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# Two Polypeptide Products of the *Escherichia coli* Cell Division Gene *ftsW* and a Possible Role for FtsW in FtsZ Function

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**Two new mutations in the cell division gene *ftsW* have been isolated and characterized. The *ftsW263*(Ts) mutation results in a block to division at the initiation stage, similar to that previously observed with the *ftsW201*(Ts) mutation. The *ftsW1640*(Ts) mutation, however, causes a block to division at a later stage. The *ftsW201* and *ftsW263* mutants were shown to be phenotypically sensitive to the genetic background and growth conditions and are possibly *relA* dependent. Immunofluorescence microscopy showed that the FtsZ protein can localize to presumptive division sites in strains carrying *ftsW*(Ts) mutations at the nonpermissive temperature, suggesting that FtsW is unlikely to be specifically required for the localization of FtsZ to the division site. Examination of the localization of FtsZ in an *ftsW rodA* double mutant (lemon-shaped cells) revealed several classes of cells ranging from a common class where an FtsZ ring structure is absent to a class where FtsZ forms a complete ring at the midpoint of a lemon-shaped cell, suggesting a role for FtsW in the establishment of a stable FtsZ-based septal structure. We further demonstrate that two FtsW peptides, FtsWL (large) and FtsWS (small), can be identified and that the expression of *ftsWS* is sufficient for complementation of *ftsW*(Ts) mutations.**

The initiation of cell division in *Escherichia coli* involves the formation of a ring of the GTP-binding (9, 27, 29), tubulin-like (10, 27a) FtsZ protein around the inner membrane of the cell at the mid-cell division site (5, 24, 36). Other division proteins such as FtsA, FtsQ, and FtsI are believed to act after the formation of the FtsZ ring (1, 3). The product of the recently identified *ftsK* gene is believed to be required at a very late stage in division (4).

*ftsW* was originally identified and sequenced by Ikeda et al. (15, 16). The peptide sequence as deduced from the DNA sequence showed (i) homology to the *Bacillus subtilis* SpoVE protein, which is required for asymmetric division during sporulation, and (ii) similarity to the RodA protein, which is required for elongation and maintenance of the rod shape in *E. coli* (15, 25). Genetic analysis of the *ftsW201*(Ts) allele revealed that this mutation caused a block to division at an early stage (20), comparable to that caused in strains carrying the *ftsZ84*(Ts) allele, using the same genetic analysis (3). We therefore suggested a role for FtsW at the initiation stage of cell division which could involve an interaction with FtsZ (20). We also identified the FtsW peptide as the product of translation of *ftsW* from the first of two possible, and in-frame, start codons (15, 20). In this paper, we report on the isolation and characterization of two new *ftsW*(Ts) alleles, the identification of a smaller FtsW peptide, and the detection of FtsZ localization in *ftsW* mutants.

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## MATERIALS AND METHODS

**Bacterial strains and growth media.** All bacterial strains are derivatives of *E. coli* K-12 and are listed in Table 1. Oxoid no. 2 nutrient broth (NB), Luria broth (LB), and LB without added sodium chloride (LBNS) were used. Minimal medium agar (0.2 g of $MgSO_4 \cdot 7H_2O$, 2 g of citric acid, 10 g of $K_2HPO_4$, 3.5 g of $NH_4NaPO_4 \cdot 4H_2O$, and 1.5 g of agar per liter) supplemented with glucose (0.4%) and thiamine (1 μg $ml^{-1}$) was used. MMCAA is minimal medium agar with Casamino Acids (0.2%). Antibiotics were added when required at the following concentrations: ampicillin, 100 μg $ml^{-1}$; tetracycline, 10 μg $ml^{-1}$; chloramphenicol, 20 μg $ml^{-1}$; and kanamycin, 25 μg $ml^{-1}$. Standard molecular techniques were used throughout (30).

**Isolation of *ftsW263*(Ts) and *ftsW1640*(Ts) alleles.** Localized mutagenesis of the *mra* (*dcw*) region at 2 min on the *E. coli* chromosome was carried out by using hydroxylamine and a P1 lysate that was prepared on strain C600 *leu*::Tn*10*($Tc^r$) as described previously for the isolation of the *ftsW201*(Ts) allele (13, 20). The *ftsW263*(Ts) mutation was isolated in strain OV2, whereas *ftsW1640*(Ts) was isolated in strain W3110.

**Cloning and sequencing of *ftsW263* and *ftsW1640*.** We amplified *ftsW263* and *ftsW1640* from the genomes of strains MK263 and MK1640, respectively. Two primers were used in the amplification process: G3309 (−70 5′GCCTTGATGAATTCAAGAACT3′ −50) and 234X (+1485 5′TGTCCACCGGATCCGCCTGC3′ +1466). The base numbers are in reference to the first possible start codon (MET1) of the *ftsW* coding sequence, and the underlined sequences are restriction sites for *Eco*RI and *Bam*HI, respectively. Inocula of MK263 and MK1640 were grown overnight at 30°C in LB. Cells were pelleted from 10 μl of each culture and were washed three times with sterile distilled water before resuspension in a final volume of 100 μl of sterile distilled $H_2O$. Cell suspensions were boiled for 10 min before 20 μl was used as the substrate for DNA amplification. The amplification reaction mixture (100 μl) contained 200 μM each dATP, dTTP, dCTP, and dGTP, bovine serum albumin (100 μg $ml^{-1}$), 1× *Taq* polymerase reaction buffer, and 2 U of *Taq* polymerase (Promega). Thirty cycles of treatment at 94°C (1 min), 45°C (1 min), and 72°C (1 min) were applied to the reaction mixture with a Hybaid thermocycler. The ~1.4-kbp product from each reaction was purified and restricted with *Eco*RI and *Bam*HI and cloned into the *Eco*RI and *Bam*HI sites of the polylinker of M13mp19. Cloned *ftsW263* and *ftsW1640* were sequenced 5′→3′ by using a T7 sequencing kit. Mutations that were found were confirmed by repeating the amplification procedure from the genomes of both strains and subsequent sequencing of the mutation sites. This eliminated the possibility of those mutations having been introduced during the amplification procedure in vitro.

**Construction of plasmids.** Plasmids pUC19WWT, pNdW, and pJF118HEW have been described before (20). Plasmid pKHS1 (Fig. 1b) is isogenic to pUC19WWT (Fig. 1a) but carries a TAG (amber) codon at amino acid position 21 (TGG [tryptophan]) relative to MET1. The TAG codon was introduced by

TABLE 1. Bacterial strains used

| Strain | Relevant marker | Other marker(s) | Source or reference |
|---|---|---|---|
| W3110 | *sup*$^0$ | | Laboratory stock |
| W3110*leu*::Tn*9* | *leu*::Tn*9* | As for W3110 but *leu* Cm$^r$ | Laboratory stock |
| OV2 | *supF81*(Ts) | *ara*(Am) *lac*(Am) *galK*(Am) *galE trp*(Am) *ilv his thy tyrT leu tsx*(Am) *reA1 spoT1* | Laboratory stock |
| CF1944 | *relA251*::*kan* | W3110 background | M. Cashel (37) |
| OV2201Tn*9* | *ftsW201*(Ts) *leu*::Tn*9* | As for OV2 but Cm$^r$ | 20 |
| W3110-52 | *rodA5211*(Ts) | Tn*10*(Tet$^r$) in the 15-min region of the chromosome | P1(SP5211Tn*10*) (31) × W3110 |
| MED263 | *leu*::*Tn*10 *ftsW263*(Ts) | OV2 background, Tet$^r$ | This work |
| OV2263 | *leu*::Tn*10 ftsW263*(Ts) | As for MED263 | P1(MED263) × OV2 |
| OV2263Tn*9* | *leu*::Tn*9* | As for OV2263 but Cm$^r$ | P1(W3110*leu*::Tn*9*) × OV2263 |
| OV263R | *ftsW263*(Ts) *rodA5211*(Ts) | As for OV2263Tn*9*, Tet$^r$ | P1(W3110-52) × OV2263Tn*9* |
| W263 | *leu*::Tn*10 ftsW263*(Ts) | As for W3110 but *leu* Tet$^r$ | P1(MED263) × W3110 |
| MK263 | *ftsW263*(Ts) | As for W3110 | P1(W3110) × W263 |
| MD1640 | *leu*::Tn*10 ftsW1640*(Ts) | As for W3110 but Tet$^r$ | This work |
| W1640 | *leu*::Tn*10 ftsW1640(Ts)* | As for MD1640 | P1(MD1640 × W3110) |
| MK1640 | *ftsW1640*(Ts) | As for W1640 | P1(W3110) × W1640 |
| MK1640-52 | *ftsW1640*(Ts) | As for MK1640 but Tet$^r$ | P1(W3110-52) × MK1640 |

site-directed mutagenesis using custom-made oligonucleotide S2339 (+50 5′ GTATCCTGGTCTAGATCTCCACGG 3′ +73; base numbers are in reference to MET1, and the amber codon is underlined) and uracil-enriched single-stranded *ftsW* template M13W (20), following the method of Kunkel et al. (21, 22). Success of the mutagenesis was confirmed by DNA sequencing. A 1.8-kbp *Eco*RI-*Hin*dIII fragment was subcloned from successfully mutagenized M13W into the *Eco*RI and *Hin*dIII sites of pUC19 to give pKHS1. pKHS2 (Fig. 1d) is a clone of the smaller of the two open reading frames in *ftsW* and was constructed as follows. An *Nde*I recognition sequence was introduced at the second possible start codon (MET2) of *ftsW* by site-directed mutagenesis using custom-made oligonucleotide S6831 (+79 5′AAGGGCTGGCATATGGGCTCGC3′ +100). The base numbers are in reference to MET1, and the underlined sequence is the restriction site for *Nde*I. Thus, the smaller open reading frame, *ftsWS*, was subcloned as an *Nde*I-*Eco*RI fragment into the *Nde*I and *Eco*RI sites of pT7-7 (32) to produce pKHS2. A *Bgl*II-*Eco*RI fragment was subcloned from pKHS2 into pJF118HE (12) to give pKHS3 (Fig. 1e) in which the expression of *ftsWS* is driven by the isopropylthiogalactopyranoside (IPTG)-inducible $P_{tac}$ promoter. The *ftsWS* gene was overexpressed in strain BL21(DE3)pLysS (32) carrying plasmid pKHS2, in which the expression of *ftsWS* can be driven from the T7 promoter. Cells of BL21(DE3)pLysS carrying plasmid pET-3c (32), pNdW (20), pT7-7 (32), or pKHS2 were grown in Spizizen's salts medium supplemented with glucose (0.4%) and thiamine (1 μg ml$^{-1}$) at 37°C until they reached an optical density at 600 nm ($OD_{600}$) of 0.7. Then 0.5 ml was removed from each culture, and expression of the T7 RNA polymerase was induced by the addition of IPTG at a final concentration of 0.5 mM. Incubation was continued for 30 min before rifampin was added to a final concentration of 200 μg ml to inhibit host RNA polymerase. After 45 min, each of the samples was pulse-labeled with 5 μCi of [$^{35}$S]methionine (ICN Flow) for 5 min, after which cells were cooled on ice, harvested, and resuspended in 200 μl of sample buffer (20). Samples were incubated at 37°C for 2 h before being loaded on sodium dodecyl sulfate (SDS)–

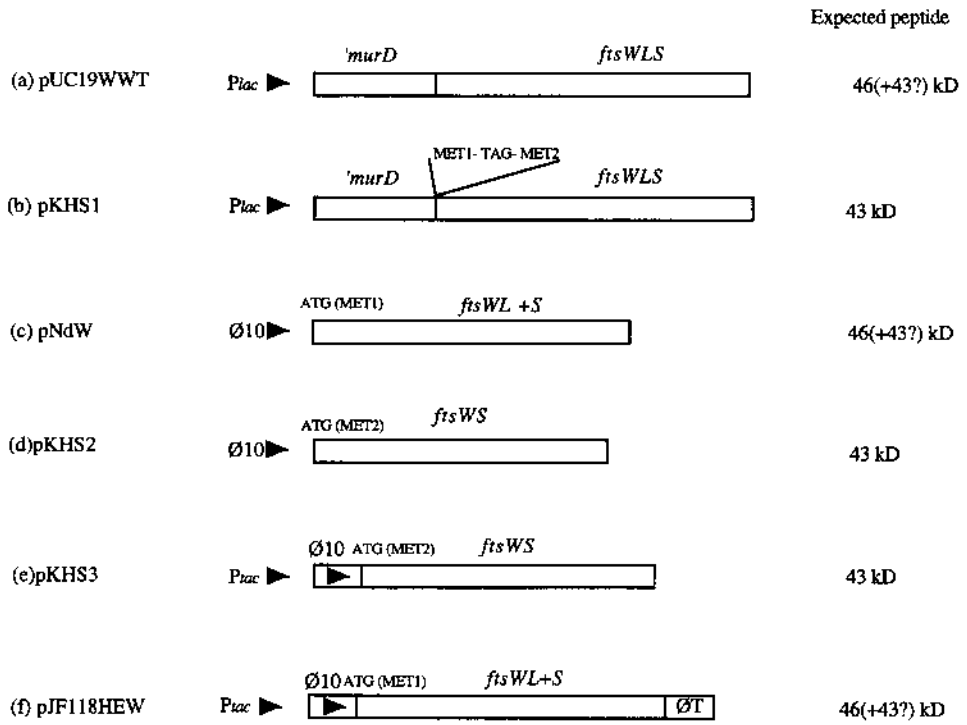


FIG. 1. Chromosomal inserts in plasmids used in this study. Arrowheads represent promoters, and ØT represents the T7 terminator. Plasmids a, c, and f have been described before (20) and are included for comparison.

TABLE 2. Growth of *ftsW* mutant strains at 30 and 42°C on different media

| Strain | Genetic background | Growth on plates containing[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | LB | | LBNS | | NB | | MMCAA | |
| | | 30°C | 42°C | 30°C | 42°C | 30°C | 42°C | 30°C | 42°C |
| OV2201*Tn9* | OV2, *ftsW201* | + | + | + | + | + | − | + | − |
| MK1 | W3110, *ftsW201* | + | + | + | + | + | + | + | − |
| OV2263Tn*9* | OV2, *ftsW263 leu*::Tn*9* | + | − | + | − | + | − | ND | ND |
| MK263 | W3110, *ftsW263* | + | + | + | − (43°C) | + | + | + | − |
| MD1640 | W3110, *ftsW1640 leu*::Tn*10* | + | − | − | − | + | − | − | − |
| MK1Δ*relA* | MK1, *relA*::*kan* | + | − | + | − | + | − | ND | ND |
| MK263Δ*relA* | MK263, *relA*::*kan* | + | − | + | − | + | − | ND | ND |
| OV2201Tn*9*(pKHS3) | OV2, *ftsW201* | ND | ND | ND | ND | + | + | ND | ND |
| OV2263Tn*9*(pKHS3) | OV2, *ftsW263* | ND | ND | ND | ND | + | + | ND | ND |
| MK1640(pKHS3) | W3110, *ftsW1640* | ND | ND | ND | ND | + | + | ND | ND |

[a] Inowla were streaked in duplicates and incubated at the indicated temperatures. +, growth; −, no growth; ND, not determined. In all cases of no growth, strains were complemented by plasmid pJF118HEW.

10% polyacrylamide gels. Gels were stained, destained, dried, and exposed to Dupont X-ray films for 12 h. Films were developed with an XGRAPH automatic developer to visualize labeled proteins.

**Localization of FtsZ by immunofluorescence.** We examined the localization of FtsZ in strains carrying the *ftsW201* mutation in two genetic backgrounds, OV2 (strain OV2201Tn*9*) and W3110 (strain MK1). An overnight culture of strain OV2201Tn*9* (in NB) was used to inoculate 25 ml of NB at a dilution of 1:500. The culture was grown in a shaking water bath (30°C) for 2 h, by which time the $OD_{600}$ had reached 0.03 to 0.05 and the cells were presumed to be in the exponential phase of growth. A 1-ml sample was taken from this culture and fixed immediately as described by Addinall et al. (1). The culture was then shifted to 42°C for 60 min, allowing for approximately two mass doublings. A 1-ml sample was taken at this point, and the cells were immediately fixed. The procedure for cell fixation and further immunofluorescence detection of FtsZ were the same as described recently (1). To prepare cells of MK1 for immunofluorescence microscopy, the same procedure was followed except that we used LB instead of NB for culturing strain MK1 since this strain does exhibit an *fts* phenotype in LB if shifted during exponential phase from 30 to 42°C (see below).

## RESULTS

**Characterization of *ftsW263*(Ts) and *ftsW1640*(Ts).** We have previously reported the isolation of the *ftsW201* allele, which, at 42°C, caused a block to division at an early stage (20). We used the same approach of localized mutagenesis to isolate two new *ftsW* mutant strains. Of several temperature-sensitive strains isolated and tested for complementation with plasmid pJF118HEW, only strains MED263 and MD1640 were complemented for growth on NB agar plates at 42°C and were further characterized. We demonstrated that the mutation in strain MED263 was linked to *leu* by cotransducing the mutation with *leu*::Tn*10* into a fresh OV2 background to give strain OV2263. The *leu*::Tn*10* in strain OV2263 was displaced by transducing the strain to chloramphenicol resistance by using a P1 lysate that was prepared on strain W3110 *leu*::Tn*9*, yielding strain OV2263Tn*9*. In all P1 transductions, *ftsW* alleles cotransduced with *leu* with a frequency in the range of 50 to 70%, consistent with the location of *ftsW* in the *dcw* cluster. Both strains OV2263 and OV2263Tn*9* were complemented with plasmid pJF118HEW for growth on NB agar plates at 42°C. Further characterization of the *ftsW263* allele in the OV2 background was carried out with strain OV2263Tn*9*. Strain OV2263Tn*9* is temperature sensitive for growth on LB, LBNS, and NB agar plates at 42°C (Table 2), and a temperature shift from 30 to 42°C of exponentially growing cultures of OV2263Tn*9* in LB results in a block to division and filamentation (Fig. 2a and b). Lysis was observed in the culture 2 h after the temperature shift. We examined whether the block in division in strain OV2263Tn*9* is also at the initiation stage, as we have reported previously for the *ftsW201* allele (20). To do this, we introduced the *rodA5211*(Ts) mutation (31) into strain OV2263Tn*9* by P1 transduction from strain W3110-52. Transductants were selected on LB-tetracycline agar plates at 30°C, were patched onto LB-tetracycline agar plates at 30 and 42°C, and incubated overnight. All transductants were temperature sensitive, as expected, and several were examined microscopically for loss of shape due to the *rodA5211* mutation. One such transductant, designated OV263R, was used in temperature shift experiments. Strain OV263R was grown in LB at 30°C, using a small inoculum from an overnight culture (LB, 30°C). The culture was shifted to 42°C when it reached an $OD_{600}$ of 0.05. After 90 min of incubation at 42°C, cells exhibited a lemon-like morphology (Fig. 2e) similar to that observed with the *ftsW201*(Ts) (20) and *ftsZ84*(Ts) (3) mutants, indicating a block to division at an early stage.

The *ftsW1640*(Ts) mutation was isolated in the prototrophic W3110 strain. The original isolate, strain MD1640, was temperature sensitive for growth on LB and NB agar plates at 42°C and was not viable on LBNS plates at either 30 or 42°C (Table 2), although revertants appeared on LBNS agar plates at 30°C with low frequency. The *ftsW1640* allele was moved into a fresh W3110 background by P1 transduction using linkage to *leu*::Tn*10*, yielding strain W1640. Strain MK1640 was constructed by transducing strain W1640 to $leu^+$, using a P1 lysate that was prepared on strain W3110, and screening for Ts transductants. In all P1 transductions, the Ts mutation cotransduced with *leu* at a frequency of 50 to 70%, which is consistent with the location of *ftsW* in relation to the *leu* operon. A temperature shift of exponentially growing cells of MK1640 in LB from 30 to 42°C resulted in filamentation (Fig. 2c and d), and lysis was observed 2 h after the temperature shift. We introduced the *rodA5211*(Ts) allele into strain MK1640 by P1 transduction (as described above for strain OV2263Tn*9*) to give strain MK1640-52. The predominant phenotype of strain MK1640-52 in LB after a temperature shift for 90 min from 30 to 42°C is indicative of a late block to division (Fig. 2f) similar to that observed with *ftsQ*, *ftsA*, and *ftsI* mutants (2), although some lemon-shaped cells were also present. At 42°C, cells of strain MK1640-52 also produced irregular morphologies such as short fat filaments and lemon-shaped cells with long filamentous tails (data not shown). In all backgrounds used, strains carrying either the *ftsW263*(Ts) or *ftsW1640*(Ts) allele were complemented for growth and division under nonpermissive conditions by plasmids pJF118HEW and pU19WWT but not by cloning vectors pJF118HE and pUC19, suggesting that the mutations lie within *ftsW*.

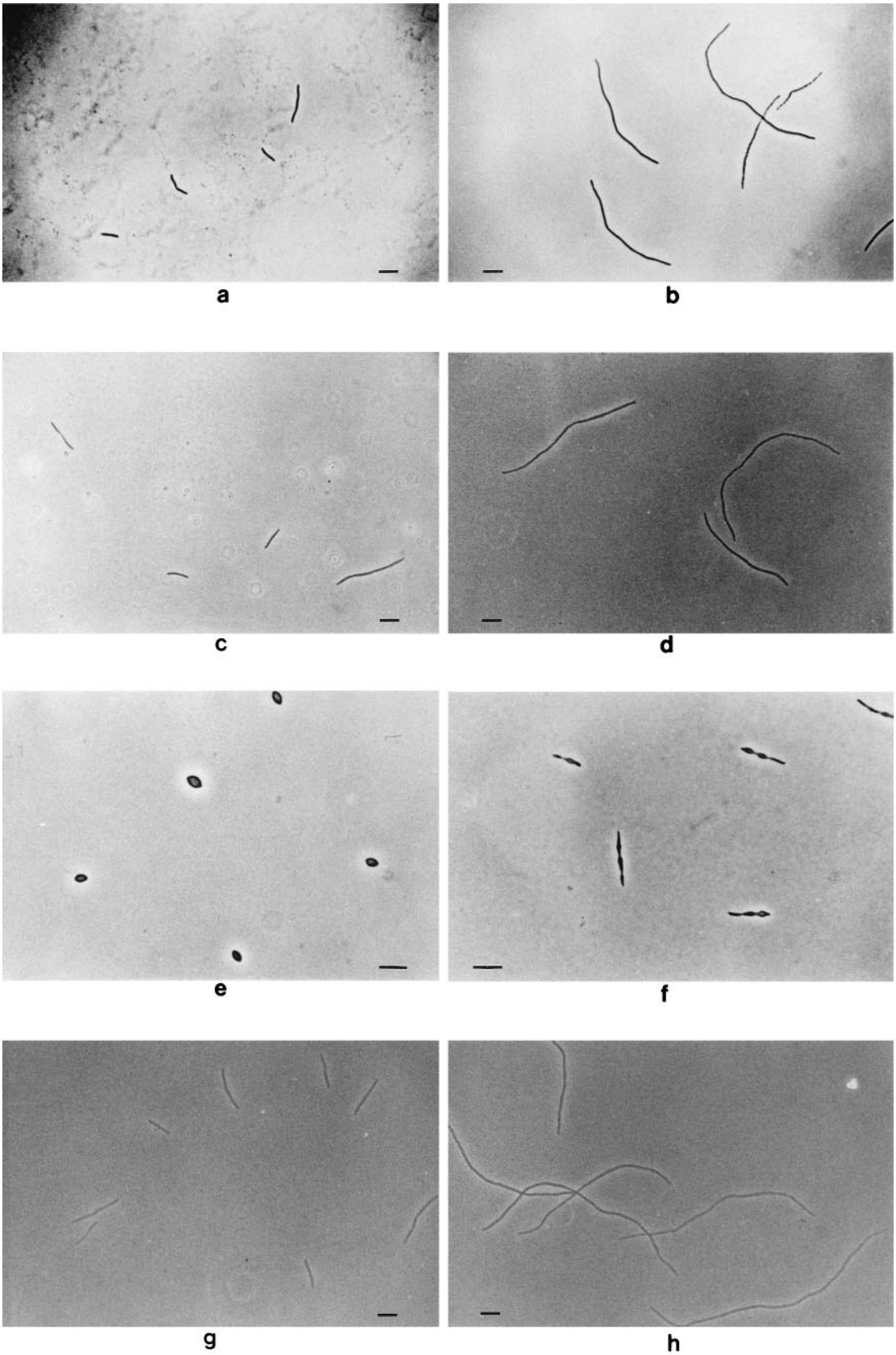


FIG. 2. Micrographs of *E. coli* cells grown in LB. (a and b) Strain OV2263Tn*9* (*ftsW263*) at 30°C (a) and 42°C (b); (c and d) strain MK1640 (*ftsW1640*) at 30°C (c) and 42°C (d); (e) strain OV263R at 42°C; (f) strain MK1640-52 at 42°C; (g and h) strain MK1 (*ftsW201*) at 30°C (g) and 42°C (h). Photographs were taken after 1.5 h of incubation at the indicated temperatures. Bars, 5 μm.

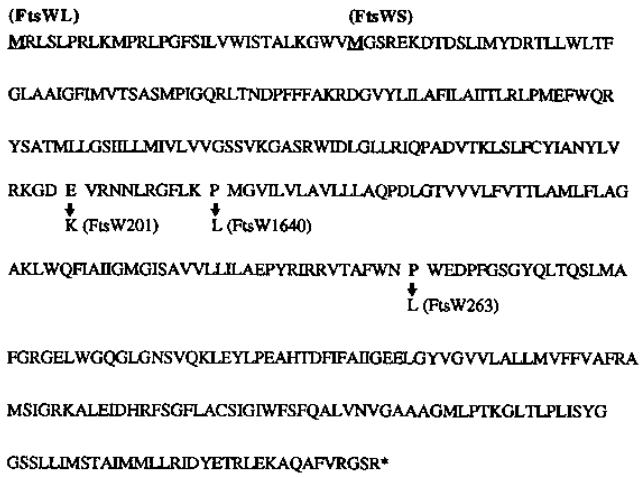


FIG. 3. Amino acid sequence of FtsWLS. The two start methionines are shown in boldface and underlined, and changes due to mutations *ftsW201*, *ftsW263*, and *ftsW1640* are indicated.

**The *ftsW201*(Ts) and *ftsW263*(Ts) phenotypes are *relA* sensitive.** Upon attempting to transduce the *ftsW201* allele into strain W3110, we failed to recover any temperature-sensitive transductants by screening on NB agar plates at 30 and 42°C. However, by screening transductants on MMCAA plates, temperature-sensitive division mutants were recovered with the expected linkage to $leu^+$ (approximately 70%). This showed that differences between the genotypes of strains OV2 and W3110 influence the phenotype of the *ftsW201* allele. Strain OV2 (Table 1) carries *relA1* and *spoT1* mutations. We therefore introduced a *relA*::*kan* deletion into strain MK1 (one of the temperature-sensitive transductants obtained on MMCAA plates) by P1 transduction (Table 1). The introduction of the *relA*::*kan* deletion made strain MK1 temperature sensitive on LB, LBNS, and NB agar plates. Similarly, the *relA*::*kan* deletion made strain MK263 temperature sensitive on LB and NB agar plates. Strains MK1Δ*relA* and MK263Δ*relA* were complemented by plasmid pJF118HEW but not by the cloning vector pJF118HE (Table 2). These results suggest that the phenotypes of *ftsW201* and *ftsW263* are in some way sensitive to the levels of (p)ppGpp in the cell. Interestingly, although strain MK1 is not temperature sensitive for growth on LB agar plates at 42°C, a shift of exponentially growing cells in LB from 30 to 42°C ($OD_{600}$ of ≤0.05) resulted in filamentation (Fig. 2g and h), suggesting that the *ftsW201* phenotype is also sensitive to differences between solid and liquid media in the W3110 background.

**Sequencing of *ftsW263* and *ftsW1640*.** We cloned both of the new *ftsW* alleles in M13mp19 and sequenced them as described in Materials and Methods. We found that the mutation in *ftsW263* is a single-base substitution (C→T) in codon 253 (CCG→CTG; $Pro_{253}$ to $Leu_{253}$). A similar mutation was found in *ftsW1640* in codon 181 (CCG→CTG; $Pro_{181}$ to $Leu_{181}$ [Fig. 3]). The mutations are consistent with the mutagenic agent being hydroxylamine.

**The identification of FtsWS.** We have previously demonstrated that translation of *ftsW* from MET1 results in the production of a single, detectable, stable peptide with an apparent molecular size of 32 kDa (20). We will refer to this peptide as FtsWL (large) to distinguish it from a possible smaller peptide with the start codon MET2, which we will refer to as FtsWS. We also demonstrated that pJF118HEW is capable of fully complementing all three temperature-sensitive *ftsW* mutants (Table 2). However, we could not eliminate the possibility that complementation is due to small and otherwise undetectable amounts of FtsWS being produced from plasmid pJF118HEW. We therefore constructed plasmid pKHS1, in which the expression of *ftsW* is driven from $P_{lac}$. pKHS1 is isogenic to plasmid pUC19WWT except for the presence of a stop codon TAG (amber) instead of the codon TGG ($Trp_{21}$). Therefore, in a suppressor-free strain ($sup^0$), the translation of *ftsWL* would be terminated at this stop codon whereas the translation of *ftsWS* should, in theory, take place. Strain MK1640($sup^0$) was transformed with pUC19, pUC19WWT, and pKHS1 and growth tested at 42°C on NB agar plates. As expected, the cloning vector pUC19 did not complement strain MK1640, whereas both pUC19WWT and pKHS1 did complement the strain for growth and division at the nonpermissive temperature. Thus, FtsWS is fully functional in complementing this mutation.

We proceeded to attempt the specific overexpression of *ftsWS*. Toward this end, we constructed plasmid pKHS2, in which the start codon of *ftsWS* (MET2) was fused to the T7 gene 10 promoter and ribosome-binding site. Both pNdW and pKHS2 were transformed into strain BL21(DE3)pLysS (32), in which IPTG induces the expression of T7 RNA polymerase, and overexpression experiments were carried out as described previously (20, 32). Plasmid pNdW produced a peptide with an apparent mobility corresponding to 32 kDa (Fig. 4, lane 2), as previously observed for FtsWL (20). Plasmid pKHS2 produced a smaller peptide of between 30 and 31 kDa in size (Fig. 4, lane 4) as calculated from SDS-polyacrylamide gel electrophoresis (PAGE) analysis. The theoretical difference in size between FtsWL and FtsWS is 3.4 kDa (15), which is larger than that calculated from the SDS-PAGE data. We have demonstrated previously that FtsWL has an unusual mobility on SDS-PAGE and that the calculated sizes of FtsW peptides (fused to T7 the gene 10 protein or with internal deletions) were not in agreement with their mobilities on SDS-PAGE (20). Since plasmid pKHS2 was engineered to effect translation from MET2 and since the peptide product is clearly smaller than that produced from pNdW, we assume that this peptide is FtsWS.

**Localization of FtsZ to the division site in the *ftsW201* mutant.** Immunofluorescence microscopy has recently been used to demonstrate that the FtsZ protein in both *B. subtilis* and *E. coli* is present at the division site (1, 23). This is in agreement with the established role of FtsZ, in particular the ability of this

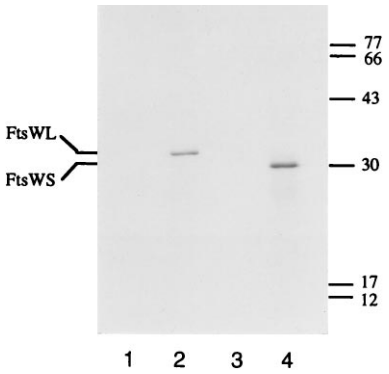


FIG. 4. Overexpression of *ftsWL* and *ftsWS*. Shown are autoradiograms of polypeptides pulse-labeled with [$^{35}$S]methionine and separated by SDS-PAGE as described previously (20). Lane 1, pET-3c; lane 2, pNdW; lane 3, pT7-7; lane 4, pKHS2. Numbers on the right indicate the molecular sizes (in kilodaltons) of protein markers.

protein to form a ring structure at the division site which remains at the leading edge of the septum during division (24). Since the *ftsW201* and *ftsW263* mutations result in an early block to division, we decided to examine strains carrying the *ftsW201* mutation for the location of FtsZ at permissive (30°C) and nonpermissive (42°C) temperatures. FtsZ was detected at division sites as a line of fluorescence across the width of the cell in strain OV2201Tn*9* at 30°C, with most cells having one ring (Fig. 5a and b). These micrographs also show a filament at 30°C with two (possibly three) Z rings, demonstrating that the *ftsW201* allele is partially defective even at the permissive temperature. At 42°C, the Z ring was also detected in OV2201Tn*9* filaments (Fig. 5c and d), and the distribution of rings in cell units ranged from one ring per cell to six rings per cell, with the majority of filaments containing one to three rings per filament (data not shown). The pattern of Z-ring formation was very similar in strain MK1, where the majority of cells had one Z ring per unit at 30°C (Fig. 5e and f) but a range of Z-ring distribution in filaments at 42°C, with the majority of filaments having one to four rings per filament (Fig. 5g and h). Therefore, although these cells form smooth-sided filaments at 42°C, in contrast to *ftsQ*, *-A*, and *-I* mutants (3, 20), Z-ring formation appears to be unaffected. These results suggest that it is unlikely that FtsW is required specifically for the localization of FtsZ to the division site.

We then examined the localization of FtsZ in an *ftsW rodA* double mutant. For this purpose, we looked at the recently isolated *ftsW263* allele in conjunction with the *rodA5211* allele. Strain OV263R was grown in LB at 30°C, and exponentially growing ($OD_{600}$ of 0.05) cells were shifted to 42°C for 90 min, which is the time required for the full development of the *fts rodA* double-mutant phenotype. Cells, which were lemon shaped, were immediately fixed and processed for immunofluorescence staining as described by Addinall et al. (1). The lemon-shaped phenotype is, so far, unique to the *ftsZ84 rodA*, *ftsW201 rodA*, and *ftsW263 rodA* mutants and contrasts with the phenotypes of strains with *ftsA*, *ftsQ*, *ftsI*, and *ftsK* mutations combined with the *rodA* mutation. We observed lemon-shaped cells in the *ftsW263 rodA* strain at 42°C, differing in the degree of localization of the FtsZ protein. The most common class (50 to 60%) was lemon-shaped cells with diffused fluorescence but without a Z ring (Fig. 5i and j), but we also found lemon-shaped cells which appeared to have a full Z ring around the mid-cell (Fig. 5k and l). Between these two extremes, we observed localization of the FtsZ protein ranging from a single spot to half a ring (an arc) spanning half the circumference of the cell (data not shown).

## DISCUSSION

The earliest detectable sign of initiation of division in *E. coli* is the formation of the Z ring at the division site. The formation of the Z ring was first detected by Bi and Lutkenhaus, using immunogold labeling coupled with electron microscopy (5). Subsequent studies from the same laboratory demonstrated that in *ftsQ*, *ftsA*, and *ftsI* division mutants, regularly spaced Z rings form at the nonpermissive temperature, confirming that the actions of FtsA, FtsQ, and FtsI in the division process are required after that of FtsZ (1). This is in agreement with earlier genetic studies in which *fts* mutations were combined with the *rodA5211*(Ts) mutation to enhance the effect of septum formation on morphology (3).

In a previous study, we reported the isolation of the *ftsW201* (Ts) allele, which caused an early block to division like that seen when FtsZ action is blocked. This finding, combined with the hypersensitivity of the strain OV201Tn*9* to increased levels of FtsZ, led us to suggest that interaction between FtsW and FtsZ may take place during the initiation stage of cell division (20). In the current study, we have demonstrated that a new allele, *ftsW263*, also results in an early block to division, whereas another new allele, *ftsW1640*, causes a block to division after the initiation stage. Although the localization of FtsW in the membrane has not been demonstrated, the hydrophobicity profile of FtsW suggests very strongly that it is an integral membrane protein (15). The requirement for FtsW at the initiation of division raised the possibility that it functions to localize FtsZ; that is, it could be a membrane target which FtsZ would recognize to initiate ring formation. However, no homolog of FtsW is present in the completely sequenced genome of the peptidoglycan-lacking prokaryote *Mycoplasma genitalium*, although this genome does contain an *ftsZ* homolog (11). The absence of an FtsW homolog in *M. genitalium* argues against FtsW being absolutely essential for the localization of FtsZ to the division site.

We therefore examined the localization of FtsZ in strains OV2201Tn*9* and MK1, both of which carry the *ftsW201*(Ts) allele. We have demonstrated that the Z ring can form in these mutants under nonpermissive conditions, making it unlikely that FtsW is required for the targeting of FtsZ to the division site. We further examined the localization of FtsZ in *ftsW263 rodA* double mutants, where lemon-shaped cells form at the nonpermissive temperature with no evidence of cell constriction, in clear contrast to the phenotypes of *rodA* cells carrying the *ftsA*, *ftsQ*, *ftsI*, or *ftsK* mutation. Most of these lemon-shaped cells showed little or no FtsZ localization, but lemon-shaped cells with FtsZ structures ranging from a spot to a full ring were also found. The contrast between the *ftsZ84 rodA* (2), *ftsW201 rodA* (20), and *ftsW263 rodA* (this work) phenotypes and the other *fts* (*ftsI*, *-Q*, and *-A* [2] and *ftsW1640* [this work]) *rodA* phenotypes may reflect the stability of the FtsZ-based septal structure. That is, the presence of regularly spaced constrictions in an *fts rodA* double mutant (as in the case of *ftsI*, *ftsQ*, *ftsA*, *ftsK*, and *ftsW1640*) suggests resistance of the division site to the loss of cell shape caused by the *rodA*(Ts) mutation. In contrast, a lemon-shape phenotype (as in the case of *ftsZ84*, *ftsW201*, and *ftsW263*) demonstrates that the division site is not resistant to the *rodA*(Ts)-mediated loss of shape and that a stable FtsZ-mediated septal structure is difficult to achieve. These observations allow us to make a distinction between three events in the initiation of division. The first is the localization of FtsZ to the membrane, which would theoretically involve specific recognition of a target molecule (as yet unknown) by FtsZ, which represents the nucleation site (2, 24). The second stage of initiation would involve the polymerization of FtsZ to form a ring, and then the third stage of initiation would be constriction of the FtsZ-based structure. Constriction can, however, be initiated before polymerization is complete (2). We are suggesting that FtsW is required for constriction of the Z ring to begin. A role for FtsW in the stabilization or constriction of the FtsZ ring would agree with our observations on the *ftsW201* and *ftsW263* mutants thus far (but does not exclude the involvement of other division proteins in this process). Recently, Addinall et al. (1) reported that in fast-growing *E. coli* cells, the FtsZ ring can be detected in 92% of cells, whereas only 45% of cells showed invagination of the cell wall, and suggested that a time period is required for the maturation of the FtsZ ring into an active complex involving other division proteins. Our analysis of the phenotypes of the *ftsW201* and *ftsW263* alleles agrees with such a notion and suggests that FtsW could be central to the formation and activation of such a complex or "divisome." The *ftsW1640*(Ts) mutation, however, appears to block division at a later stage

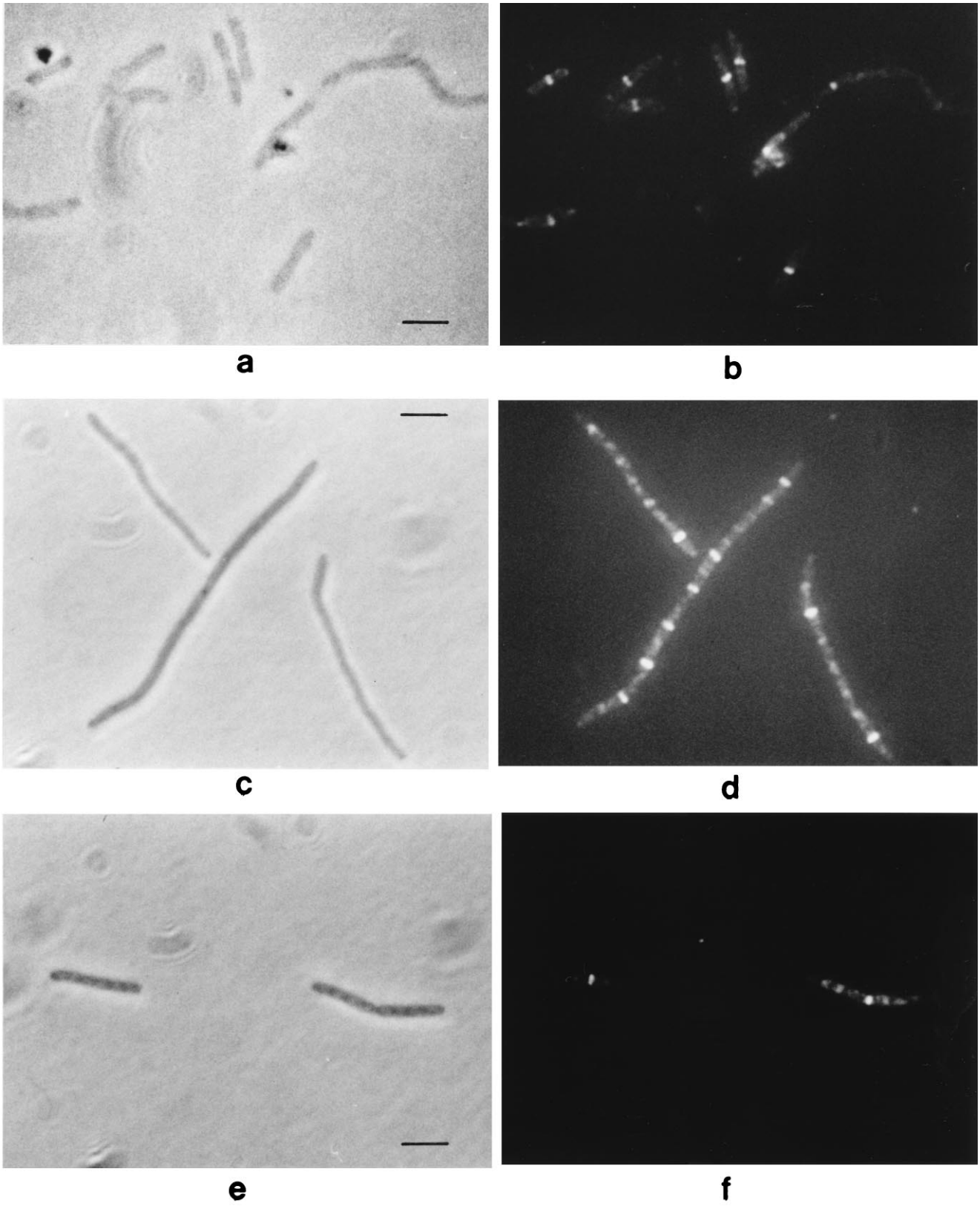


FIG. 5. FtsZ localization in *ftsW* and *ftsW rodA* mutants. Panels a, c, e, g, i, and k are phase-contrast micrographs; panels b, d, f, h, i, and j are corresponding immunofluorescence micrographs. Shown are strain OV2201Tn*9* (*ftsW201*) at 30°C (a and b) and at 42°C (c and d), strain MK1 (*ftsW201*) at 30°C (e and f) and at 42°C (g and h), and strain OV263R (*ftsW263 rodA*) at 42°C showing no Z ring (i and j) and a Z ring (k and l). Bars, 5 μm.

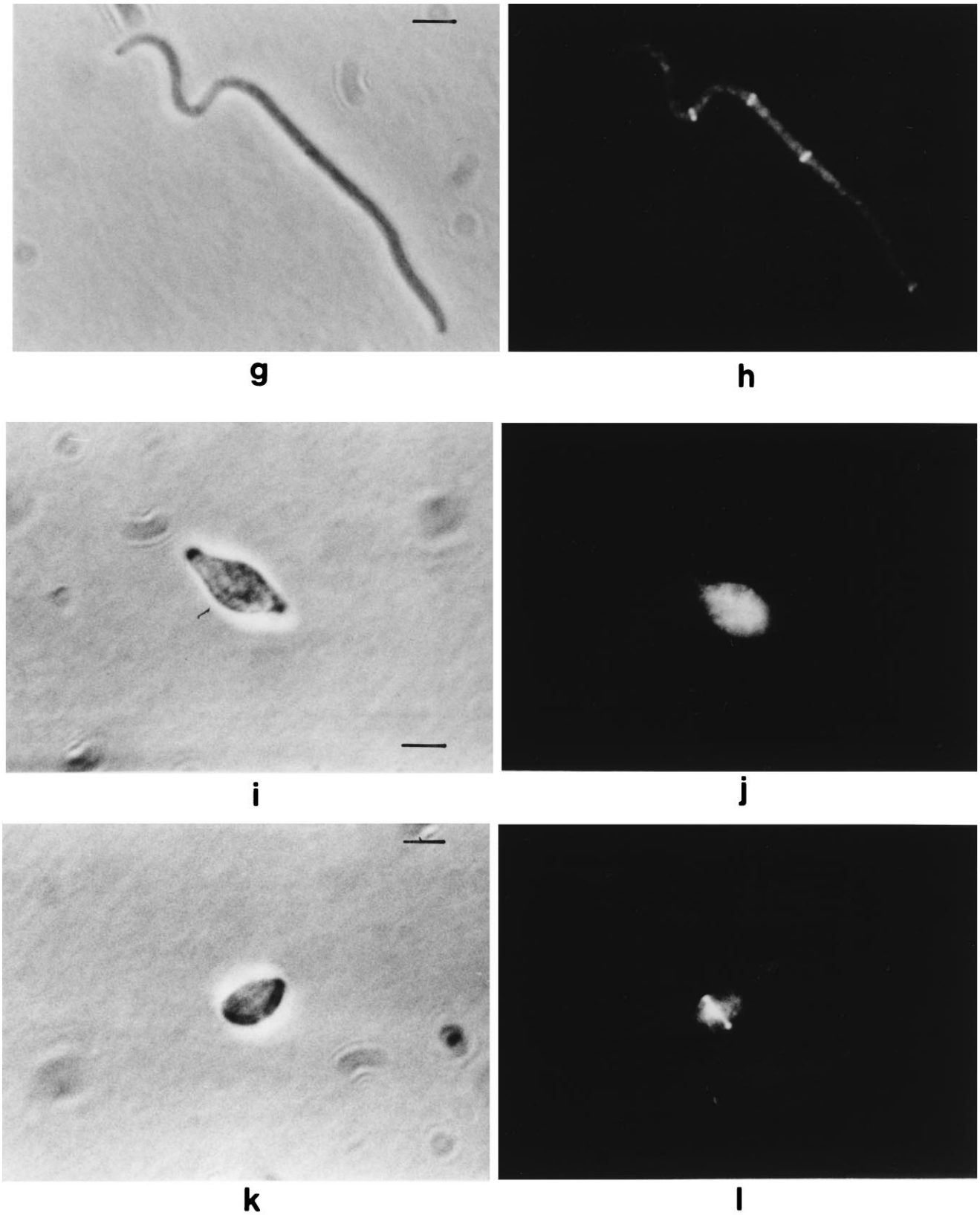


FIG. 5—*Continued.*

than that caused by either the *ftsW201*(Ts) or the *ftsW263*(Ts) mutations, and therefore the division block in this mutant is presumed to be postinitiation, suggesting that the full function of FtsW is required throughout septation and not only at the initiation stage. We have previously suggested that FtsW is unlikely to act in concert with PBP3 (as was hypothesized by Ikeda et al. [15]) because the *ftsW201* mutation results in an early block to cell division (20) unlike that which is caused by mutations in the *ftsI* gene which encodes PBP3 (3). However, our finding that a new mutation in *ftsW*, *ftsW1640*, results in a postinitiation block to cell division does allow for the possibility of interaction between FtsW and other late-acting division proteins, including PBP3. Topology prediction using TopPredII (8) suggests that the *ftsW201*, *ftsW263*, and *ftsW1640* mutations lie in regions of the gene encoding cytoplasmic, periplasmic, and transmembrane sections of the protein, respectively. The locations of the various mutations which we have isolated may reflect the way in which this protein performs its function. Further work is under way to determine the topology of the corresponding amino acids in FtsW protein in the cell membrane.

The *ftsW201* and *ftsW263* mutations demonstrated sensitivity to both genetic background and growth conditions. Because strain OV2 carries the *relA1* and *spoT1* alleles, we investigated whether the phenotypes of *ftsW201* and *ftsW263* were *relA* sensitive. We have demonstrated this to be the case. The *relA* gene codes for the RelA protein, which is a (p)ppGpp synthetase, while the SpoT protein, encoded by *spoT*, is believed to be a bifunctional enzyme mainly required for the hydrolysis of (p)ppGpp but also capable of synthesizing the nucleotide (14, 37). Although many uncertainties remain about the exact role of (p)ppGpp in *E. coli*, the broad consensus is that these nucleotides regulate the transcription of rRNA genes. The effect of (p)ppGpp is most evident during amino acid starvation, when accumulation of this nucleotide results in inhibition of transcription of rRNA genes and consequently affects ribosomal synthesis, protein synthesis, and growth rate in what is known as the stringent response (6, 7). A link between the levels of (p)ppGpp and cell division whereby (p)ppGpp could act as a positive regulator of the expression of one or more of the division genes has been proposed. This hypothesis is based on the observations that (i) stable spherical *E. coli* cells which can be obtained by inactivation either of *pbpA* or of *rodA* show unusually high levels of (p)ppGpp (19, 34) and (ii) overexpression of either *ftsQAZ* (but not *ftsQA*) or *relA* does result in stable spherical growth in cells challenged with mecillinam, a specific inhibitor of PBP2 (35). *ftsW201* and *ftsW263* mutants show *relA* sensitivity on rich medium plates, which suggests a sensitivity to (p)ppGpp under these conditions. We cannot offer an explanation for this *relA* sensitivity or for the sensitivity of the *ftsW201* and *ftsW263* to growth conditions other than that (p)ppGpp, directly or indirectly, might influence the function of the FtsW protein(s) and/or the expression of the *ftsW* gene. A study of the activity and regulation of *ftsW* promoter under various growth conditions and in different *relA* backgrounds is necessary before any firm conclusions can be drawn.

We have previously identified the FtsW peptide (414 amino acids) by engineering the translation of the *ftsW* message from MET1 (20). This peptide appeared to be functional, as demonstrated by complementation of *ftsW* mutations. However, we could not eliminate the possibility that a trace amount of FtsWS, too low to be visible on gels, was actually responsible for this complementation. In the present study, we have investigated the existence of a smaller FtsW protein. By using an approach similar to that which we have employed previously, we were able to overexpress and detect a smaller FtsW peptide (384 amino acids), the translation of which begins at MET2. FtsWS is capable of complementing all of our *ftsW*(Ts) mutations. In addition, initiation of translation from MET1 is not compulsory for initiation of translation at MET2, as shown by the ability of pKHS1, in which translation from MET1 is terminated by an amber mutation, to complement the *ftsW1640* allele in a $sup^0$ genetic background. Whether both proteins are produced in normal cells and whether they have individual roles in the division process remain to be seen.

From our previous and current studies on the *ftsW* gene and its products, we conclude that one or another form of FtsW is required during the initiation of division, that it contributes to the stabilization of the FtsZ ring during initiation, and that FtsW is probably required throughout septation since the *ftsW1640* mutation causes a postinitiation block to cell division. Our interpretations are derived from the data which we have obtained in analyses of temperature-sensitive division mutants. Theoretically, we cannot rule out that the mere presence of one or more known Fts proteins may indeed be required for the localization of FtsZ to division sites, particularly since little is known about the stability and the function of temperature-sensitive Fts proteins. Future experiments using different or improved tools may allow for different interpretations.

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