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The Proper Ratio of FtsZ to FtsA Is Required for Cell Division To Occur in *Escherichia coli*

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Interactions among cell division genes in *Escherichia coli* were investigated by examining the effect on cell division of increasing the expression of the *ftsZ*, *ftsA*, or *ftsQ* genes. We determined that cell division was quite sensitive to the levels of FtsZ and FtsA but much less so to FtsQ. Inhibition of cell division due to an increase in FtsZ could be suppressed by an increase in FtsA. Inhibition of cell division due to increased FtsA could be suppressed by an increase in FtsZ. In addition, although wild-type strains were relatively insensitive to overexpression of *ftsQ*, we observed that cell division was sensitized to *ftsQ* overexpression in ftsI, *ftsA*, and *ftsZ* mutants. Among these, the *ftsI* mutant was the most sensitive. These results suggest that these gene products may interact and that the proper ratio of FtsZ to FtsA is critical for cell division to occur.

Cell division is a complex process that requires the temporal and spatial coordination of the activities of multiple genes. Among the cell division genes identified in *Escherichia coli*, the *ftsZ*, *ftsA*, *ftsQ*, and *ftsI* genes have been the most studied (reviewed in references 6 and 15). They are located in a large gene cluster involved in cell wall biosynthesis and cell division located at 2 min on the *E. coli* genetic map. Each of these *fts* genes has been shown to be essential for cell division and cell viability.

Details are beginning to emerge about the role of the *fts* genes in cell division. The *ftsZ* gene, which encodes a 40-kDa protein that fractionates as a cytoplasmic protein (19, 24, 31), plays a key role in cell division. Recent immunolocalization studies demonstrated that FtsZ forms a dynamic ring structure at the leading edge of the constriction during cell division (7). The formation of this FtsZ ring is the earliest identified step in the division process. This unique localization of FtsZ during septation implies a specific role for FtsZ in cell division. In addition, accumulated evidence has suggested that FtsZ is the target of endogenous cell division inhibitors, SulA and MinCD, and its level determines the frequency of cell division (3–5, 13).

The products of the *ftsA*, *ftsI*, and *ftsQ* genes are thought to function after *ftsZ* in cell division (2). The *ftsA* gene product is a 45-kDa protein that is peripherally associated with the cytoplasmic membrane (23). Some evidence suggests that it is a component of the septum and interacts with PBP3, the *ftsI* gene product (27, 28). PBP3 is one of the four high-molecular-weight penicillin-binding proteins and is specifically required for cell division (25, 26). It is also the only essential cell division protein with defined biochemical activities. It has both transpeptidase and transglycosylase enzymatic activities (20). PBP3 is an integral membrane protein with a simple topology. It has a noncleavable signal sequence near the amino-terminal end that mediates transport of the enzymatic domain to the periplasm and anchors it to the cytoplasmic membrane (9). FtsQ, about which less in known, is a 31-kDa integral membrane protein with the same topology as PBP3 (11).

Inhibition of *ftsZ* function, either in *ftsZ* mutants at the nonpermissive temperature or following induction of inhibitors that block FtsZ function, leads to the formation of filaments that show no sign of constrictions (2, 15). In contrast, filaments formed by *ftsA* and *ftsI* mutants have some indentations along the filament that could be aborted or stalled division attempts (15). Depending upon the conditions, *ftsQ* gene defects result in filaments with or without constrictions (11). On the basis of the morphological characteristics of these mutants, it has been argued that in septum formation, *ftsZ* functions first, followed by *ftsA* and *ftsI*, with *ftsQ* functioning throughout the process (2, 15).

Some evidence exists that for the proper functioning of the division machinery these *fts* gene products have to be present at the appropriate level. Thus, artificial elevations of one or more of these gene products that are required for division can lead to a block to the division process. This has been shown most clearly for FtsZ (32) but also for FtsA (30), PBP3 (the *ftsI* gene product) (17), and FtsQ (11). Tenfold overproduction of FtsZ blocks cell division, whereas a much greater overproduction of FtsQ is needed to block division. The amount of FtsA and FtsI required to block division has not been quantitated.

In this report, we provide evidence that the ratio of FtsZ to FtsA is critical for division, suggesting an interaction between FtsZ and FtsA. In addition, we present evidence for interactions between FtsQ and FtsZ, FtsA, and PBP3. The significance of these interactions in cell division will be discussed.

MATERIALS AND METHODS

Bacterial strains and media. The bacterial strains are listed in Table 1. All the bacterial strains were grown on L agar plates or in L broth supplemented with thymine (50 μg/ml) and the appropriate antibiotics as in earlier studies (12). Minimal salts medium supplemented with 0.5% glucose and 1 mg of thymine per ml was used for examination of the effect of overproduction of FtsQ on cell division.

Phage. Phage λKD5 contains a promoterless *ftsZ* gene cloned downstream of the *tac* promoter obtained from pKK223-3 (10). This phage was constructed in several steps. First, the *ftsZ* gene was obtained without any promoters. This was done by cloning a small *Hae*III-*Eco*RI fragment

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TABLE 1. Bacterial strains

Strain	Relevant marker(s)	Source or reference
W3110	Prototroph	Laboratory collection
MC4100	F⁻ Δ*lacU69 araD136 relA rpsL thi*	7
MC4100T	*leu*::Tn*10*	8
MCZ84	MC4100T *ftsZ84*	8
MCZ26	MC4100T *fts26*	8
MCA12	MC4100T *ftsA12*	8
MCA27	MC4100T *ftsA27*	8
MCI23	MC4100T *ftsI23*	8
MCQ1	MC4100T *ftsQ1*	8
JFL110	*ftsA12 recA*	19

that contains the 5′ end of the *ftsZ* gene including the ribosome binding site into pUC9 (29). The *ftsZ* gene was then reconstituted by cloning into this plasmid an *Eco*RI fragment from λ16-25#9 (33) that contains the 3′ end of the *ftsZ* gene and the *envA* gene with a Tn*5* insertion. This plasmid was designated pZP4. A *Hin*dIII fragment containing just the *ftsZ* gene was obtained from pZP4 and cloned into pKD100 downstream of the *tac* promoter to give pKD101. pKD100 contains the *tac* promoter on a *Bam*HI fragment, obtained from pKK223-3, cloned into pSR600 upstream of the *lacZ* gene. pSR600 was provided by Sue Rockenbach and contains the *lacZ* gene in a pBR322 derivative which also contains the *bla* gene. The *ftsZ* gene downstream of the *tac* promoter was crossed from pKD101 to λ1034 by in vivo recombination to give λKD5. λ1034 contains the 3′ end of the *lacZ* gene and a portion of the *bla* gene which allows recombination with plasmids that contain these two genes.

Plasmids. The plasmids used in this study are depicted in Fig. 1. pZAQ has been described previously (32). pKD9 has the *Bgl*II-*Cla*I fragment from pZAQ cloned into pACYC184 between the *Bam*HI and *Cla*I sites. pKD9K was constructed by inserting the *Eco*RI fragment containing the *kan* gene from pUC4K (29) at the *Eco*RI site in the *ftsZ* gene in pKD9. pKD122 has the *Bam*HI-*Eco*RI fragment from pZAQ cloned into pJF118HE (16) at the polylinker site downstream of the *tac* promoter. pKD130 was constructed by ligating the *Pst*I-*Bgl*II fragment from pZAQ with pJF118HE digested with *Pst*I and *Bam*HI. pKD135 has the sequence from the *Pst*I site upstream of *ftsQ* to the *Eco*RI site in *ftsZ* cloned into pBR322. The *Bam*HI fragment from pUC4K containing the *kan* gene was inserted in pKD135 at either the *Bgl*II site in *ftsA* or the *Bam*HI site in *ftsQ*, resulting in pKD135AK and pKD135QK, respectively.

Immunoblotting. The levels of the FtsZ protein in the cell were determined by immunoblotting as described previously (32).

Photomicroscopy. Cells sampled for photomicroscopy were fixed in 10% formaldehyde. A thin layer of 1% agarose was made on a glass slide, and the fixed cells were spotted on it. The cells were examined and photographed by using phase-contrast microscopy.

RESULTS

Increased level of FtsA can suppress filamentation caused by overproduction of FtsZ. We first observed that when pKD9, which contains the *ftsZ* gene cloned into pACYC184, was introduced into an *ftsA12* mutant, JFL110, the strain grew poorly and the cells were very filamentous. Introduction of pKD9 into the wild-type strain W3110 (Fig. 2A, compare

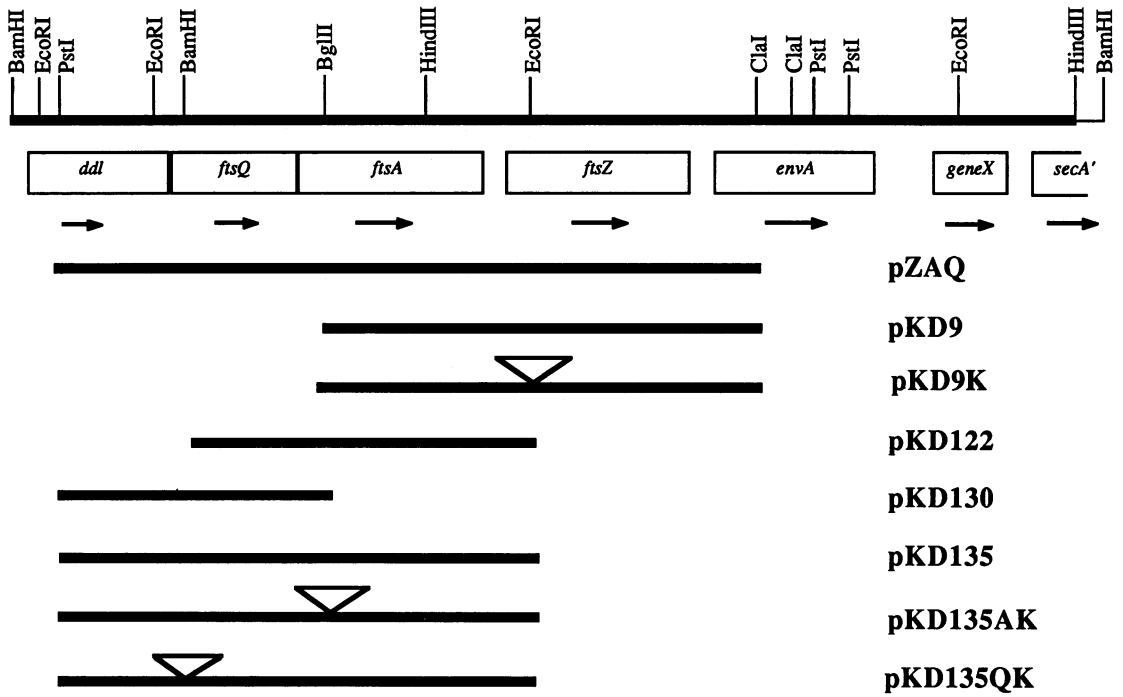

FIG. 1. Diagram of plasmids used in this study. The positions of the genes are indicated below the restriction map. The restriction fragment contained within each of the plasmids is indicated by the solid horizontal line. The presence of the *kan* insert is indicated by an open triangle.

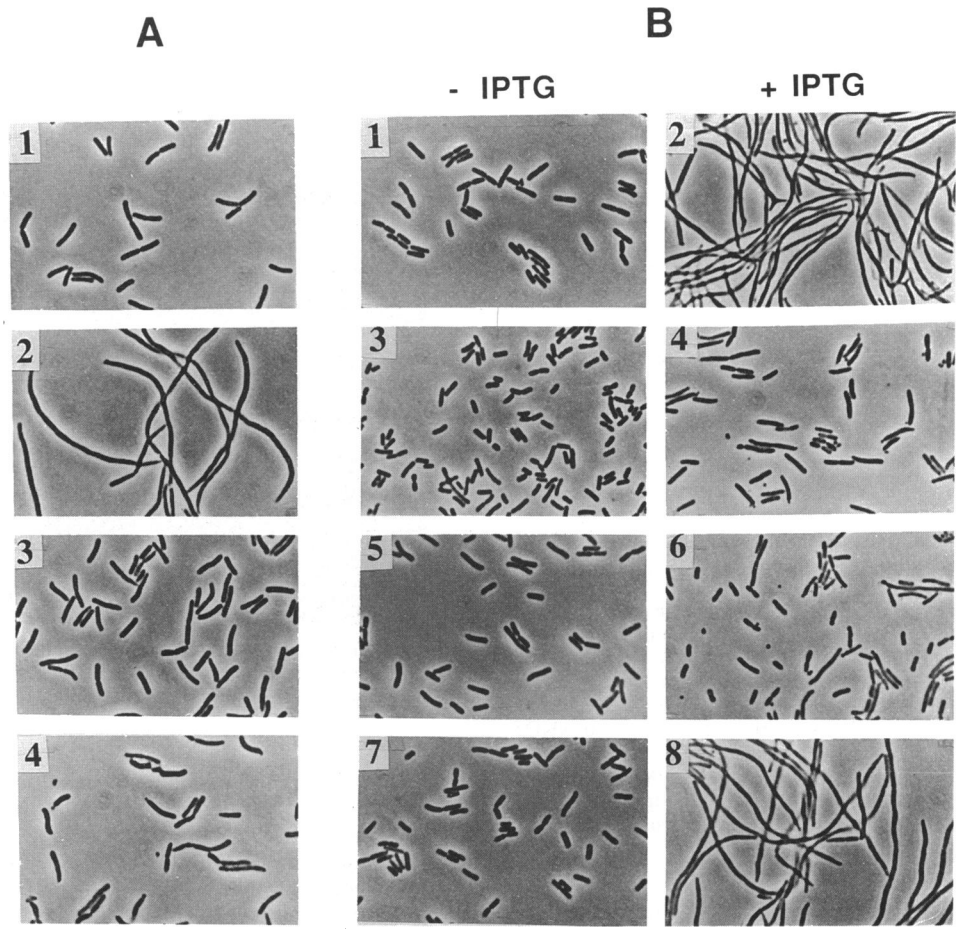

FIG. 2. The effect of increased FtsZ on cellular morphology and suppression by overexpression of *ftsA*. (A) Effect of various *ftsZ*-containing plasmids on cellular morphology: panel 1, W3110; panel 2, W3110(pKD9); panel 3, W3110(pKD9K); panel 4, W3110(pZAQ). (B) *ftsA*-containing plasmids can suppress the inhibitory effect of induction of *ftsZ* from λKD5: panels 1 and 2, W3110(λKD5)(pBR322); panels 3 and 4, W3110(λKD5)(pKD135); panels 5 and 6, W3110(λKD5)(pKD135QK); panels 7 and 8, W3110(λKD5)(pKD135AK).

panels 1 and 2) resulted in a similar phenotype, although the filamentation was not as severe and became less obvious after several passages. This is in contrast to the effect of pZAQ, a pBR322 derivative that also contains the *ftsZ* gene but actually causes enhanced division activity in the form of a minicell phenotype (Fig. 2A, panel 4) (32). To prove that the *ftsZ* gene carried on pKD9 was the gene responsible for the division inhibition, the *ftsZ* gene on this plasmid was inactivated by inserting the Kan^r cartridge at the *Eco*RI site near the 5′ end of the gene. The resultant plasmid, pKD9K, had no effect on cell growth or morphology when introduced into JFL110 or W3110 (Fig. 2A, panel 3). This confirmed that the filamentation caused by pKD9 was due to the intact *ftsZ* gene on the plasmid. In addition, as noted above, the inhibitory effect caused by *ftsZ* was more obvious in the *ftsA12* mutant than in the wild-type strain. This is most likely due to reduced FtsA activity in the mutant even at its permissive temperature. This raised the possibility that inhibition caused by increased FtsZ is due to an FtsA deficiency.

It is known that cell division is inhibited when the FtsZ level is increased 10-fold over the normal level (32). Therefore it was a possibility that the filamentation we observed with pKD9 resulted from too much *ftsZ* expression. However, this seemed unlikely since pZAQ would have a higher copy number than the pACYC184 derivative pKD9. In addition, pZAQ carries more DNA sequences upstream of *ftsZ* than pKD9, sequences known to contain promoters that increase *ftsZ* expression (18, 33). As expected, pZAQ produced more FtsZ than pKD9 as measured by Western blot (immunoblot) (Fig. 3A). This apparent contradiction could be explained if the intact *ftsA* or *ftsQ* gene or both, which are present on pZAQ but not on pKD9, relieve the inhibitory effect on cell division due an increased amount of FtsZ.

To determine whether this was the case and to determine which gene or genes, *ftsA* or *ftsQ* or both, were responsible for the suppression of filamentation caused by overproduction of FtsZ, pKD135, pKD135AK, and pKD135QK were constructed (Fig. 1). The plasmid pKD135 has the *ftsQ* and *ftsA* genes, but not *ftsZ*, cloned in pBR322 and should produce about the same amount of FtsA and FtsQ as pZAQ but no FtsZ. pKD135AK and pKD135QK are the same as pKD135 except that the *ftsA* and *ftsQ* genes, respectively, were inactivated by *kan* insertion. W3110 was transformed with these plasmids, and the transformants were then lysogenized with an *ftsZ*-expressing phage, λKD5. This phage has *ftsZ* under the *tac* promoter control and causes filamentation of W3110 containing the control plasmid pBR322 upon

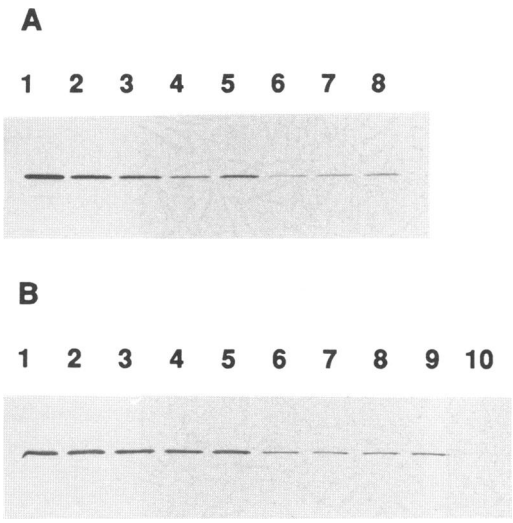

FIG. 3. Determination of the levels of FtsZ. The amount of FtsZ was assessed by immunoblotting whole cell extracts. (A) Extracts of exponential cultures of W3110 containing the following plasmids (each lane contains the same amount [0.1 optical density units] of material obtained at an optical density of 540 nm, unless otherwise indicated): lane 1, pZAQ; lane 2, pZAQ (diluted 1:2); lane 3, pKD9; lane 4, pKD9 (diluted 1:2); lane 5, pZAQ (diluted 1:4); lane 6, pKD9 (diluted 1:4); lane 7, pKD9K; lane 8, no plasmid. (B) Cell extracts obtained 2 h after induction of *ftsZ* with IPTG from a W3110 λKD5 lysogen containing the following plasmids: lane 1, a nonlysogen control containing pZAQ; lane 2, pBR322; lane 3, pKD135; lane 4, pKD135QAK; lane 5, pKD135QK; lanes 6 to 9, a repeat of lanes 2 to 5 except diluted 1:4; lane 10, a nonlysogen control.

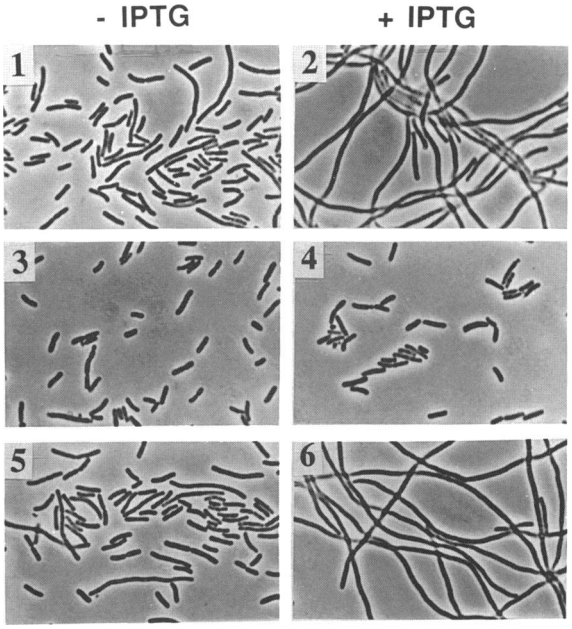

FIG. 4. Increased FtsZ can suppress filamentation due to overexpression of *ftsA*. Panels: 1 and 2, W3110(pKD122)(pACYC184); 3 and 4, W3110(pKD122)(pKD9); 5 and 6, W3110(pKD122)(pKD9K).

induction with IPTG (isopropyl-β-D-thiogalactopyranoside) (Fig. 2B). W3110 (λKD5), containing pKD135, did not show a filamentation phenotype after 2 h of induction but actually produced many minicells with a frequency comparable to that induced by pZAQ. Cells containing pKD135QK also produced many minicells and no long filaments following induction, although the cell length was a little more heterogeneous than observed with pKD135. In contrast, W3110 harboring pKD135AK formed long filaments under the same conditions. The FtsZ level in these cells carrying the different plasmids was measured 2 h after IPTG induction, and no difference was detected (Fig. 3B). These results demonstrated that increasing the gene dosage of *ftsA* by using a multicopy plasmid can suppress filamentation caused by overproduction of FtsZ. The slightly less efficient suppression by pKD135QK compared with that by pKD135 is probably due to a slight inhibitory effect of the *kan* insertion on the expression of *ftsA* from the promoters upstream of the insertion site. Consequently, a little less FtsA would be produced by pKD135QK than by pKD135. Although the filamentation caused by increased FtsZ can be suppressed by increasing FtsA, the filaments did not have the typical indented morphology characteristic of an *ftsA* defect. Surprisingly, the filaments have a morphology characteristic of an *ftsZ* defect. These results also indicate that cell division is more sensitive to inhibition by increased FtsZ than previously reported.

Increased level of FtsZ can suppress filamentation caused by overproduction of FtsA. It has been reported that overproduction of FtsA can inhibit cell division (30). The filaments induced by overproduction of FtsA appeared very smooth, much like FtsZ-deficient filaments. This is quite different from the appearance of filaments resulting from defects in *ftsA*, which have characteristic indentations along the length of the filament. In the above experiments, it was noticed that W3110 containing pKD135 and pKD135QK produced obviously longer cells than W3110 with pKD135AK or W3110 alone (data not shown). This suggested that the amount of *ftsA* expressed from pKD135 or pKD135QK caused some degree of inhibition of cell division. Again, cells transformed with pZAQ, which is different from pKD135 only in that it also expresses *ftsZ*, are small and produce many minicells. Together these observations suggest that overproduction of FtsA might adversely affect FtsZ function and this effect might be suppressed by increasing the FtsZ level in the cell. To test this possibility, pKD122, which has the *ftsA* gene under *tac* promoter control, was constructed and introduced into W3110. This plasmid caused a filamentation phenotype even without IPTG induction, presumably because of the basal level of expression of *ftsA* from the *tac* promoter. Induction of this strain with 0.1 mM IPTG resulted in long, smooth filaments, as has been reported (30) (Fig. 4). This strain was then transformed with pKD9, which is compatible with pKD122 and produces a three- to fourfold increase in FtsZ. As shown above, this plasmid alone causes filamentation (particularly in initial transformants). However, the cells containing both pKD122 and pKD9 were obviously shorter and produced some minicells. More dramatically, at 2 h after induction with 0.1 mM IPTG, the control culture containing pKD122 and pACYC184 was very filamentous. In contrast, the experimental culture containing pKD122 and pKD9 produced minicells and the cells were only a little elongated. Substitution of pKD9 by pKD9K (*ftsZ::kan*) led to cells as filamentous as the control (Fig. 4). We concluded that increasing FtsZ in the cell can suppress the filamentation caused by overproduction of FtsA.

Overproduction of FtsQ causes filamentation in *ftsZ*, *ftsA*, and *ftsI* mutants. Earlier studies have shown that up to 200-fold overproduction of FtsQ had no observable effect on

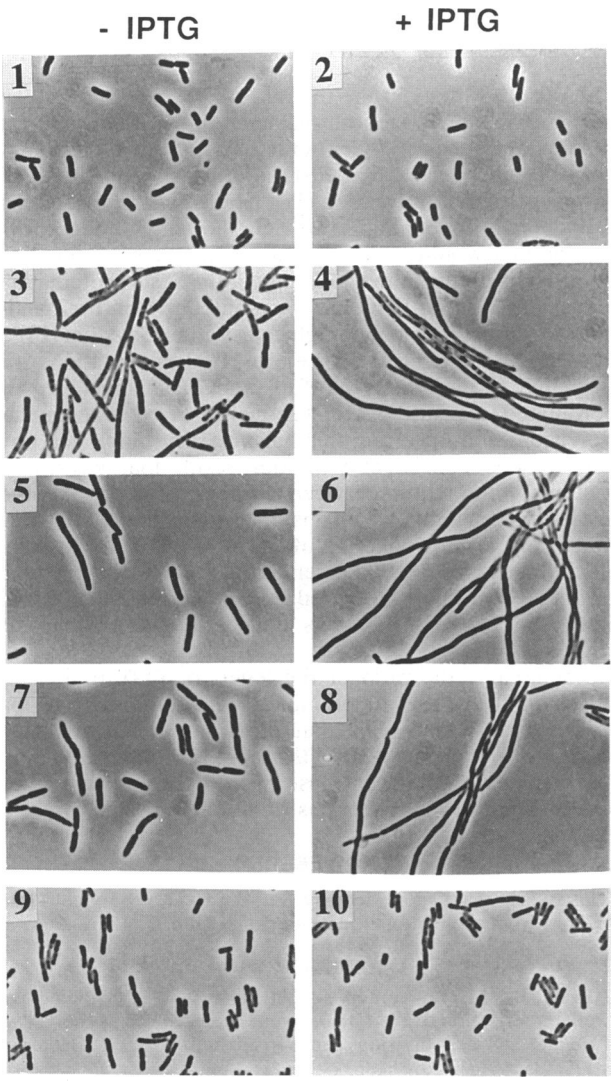

FIG. 5. Increased expression of *ftsQ* causes filamentation in *ftsZ*, *ftsA*, and *ftsI* mutants. Isogenic derivatives of MC4100 containing pKD130 were examined to determine the effect of *ftsQ* overexpression. The strains were as follows: panels 1 and 2, MC4100(pKD130); panels 3 and 4, MCZ84(pKD130); panels 5 and 6, MCA27(pKD130); panels 7 and 8, MCI23(pKD130); panels 9 and 10, MCQ1(pKD130).

cellular morphology when cells were grown in L broth (11). Only in minimum medium did the FtsQ overproduction cause the formation of filaments. We constructed pKD130, a multicopy plasmid that has the *ftsQ* gene under control of the *tac* promoter. With maximum IPTG induction, the wild-type host showed no noticeable changes in growth or morphology in L broth but formed constricted filaments in minimal medium, consistent with the earlier observations. However, when this plasmid was introduced into several *fts* mutants, IPTG induction resulted in filamentation of these mutants even in L broth at the permissive temperature (Fig. 5). The inhibitory effect on cell division caused by overproduction of FtsQ was most dramatic in the *ftsI23* mutant (Fig. 5). Only in this mutant did FtsQ overproduction cause lethality (data not shown). In *ftsA* and *ftsZ* mutants, FtsQ overproduction did not cause lethality but filamentation was still extensive. The filaments formed in the *ftsI* mutant had some constrictions along the cell length and could easily be distinguished from those filaments formed in the *ftsZ* and *ftsA* mutants. In contrast, in the *ftsQ* mutant, the overproduction of *ftsQ* had no noticeable effect. Thus, the presence of mutant *ftsI*, *ftsA*, or *ftsZ* gene products sensitizes the division apparatus to overproduction of FtsQ.

DISCUSSION

The results presented in this report further emphasize that a proper balance must be maintained between various division proteins to ensure the normal operation of the division machinery. We demonstrated that division is very sensitive to the ratio of FtsZ to FtsA. Increasing the FtsA level led to a block in cell division that was due to insufficient FtsZ activity since the block could be overcome by simply increasing FtsZ. Also, the morphology of the filaments (smooth) produced was typical of an FtsZ deficiency. Increasing the level of FtsZ led to a block to cell division that was due to an apparent deficiency of FtsA since the block could be overcome by simply increasing the level of FtsA. However, the morphology of these filaments (smooth) was typical of an FtsZ deficiency and not an FtsA deficiency (indented morphology). This implies that FtsZ overproduction interferes with its own function and that this can be suppressed by FtsA overproduction.

The simultaneous increase in FtsZ and FtsA increased the division capacity as evidenced by the minicell phenotype produced. The level of FtsZ appeared to be rate limiting for division since increasing the level of FtsZ led first to a minicell phenotype and then at higher levels to filamentation. In contrast, increasing the level of FtsA never resulted in a minicell phenotype, only filamentation. This suggests that increases in FtsA lead to an FtsZ deficiency while small increases in FtsZ can induce minicell formation without interfering with FtsZ function.

Previously we had shown that increasing the level of FtsZ about 10-fold would block cell division (32). However, construction of plasmids like pKD9, which is reported here, that should not produce this high a level of FtsZ and yet caused division interference caused us to further examine this issue. Our results show that even a three- to fourfold increase in FtsZ interferes with division. This interference of cell division can be completely suppressed by raising the level of FtsA. Since many of the plasmids we used previously contained both the *ftsA* and *ftsZ* genes it would explain our previous results, which suggested that the level required for inhibition by FtsZ may be higher. It is in part also explained by the fact that the 10-fold figure was arrived at following short periods of induction of *ftsZ* under control of the *lac* promoter and not steady-state conditions (32).

These results obtained with FtsA and FtsZ overproduction contrast with the results obtained with FtsQ overproduction. As previously reported (11) we only observed an effect of the increased expression of *ftsQ* in minimal medium in wild-type strains; no effect was seen in rich medium. However, when *ftsQ* was overexpressed in various mutant backgrounds at the permissive temperature, effects on cell division were readily observed. The most sensitive mutant appears to be the *ftsI23*(Ts) mutant, in which overexpression of *ftsQ* not only leads to filamentation but is lethal as well. Overexpression in various *ftsZ* or *ftsA* mutants also leads to filamentation, whereas overexpression in the *ftsQ1* mutant has no detectable effect. It appears that the heterologous mutant proteins render cells more sensitive to the effects of

overexpression of *ftsQ*. Similar results have also been observed with an increased gene dosage of *ftsI* (17). Multicopy expression of *ftsI* in wild-type or *ftsI* mutant cells had little effect on cell division; however, overexpression in *ftsZ84*, *ftsA13*, or *ftsQ1* mutants led to a filamentation phenotype.

Although the *ftsZ*, *ftsA*, and *ftsQ* genes are closely linked on the chromosome and transcription initiated from promoters upstream of the proximal *ftsQ* gene can transcribe through the distal *ftsZ* gene, the actual concentrations of the products of these three genes in the cell are dramatically different (33). It has been estimated that a cell has approximately 20,000 molecules of FtsZ (8, 24), 200 molecules of FtsA (21), and 50 molecules of FtsQ (11). These different levels are achieved by the different efficiencies of the ribosome binding sites for the three genes (21), and the distal *ftsZ* gene is expressed from additional promoters within *ftsA* (33). These quite different levels of these gene products suggests that these three division proteins play very different roles in cell division. The tandem arrangement of these genes may help to ensure that the proper balance between these proteins is maintained. The necessity of maintaining the proper ratio of FtsA to FtsZ may explain why these genes are linked in organisms as distantly related as *E. coli* and *Bacillus subtilis* (1).

During cell division, FtsZ forms a dynamic ring at the division site, and it was suggested that this may function as a cytoskeletal element during division, a role consistent with the amount of FtsZ in the cell (7). The diameter of the ring decreases as cell division progresses until septation is completed, when the FtsZ ring is disassembled. This ring is presumably membrane associated since the FtsZ is located at the inner surface of the cytoplasmic membrane in immunolocalization studies. Hypothetical roles for the FtsZ ring structure include determination of the division site, generation of a centripetal force that leads the cell wall growth inward, and activation of division-specific biosynthetic machinery. To carry out any of these roles the FtsZ ring must interact with the membrane through interactions with one or more specific proteins. A possible candidate for one of these proteins is FtsA. FtsA is known to be a peripheral membrane protein (23), and our results suggest that it functionally interacts with FtsZ. Also, on the basis of morphological observations it has been argued that FtsA is a component of the septum (28). Thus, FtsA may be a link between the FtsZ ring and the membrane.

Provided that FtsZ plays an essential role in activation of the septal biosynthetic apparatus, it must somehow communicate with the components of this apparatus, at least one of which is PBP3. It may interact through FtsA or directly interact with one or a few key components that have a topology like that of PBP3 and FtsQ. It is quite possible that communication between FtsZ and the septum-specific PBP3 could occur through FtsA since it has been proposed that FtsA and PBP3 interact (27). Our observation that *ftsZ*, *ftsA*, and *ftsI* mutants are more sensitive to division inhibition by overproduction of FtsQ than wild-type strains provides evidence for a functional interaction between these cell division proteins. It has also been shown that increased gene dosage of *ftsI* is detrimental to division in *ftsZ*, *ftsA*, and *ftsQ* mutants, suggesting a functional interaction between these proteins (17). Interactions between these proteins has been inferred from other studies as well (14). In addition, our recent immunolocalization studies showed that inactivation of either FtsA, FtsQ, or PBP3 affected FtsZ ring formation or its stability (8). In addition, we have observed that several *ftsZ* mutants produce cells with altered cell pole morphology (7a). This phenotype could be explained as a result of an altered interaction between these mutant FtsZ proteins and PBP3.

Less is known about the role of FtsQ in cell division. The *ftsQ1* mutant forms smooth filaments at 42°C but filaments with constrictions at 37°C. On the basis of this morphology, it has been suggested that FtsQ is required throughout the process of septum formation (11). The amino acid sequence of FtsQ has no homology to PBP3 or any other known proteins that would give a clue to its biochemical function. On the basis of the known topology of the FtsQ protein it has been suggested that FtsQ may be a link between FtsZ and a penicillin-insensitive peptidylglycan synthetic system (PIPS) (22). PIPS is thought to act before PBP3 in septation. It is likely that the activities of PIPS and PBP3 must be well coordinated to ensure the right sequence of events occurring at division, and upsetting this balance may block cell division. This is consistent with our result that suggests a functional interaction between FtsZ and FtsQ. Another suggestion from our results is that FtsQ and PBP3 may interact too. Overproduction of FtsQ in *ftsZ* or *ftsA* mutant cells produced a smooth filamentation, whereas overproduction in the *ftsI23* mutant produced constrictions that suggested the filamentation was due to a decreased PBP3 activity.

In summary, the ratio of the levels of FtsZ to FtsA is critical for the proper functioning of the cell division apparatus. The results suggest that FtsA has a critical role in formation or stability of the FtsZ ring; however, the nature of the interaction between these two proteins as well as the other division proteins remains to be determined.

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