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The FtsQ Protein of *Escherichia coli*: Membrane Topology, Abundance, and Cell Division Phenotypes Due to Overproduction and Insertion Mutations

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The *ftsQ* gene is one of several genes thought to be specifically required for septum formation in *Escherichia coli*. Published work on the cell division behavior of *ftsQ* temperature-sensitive mutants suggested that the FtsQ product is required throughout the whole process of septum formation. Here we provide additional support for this hypothesis based on microscopic observations of the cell division defects resulting from insertional and temperature-sensitive mutations in the *ftsQ* gene, and constitutive overexpression of its gene product. On the basis of the published, predicted amino acid sequence of the FtsQ protein and our analysis of fusion proteins of the FtsQ protein to bacterial alkaline phosphatase, we conclude that FtsQ is a simple cytoplasmic membrane protein with a ~25-amino-acid cytoplasmic domain and a ~225-amino-acid periplasmic domain. We estimate that the FtsQ protein is present at about 22 copies per cell.

Cell shape in *Escherichia coli* is determined by the rigid murein cell wall layer located between the inner and outer membranes. The final steps in the synthesis of the cell wall occur in this periplasmic compartment, catalyzed at least in part by a set of penicillin-binding proteins (PBPs) (for a review, see reference 24). Whereas most of these PBPs appear to be required for normal growth and elongation of the cell, PBP3 is required only for cell wall biogenesis during septation. PBP3, the *ftsI* or *sep* gene product, has enzymatic activities capable of polymerizing glycan subunits into the cell wall as well as cross-linking adjacent glycan chains to provide rigidity to the cell wall (9). Several other genes that are specifically required for septum formation have been identified among mutations that result in filamentation temperature-sensitive (Fts) phenotypes (for a review, see reference 7).

Determining the cellular locations of the protein products encoded by these genes is an important step toward learning their function. Proteins with periplasmic locations or membrane proteins with periplasmic domains, such as PBP3 (3), may catalyze the necessary changes in the cell envelope associated with septation. The membrane anchor of transmembrane proteins may function simply to localize such cell division proteins close to the outer surface of the cytoplasmic membrane. Alternatively, transmembrane proteins having both cytoplasmic and periplasmic domains may mediate the process by which cytoplasmic events influence the activity of cell division proteins located in the periplasm. Proteins with predominantly cytoplasmic locations may be involved in the regulation of the cell division process. The cytoplasmic FtsZ protein, for example, regulates the frequency of cell division (2, 13) and is a key element in arresting cell division as part of the response of the SOS system to DNA damaging agents (8, 12, 14). Some of the proteins encoded by the *minB* locus are also thought to act through the *ftsZ* protein to ensure specificity of septum localization (6).

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The *ftsQ* gene is located at 2 min on the bacterial chromosome in a cluster of cell division genes involved in septum formation and synthesis of cell wall components (1). While its function remains unknown, the FtsQ protein is thought to be required throughout the whole process of septum formation. This hypothesis is based on the rapidity with which cell division ceases upon shifting an *ftsQ* temperature-sensitive strain to a restrictive temperature (1, 7). The DNA sequence of the *ftsQ* gene has been determined previously (25), and recently its product has been overproduced and identified as a membrane protein in maxicells (28). Here we provide an analysis of the topology of the FtsQ protein in the cytoplasmic membrane. To learn more about the requirement for FtsQ protein during the process of septum formation, we examine the phenotypes associated with mutations in the *ftsQ* gene and with constitutive overexpression of its gene product.

MATERIALS AND METHODS

Media and chemicals. Standard rich bacterial growth media were LB liquid broth (20) and TYE agar, which differs from LB agar in that it contains 8 g of NaCl per liter. Defined minimal medium was M63 (20) plus 18 amino acids (no methionine or cysteine), thiamine (1 μg/ml), and 0.2% of the specified carbon source. Ampicillin (200 μg/ml), kanamycin (40 μg/ml), and tetracycline (20 μg/ml) were used as needed at the concentrations given unless indicated otherwise. XP (5-bromo-5-chloro-3-indolyl phosphate) obtained from Bachem Fine Chemicals (Torrance, Calif.) was used at 40 μg/ml as an indicator of alkaline phosphatase (AP) activity on plates. L-Arabinose was purchased from Pfanstiehl Laboratories Inc. (Waukegan, Ill.), and restriction enzymes and T4 DNA ligase were from New England BioLabs, Inc. (Beverly, Mass.)

Bacteria, bacteriophages, and plasmids. Bacterial strains are listed in Table 1. The *ftsQ*(Ts) allele was moved from strain TOE-1 (1) into our strain background by P1 transduction crosses, using a closely linked Tn*10* insertion in *leu*. The *recA*::*cat* allele taken from strain BW10724, a gift of Barry

TABLE 1. Bacterial strains

E. coli strain	Genotype[a]	Reference or source
CC118	F⁻ *araD139* Δ(*ara-leu*)*7697* Δ*lacX74* Δ*phoA20 galE galK thi rpsE rpoB argE*(Am) *recA1*	17
KS272	F⁻ Δ*lacX74 galE galK thi rpsL* Δ*phoA* (*PvuII*)	29
MJC39	KS272 *zec*::Tn*10-992 motB*::Tn*phoA*	This study
MJC127	KS272 *ftsQ*(Ts)	This study
MJC225	KS272 *polA*(Ts-12) *zig*::Tn*10* (λ16-25)	This study
MJC231	KS272 *ftsQ*::Tn*phoA50 leu*::Tn*10* (λ16-25)	This study
MJC251	KS272 *ftsQ*::Tn*phoA187 leu*::Tn*10* (λ16-25)	This study
MJC252	KS272 *ftsQ*::Tn*phoA80 leu*::Tn*10* (λ16-25)	This study
MJC255	KS272 *leu*::Tn*10* (λ16-25)	This study
MJC261	KS272 *phoR recA*::*cat*	This study
MJC263	KS272 *phoR phoA*⁺ *recA*::*cat*	This study
MJC265	KS272 *ftsQ*::Tn*phoA50*/pMJC103	This study
MJC266	KS272 *ftsQ*::Tn*phoA80*/pMJC103	This study
MJC267	KS272 *ftsQ*::Tn*phoA187*/pMJC103	This study

[a] Three different Tn*phoA*ΔIS*50*R insertions occurring at amino acids 50, 80, and 187 in the FtsQ protein were crossed from plasmids to the chromosome as described in Materials and Methods; these insertion mutations are named *ftsQ*::Tn*phoA50*, *ftsQ*::Tn*phoA80*, and *ftsQ*::Tn*phoA187*, respectively.

Wanner, was introduced into new strains by P1 transduction crosses, selecting with 10 μg of chloramphenicol per ml. The *motB*::Tn*phoA* insertion was obtained on a multicopy plasmid from S. Parkinson. The fusion joint is at amino acid 94 of MotB. Plasmids are shown in Fig. 1. The lambda transducing phages for the *ftsQ* region, λ16-25 and λ16-2, have been described previously (16).

Isolation and detection of AP fusion proteins. Tn*phoA* insertions in plasmid pZQ (in strain CC118) were isolated with λTn*phoA* by a procedure similar to that previously described (26). DNA sequence analysis was performed as previously described (30a). Procedures for radioactive labeling, immunoprecipitating, and visualizing proteins were done as described previously (10, 29), except that proteins were not precipitated with trichloroacetic acid prior to immunoprecipitation. Protein secretion was blocked by adding 2 mM sodium azide, which inhibits the action of the *secA* protein (23), for 2 min prior to the labeling experiment. AP enzymatic activity was measured as the rate of *p*-nitrophenyl phosphate hydrolysis as previously described (4), except that permeabilized cells (19) incubated at 30°C were used.

Specific activity of FtsQ-AP fusion proteins. To calculate the specific activity of FtsQ-AP fusion proteins relative to native AP, serial dilutions of the samples shown in Fig. 2 were prepared and fractionated on 10% polyacrylamide-sodium dodecyl sulfate gels (data not shown). In strain MJC263, the native AP protein is constitutively made due to the *phoR* mutation and serves as an internal standard (Fig. 2). Densitometric measurements were made from different length exposures of these autoradiograms. After correcting for the number of methionine residues in each of the FtsQ-AP fusion proteins and native AP, we calculated their relative synthesis rates. The enzymatic activity for native bacterial AP was measured directly in the AP constitutive strain MJC263, transformed with the pZQ control plasmid. The AP enzymatic activity produced by each of the FtsQ-AP fusion proteins was measured instead in the strain MJC261 transformed with pZQ::Tn*phoA* plasmids. This strain is isogenic to MJC263 but does not produce the native AP as a result of the Δ*phoA*(*Pvu*II) deletion, thus allowing accurate

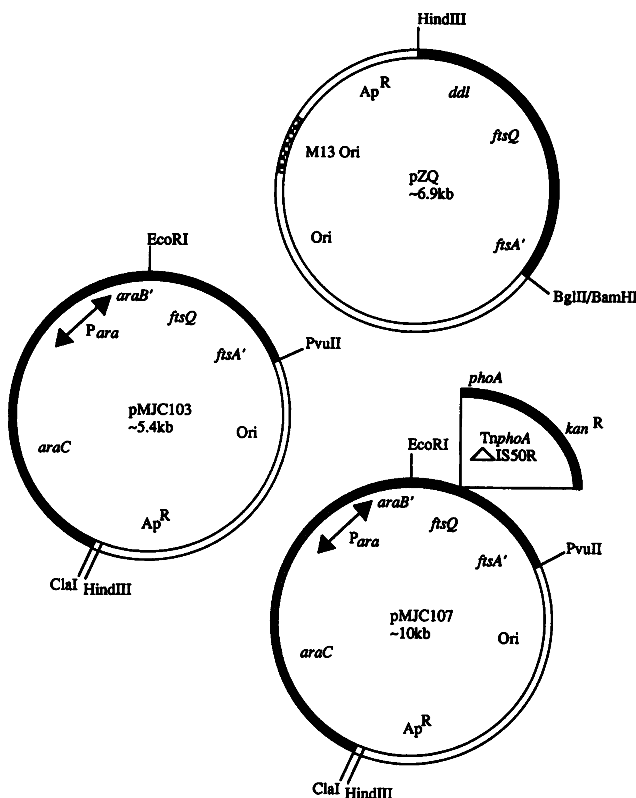

FIG. 1. Plasmid constructions. (i) pZQ. Plasmid pZQ contains the 2.4-kb *Hin*dIII-*Bgl*II DNA fragment from the λ16-2 *ftsQ* transducing phage cloned into a derivative of pBR322 that contains an F1 phage replication origin (pZ150 [36]). The insert on plasmid pZQ encodes the *E. coli ddl* gene, the *ftsQ* gene, and a fusion protein containing the first 41 amino acids of the *ftsA* protein fused to the remaining C-terminal portion of the tetracycline resistance gene on pBR322. (ii) pMJC103. Plasmid pMJC103 contains a 1-kb *Eco*RI-*Pvu*II fragment from pZQ which includes the entire coding region of the *E. coli ftsQ* gene and its putative Shine-Dalgarno sequence. This DNA fragment was cloned into plasmid pDB27 (obtained from David Blair, Harvard University) that had been digested with restriction enzymes *Eco*RI and *Pvu*II, thus placing the *ftsQ* gene under the regulation of the *E. coli araBAD* promoter. pMJC103 retains the translational controls of the *araB* gene and 5 amino acids of *araB* located before the *Eco*RI site. Although we have confirmed by DNA sequencing that the *ftsQ* gene is inserted out of frame with *araB'*, it is possible that the presence of the additional *araB* Shine-Dalgarno sequence increases the translational efficiency of the *ftsQ* gene (7a). The *Cla*I-to-*Pvu*II segment of pMJC103 containing the Ap^r gene is identical to the corresponding segment of pBR322, except that the pBR322 *Eco*RI restriction site is changed to a *Hin*dIII site. (iii) pMJC107. A Tn*phoA*ΔIS*50*R insertion at amino acid 50 of FtsQ was transferred to plasmid pMJC103 by homologous recombination to create plasmid pMJC107. In this plasmid, expression of the FtsQ-AP fusion protein is under control of the *araBAD* promoter. The plasmid pMJC107 was shown by restriction mapping and DNA sequencing of the FtsQ-AP fusion junction to contain the Tn*phoA*ΔIS*50*R insertion at the proper position in the *ftsQ* gene.

measurement of the AP enzymatic activity produced by an FtsQ-AP fusion protein. Specific activity was determined as FtsQ-AP enzymatic activity relative to that of native AP/FtsQ-AP protein synthesis rate relative to that of native AP.

Transfer of insertions from plasmids to the chromosome. Tn*phoA* transposon insertions in the *ftsQ* gene on plasmid pZQ were stabilized by constructing an internal deletion of

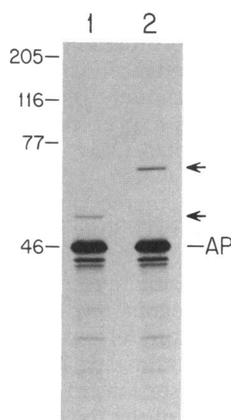

FIG. 2. Protein synthesis rate of FtsQ-AP fusion proteins. Strain MJC263 transformed with FtsQ-AP fusion protein-producing plasmids was grown in M63 minimal medium containing 0.2% glycerol, 18 amino acids (no methionine or cysteine), and 20 μg of ampicillin per ml at 37°C. Proteins were labeled by growing strains for 2 min in the presence of [^{35}S]methionine and were immunoprecipitated with antisera raised against bacterial AP. Positions of molecular mass markers (in kilodaltons) are indicated to the left. In addition to the FtsQ-AP fusion protein band in each lane, a band for native AP is present as a result of its constitutive production in strain MJC263 (*phoR phoA*$^+$). This band serves as an ideal internal standard for calculations of specific activity (see Materials and Methods). The position of the AP band is labeled, and the arrows identify the position of the two FtsQ-AP fusion proteins. Lane 1, MJC263/pZQ::Tn*phoA80*; lane 2, MJC263/pZQ::Tn*phoA187*.

Tn*phoA* that removes most of the IS*50*R element but retains the kanamycin resistance (Kan^r) gene (Tn*phoA*ΔIS*50*R). The deletions, constructed in vitro on pZQ::Tn*phoA* plasmids, remove the DNA extending from the *Sma*I site at position 4434 on the Tn*phoA* map to the *Hpa*I site at position 7551.

Plasmids containing three different pZQ::Tn*phoA*ΔIS*50*R insertions were transformed into strain MJC225 [*polA*(Ts)], and ampicillin-resistant (Amp^r) (200 μg/ml) colonies were selected and purified at the permissive temperature of 30°C. Integrants of this plasmid into regions of homology on the bacterial chromosome were selected by restreaking these transformants at 42°C on plates containing kanamycin (40 μg/ml). Several independent integrants were pooled. A phage P1 lysate was prepared on this pool, and Kan^r *leu*$^+$ transductants were selected in a cross to a recipient strain that was *polA*$^+$ *leu*::Tn*10* (about 67% linked to *ftsQ*) and contained an additional copy of the *ftsQ* gene on phage λ16-25 integrated at the chromosomal *att*λ site. All of these transductants also retained at least a low level of ampicillin resistance (30 μg/ml), suggesting either that the entire plasmid was still integrated into the chromosomal copy of *ftsQ* or that the plasmid had been excised but remained in the transductant as a free plasmid. To screen for those chromosomes in which the plasmid had been excised, leaving behind the Tn*phoA*ΔIS*50*R insertion, we prepared P1 lysates on these strains. These lysates were used to transduce KS272 (λ16-25) to kanamycin resistance, and these transductants were then screened for ampicillin sensitivity.

Derivatives of these ampicillin-sensitive transductants, representing three different sites of Tn*phoA*ΔIS*50*R insertion (at amino acids 50, 80, and 187 of FtsQ) display three properties consistent with their containing an *ftsQ*::Tn*phoA*ΔIS*50*R insertion. First, the cotransduction frequency of Kan^r and *leu*::Tn*10* from P1 lysates made on these strains was similar to the cotransduction frequency of *ftsQ*(Ts) and *leu*::Tn*10* (see Results), provided that the recipient strain had an additional copy of *ftsQ*$^+$ provided by λ16-25. Second, if the recipient did not contain a second copy of the *ftsQ*$^+$ gene, we failed to see Kan^r transductants among those selected for tetracycline resistance (Tet^r). Third, each of these insertions could be transduced by P1 crosses into strain KS272/pMJC103, in which the only gene from the 2-min region that is present in extra copies is the *ftsQ* gene itself. Since many of the genes in the 2-min region are either known or thought to be essential, it is unlikely that the insertions are associated with additional chromosomal rearrangements.

The Tn*phoA* insertion in the *motB* gene (provided by S. Parkinson) was on plasmid pSYC917. Since this plasmid was very unstable, we were able to cross the Tn*phoA* insertion onto the chromosome directly in the *polA*$^+$, motile strain KS272. Strain KS272/pSYC917 was plated at high density selecting only for kanamycin resistance. After overnight growth, the cells were harvested and plated under the same conditions. This cycle was repeated several times. Finally, cells were plated for single colonies on plates containing kanamycin and screened for low-level AP activity, ampicillin sensitivity, lack of motility, and cotransduction of the Kan^r gene with a Tn*10* linked to *motB*.

Photomicroscopy. Cells sampled for photomicroscopy were fixed directly in growth medium by the addition of glutaraldehyde to 0.5%. Fixation was for about 1 h on ice; cells were then washed, resuspended at appropriate concentrations in M63 salts, and stored at 8°C. Cells were visualized and photographed, using phase-contrast conditions. Estimates of the difference in average cell length between KS272 and MJC127 growing at 30°C were derived by measuring cells in photographs printed at the same magnification.

RESULTS

Location of the FtsQ protein. To identify periplasmic domains in the FtsQ protein, we constructed protein fusions of FtsQ to bacterial AP, using transposon Tn*phoA* (17). Since bacterial AP must be exported to the periplasm to be enzymatically active, these protein fusions can be used both to identify proteins which rely on a cleavable signal sequence for their export to the periplasm or outer membrane and to locate periplasmic domains of proteins inserted in the cytoplasmic membrane.

Strains with random Tn*phoA* insertions in plasmid pZQ (Fig. 1) containing the *ftsQ* gene were isolated. The fusion junctions of insertions that produced detectable AP enzymatic activity were determined by DNA sequencing. Nine of these insertions were located within the *ftsQ* gene, producing FtsQ-AP fusion proteins that contain from 50 to 212 amino acids from the amino terminus of the FtsQ protein. The strains making these fusion proteins each produce about 15 U of AP enzymatic activity (Table 2). Two FtsQ-AP fusion proteins (Fig. 2) have a specific activity (units of AP enzymatic activity/protein synthesis rate) approximately equivalent to that of the native AP enzyme (Table 2). From this analysis, we conclude that the FtsQ protein contains an efficient periplasmic export signal. From the distribution of enzymatically active fusion proteins (Table 2), we consider it likely that amino acids 50 to 212 of the FtsQ protein are located in the periplasm.

The model for location and membrane topology of the FtsQ protein is shown in Fig. 3. Hydrophobicity analysis of the FtsQ protein (35) reveals a hydrophobic segment from

TABLE 2. Location of fusion junctions and AP enzymatic activity of strains with Tn*phoA* insertions in pZQ

Plasmid	Fusion joint in FtsQ (amino acid no.)[a]	AP enzymatic activity (U)[b]	Sp act[c]
pZQ	None	0.2	NT
pZQ::Tn*phoA50*	50	7	NT
pZQ::Tn*phoA80*	80	15	1.4
pZQ::Tn*phoA91*	91	15	NT
pZQ::Tn*phoA107*	107	12	NT
pZQ::Tn*phoA113*	113	12	NT
pZQ::Tn*phoA159*	159	13	NT
pZQ::Tn*phoA164*	164	14	NT
pZQ::Tn*phoA187*	187	14	1.3
pZQ::Tn*phoA212*	212	14	NT

[a] The fusion joint (determined from DNA sequence) specifies the number of amino acids derived from the amino terminus of the FtsQ protein in the FtsQ-AP fusion protein.

[b] AP activity was measured from a minimum of three separate cultures of KS272 transformed with each of these plasmids and grown at 37°C to mid-logarithmic phase in LB broth supplemented with 200 μg of ampicillin per ml. Standard deviation measures indicate that only the AP enzymatic activity for the strain producing the FtsQ-AP fusion protein containing 50 amino acids from FtsQ is significantly different from those of the strains producing fusion proteins containing 80 and greater amino acids from FtsQ.

[c] Specific activity for FtsQ-AP fusion proteins is expressed relative to native AP, as described in Materials and Methods. NT, Not tested.

amino acids 25 to 49 which could function as a membrane-spanning segment. Consistent with this location for FtsQ, we find that the FtsQ-AP fusion protein containing 187 amino acids of FtsQ fractionates with the pellet in an osmotic shock cell fractionation experiment (18), under conditions where periplasmic proteins are released into the supernatant (data not shown). Also consistent with this model are our experimental results which show that the FtsQ protein is unlikely to contain a cleavable signal sequence. Inhibition of protein secretion with 2 mM sodium azide (23) did not affect the polyacrylamide gel migration of an FtsQ-AP fusion protein containing 80 amino acids of FtsQ. Specifically, we did not detect a larger precursor for the FtsQ-AP fusion protein. In these same cells, we readily detected the larger precursor forms of both ribose-binding protein and maltose-binding protein, periplasmic proteins which rely on cleavable signal sequences for their export (data not shown).

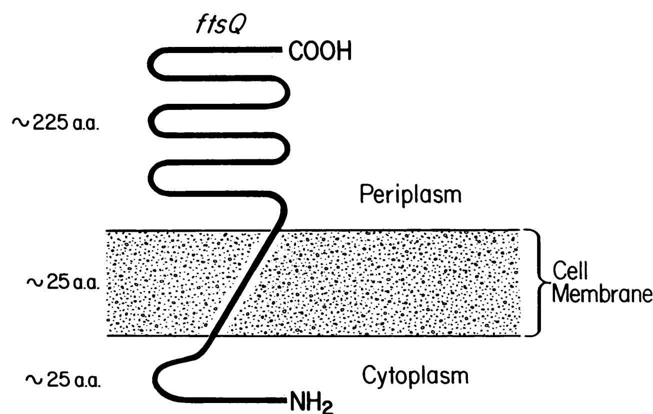

FIG. 3. Proposed model for FtsQ protein membrane topology. Support for this model for FtsQ protein membrane topology is provided in the text. The amino-terminal ~25 amino acids of FtsQ are cytoplasmic, part or all of amino acids ~25 to 49 are embedded in the cytoplasmic membrane, and the remaining ~225 amino acids are periplasmic.

Estimate of FtsQ abundance. The FtsQ protein has been difficult to detect and has only recently been visualized in maxicells in which it is expressed under the control of a strong inducible promoter (28). The protein fusion constructs we have made in which FtsQ is fused to bacterial AP are enzymatically active, thereby making it possible for us to estimate the amount of FtsQ protein produced by measuring AP enzymatic activity.

Tn*phoA* insertions creating protein fusions of FtsQ to AP were crossed by homologous recombination from plasmids into the chromosomal copy of the *ftsQ* gene, using a *polA* temperature-sensitive strain (see Materials and Methods). As we expected these insertions to be lethal (see below), we provided a second copy of the *ftsQ* region, using the λ16-25 *ftsQ* transducing phage integrated at the *att*λ site.

AP enzymatic activities of strains with Tn*phoA* transposon insertions in the *ftsQ* gene were measured and compared with the enzymatic activity produced by a strain with a chromosomal Tn*phoA* transposon insertion in the periplasmic domain of the simple membrane protein MotB (5). The copy number of the MotB protein is estimated to be 150 ± 70 copies per cell (34). The strain MJC39 producing the MotB-AP fusion protein has about 22 U of AP enzymatic activity in M63 minimal glycerol medium at 34°C (prevents catabolite or high-temperature repression of genes in the flagellar regulon). Under these same growth conditions, the AP enzymatic activity from strains producing an FtsQ-AP fusion protein is 3.2 U. This number is derived as an average of the enzymatic activity from strains MJC252 (2.9 U) and MJC251 (3.5 U), which produce an FtsQ-AP fusion protein containing 80 and 187 amino acids of FtsQ, respectively. Comparison to MotB, therefore, indicates that FtsQ-AP fusion proteins are present in about 22 copies per cell and suggest that this is the level of expression of FtsQ.

Cell division in the *ftsQ* temperature-sensitive mutant. The cell division defects resulting from the *ftsQ* temperature-sensitive mutation have been previously reported. Depending on the strain background and growth conditions, either nonseptate filaments (1) or filaments with partial septa along their length (30) accumulate after a shift to the restrictive temperature. These different phenotypes could be due simply to different amounts of residual function of the FtsQ mutant product. To test this hypothesis, we examine the cell division defects in the *ftsQ* temperature-sensitive mutant strain at different temperatures at which different amounts of residual function may be expected. Also, to get a clearer indication of the null phenotype, we examine the cell division defect resulting from insertion mutations in the *ftsQ* gene (see section below).

The *ftsQ* temperature-sensitive mutant strain MJC127 (Table 1), growing at 30°C (Fig. 4b), has an average cell length approximately 150% that of the isogenic $ftsQ^+$ strain KS272 (Fig. 4a). As previously reported (1, 30), we assume that the longer cells result from a reduction or possibly altered function of the FtsQ mutant protein at 30°C. The filaments that are formed when MJC127 is shifted to 37°C for about two mass doublings (Fig. 4c) have clearly visible septa along their length. To ascertain whether these septa are capable of continuing on to complete cell division, we observed these filaments after a shift down to the permissive temperature of 25°C by time-lapse photomicroscopy on agar slabs. Partial septa in these filaments efficiently matured into sites of cell division following the shift to the permissive temperature (data not shown). We conclude that at 37°C

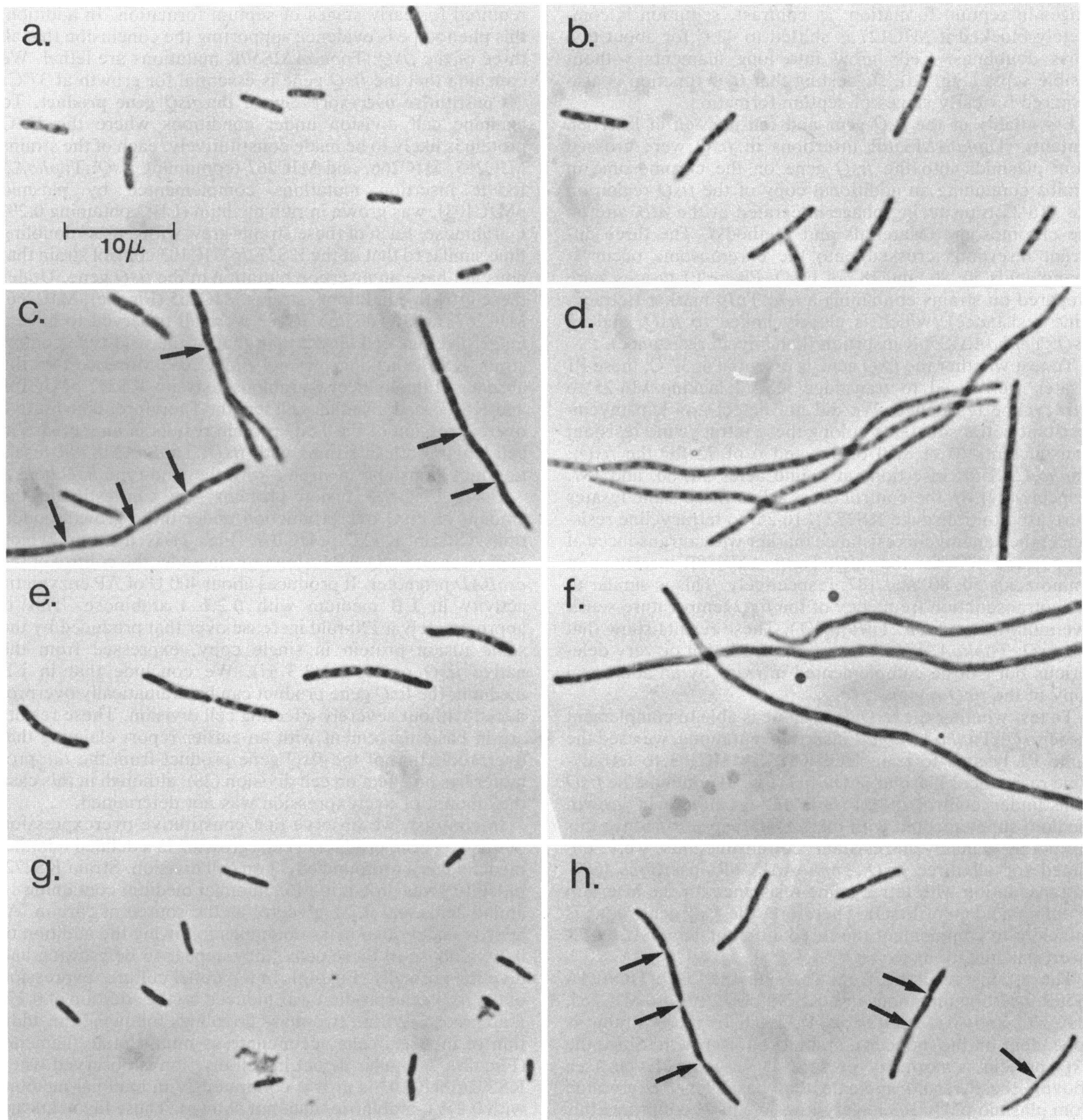

FIG. 4. Cellular morphologies. Samples of cells grown to early or mid-logarithmic phase under the conditions listed below were imaged with phase-contrast microscopy, photographed, and printed at the same magnification. Arrows in panels c and h point to partially completed septa. (a) Strain KS272 (*ftsQ*$^+$) grown in LB rich broth at 30°C. (b) Strain MJC127 [*ftsQ*(Ts)] grown in LB rich broth at 30°C. (c) Strain MJC127 [*ftsQ*(Ts)] grown in LB rich broth at 30°C, then shifted to 37°C for approximately two mass doublings. (d) Strain MJC127 [*ftsQ*(Ts)] grown in LB rich broth at 30°C, then shifted to 42°C for approximately two mass doublings. (e) Strain MJC265 (*ftsQ*::Tn*phoA50*/pMJC103) grown in LB rich broth plus 0.2% L-arabinose to stationary phase at 37°C, washed, inoculated at a 1/250 dilution into the same medium, and grown for 140 min at 37°C. (f) MJC265 (*ftsQ*::Tn*phoA50*/pMJC103) grown in LB rich broth plus 0.2% L-arabinose to stationary phase at 37°C, washed, inoculated at a 1/250 dilution into the same medium lacking L-arabinose, and grown for 140 min at 37°C. (g) Strain KS272/pMJC103 grown in M63 minimal medium containing 18 amino acids (no methionine or cysteine), 40 μg of ampicillin per ml, and 0.2% glycerol to an optical density at 600 nm of 0.07. Glucose was then added to 0.2%, and the cells were grown at 37°C for 140 min. (h) Strain KS272/pMJC103 grown in M63 minimal medium containing 18 amino acids (no methionine or cysteine), 40 μg of ampicillin per ml, and 0.2% glycerol to an optical density at 600 nm of 0.07. L-Arabinose was then added to 0.2%, and the cells were grown at 37°C for 140 min.

the *ftsQ* temperature-sensitive mutant is defective either throughout the entire septation reaction or at a late stage or stages in septum formation. In contrast, septation is completely blocked if MJC127 is shifted to 42°C for about two mass doublings. Cells grow into long filaments without visible septa (Fig. 4d), suggesting that *ftsQ* function is also required for early stages of septum formation.

Essentiality of the *ftsQ* gene and cell division of insertion mutants. Tn*phoA*ΔIS*50*R insertions in *ftsQ* were crossed from plasmids into the *ftsQ* gene on the chromosome in strains containing an additional copy of the *ftsQ* region on the λ16-25 transducing phage integrated at the *att*λ site on the chromosome (Materials and Methods). The three different insertions crossed onto the chromosome occur at amino acids 50, 80, and 187 of FtsQ. Phage P1 lysates were prepared on strains containing a *leu*::Tn*10* marker (tetracycline resistance), which is closely linked to *ftsQ*, and an *ftsQ*::Tn*phoA*ΔIS*50*R insertion (kanamycin resistance).

To test whether the *ftsQ* gene is essential at 37°C, these P1 lysates were used to transduce KS272 lacking λ16-25 to tetracycline resistance. We did not detect any kanamycin-resistant cotransductants among these tetracycline-resistant transductants (0 of 8, 0 of 36, and 0 of 72 for the *ftsQ*::Tn*phoA*ΔIS*50*R insertions at amino acids 50, 80, and 187, respectively). In the control cross when these same lysates were used to transduce KS272 (λ16-25) to tetracycline resistance, the kanamycin resistance marker was cotransduced at 63, 53, and 47% for the *ftsQ*::Tn*phoA*ΔIS*50*R insertions at amino acids 50, 80, and 187, respectively. This is similar to the cotransduction frequency of the *ftsQ* temperature-sensitive mutation and *leu*::Tn*10* (63%). These results show that the *ftsQ*::Tn*phoA*ΔIS*50*R insertions are lethal or very deleterious but can be complemented in *trans* by an additional copy of the *ftsQ* region.

To test whether the *ftsQ* gene alone is able to complement these *ftsQ*::Tn*phoA*ΔIS*50*R insertion mutations, we used the same P1 lysates to transduce KS272/pMJC103 to tetracycline resistance. Plasmid pMJC103 (Fig. 1) contains the *ftsQ* gene under control of the *araBAD* promoter. In growth medium supplemented with 0.2% L-arabinose to induce this promoter, kanamycin-resistant cotransductants were obtained for all three *ftsQ*::Tn*phoA*ΔIS*50*R insertions (58% cotransduction with tetracycline resistance for the insertion at amino acid 50 of FtsQ). Therefore, the *ftsQ* gene alone is sufficient to complement the three different Tn*phoA*ΔIS*50*R insertions mutations in *ftsQ*.

The strains resulting from crossing the *ftsQ*::Tn*phoA*ΔIS*50*R insertion mutations into KS272/pMJC103 are MJC265, MJC266, and MJC267 (Table 1). Each of these strains is dependent on the inducer L-arabinose for growth. Since the *ftsQ* protein is normally present at very low levels (see above), these results indicate that basal level expression from plasmid pMJC103 is very low. We have confirmed this by measuring AP enzymatic activity from a strain which expresses an FtsQ-AP fusion protein under the control of the *araBAD* promoter. Strain KS272/pMJC107 (Fig. 1) produces only 0.7 U of AP enzymatic activity. Higher basal level expression probably accounts for the observation that a plasmid in which the *ftsQ* gene is expressed under control of the *tac* promoter is able to complement an *ftsQ* temperature-sensitive mutation in the absence of inducer (28).

To examine cell division in the absence of functional FtsQ product, strains MJC265, MJC266, and MJC267 were grown to stationary phase in rich growth medium containing L-arabinose and then inoculated into similar medium lacking this inducer. In medium lacking L-arabinose, each of these strains grew as long filaments without visible septa (Fig. 4f; also data not shown), confirming that the FtsQ protein is required for early stages of septum formation. In addition, this phenotype is evidence supporting the conclusion that all three of the *ftsQ*::Tn*phoA*ΔIS*50*R mutations are lethal. We conclude that the *ftsQ* gene is essential for growth at 37°C.

Constitutive overexpression of the *ftsQ* gene product. To examine cell division under conditions where the FtsQ protein is likely to be made constitutively, each of the strains MJC265, MJC266, and MJC267 (containing *ftsQ*::Tn*phoA*ΔIS*50*R insertion mutations complemented by plasmid pMJC103), was grown in rich medium (LB) containing 0.2% L-arabinose. Each of these strains grew with a mass doubling time similar to that of the KS272/pMJC103 control strain that does not have an insertion mutation in the *ftsQ* gene. Under these growth conditions, strains MJC265 (Fig. 4e), MJC266, MJC267, and KS272/pMJC103 were all observed to have a longer average cell length than that of the wild-type control strain KS272 lacking plasmid pMJC103. Moreover, in the absence of the inducer L-arabinose, strains KS272/pMJC103 and KS272 had a similar cell length. Therefore, constitutive overexpression of the FtsQ protein results in an increase in cell length both in strains with *ftsQ*::Tn*phoA*ΔIS*50*R insertion mutations and in strains with the wild-type *ftsQ* gene.

Using FtsQ-AP fusion proteins, we can estimate the amount of FtsQ overproduction under these growth conditions. Strain KS272/pMJC107 (Fig. 1) is a strain which expresses an FtsQ-AP fusion protein under the control of the *araBAD* promoter. It produces about 400 U of AP enzymatic activity in LB medium with 0.2% L-arabinose. This is approximately a 120-fold increase over that produced by the same fusion protein in single copy, expressed from the native *ftsQ* promoter (3.3 U). We conclude that in LB medium, the *ftsQ* gene product can be dramatically overproduced without severely affecting cell division. These results are in basic agreement with an earlier report claiming that overproduction of the *ftsQ* gene product from the *tac* promoter has no effect on cell division (28), although in this case the amount of overexpression was not determined.

In contrast, we observe that constitutive overexpression of the *ftsQ* gene product in cells growing in a defined minimal medium has a profound effect on cell division. Strain KS272/pMJC103 was grown in M63 minimal medium containing 18 amino acids and 0.2% glycerol as the source of carbon. At approximately two mass doublings following the addition of 0.2% glucose to these cells, they appear to be growing and dividing normally (Fig. 4g). In a parallel culture, expression of the *ftsQ* gene product was induced by the addition of 0.2% L-arabinose. Within two mass doublings following the addition of inducer, cells accumulate as multiseptate filaments (Fig. 4h). A similar defect in cell division is observed when KS272/pMJC103 is grown continuously in minimal medium with 0.2% L-arabinose (data not shown). These filaments are similar to those produced when the *ftsQ* temperature-sensitive mutant is grown at a temperature at which cell division is only partially blocked (Fig. 4c). Therefore, overproduction of the *ftsQ* gene product also appears to cause a defect in cell division at a stage or stages beyond the initiation of septum formation.

Once again, we can use FtsQ-AP fusion proteins to estimate the amount of FtsQ overproduction in M63 minimal medium. Induction of AP enzymatic activity reaches a steady state in strain KS272/pMJC107 within 1 h after the addition of L-arabinose to cells growing in M63 glycerol minimal medium. Overproduction is about 70-fold over that produced by the same fusion protein in single copy, ex-

pressed from the native *ftsQ* promoter (3.4 U). This fold overproduction may be slightly lower than that seen for growth in rich medium above, suggesting that some other factor is responsible for the more profound effects of FtsQ overexpression seen in the minimal medium versus the rich medium.

DISCUSSION

The process of cell division involves the ordered structural alteration of the cell envelope. Therefore, it seems likely that some of the key proteins involved in this process will be membrane proteins. Membrane-spanning segments of bacterial proteins are usually made up of about 20 amino acids which are mostly uncharged or hydrophobic (11). Furthermore, the segment closely bordering the membrane on the cytoplasmic side usually has a net positive charge (31). The FtsQ protein has a hydrophobic stretch of amino acids from positions 25 to 49, bordered by two positively charged amino acid residues near the amino-terminal junction of this stretch and two negatively charged residues at the carboxy-terminal junction (25, 35). From this sequence, it can be predicted that the FtsQ protein is inserted in the cytoplasmic membrane with amino acids 50 to 276 exposed to the periplasm (Fig. 3).

Our results showing that nine different Tn*phoA* insertion sites from amino acids 50 to 212 of the FtsQ protein produce enzymatically active FtsQ-AP fusion proteins and that the FtsQ-AP fusion proteins with either 80 or 187 amino acids of the FtsQ protein are of high AP specific activity support this simple topology. The FtsQ-AP fusion protein with 50 amino acids of FtsQ consistently has about one half the absolute enzymatic activity of the eight later fusion proteins with 80 to 212 amino acids of FtsQ (Table 2). One explanation may be that 50 amino acids of FtsQ does not provide the full export signal. Alternatively, this particular fusion protein may have lower AP activity as a result of decreased expression or a context effect of the fusion junction on AP enzymatic activity. Additional support for the model we present for FtsQ protein topology comes from cell fractionations (our unpublished results) of normal cells making an FtsQ-AP fusion protein and the results of Storts et al. (28) who show that the native FtsQ protein in maxicells cofractionates with the inner membrane fractions of the cell.

The model for membrane topology of the FtsQ protein (Fig. 3) predicts that most of the protein is localized to the periplasm. We speculate, therefore, that the FtsQ protein may have a direct, perhaps enzymatic, role in cell envelope morphogenesis. The prediction of a small cytoplasmic domain also raises the possibility that the FtsQ protein could play a role in transmitting information relating to cell division between the cytoplasm and periplasm.

The amount of FtsQ-AP fusion protein made can be estimated from AP enzymatic activity, providing that the AP moiety is exposed to the periplasm and that the enzymatic activity of AP is not inhibited by attachment of sequences to its amino terminus. We think it likely that both these conditions are met, since the specific activity of at least two of the FtsQ-AP fusion proteins is as high as that of native AP (Table 2). Although our estimate of FtsQ copy number (about 22 copies per cell), based on comparisons of FtsQ-AP fusion proteins to a MotB-AP fusion protein, is only a rough estimate, it is clear that only a small amount of FtsQ protein is required for cell division. Furthermore, this estimate of FtsQ copy number is close to estimates of the copy number for PBP3 (27), a protein of similar topology (3) whose enzymatic activity is likely to be important at the cell septum.

The range of cell division phenotypes associated with altering the FtsQ protein by mutation indicates that FtsQ protein is involved directly or indirectly throughout the whole septation process. That FtsQ is involved in early stages of septum formation is indicated by the formation of nonseptate filaments due to insertional mutations in *ftsQ* (Fig. 4f) or growth of the *ftsQ* temperature-sensitive mutant at 42°C (Fig. 4d) (1). On the other hand, growth of the temperature-sensitive *ftsQ* mutant at 37°C (Fig. 4c) or in a different strain background at 42°C (30) as well as overproduction of FtsQ (Fig. 4h) results in formation of multiseptate filaments, a phenotype consistent with a defect in later stages of the septation process.

The multiseptate filaments formed as a result of these perturbations in FtsQ function are interesting in that the spacing between adjacent septa are clearly greater than unit cell length for the isogenic $ftsQ^+$ strain (Fig. 4a) and may be slightly greater than the unit cell length for the *ftsQ* temperature-sensitive strain growing at 30°C (Fig. 4b). One possible explanation for these results is that the rate of initiation of new septa is slowed relative to the rate of cell elongation due to the alteration in FtsQ activity. In contrast, the spacing between adjacent septa in the multiseptate filaments seen in either *ftsA* mutants (15) or the *envA* mutant (*22*) appears to be at unit cell length. In these mutants, it appears that early stages of septum formation occur normally and only later stages are affected.

The *ftsQ* gene is able to carry out its essential cell division function when expressed under the control of either the *tac* (28) or *araBAD* promoters. We observe that the consequence of altered expression of *ftsQ* depends upon the conditions under which cells are grown. In rich growth medium (LB), cell division is relatively normal, although average cell length is increased, under conditions where *ftsQ* gene product is overexpressed by about 120-fold (Fig. 4e). Since the *ftsQ* gene is thought to be translated with relatively low efficiency (21), note that the overproduction we observe may be dependent in part on the presence of an additional Shine-Dalgarno sequence from *araB* (7a), which is retained in our constructs (Fig. 1). In minimal medium (M63), by contrast, cells accumulate as multiseptate filaments when the *ftsQ* gene product is overexpressed, probably to a somewhat lesser extent (Fig. 4h). These multiseptate filaments are similar to those produced from inactivation of the temperature-sensitive *ftsQ* gene product at 37°C (Fig. 4c), conditions which might be expected to reduce, but not eliminate, the function of FtsQ protein. Therefore, at the phenotypic level, too much FtsQ protein appears to have the same effect as too little.

The different effects we see due to overexpression of the *ftsQ* gene product in rich versus minimal medium do not appear to be due to large differences in the ultimate level of overexpression. However, we did observe a slower rate of accumulation of AP enzymatic activity from FtsQ-AP fusion proteins upon induction in rich versus minimal medium (unpublished results), leading to the possibility that cells can adapt in some way to overexpression of the *ftsQ* gene product. Alternatively, factors such as growth rate, osmolarity, or presence of certain nutrients could explain the differences in the two media. Either the effects of different media or the increased basal level of expression from the *tac* promoter could account for the failure to see a cell division defect caused by constitutive overexpression of the *ftsQ* gene product in an earlier report (28).

The production of multiseptate filaments due to constitutive overexpression of the *ftsQ* gene product is different from the phenotypes seen as a result of overexpression of other cell division gene products. Overproduction of FtsA protein results in production of nonseptate filaments (32), even though a variety of *ftsA* mutants produce multiseptate filaments (7). Moderate levels of overexpression of the *ftsZ* gene product result in the production of minicells, presumably because of activation of cell division at the cell poles, which are the previous sites of cell division. Still higher levels of overexpression of *ftsZ* result in a complete block in cell division and the accumulation of nonseptate filaments (33). Other proteins which inhibit cell division can act through the *ftsZ* protein, resulting in the production of nonseptate filaments, either as part of a response to DNA damage (8) or as part of a mechanism to specify septum localization (6).

Since FtsQ protein produced under the control of foreign promoters is functional in vivo, we consider it unlikely that the *ftsQ* gene must be transcribed only at a specific time during the cell cycle, for instance to control the initiation of septum formation. We cannot rule out the possibility that under these conditions *ftsQ* production or activity is still tightly regulated in the cell cycle by mechanisms such as translational controls (21), interaction with other proteins, or posttranslational modification. Whether the normal complement of FtsQ protein or the overproduced amount is specially localized to sites of septum formation (septum and cell poles) remains an interesting question.

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