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FtsA Is Localized to the Septum in an FtsZ-Dependent Manner

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The localization of the cell division protein FtsA in *E. coli* was examined. FtsA was found to localize to the septum in a ring pattern as previously shown for FtsZ. The localization of FtsA was completely dependent on the localization of FtsZ. Under a variety of conditions that prevented formation of the Z ring, FtsA was unable to localize. In mutants where FtsZ forms structures in addition to Z rings, the pattern of FtsA duplicated these structures. These results suggest that the Z ring recruits FtsA to the septum.

Cell division in *Escherichia coli* is a complex process that involves the formation of a septum at the correct time and place to generate newborn cells that are nearly equivalent (20, 22). Through genetic studies, a number of genes—*ftsA, ftsI, ftsL, ftsQ, ftsN, ftsW*, and *ftsZ*—which are essential for this process have been identified (8). Among these genes, *ftsZ* and *ftsA* encode cytoplasmic proteins whereas the others encode membrane proteins. A key problem in the study of cell division is how the activities of these products are spatially regulated to produce a septum.

A crucial component of the division apparatus in bacteria is the Z ring, which is formed by the tubulin-like protein FtsZ (7). The Z ring localizes to the predivisional site and contracts during septation at the leading edge of the septum. It has been suggested that the Z ring is a cytoskeletal element formed through self-assembly, which localizes septal growth (7, 28). Consistent with this model, FtsZ has been shown to undergo GTP-dependent assembly into filaments in vitro (12, 23, 30), and an *ftsZ* mutant that produces Z spirals, as well as rings, has spiral and normal-shaped septa (2).

Genetic evidence suggests that FtsA may also be a component of the septum, possibly interacting with FtsZ as well as the *ftsI* gene product, PBP3 (33, 34). FtsA has homology to the ATPase domain of a diverse set of protein families that include actin, DnaK, and sugar kinases, suggesting that it is an ATPase (11). Although an ATPase activity for FtsA has not been demonstrated, about 30% is phosphorylated in vivo at a position corresponding to the autophosphorylation site of DnaK (31). However, the importance of this phosphorylation and the biochemical function of FtsA remain to be elucidated.

Morphological evidence indicates that *ftsZ* acts earlier than other known cell division genes (5, 32). Consistent with this, examination of FtsZ localization in several *fts* mutants suggests that Z ring biogenesis is independent of *ftsA, ftsI*, and *ftsQ*, indicating that the products of these genes act after the Z ring is formed (1). However, the ratio of FtsZ to FtsA is important for cell division to occur (17, 21). If either protein is in excess, division does not occur, whereas if the levels of both are high, excess division occurs in the form of minicells.

To determine if additional proteins are localized to the division site and if the Z ring functions to recruit these proteins to the septum, we examined the localization of FtsA in wild-type cells and a variety of mutants. We found that FtsA was localized to the septum and that this localization was dependent upon Z ring formation.

MATERIALS AND METHODS

Bacterial strains and plasmids. (i) Strains. MC4100T (*leu*::Tn*10*), MCZ84 [*leu*::Tn*10 ftsZ84*(Ts)], MCZ26 [*leu*::Tn*10 ftsZ26*(Ts)], MCA6 [*leu*::Tn*10 ftsA6* (Ts)], MCA12 [*leu*::Tn*10 ftsA12*(Ts)], MCA27 [*leu*::Tn*10 ftsA27*(Ts)], MCQ1 [*leu*::Tn*10 ftsQ1*(Ts)], and MCI23 [*leu*::Tn*10 ftsI23*(Ts)] are all derivatives of *Escherichia coli* K-12 strain MC4100 (7, 14) and were described previously (9, 18). BEF4 F′ *lacI*^{q} Tn*9* (λKD5) was constructed from BEF4 (16), an *srl*::Tn*10, recA56* derivative of the wild-type *E. coli* K-12 strain W3110 (17). JFL110 F′*lacI*^{q} Tn*9* was used as a donor to transfer F′ *lacI*^{q} Tn*9* (37) into BEF4 by spot-mating (29). The resulting strain, BEF4 F′, was lysogenized with λKD5, which contains an IPTG-inducible copy of the *ftsZ* gene (17). Strain KJB24 is a *rodA*$_{sui}$ derivative of W3110 (6). JKD31(pKD3C) carrying a *kan* insertion in the chromosomal copy of *ftsA* was constructed basically as described previously for a strain in which *kan* was inserted in *ftsZ* (16). The *kan* element from pUC-4K was cloned into the *Bam*HI site located just inside the 5′ end of the *ftsA* gene in plasmid pZAQ (37). The resultant plasmid, pZAQ-AK, was linearized and used to transform V355.594 (*recD*) (pKD3C) to kanamycin resistance in the presence of ampicillin. Kanamycin-resistant colonies were screened for temperature sensitivity in the absence of ampicillin. Such colonies were candidates for having the *kan* insertion in *ftsA* on the chromosome or the plasmid. One of these strains was complemented by pZAQ but not pZAQ-AK, confirming that *ftsA* was disrupted. The *ftsA*::*kan* was transduced to MC4100(pKD3C), which was made *recA56* by cotransduction with *srl*::Tn*10* with P1 grown on JC10240 (16). All strains were grown in L broth as previously described (7, 18).

(ii) Plasmids. Plasmid pKD3C carries the *ftsA* and *ftsZ* genes on a temperature-sensitive replicon (15). It was constructed by replacing the ampicillin resistance gene on pKD3 with the chloramphenicol resistance gene from pBR325 (16). Plasmid pFAD38 carrying an inducible *sulA* gene was constructed as follows. The *sulA* gene was cloned by PCR into the vector pQE-32 (Qiagen), such that *sulA* was fused in frame to the six-histidine "tag" to give pFAD37. The six-histidine-tagged *sulA* gene was excised from pFAD37 (excluding the p_{TAC} promoter) and inserted into pBAD18 (24), putting it under the control of the p_{BAD} promoter, to give pFAD38. A control plasmid, pFAD8, was constructed by cloning the equivalent region (encoding the six-histidine tag and the polylinker) from pQE-32 into pBAD18. MC4100T containing pFAD38 formed long filaments after induction for 1 h with 0.1% arabinose, whereas MC4100T containing either pBAD18 or pFAD8 continued to divide normally under these conditions.

FtsA antisera. To obtain FtsA for raising antisera, the *ftsA* gene was cloned into the expression vector pUC19 (35) by PCR. Primers were designed to introduce a consensus ribosome-binding site upstream of the *ftsA* initiation codon. The resulting plasmid, pUC19A, was transformed into W3110 containing F′ *lacI*^{q} Tn*9*. This strain overexpressed *ftsA* when induced with isopropyl-β-D-thiogalactopyranoside (IPTG). The overproduced FtsA came down in a high-speed centrifugation to remove membranes; however, it did not appear to be membrane bound, because it was not solubilized by treatment with Sarkosyl, which was used to solubilize the inner membrane. Following extraction of the membrane pellet with Sarkosyl, the FtsA was solubilized with 8 M urea and the outer membrane was removed by high-speed centrifugation. The supernatant containing the FtsA was separated by preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The band corresponding to FtsA was cut out of the gel, and the FtsA was electroluted and sent to Cocalico Biologicals, Inc., for preparation of rabbit antiserum. The antiserum obtained was checked by immunoblotting (as described previously [37]) of a wild-type cell lysate and then affinity purified as follows. First, we wanted to specifically remove antibodies to EF-Tu (since this protein runs very close to FtsA on SDS-PAGE gels) and FtsZ. Even

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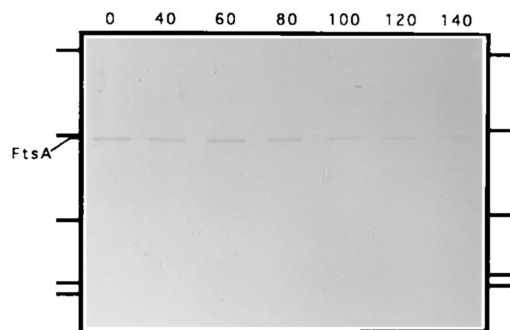

FIG. 1. Specificity of the antibodies used in this study. A culture of JKD31 (pKD3C) was shifted from 30 to 42°C at time zero. This strain has the chromosomal *ftsA* gene disrupted and has wild-type *ftsA* carried on the temperature-sensitive replicon, pKD3C. Samples were taken at the indicated times (min) and immunoblotted with the affinity-purified FtsA antibodies. The positions of molecular weight markers are indicated at the sides. They are, from the top, phosphorylase *b* (97,400 molecular weight), ovalbumin (45,000), carbonic anhydrase (31,000), trypsin inhibitor (21,500), and lysozyme (14,400).

though no anti-FtsZ antibodies were obvious from the original Western blot, we took this approach to make sure that no localization could be attributed to FtsZ antibodies. The antiserum was therefore incubated for 1 h at 4°C with EF-Tu (the high-speed supernatant of a wild-type cell lysate was blotted onto nitrocellulose, and the EF-Tu band was excised), FtsZ, and some unidentified contaminating proteins from the original immunoblot (these were excised from a total wild-type cell lysate which had been transferred onto nitrocellulose). The resulting antiserum was incubated at room temperature for 4 h with purified FtsA immobilized on nitrocellulose. This filter was washed three times, for 15 minutes each, with phosphate-buffered saline (PBS) (10 mM Na_3PO_4 [pH 7.4], 150 mM NaCl, 15 mM KCl) containing 0.1% Tween (Sigma) and then once with PBS. The bound anti-FtsA antibody was eluted into 0.7 ml of 0.2 M HCl by incubation for 15 min at room temperature. This eluate was neutralized with 0.3 ml of 1 M K_2PO_4 and then dialyzed overnight against PBS at 4°C and stored at −70°C.

Immunofluorescent staining. Cells were prepared for immunofluorescent staining exactly as described previously (1, 2). Labeling was performed with either affinity-purified anti-FtsZ antibody as before (1) or an appropriate dilution of the affinity-purified anti-FtsA antibody as a primary antibody and a secondary antibody conjugated to the fluorophore Cy3 (Jackson Immunoresearch). Observation and photography were also done as previously described (1), except that for some samples, a filter block with a 517- to 552-nm excitation filter and a 590-nm barrier filter (XF34; Omega Optical) was used, giving a much stronger signal with the Cy3 fluorochrome. This was particularly useful for observation of FtsA localization, which was consistently weaker than the signal for FtsZ.

RESULTS

FtsA localizes to the division site. Antibodies to FtsA were affinity purified as described in Materials and Methods. To test the quality of the purified antibodies, we used strain JKD31 (pKD3C), which has the chromosomal *ftsA* gene disrupted by insertion of a *kan* element and wild-type *ftsA* supplied on a temperature-sensitive replicon. Shifting a culture of this strain from 30 to 42°C resulted in filamentation and cell death, indicating that *ftsA* was essential in *E. coli*. Immunoblot analysis of samples before and after the shift revealed a single band that decreased in intensity with time at 42°C (Fig. 1). This result confirmed that the antibodies were specific for FtsA.

Immunofluorescence microscopy was used to investigate the distribution of FtsA in the wild-type strain MC4100T. FtsA localization was observed as a thin band of fluorescence at the cell center, perpendicular to the long axis of the cell (Fig. 2A and B). This was identical to the pattern previously observed for FtsZ (1, 27) and therefore is likely to represent FtsA localized in a ring pattern. Cells undergoing septation showed shorter fluorescent bands, and some small cells contained no distinct localization of fluorescence (Fig. 2A and B). This indicated that FtsA is localized at the leading edge of the invaginating septum and that there is a part of the cell cycle where FtsA is not localized. Again, these observations are very similar to those obtained for FtsZ (1). The number of bands of FtsA was compared to the number of Z rings by parallel immunofluorescence labeling of the same wild-type culture. They were found at similar frequencies, with Z rings being slightly more common (Table 1).

FtsA localization requires Z rings. It has been proposed that a function of the Z ring is to recruit other division proteins to the division site (1, 7, 28). Since FtsA and FtsZ are thought to interact, we tested whether FtsA localization might require the presence of a Z ring by looking for FtsA localization in situations where Z rings are not formed. *ftsZ84*(Ts) and *ftsZ26*(Ts)

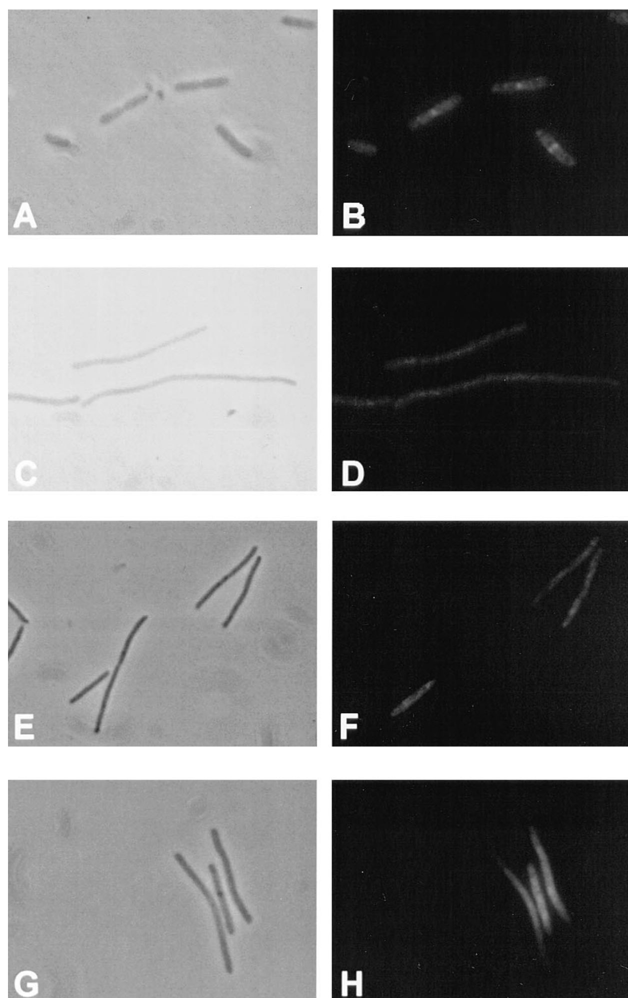

FIG. 2. FtsA localization in wild-type cells and under conditions where Z rings do not form. The panels on the left are phase-contrast photomicrographs, and those on the right are immunofluorescence photomicrographs. Immunofluorescence with affinity-purified anti-FtsA antibody was performed on MC4100T (wild type [A and B]), MCZ84 grown at 42°C for 30 min (FtsZ inactivated [C and D]), MC4100T containing pFAD38 after growth with 0.1% arabinose for 1 h (SulA overproduced [E and F]), and BEF4 F′ (λKD5) after growth with 90 μM IPTG for 2 h (FtsZ overproduced [G and H]). (A and B) Five cells are visible, three of which have bands of fluorescence, corresponding to localization of FtsA. The cell on the lower right, with no sign of division, has a wide band, whereas the two central cells with constrictions have narrower bands. The small cell on the left and the one in the upper right corner have no FtsA localization. (C to H) Cells are shown under three different conditions where Z ring formation is prevented. None of them has any distinct localization of FtsA. (Note that one cell in panel F shows very little background fluorescence, presumably due to insufficient permeabilization.)

TABLE 1. Comparison of FtsA and FtsZ localization in dividing cells

Strain	Primary antibody	% of total no. of cells with:		Mean no. of rings per cell	No. of cells counted
		No bands	One band		
MC4100T[a]	Z	8	92	0.92	932
	A	16.5	83.5	0.835	240
MCZ84[b]	Z	22.4	77.6	0.776	185
	A	29.3	70.7	0.707	172

[a] The data for FtsZ localization in MC4100T come from reference 1 and are averaged over a number of experiments. In all cases, MC4100T growing at 30°C in L broth had a generation time of approximately 35 min.

[b] MCZ84 was grown at 30°C with a generation time of approximately 45 min. We have shown previously that the percentage of wild-type cells with a Z ring decreases as the growth rate decreases (3). This probably accounts for the differences shown here between MCZ84 and MC4100T.

mutants do not form Z rings at a detectable frequency during growth at the nonpermissive temperature (1). Immunofluorescence performed with FtsA antibodies in strain MCZ84 grown at the permissive temperature showed a similar pattern of fluorescence to that observed in wild-type cells (Table 1); however, at the nonpermissive temperature, no localization of FtsA was observed (Fig. 2C and D). Strain MCZ26 grown at the nonpermissive temperature also lacked any distinct FtsA localization (data not shown). These results indicate that in the absence of a functional FtsZ, the FtsA protein cannot localize.

Z ring formation in wild-type cells is prevented by overproduction of the cell division inhibitor SulA (10). To examine the effect of SulA overexpression on FtsA localization, a plasmid carrying an inducible *sulA* gene under the control of the arabinose promoter (pFAD38; see Materials and Methods) was introduced into strain MC4100T. In the absence of induction, cells grew and divided normally and FtsA was distributed in bands in most cells as described for MC4100T (data not shown). When SulA expression was induced by the addition of arabinose, cells filamented and no fluorescent bands corresponding to localized FtsA were visible (Fig. 2E and F). There were also no Z rings present under these conditions (data not shown), confirming previous results obtained by the less sensitive immunoelectronmicroscopy technique (10).

Expression of FtsZ severalfold above the physiological level blocks division and produces smooth-sided filamentous cells (17, 37). We have observed that under these conditions, Z ring formation is prevented (data not shown). We examined FtsA distribution in filamentous cells caused by FtsZ overexpression and found again that there was no FtsA localization (Fig. 2G and H). Therefore, FtsA localization fails to occur in two situations in which wild-type FtsZ is prevented from forming rings.

The above results indicate that FtsA localization cannot occur under conditions where Z ring formation is prevented. This would be expected if FtsA localization depends upon prior Z ring formation. Thus, FtsA could be recruited to the division site by a direct interaction with FtsZ or indirectly through the formation of a nascent septum induced by Z ring formation.

The pattern of FtsA localization is determined by FtsZ. To further test whether FtsA localization depends upon FtsZ, we looked at the distribution of FtsA in situations where nonring Z-structures are formed. In strain MCZ26 growing at the permissive temperature, Z spirals are formed at a high frequency (2). Immunofluorescence performed with FtsA antibodies in strain MCZ26 growing at the permissive temperature showed that FtsA was also frequently localized in spiral patterns (Fig. 3A to D). In general, FtsA spirals were shorter and fainter than Z spirals observed in a parallel experiment. In spherical *E. coli* mutant strains such as KJB24, arc-shaped precursors to Z rings can be observed (2). Arc-shaped localization of FtsA was also observed in KJB24 (Fig. 3E and F). These results, in addition to those obtained with wild-type cells, indicate that the pattern of FtsA localization very specifically mimics that of FtsZ. In particular, the results with strain MCZ26, in which a single *ftsZ* mutation (8) causes both FtsZ and FtsA to be localized in spiral and ring patterns at the permissive temperature (2; this work) while preventing the localization of both at the nonpermissive temperature (1; this work), provide particularly good evidence that the presence and pattern of FtsA localization are completely dependent on FtsZ.

FtsA localization in other cell division mutants. Z rings can form in the absence of the full function of the cell division

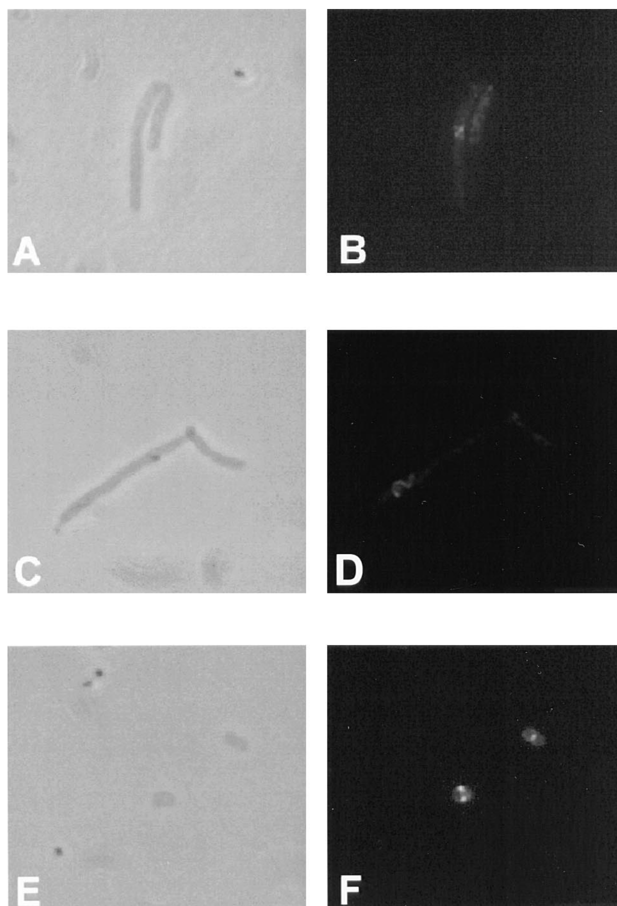

FIG. 3. FtsA localized in spiral and arc patterns. The panels on the left are phase-contrast photomicrographs, and those on the right are immunofluorescence photomicrographs. Immunofluorescence with affinity-purified antibody to FtsA was performed on strains MCZ26 (A to D) and KJB24 (E and F) grown at 30°C. (B and D) Spiral localization of FtsA in MCZ26, similar to that previously reported for FtsZ in this strain (2). (F) Arc-shaped localization of FtsA in KJB24 as reported previously for FtsZ (2). The central cell shows two bright dots diametrically opposite each other. These are the arms of an arc of fluorescence pointing toward the observer. A very faint line between the two dots is the back of the arc. When the plane of focus was altered slightly in one direction, the line was seen more clearly, connecting the dots (data not shown). When the focus was altered in the other direction, the dots were not observed to connect. In panels E and F, the right-hand cell is constricting and shows a narrow band of FtsA localization as shown for wild-type cells (Fig. 2).

proteins FtsA, FtsI, and FtsQ (1). This supports the idea that FtsZ acts early in cell division, with these other cell division proteins acting later in the process (5, 32). It is also good evidence that FtsA, FtsI, and FtsQ are not required for Z ring formation (1). Therefore, the filamentous cells caused by *ftsA* mutations (three have been examined [1]), contain Z rings at division sites which are arrested at the earliest stage of division for which FtsA is required. The same can be said for FtsI and FtsQ (1).

It is possible that the correlation between FtsZ and FtsA localization is due to the interaction of FtsA with another protein which is recruited to the division site by FtsZ. Loss of function of such a protein would be expected to cause a division defect and prevent FtsA localization without affecting the Z ring. We therefore investigated the pattern of FtsA localization in a number of cell division mutants. In strains MCA12, MCI23, and MCQ1 (containing mutations in *ftsA, ftsI*, and *ftsQ*, respectively) grown at the permissive temperature, FtsA was localized as a single fluorescent band at the midcell of the majority of cells (data not shown), similar to results previously obtained for FtsZ (1). FtsA localization in MCI23 and MCQ1 filamentous cells, induced by growth at the nonpermissive temperature, was also similar to the pattern of FtsZ localization (1). That is, in most cells, multiple bands of FtsA were observed at regular intervals along the cell length (Fig. 4A to D). For both strains, the number of bands of FtsA and Z rings per cell were compared by parallel immunofluorescent labeling of the same culture and were found to mimic one another very closely (Fig. 5). Again, the Z rings were very slightly more abundant.

In MCA12 filamentous cells, caused by growth at the nonpermissive temperature, FtsA did not show any specific localization (Fig. 4E and F; Fig. 5), in stark contrast to results with FtsZ localization (Fig. 4G and H; Fig. 5) (1). When the same experiment was performed with two additional FtsA mutant strains (MCA6 and MCA27), similar results were obtained; that is, FtsA localization was abolished at the nonpermissive temperature whereas Z rings were observed at many positions along the cell length (Fig. 4I to L). Filaments of MCA27 contained some bands of FtsA, but these were rare, while the frequency of Z rings was comparable to that observed in other *ftsA* mutants (not shown).

From the above results, it is apparent that FtsA can be localized in the absence of fully functional FtsQ and FtsI proteins. In contrast, in *ftsA* mutant strains at the nonpermissive temperature, this localization is abolished even though Z rings can still form. These results provide evidence that FtsA is not recruited to the septum by either FtsQ or FtsI and also that the function of FtsA in division is to interact with the Z ring. That is, filamentation due to an FtsA defect correlates with a lack of FtsA localization to the Z ring. To support this conclusion, we performed a Western blot analysis with purified anti-FtsA antiserum on MCA12, MCA6, and MCA27 cell lysates prepared before and after a shift to the nonpermissive temperature. In all three strains, the FtsA band maintained its intensity well past the time at which cells were examined for immunofluorescence (data not shown). These results indicate that the lack of FtsA localization is not due simply to the disappearance of the mutant FtsA proteins by rapid degradation at the nonpermissive temperature.

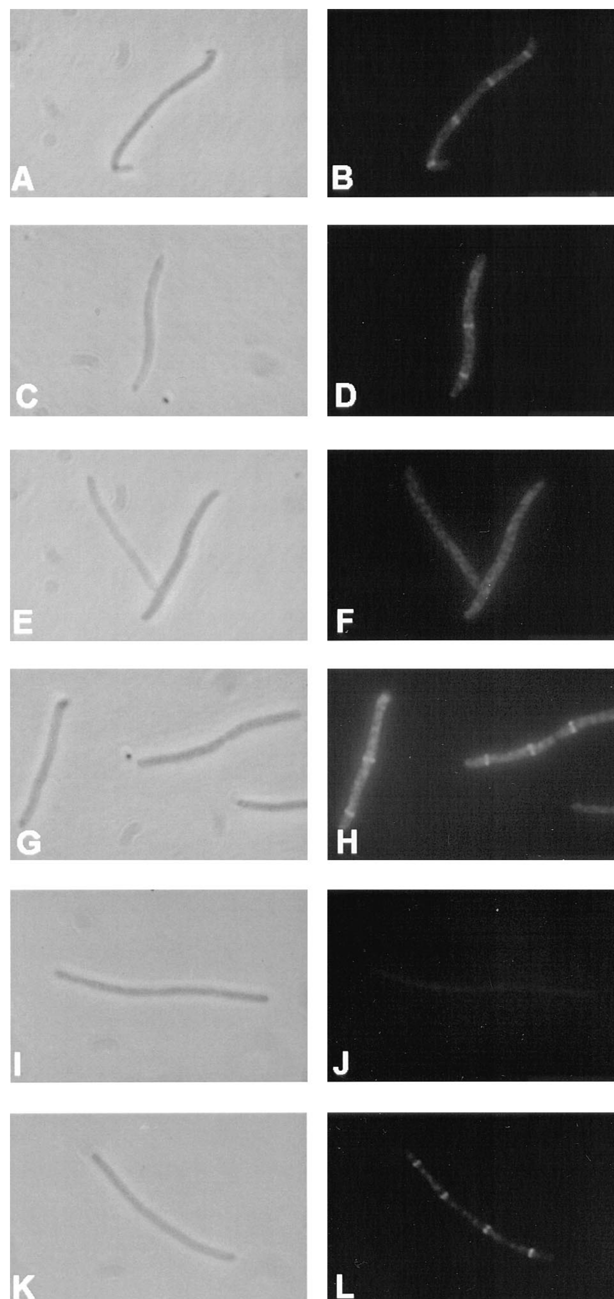

FIG. 4. FtsA and FtsZ localization in *ftsI, ftsQ*, and *ftsA* division mutants. The panels on the left are phase-contrast photomicrographs, and those on the right are immunofluorescence photomicrographs. Immunofluorescence with affinity-purified anti-FtsA antibody was performed on strains MCQ1 (A and B), MCI23 (C and D), MCA12 (E and F), and MCA6 (I and J) grown at 42°C for 30 min. Cells from the same samples of MCA12 (G and H) and MCA6 (K and L) were also labeled with affinity-purified antibody to FtsZ. (B and D) Multiple bands of FtsA localization in *ftsQ* and *ftsI* mutants at the nonpermissive temperature. (F and J) No FtsA localization is observed in two different *ftsA* mutants at the nonpermissive temperature. (H and L) Cells from the same samples of MCA12 and MCA6 have multiple Z rings as reported previously (1).

DISCUSSION

Cell division involves the localized action of a number of gene products to form a septum. Previous work has shown that FtsZ localizes to the division site in a pattern designated the Z ring. The main conclusions of this study are that (i) FtsA, another essential cell division protein in *E. coli*, is also localized to the division site and that (ii) the localization of FtsA is dependent upon the localization of FtsZ, whether as a ring, spiral, or arc. It is unlikely that FtsA, which is estimated to be present at approximately 200 molecules per cell (36), forms a structure as suggested for the Z ring (7). Our results are en-

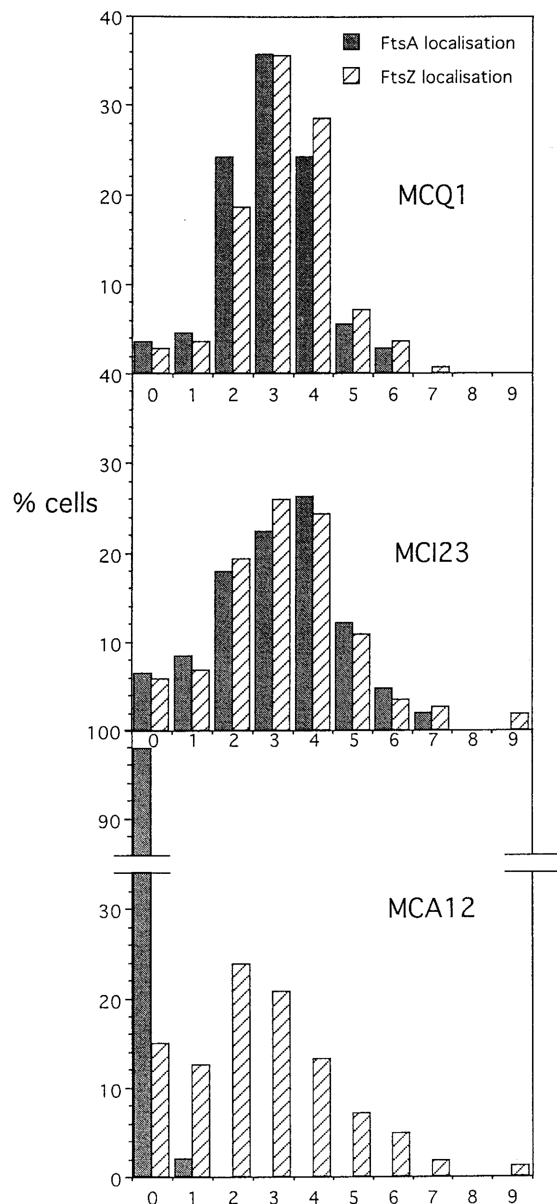

FIG. 5. Comparison of the frequency of FtsA and FtsZ localization in division mutants. Log-phase cultures of strains MCQ1, MCI23, and MCA12, growing at 30°C, were shifted to 42°C for 30 min and then processed for immunofluorescence. For each strain, cells were separately labeled with affinity-purified anti-FtsA and anti-FtsZ primary antibodies. The number of fluorescent bands in each cell were counted, and the results for FtsA and FtsZ are compared in histograms. It can be seen that for both MCQ1 and MCI23, the frequency of FtsA (shaded boxes) and FtsZ (striped boxes) localization is very similar, with the values for FtsA being slightly lower. The average number of Z rings per cell for MCQ1 was 3.22 (number of cells scored = 141), and the average number of fluorescent FtsA bands per cell was 2.99 (number of cells scored = 112); for MCI23, these numbers were 3.26 (total of 120), and 3.18 (total of 107) respectively. FtsA localization was virtually undetectable in MCA12 (just two faint bands in 100 cells), whereas the frequency of FtsZ localization was comparable to that in the other division mutants (the number of Z rings per cell was 2.61 [total of 168], and the number of fluorescent FtsA bands per cell was 0.02 [total of 100]).

tirely consistent with the Z structures recruiting FtsA to the division site. Importantly, we were able to use the immunofluorescence technique to localize FtsA, even though it is much less abundant than FtsZ. Thus, this technique may work to localize other cell division proteins that are also less abundant than FtsZ.

Our initial studies with a wild-type strain revealed that the frequency of cells in which FtsA was localized correlated approximately with the frequency of cells positive for Z rings. In fact, this was true under a variety of conditions where both FtsA and FtsZ were localized, but FtsZ localization was consistently slightly more frequent (Table 1; Fig. 5). We attribute this to reduced sensitivity in detecting FtsA localization compared to FtsZ localization, due to the relative abundance of the two proteins in the cell. However, it could be interpreted as an indication that FtsA localization lags behind Z ring formation.

Examination of several conditions known to block Z ring formation revealed that FtsA also failed to localize. These conditions included two *ftsZ*(Ts) mutants at the nonpermissive temperature and overproduction of the cell division inhibitor SulA, which is known to interact directly with FtsZ (25, 26). In addition, overproduction of FtsZ, which is known to inhibit cell division (37), blocked Z ring formation and also prevented localization of FtsA. Further evidence that FtsZ recruits FtsA to the division site came from examining situations in which nonring Z structures are observed. Perhaps the most convincing is the *ftsZ26*(Ts) mutant, in which Z spirals and rings are observed at the permissive temperature (2). We observed both spiral and ring patterns of FtsA localization as well. In a spherical mutant where arcs are formed as precursors to Z rings (2), we also observed arcs of FtsA localization that appear to be precursors to rings of FtsA. Thus, FtsA localization mimics FtsZ localization.

In *ftsQ* and *ftsI* filaments, both FtsZ and FtsA rings were observed (1; this work). In several *ftsA* mutants grown at the nonpermissive temperature, FtsZ rings were detected, as reported previously (1), but localization of FtsA was not observed. Quantitative immunoblotting revealed that at the time of sampling, the levels of the mutant FtsA proteins had not detectably decreased. Thus, the failure of these *ftsA* mutants to divide correlated with the inability of the proteins to localize to the Z ring.

How do proteins localize to the division site? A model has been proposed to account for the localization of FtsZ (7, 28). In this model, it is suggested that FtsZ self-assembles into the Z ring in response to a nucleation signal. This model is supported by the finding that FtsZ can undergo GTP-dependent self-assembly in vitro (12, 23, 30). The results presented here are entirely consistent with FtsA being recruited to the division site by the Z ring. Thus, we do not think that FtsA itself makes a structure but that it complexes with FtsZ in the cytoplasm or very soon after Z ring formation. This model is supported by evidence for a direct interaction between FtsZ and FtsA from studies involving the yeast two-hybrid system and biochemical studies with an FtsZ affinity column (13). These studies suggest that FtsZ and FtsA can interact in the cytoplasm to form a complex, raising the possibility that they simultaneously localize to the division site. However, our earlier studies, repeated here, demonstrate that Z rings can form in the absence of functional FtsA, and here we show that no FtsA is localized under those conditions. Thus, Z rings can form in the absence of FtsA localization.

The recruitment of FtsA to the division site raises the possibility that other division proteins are also localized and recruited to the division site by interaction with the Z ring. Proteins such as FtsI (PBP3) could interact directly with FtsZ or FtsA through their cytoplasmic, N-terminal domain, which in the case of FtsI is essential (19). Others, such as FtsN and FtsQ, must not act directly, since their N-terminal cytoplasmic domains are not essential (19). Having an antiserum to these

other proteins would allow the localization of these proteins to be tested provided that there is sufficient sensitivity. Also, simultaneous labeling of the division proteins would provide more information about the relative timing of localization events.

It is not clear what role FtsA plays in cell division or in the functioning of the FtsZ ring. In *E. coli* it is clearly essential, although in *Bacillus subtilis* it is not (4). However, *ftsA* null mutants of *B. subtilis* are extremely filamentous, implying that division was occurring very inefficiently. Two possibilities for FtsA function are that FtsA acts as a link between FtsZ and other cell division proteins and that FtsA enhances the dynamics of the Z ring. Our localization studies, however, indicate that the Z ring formation occurs in the absence of FtsA. Thus, it is somewhat surprising that the ratio of FtsZ to FtsA is important for division to occur. It is possible that overproduction of FtsA ties up FtsZ in a complex that is not capable of localization. Indeed, we have observed that overproduction of FtsA prevents Z ring formation (3). We also observed that overproduction of FtsZ itself prevents FtsZ localization. The explanation for this is not known, although the effect can be suppressed by increasing the amount of FtsA (17).

In summary, FtsA is localized to the division site, and this is entirely dependent upon FtsZ. We favor a model in which FtsA is recruited to the division site by a direct interaction with the Z ring. Thus, the Z ring could function, in part, by recruiting other proteins such as FtsA, thus confining their activities to the septum.

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