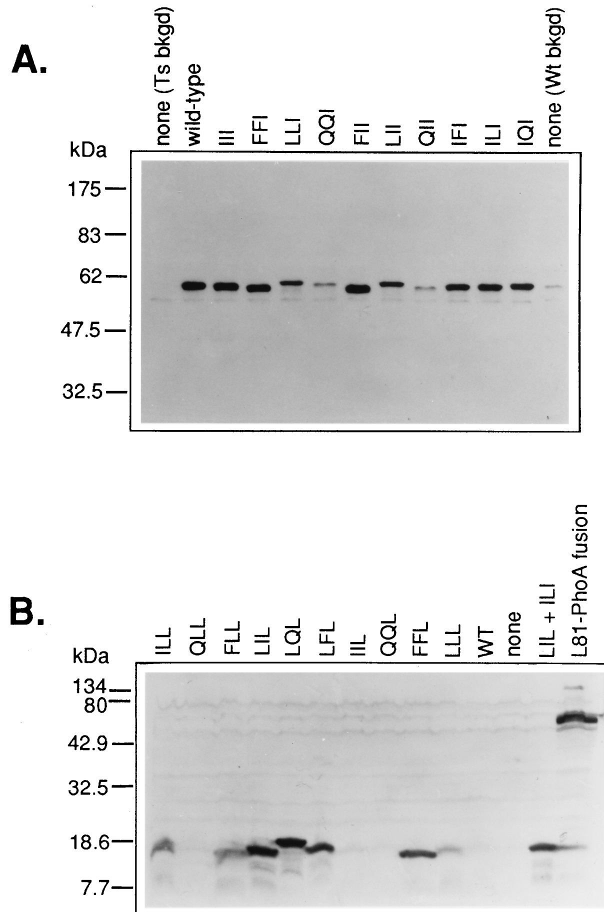

FIG. 4. Steady-state levels of FtsI (A) and FtsL (B) swap proteins as determined by Western blotting. Molecular mass standards are indicated at the left of each blot. The proteins expressed are indicated above each lane. none, cells carrying the vector pBAD18 without any insert. In the case of FtsI, vector alone was examined in both the Ts mutant LMG64 (far left) and wild-type strain KS272 (far right) to allow the steady-state levels of chromosomally expressed Ts and wild-type (Wt) FtsI proteins to be compared with the levels of expression of swap proteins. All FtsI swaps were induced with 0.002% arabinose, except for QQI and QII, which were weakly expressed and were induced with 0.2% arabinose. All FtsL swaps and the FtsL81-PhoA fusion protein were induced with 0.2% arabinose. The lane marked LIL + ILI was included because this combination of the two swaps was tested to see whether they would complement an FtsL depletion strain when expressed in the same cell. They did not.

an *ftsL* null mutation and fusions of *phoA* (encoding alkaline phosphatase) to *ftsL* in this plasmid give high levels of alkaline phosphatase activity (see below), FtsL is obviously expressed. The alkaline phosphatase activity levels of fusions to wild-type FtsL and to the FtsL engineered with restriction sites in the gene are similar to those of fusions derived from swaps for which we can readily detect protein (data not shown). The inability to detect wild-type FtsL in these experiments raises the possibility that wild-type FtsL is unstable while the swap constructs are stable.

Although we do not have antibody to FtsQ, since all of our FtsQ swap constructs complemented an *ftsQts* mutant, they must be expressed (Table 2 and below). We specifically designed the swap constructs so as to make it likely that the topology of each protein would be such that the periplasmic domain of the original protein was maintained in the periplasm. Nevertheless, we tested a number of the FtsI swaps to verify this outcome. The reason for focusing on FtsI is that

TABLE 2. Complementation, protein detection, and membrane topology of swap constructs

Plasmid	Complementation[a]			Detection of protein[b]	Correct topology[c]	Filamentation in Ts strains at 42°C[d]
	Ts strains in rich medium	Ts strains in M63 medium	Transduction in M63 medium			
FtsI (wild type)	+++	+++	+++	Yes	Yes	−
I_{Eag}/I/I	+++	+++	+++	Yes	ND	+
I/I_{Msc}/I	+++	+++	+++	Yes	ND	+
I_{Eag}/I_{Msc}/I (parent of I swaps)	++	++	++	Yes	ND	+
I/I_{Stu}/I	−	−	−	Yes	ND	+++
F/F/I	−	−	−	Yes	Yes	+++
Q/Q/I	−	−	−	Yes (LL)	ND	+++
L/L/I	−	+	−	Yes	ND	+++
L/I/I	−	+	−	Yes	Yes	+++
Q/I/I	−	+	−	Yes (LL)	ND	+++
F/I/I	−	−	−	Yes	ND	+++
I/L/I	−	−	−	Yes	ND	+++
I/Q/I	−	−	−	Yes	Yes	+++
I/F/I	−	−	−	Yes	ND	+++
L/I/L + I/L/I	−	−	−	Yes	ND	+++
ss/I_{perip}	−	−	ND	Yes[e]	Yes[e]	+++
pBAD18	−	−	−	No	NA	+++
FtsQ (wild type)	+++	+++	+++	ND	ND	−
Q_{Eag}/Q_{Msc}/Q (parent of Q swaps)	+++	+++	+++	ND	ND	−/+
I/I/Q	+	++	−	ND	ND	++
L/L/Q	+	++	−	ND	ND	++
F/F/Q	+	++	−	ND	ND	++
I/Q/Q	+	++	−	ND	ND	++
L//Q/Q	+	++	−	ND	ND	++
F/Q/Q	+	++	−	ND	ND	++
Q/I/Q	++	++	+++	ND	ND	+
Q/L/Q	++	++	+++	ND	ND	+
Q/F/Q	++	++	+++	ND	ND	+
ss/Q_{perip}	−	−	ND	ND	ND	+++
pBAD18	−	−	−	ND	NA	+++
FtsL (wild type)	NA	NA	+++	No	Yes	−
L_{Eag}/L_{Msc}/L (parent of L swaps)	NA	NA	+++	Trace	Yes	−
I/I/L	NA	NA	−	Trace	Yes	NA
Q/Q/L	NA	NA	−	No	Yes	NA
F/F/L	NA	NA	−	Yes	Yes	NA
I/L/L	NA	NA	−	Yes (LL)	Yes	NA
Q/L/L	NA	NA	−	No	ND	NA
F/L/L	NA	NA	−	Yes (LL)	Yes	NA
L/I/L	NA	NA	−	Yes	Yes	NA
L/Q/L	NA	NA	−	Yes	Yes	NA
L/F/L	NA	NA	−	Yes	Yes	NA
L/I/L + I/L/I	NA	NA	−	Yes	ND	NA
pBAD18	NA	NA	−	No	NA	NA

[a] Complementation of Ts mutants was determined by efficiency of colony formation and colony size at the nonpermissive temperature (42°C) in Ts strains harboring the different swap plasmids. Complementation by transduction was done with P1, crossing *ftsL*, *ftsI* or *ftsQ* null mutations onto the chromosome of wild-type strains harboring the different swap plasmids. ss, signal sequence; perip, periplasmic domain; ND, not determined; NA, not applicable.
[b] By Western blotting (see Fig. 4). Some data not shown. LL, low levels.
[c] For FtsI periplasmic domain constructs, correct topology (i.e., periplasmic domain in the periplasm) was determined by binding of FLU-C_6-APA to swap proteins in permeabilized cells (see Fig. 5 and 6). For FtsL periplasmic domain constructs, the periplasmic domain of the L81 *ftsL-phoA* fusion containing 23 residues of the FtsL periplasmic domain fused to mature alkaline phosphatase (AP) was used to replace the FtsL periplasmic domain in the swap plasmids. Alkaline phosphatase activity levels for the FtsL-swap-AP fusions were determined as described elsewhere (18) and were correlated with the amounts of protein observed in Western blots (Fig. 4 and data not shown).
[d] Early-log-phase cultures of Ts strains were grown at the permissive temperature in rich medium and were shifted to the nonpermissive temperature under inducing conditions.
[e] Production and periplasmic localization of this protein have been previously reported (4).

this verification was facilitated by the penicillin-binding properties of the periplasmic domain of the protein (38). We were able to use a fluorescent penicillin derivative (FLU-C_6-APA) (23, 33) that is membrane impermeant to show that the penicillin-binding domain of FtsI lies outside the cytoplasmic membrane rather than inside the cytoplasm. Specifically, we compared wild-type FtsI with a cytoplasmically localized version of FtsI in which the MBP (lacking its signal sequence) was fused to the periplasmic domain of FtsI. Spheroplasts derived from cells expressing FtsI or MBP-FtsI were incubated with FLU-C_6-APA for 20 min, at which time the spheroplasts were lysed and incubation continued for an additional 20 min. Samples

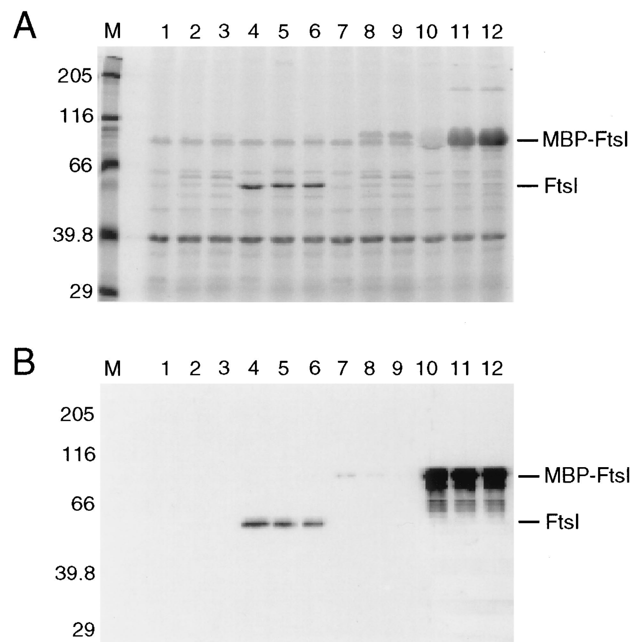

FIG. 5. Use of penicillin-binding assay to determine protein topology. (A) Fluorescence image; (B) Western blot. KS272 (wild-type) cells carrying plasmids capable of expressing wild-type FtsI (pLD139) or a cytoplasmically localized fusion of FtsI to the MBP (pDSW156) were converted to spheroplasts and incubated for 20 min in the presence of 20 μM FLU-C_6-APA (lanes 1, 4, 7, and 10). Spheroplasts were then lysed and incubated for an additional 10 (lanes 2, 5, 8, and 11) or 20 (lanes 3, 6, 9, and 12) min. Proteins were separated by SDS-PAGE and were detected by fluorescence imaging or Western blotting with an antipeptide antibody against FtsI. Lanes 1 to 3 and 7 to 9 are from uninduced cultures; lanes 4 to 6 and 10 to 12 are from induced cultures. Molecular mass standards are indicated at the far left.

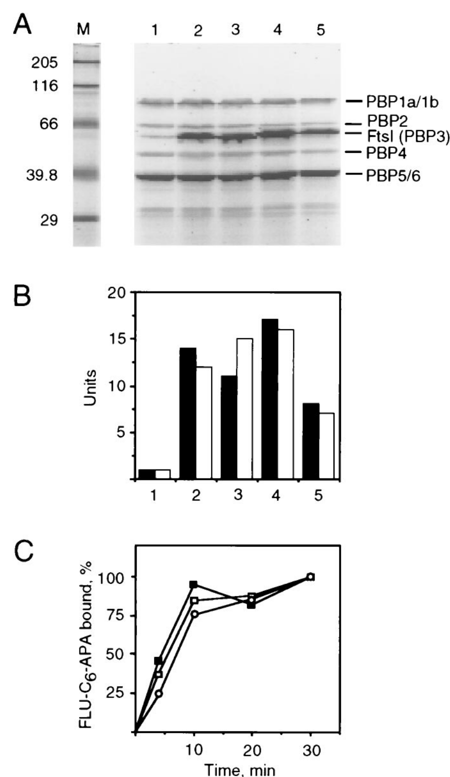

FIG. 6. Penicillin-binding activities of three FtsI swap proteins. (A) Fluorescence image of gel. KS272 derivatives carrying various plasmids as indicated below were induced for 1 h with 0.002% arabinose and were then assayed for binding of FLU-C_6-APA as described in Materials and Methods. Lanes: 1, pBAD18-Kan (vector); 2, pLD139 (FtsI); 3, pLD140 (FFI); 4, pLD141 (LII); and 5, pLD142 (IQI). Molecular mass standards are indicated at the far left, and several PBPs are identified at the far right. (B) Comparison of amounts of FLU-C_6-APA bound to amounts of FtsI protein. The fluorescence signal in the FtsI band in panel A was quantitated (filled bars), and a portion of each reaction mixture was used to determine the relative levels of FtsI protein by Western blotting (open bars). Values are arbitrary units normalized to the signal obtained from chromosomally expressed FtsI (KS272/pBAD18-Kan). (C) Kinetics of binding of FLU-C_6-APA to FFI (filled squares; KS272/pLD140), FtsI expressed from a plasmid (open squares; KS272/pLD139), and chromosomally encoded FtsI (open circles; KS272/pBAD18-Kan). Assays were done with permeabilized cells that had been induced for 1 h with 0.002% arabinose. Values are normalized by setting the final time point to 100%. The endpoints attained by FFI and plasmid-encoded FtsI were 24- and 13-fold, respectively, higher than that of chromosomally encoded FtsI in this experiment.

were removed at various time points and analyzed for fluorescein-labelled protein by SDS-PAGE and amounts of FtsI or MBP-FtsI by Western blotting (Fig. 5). FtsI bound FLU-C_6-APA in spheroplasts, and labelling did not increase noticeably after lysis of spheroplasts. In contrast, the cytoplasmically localized MBP-FtsI hybrid protein was labelled only after spheroplasts were lysed.

To ask whether the swap proteins had the correct membrane topology and retained transpeptidase activity, three plasmid-borne swap proteins (FFI, LII, and IQI) were compared to plasmid-borne wild-type FtsI and chromosomally encoded wild-type FtsI in a penicillin-binding assay. Cells were incubated for 30 min in the presence of FLU-C_6-APA and then samples were removed to determine the extent of labelling and the amount of each FtsI protein (by Western blotting [Fig. 6]). In each case, the amount of FLU-C_6-APA bound was directly proportional to the amount of FtsI protein. In a separate experiment, we found that the kinetics of labelling were similar for FFI and wild-type FtsI (Fig. 6). Taken together, our results suggest that substitution of the amino-terminal cytoplasmic domain and/or transmembrane segment of FtsI does not have major effects on the periplasmic localization or on the conformation of the periplasmic domain. The latter observation is consistent with previous evidence that a soluble form of FtsI created by deleting the cytoplasmic domain and MSS has normal penicillin-binding properties (13, 16).

Again, since all of the FtsQ constructs complemented an *ftsQts* mutation, they must express enough protein with the proper topology to allow such complementation. Since we had available no specific test for FtsL topology, we generated fusions of FtsL, FtsL engineered with restriction sites, and the FtsL swaps to alkaline phosphatase using a fusion previously shown to be a good indicator of proper topology for FtsL (*ftsL-phoAL81*) (18). In these hybrid proteins, alkaline phosphatase is fused to residue 24 of the FtsL periplasmic domain. All swaps, except for LFL and QLL, were tested and gave high levels of alkaline phosphatase expression, as observed on indicator plates containing 5-bromo-3-chloro-indolyl-phosphate. We believe that given (i) the results with alkaline phosphatase fusions and (ii) the care with which we generated our constructs so as to ensure proper topology, these constructs are also highly likely to assume the correct arrangement in the membrane. The considerable experience in predicting topology of constructs in numerous other studies reinforces this conviction (25, 29, 40).

Complementation by swap constructs: swaps containing the FtsI periplasmic domain. We first tested complementation of an *ftsI*(Ts) mutation by plasmids encoding swap constructs in

which either the amino-terminal cytoplasmic domain or the transmembrane segment of FtsI or both were replaced by the analogous domains from the other membrane proteins. We tested complementation for growth at 42°C on both rich and minimal media and at different levels of expression of the swap constructs by varying the arabinose concentration in the media. Growth was assessed by streaking out on solid medium. On rich medium, we found essentially no complementation by swap constructs. (High-level expression of the QII hybrid reproducibly showed some growth in the heavy part of the streak, but no isolated colonies grew up, even upon restreaking.) However, in minimal medium, with various results, we did see growth of certain strains. In one case, this occurred even at the very low levels of expression of the swap that resulted from the absence of arabinose and the presence of glucose. There was also some curious variation in the relative abilities of wild-type *ftsI* and the *ftsI* construct with the engineered restriction sites to complement. This may be due to differences in the levels of the two proteins.

Thus, while under many conditions the *ftsI* swap constructs did not appear to be functional, these studies utilizing the *ftsI*(Ts) mutation yielded ambiguous results. It seemed possible that interactions between the altered FtsI of the Ts mutant and the hybrid FtsI molecules might either rescue the function of the FtsI(Ts) mutant protein or generate dimers that were functional. Therefore, we decided to utilize an *ftsI* null mutant for further studies. The null mutation chosen is one in which the transposon Tn*phoA* is inserted in the *ftsI* gene in such a way that alkaline phosphatase is fused to a codon (Asp122) in the periplasmic domain of FtsI (7a). This strain is unable to grow unless a complementing plasmid carrying the *ftsI* gene is present. When the complementing *ftsI* gene is under the control of the arabinose promoter, shutting off this promoter results in a severe cell division defect, and cells produce filaments that eventually lyse.

We determined whether swap constructs would complement the *ftsI* null mutation as follows. Plasmids encoding wild-type *ftsI* and each of the swaps were transformed into strain LMG194, which carries a wild-type *ftsI* gene and a Tn*10* insertion in *leu*, which is 75% linked to *ftsI* by P1 transduction. A P1 transducing lysate made on a Leu^+ strain carrying the *ftsI* null mutation was then used to transduce the strains harboring these plasmids. Since the transposon Tn*phoA* includes a kanamycin resistance gene, we could monitor transfer of the null mutation into the recipient strains by selecting for kanamycin resistance and leucine prototrophy. We found that the strains carrying the *ftsI* wild-type gene and the *ftsI* gene with engineered restriction sites could be transduced to kanamycin resistance on minimal medium, while none of the swap construct strains yielded transductants. Thus, it appears that none of the swap constructs are able to complement an *ftsI* null mutation.

FtsQ. As with *ftsI*, we first studied complementation by FtsQ swaps using an *ftsQ*(Ts) mutation. In contrast to the initial *ftsI* studies, we found complementation of the Ts mutant by many of the constructs on both rich and minimal media (Table 2). Every swap construct was able to complement on minimal medium. In some cases, the complementation was most effective at high arabinose concentrations (high levels of swap expression), and in others it was most effective at lower levels of expression. We have already pointed out here that expression from the *ftsQ* translation initiation sites may be considerably lower than that from those of *ftsI*, *ftsL*, and *malF*. Thus, the FtsQ swaps expressed from an FtsQ amino terminus are able to complement at 0.2% arabinose, while all other swaps appear to be toxic at these levels. In general, the results suggest that constructs that contain both the transmembrane segment and the periplasmic domain of FtsQ complement better than those with only the periplasmic domain.

Given the contrasting findings in complementation studies with the *ftsI*(Ts) mutant and the *ftsI* null mutant, we proceeded to test swap constructs expressing the FtsQ periplasmic domain in a null (*ftsQ-phoA* fusion) *ftsQ* mutant. Here also, there was a difference between the two types of complementation experiments. First, we found that any swap construct in which the cytoplasmic domain of FtsQ was replaced could not complement the *ftsQ* null mutant. In contrast, constructs in which the cytoplasmic domain was intact but the FtsQ MSS was replaced were still functional. Even the construct in which the MalF MSS replaced the FtsQ MSS complemented the null mutation. Thus, the specific sequence of the MSS of FtsQ is not required for its function and may act only as a membrane-anchoring sequence.

FtsL. For FtsL, we had no Ts mutants but only an *ftsL-phoA* null mutation. In this case, alkaline phosphatase is fused to codon 24 of the FtsL periplasmic domain. A bacteriophage P1 lysate made on the null mutant was used to transduce strains carrying plasmids encoding the various *ftsL* constructs plus a chromosomal wild-type *ftsL* gene to kanamycin resistance and leucine prototrophy. While the strains carrying the wild-type *ftsL* or the *ftsL* engineered with restriction sites could be transduced to kanamycin resistance, none of the swap construct-expressing strains could.

Possible dominant effects of swap construct expression. In some cases, we observed growth-inhibitory effects at the highest level of expression of swap constructs in *ftsI*(Ts) and *ftsQ*(Ts) backgrounds (not shown). Examination of cells under these conditions showed a variable degree of filamentation. However, there was no particular pattern to these effects. It is known that, in general, overexpression of membrane proteins frequently leads to growth inhibition. Thus, the significance of these observations is not clear.

DISCUSSION

The studies of exchanges of domains between membrane proteins involved in cell division that we report here provide an approach to defining the roles of various domains of these proteins. They point to domains that are likely to be essential for the function of the proteins and suggest experiments to further explore the function of these proteins and any partners with which they may interact.

FtsI. Previous studies had suggested that at least a membrane anchor was necessary for FtsI to be functional for cell division (16, 17a). Conversion of this protein to a periplasmic location by replacement of the cytoplasmic domain and MSS by a cleavable signal sequence resulted in the inability to complement an *ftsI* mutation. Dai et al. (10) reported that replacement of the cytoplasmic and transmembrane domains of FtsI with comparable sequences from the unrelated protein MalG resulted in a noncomplementing construct, although topology and expression were not reported. Our results also suggest that both the cytoplasmic domain and the MSS are essential functional components of FtsI. The MSS appears sensitive to very slight alterations. The addition of a single amino acid or the change of an amino acid in or adjacent to the MSS reduces FtsI function. For example, introducing a *Stu*I restriction site at the 3′ end of the MSS inserts a leucine into the MSS and results in an I/I_{StuI}/I protein that cannot support cell division (Table 2). More recent studies on a variety of alterations of the MSS further support the conclusion that the sequence, length, and arrangement of this sequence is critical for FtsI function (43). The essentiality of the cytoplasmic domain indicates that it is

not simply a sequence that contributes to the anchoring of the protein in the proper orientation in the membrane. Rather, these results suggest that the cytoplasmic domain performs an important role, perhaps interacting with some other component of the cell division apparatus.

FtsL. In terms of complementation, the results with FtsI and FtsL are the same. They suggest that the FtsL cytoplasmic and MSS domains are important for the functioning of the protein. Mutants of the periplasmic domain of FtsL also interfere with the cell division process (18).

FtsQ. All constructs that retain the periplasmic domain of FtsQ complement to some degree an *ftsQ*(Ts) mutant. Again, Dai et al. (10) describe a MalG-FtsQ construct analogous to their MalG-FtsI hybrid referred to above. This construct also complemented the *ftsQ*(Ts) mutation; the expression and topology of this construct were not reported.

However, our studies of complementation of an *ftsQ* null mutant suggest that studies with the *ftsQ*(Ts) mutation are misleading. Specifically, the results with complementation of the null mutant by swaps of the cytoplasmic domain of FtsQ, as with FtsI and FtsL, show that this domain is essential to the function or localization of FtsQ. In contrast, the null mutant could be complemented by constructs in which only the MSS of FtsQ was replaced. The fact that the substitution of the MalF MSS allows functionality of FtsQ suggests that there are no signals in the MSS that are important for subcellular localization. That is, if, for example, the MSS of FtsQ directed it to the cell septum where it had to be localized in order to be functional in cell division, replacement of this domain with the analogous sequence of MalF, which we presume is not localized to the septum, should have eliminated this function. This conclusion must be qualified, since overexpression of the constructs may produce enough of the hybrid proteins so that a sufficient amount is localized to the cell septal region simply by random distribution. However, we did analyze complementation at low levels of induction of these constructs and still obtained positive results. Our results suggest that although the specific sequence of the FtsQ MSS is not important, an MSS is likely required for FtsQ function in cell division, since both the FtsQ periplasmic and cytoplasmic domains appear to be essential.

Cautions in swap analysis. In the analysis of swap proteins, it is obviously essential to verify that the proteins are expressed and are at least stable enough so that substantial levels of the protein are present at steady state. We have demonstrated that this is the case for all of the constructs containing the FtsI periplasmic domain by Western blotting with FtsI antibodies. Similarly, we could show the presence of greater-than-wild-type levels of many of the constructs containing the FtsL periplasmic domain using FtsL antibody. In the case of FtsQ, since all of the constructs complemented in the *ftsQ*(Ts) background, they must all be expressed.

Although the swaps were constructed so as to make it most likely that their membrane topology would be the same as that of the wild-type protein, we checked with certain swaps to determine if that was the case. With swaps containing the FtsI periplasmic domain, this was done by assaying binding of a fluorescent penicillin analog and showing that the binding was external to the cytoplasmic membrane. These findings also showed that the transpeptidase activities of these hybrid proteins were probably intact. Again, with FtsQ, the positive complementation results with the *ftsQ*(Ts) mutant made it unnecessary to do topology analysis, since these findings indicate that at least a significant fraction of the protein has the proper topology required for functionality. With FtsL, the results obtained from *phoA* fusions to FtsL and its swap derivatives strongly suggest that the hybrid proteins assumed the proper topology. Also, we have argued above that it is highly likely that FtsL constructs assumed the proper topology, given the results with FtsQ and FtsI and the way in which the constructs were designed.

We interpreted absence of complementation to mean that the segment(s) replaced in a particular construct is essential for the function of the protein. However, it is possible that in the process of replacing, for instance, a cytoplasmic domain of a protein, we subtly altered the position, structure, or orientation of the MSS. Then, it is not the cytoplasmic domain that is essential but the MSS and its particular arrangement in the membrane. This type of alternative interpretation is potentially subject to testing, since one might expect that it would be relatively easy to restore function to the hybrids by simple mutational changes. If such mutations could be found, they would show that certain of the negative complementation results would have to be reinterpreted and that the specific sequences of certain domains are not essential. Such results would also be of significant interest in terms of the organization of membrane proteins in general.

Contrasting complementation results with an *fts*(Ts) mutation and an *fts* null mutation. The failure of swap constructs with the FtsI periplasmic domain to complement a null *ftsI* mutation indicates that these proteins are not functional on their own. Yet some of them are able, under some conditions, to complement an *ftsI*(Ts) mutation. We can conceive of several possible explanations for this difference. First, if FtsI is normally dimeric, then the FtsI(Ts) protein may be able to form a dimer with certain of the swap proteins generating a partially functional heterodimer. Alternatively, overexpression of swap proteins may rescue the function of the FtsI(Ts) protein by, for example, saturating cellular proteases that might otherwise degrade the Ts protein. Since overexpression of these membrane proteins can have some deleterious effects on the cell, it is clear that they can interfere with some cellular processes.

Similar explanations may account for the differences in complementation between the *ftsQ*(Ts) mutant and the *ftsQ* null mutant.

Further work. Our results suggest that for FtsI and FtsL, the cytoplasmic, membrane, and periplasmic domains of the proteins are essential for their function. Thus, all domains of the protein may be usefully studied by mutagenic analysis. In the introduction, we suggested various possible roles for these domains. One possible role for a domain is to signal localization to the cell septum via interaction with one of the other Fts proteins or with other unidentified cellular components. By immunofluorescence microscopy, it is now possible to observe the positioning of membrane proteins such as FtsI to the cell septum (42). Thus, the effects of substituting one or another domain of this protein on its localization to the septum can be used to define the localization signals and to do mutagenic analysis of such signals.

One may also usefully pursue analysis of protein-protein interactions of different domains of these proteins. For example, the yeast two-hybrid system or an analogous bacterial system could reveal components that interact with the hydrophilic domains of FtsI and FtsL. The yeast system may not be useful for seeking interactions between MSSs, since these domains might cause problems for localization in the yeast cell and prevent the localization of hybrid protein to the cell nucleus where effects are assayed.

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