JOURNAL OF BACTERIOLOGY, June 1991, p. 3500–3506
0021-9193/91/113500-07$02.00/0

Vol. 173, No. 11

ftsZ Is an Essential Cell Division Gene in *Escherichia coli*

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Received 14 January 1991/Accepted 26 March 1991

The *ftsZ* gene is thought to be an essential cell division gene in *Escherichia coli*. We constructed a null allele of *ftsZ* in a strain carrying additional copies of *ftsZ* on a plasmid with a temperature-sensitive replication defect. This strain was temperature sensitive for cell division and viability, confirming that *ftsZ* is an essential cell division gene. Further analysis revealed that after a shift to the nonpermissive temperature, cell division ceased when the level of FtsZ started to decrease, indicating that septation is very sensitive to the level of FtsZ. Subsequent studies showed that nucleoid segregation was normal while FtsZ was decreasing and that *ftsZ* expression was not autoregulated. The null allele could not be complemented by λ16-2, even though this bacteriophage can complement the thermosensitive *ftsZ84* mutation and carries 6 kb of DNA upstream of the *ftsZ* gene.

Accumulating data suggest that the *ftsZ* gene plays a critical role in the initiation of cell division in *Escherichia coli* (19). Morphologic analysis of the *ftsZ84* filaments formed at the nonpermissive temperature and physiological studies of this mutant suggest that *ftsZ* acts earlier in the cell division pathway than other reported *fts* genes (4, 13). However, only one temperature-sensitive mutation in *ftsZ*, *ftsZ84*(Ts), has been characterized, and this mutation can be suppressed by a high salt concentration (22, 26). Nonetheless, on the basis of the conditional, lethal phenotype of the *ftsZ84* mutation, it has been assumed that *ftsZ* is an essential cell division gene.

The *ftsZ* gene has been sequenced and encodes a 40-kDa protein that is hydrophilic (33). Immunoblot analysis has revealed a cross-reacting antigen in a diverse set of bacterial species, indicating that the *ftsZ* gene is highly conserved among the eubacteria (10). Characterization of the *ftsZ* gene from *B. subtilis* revealed that the FtsZ protein has 50% amino acid identity to the *E. coli* homolog (2). A conditional, lethal, temperature-sensitive mutation [*tsI* = *ftsZ1*(Ts)] that blocks cell division at the nonpermissive temperature was located in the *Bacillus subtilis ftsZ* gene, suggesting that the gene has a similar function in this organism.

Strong evidence for a critical regulatory role for *ftsZ* in cell division came from analysis of the effects of FtsZ overproduction on cell division. A two- to sevenfold increase in the level of FtsZ in wild-type cells induces a minicell phenotype which is not accompanied by an increase in the average cell length (32). This phenotype is in contrast to the phenotype of the *min* mutant, in which minicell production is accompanied by an increase in the average cell length, suggesting that minicell formation occurs at the expense of medial divisions and that the division potential is limited (29). An increase in FtsZ alone can suppress the increased average cell length of the *min* mutant, supporting the suggestion that FtsZ determines the division potential (6). These data strongly suggest that the FtsZ level is a controlling element for the frequency of cell division. If this were true, one would expect that cell division would be very sensitive to even small decreases in FtsZ.

Although the *ftsZ84*(Ts) mutation is the only conditionally lethal mutation isolated in *ftsZ*, it is not the only *ftsZ* mutation that has been isolated. A class of mutations that suppress the sensitivity of *lon* to filamentous death following treatment with DNA-damaging agents maps in the *ftsZ* gene (7, 21). These mutations, referred to as *ftsZ*(Rsa) (formerly *sulB* or *sfiB*), alter the *ftsZ* gene product such that it is resistant to the SOS-inducible cell division inhibitor *sulA* (*sfiA*). Most of these mutations do not drastically affect the postulated essential cell division function, although several *ftsZ*(Rsa) mutations confer a slight temperature- and salt-sensitive filamentation phenotype (15, 18). However, one recently isolated *ftsZ*(Rsa) mutation, *ftsZ3*(Rsa), which was isolated in a strain diploid for *ftsZ*, cannot support cell growth in the absence of a wild-type copy of *ftsZ* (7). This is consistent with the notions that *ftsZ* is an essential gene and the *ftsZ3*(Rsa) mutation knocks out the essential *ftsZ* function.

In this study, we constructed a null allele of the *ftsZ* gene in the presence of additional copies of *ftsZ* supplied on a temperature-sensitive replicon. This allowed us to determine the phenotype of a conditional null allele and demonstrate that *ftsZ* is a cell division gene that is essential for viability in *E. coli*.

(A preliminary account of this work was presented at a European Molecular Biology Organization Workshop on the Bacterial Cell Cycle [5].)

MATERIALS AND METHODS

Bacterial and bacteriophage strains. The bacterial strains used in this investigation are listed in Table 1. Phage λ16-2 has been described previously (Fig. 1) (22). It contains a 10-kb chromosomal insert including the *ftsZ* gene. Phage λSR124 contains an *ftsZ-lacZ* operon fusion. The phage carries a 2.3-kb *Eco*RI fragment containing *ftsQ*, *ftsA*, and the 5′ end of the *ftsZ* gene cloned upstream of the *lacZ* gene. λ16-25K (*ftsZ::kan*) was obtained by recombination between λ16-25 (Fig. 1) and pJW5.2K (22).

Media and growth conditions. All strains were grown on L agar plates or in L broth supplemented with thymine (50 μg/ml) and the appropriate antibiotics (24). The antibiotics were used at the following concentrations (in micrograms per milliliter): ampicillin, 100; kanamycin, 25; tetracycline, 12.5; spectinomycin, 25; and chloramphenicol, 17. Minimal

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TABLE 1. Bacterial strains

Strain	Relevant marker	Source or reference
W3110	Prototroph	Laboratory collection
D110	W3110 *polA*	Donald Oliver
NK6923	*leu*::Tn*10*	M. Singer
KL723	F'104	B. Bachmann
BEF4	W3110 *srl*::Tn*10 recA56*	This work
BW10724	*recA*::*cat*	Donald Oliver
JKD7(pKD3)	W3110 *ftsZ*::*kan*	This work
JKD7-1(pKD3)	W3110 *ftsZ*::*kan recA56*	P1(JC10240) × JKD7 (Tet^r UV^s, then cure Tn*10*)
JKD3-1(pKD3)	W3110 *ftsZ*::*kan leu*::Tn*10*	This work
JKD3-1(pKD3)	W3110 *ftsZ*::*kan recA*::*cat leu*::Tn*10*	P1(BW10724) × JKD7 (select Cm^r screen UV*s*)
JKD7-1 (λ16-2)(pKD3)	Lysogen of JKD7-1(pKD3)	This work
JKD9(pKD3)	AMA1004 *ftsZ*::*kan*	P1(JKD7-1) × AMA1004(pKD3) (select Kan^r)
AMA1004	Δ*lac*(*I-Z*)*29*	M. Casadaban
JC10240	*srl*::Tn*10 recA56*	11

agar plates containing kanamycin were used to select progeny from a cross of JKD7-1(pKD3) and KL723(F'104). This medium selects for F'104 complementation of the leucine requirement of JKD3-1(pKD3), which is due to a *leu*::Tn*10* insertion.

Plasmids. Plasmid pJW5.2 contains a *Bam*HI-*Cla*I fragment containing the *ftsZ* and *ftsA* genes cloned into the same restriction sites in a derivative of pBR322 in which the *Eco*RI site had been filled in (Fig. 1). An *Eco*RI fragment containing the kanamycin resistance gene from pUC4K (30) was cloned into the single *Eco*RI site of pJW5.2 that is located near the 5' end of the *ftsZ* gene (Fig. 1 and 2). This plasmid was designated pJW5.2K. pKD3 (Fig. 1) contains a *Bam*HI fragment from pBEF0 (7) cloned into temperature-sensitive pSC101 derivative pEL3 (1), which was obtained from Paul March. pKD4 and pBS58 (7) contain the *ftsZ* gene and different amounts of the flanking sequence cloned into pSC101 derivative pGB2 (9) (Fig. 1). pKD4 was constructed by cloning a *Bgl*II-*Pst*I fragment from pKD3 into the *Bam*HI and *Pst*I sites in the polylinker region of pGB2.

Genetic manipulations. P1 transduction, transformation, conjugation, and lysogenization were done by standard procedures (24).

Immunoblot analysis. Immunoblots for determination of FtsZ levels were done as described previously, by using either a secondary antibody coupled to horseradish peroxidase or ^{125}I-labeled protein A (32).

Photomicroscopy. The average cell lengths of populations of cells at each time point were determined by photographing cells and measuring at least 100 cells. 4,6-Diamidino-2-phenylindole nucleoid staining and fluoresence microscopy were done essentially as described by Hiraga et al. (17).

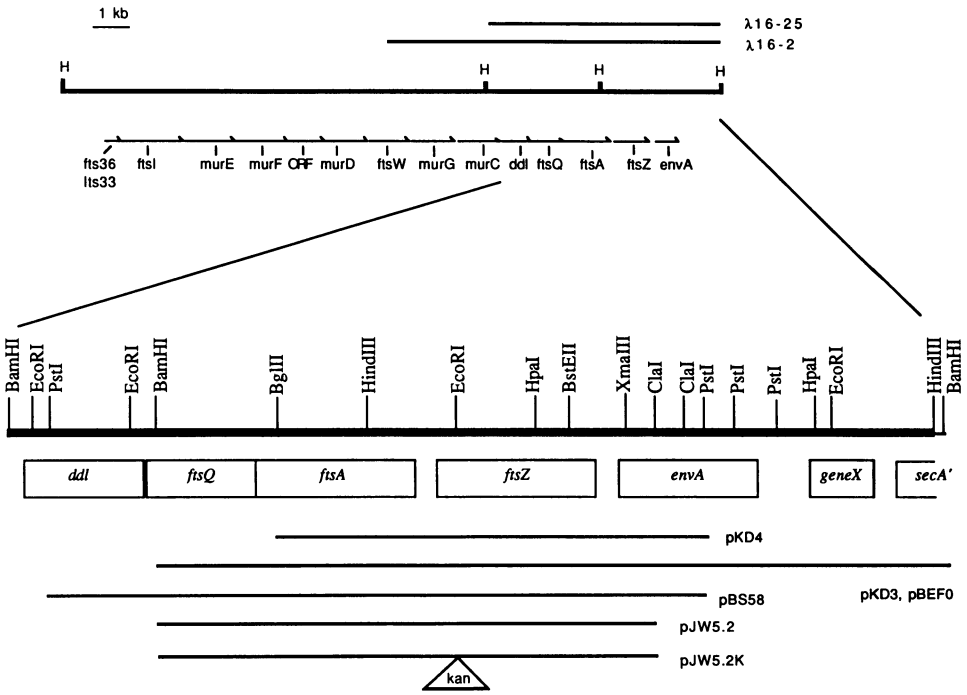

FIG. 1. Diagram of the 2-min region and the plasmids and phages used in this study. The *ftsZ* gene lies near the distal end of a large gene cluster. The extents of the inserts in the phages and plasmids used in this study are indicated by lines. The small *Hin*dIII-*Bam*HI fragment from the right end of the enlarged portion of the diagram is from λ.

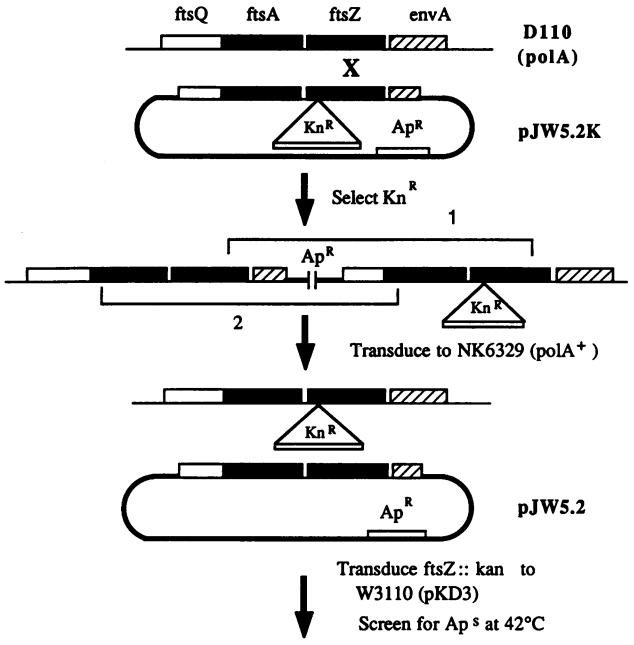

FIG. 2. Construction of a strain containing an interrupted *ftsZ* allele. Integration of pJW5.2K was obtained in a *polA* strain by selection for Kan^r. The resulting structure is shown in the center. Excision of the plasmid after transduction to a *polA*$^+$ strain (indicated by the numeral 2) would generate an intact *ftsZ* gene on the plasmid, increasing the level of *ftsZ* and leading to minicell production, and would leave the chromosomal *ftsZ* gene interrupted. *ftsZ*::*kan* was then transduced to W3110 containing pKD3 to give JKD7(pKD3). Subsequent introduction of the *recA56* mutation yielded JKD7-1(pKD3).

RESULTS

Construction of a null allele of *ftsZ*. The temperature-sensitive lethal phenotype induced by the *ftsZ84* mutation suggested that *ftsZ* is an essential gene. Preliminary attempts in our laboratory to disrupt the *ftsZ* gene in a strain with a single copy of *ftsZ* failed, supporting the idea that this gene is essential. To obtain positive evidence that *ftsZ* is essential, we attempted to disrupt the *ftsZ* gene in the presence of a second copy of *ftsZ* carried on a λ transducing phage. We chose λ16-2, since this phage carries a large chromosomal insert with approximately 6 kb of DNA upstream of *ftsZ* and has been shown to complement the *ftsZ84*(Ts) mutation under the most stringent test conditions (22, 23). A λ16-2 lysogen of a *recD* strain (28) was transformed with linearized pJW5.2K (*ftsZ*::*kan*). Kan^r transformants were obtained and screened to determine the location of the *kan* gene. In most cases, the *kan* gene was located on the transducing phage, although in rare instances the *kan* gene was located elsewhere on the bacterial chromosome, but P1 transduction revealed that it was not linked to the *ftsZ* locus at 2 min (data not shown). In another approach, an *Eco*RI-*Hpa*I restriction fragment internal to the *ftsZ* gene was cloned onto pEL3, which is temperature sensitive for replication. Insertion of this plasmid by homologous recombination would disrupt the *ftsZ* gene. W3110 (λ16-2) lysogens containing the plasmid were plated at 42°C in the presence of ampicillin to select for the plasmid. Survivors were obtained at a frequency of 10^{-4}. Of 30 survivors screened, all contained the *kan* gene on the phage. A possible explanation for the failure to target the gene disruption to the normal locus was that λ16-2 cannot provide sufficient FtsZ to complement a null allele of *ftsZ*, even though it can complement the *ftsZ84*(Ts) mutation.

TABLE 2. Complementation of the disrupted *ftsZ* allele

Strain	Relative efficiency of plating at 42°C
BEF4(pKD3)	1.0
JKD7-1(pKD3)	2.0×10^{-6}
JKD7-1(pKD4)	0.93
JKD7-1(pBS58)	1.0
JKD7-1(pKD3)F'104	1.0
JKD7-1 λ16-2(pKD3)	4.7×10^{-4}

In a separate approach, we chose to use a plasmid to provide additional copies of *ftsZ* since several of the plasmids we constructed resulted in more expression of *ftsZ* than λ16-2. A *polA* strain (16) was transformed with intact pJW5.2K (*ftsZ*::*kan*). Since this plasmid is a pBR322 derivative, it cannot replicate in this host and kanamycin-resistant survivors should result from Campbell insertion of the plasmid through homologous recombination at the *ftsZ* locus (Fig. 2). Eight Kan^r survivors were obtained, and seven of these were also ampicillin resistant. The ampicillin-sensitive strain contained a normal level of FtsZ and was not studied further. A P1 lysate was prepared on one of the Kan^r Amp^r transformants and used to transduce NK6923 (*leu*::Tn*10*) to Kan^r. Among the Kan^r transformants, 37.7% became Tet^s, indicating that the plasmid had integrated at the chromosomal *ftsZ* locus. The transductants were screened microscopically, and about 10% were observed to produce minicells. This phenotype is known to be induced by an increased level of FtsZ that results from an increased dosage of *ftsZ* (20, 32). Such a situation could arise in the transformants (NK6923 is *polA*$^+$) if the plasmid were excised from the chromosome in such a way that the disrupted allele was retained on the chromosome and the wild-type allele was on the plasmid, regenerating pJW5.2 (Fig. 2). Such a plasmid has been shown to cause minicell production (32). To confirm this result and obtain conditional expression of *ftsZ*, phage P1 was used to transduce Kan^r from a minicell-producing transductant to W3110 containing plasmid pKD3 (Fig. 1). This plasmid contains the *ftsZ* gene cloned onto temperature-sensitive replicon pEL3. Among the Kan^r transductants, 45% were Tet^r, indicating cotransduction of these two markers. Tet^s and Tet^r transductants were selected and designated JKD3(pKD3) and JKD7(pKD3), respectively. Subsequently *recA* mutations were introduced by P1 transduction to give JKD3-1(pKD3) and JKD7-1(pKD3) (Table 1).

***ftsZ* is essential for cell division and viability.** The cell morphology of JKD7-1(pKD3) appeared normal, consistent with the observation that the FtsZ level was indistinguishable from that of a strain with an intact chromosomal *ftsZ* gene. To determine whether *ftsZ* was essential, JKD7-1(pKD3) was plated at 30 and 42°C on L agar plates. The plating efficiency at 42°C was 2×10^{-6}, which is about the reversion frequency of the temperature-sensitive mutation affecting plasmid replication (Table 2). This frequency could not be increased by a high salt concentration, which is known to suppress the *ftsZ84*(Ts) mutation (data not shown). Several hundred revertants were examined, and all were Amp^r, indicating that in all instances the plasmid mutation conferring temperature-sensitive replication had reverted.

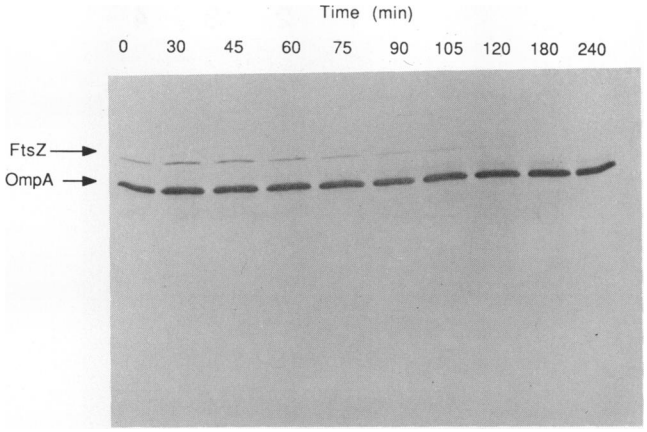

FIG. 3. The level of FtsZ in JKD7-1(pKD3) following a shift to the nonpermissive temperature. JKD7-1(pKD3) growing exponentially in L broth was shifted to 42°C at zero time. Samples were taken at the times indicated and adjusted so that an equivalent amount of cell mass was loaded in each lane. The samples were analyzed for FtsZ and OmpA contents by immunoblot analysis.

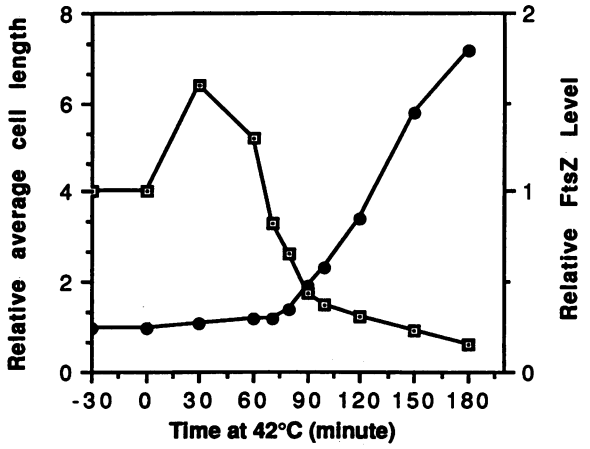

FIG. 4. FtsZ level and average cell length. JKD7-1(pKD3) was shifted to 42°C as described in the legend to Fig. 3. At various times, samples were taken and the average cell length of the population and the FtsZ level were determined. The average cell length was determined by measuring at least 100 cells in photomicrographs at each time point. The FtsZ level was determined by using radioactive protein A as the secondary reagent in immunoblots, excising the bands, and measuring the radioactivity.

This suggested that the *ftsZ* gene is essential and also revealed that bypass mutations are not readily isolated. It was still possible, however, that the *kan* insertion in *ftsZ* was polar on *envA*, an essential gene located just downstream of *ftsZ* (3), and this was responsible for the temperature sensitivity phenotype of JKD7-1(pKD3). This was possible since pKD3 contained *envA*, in addition to *ftsZ*, and thus could provide both gene products at the permissive temperature. To rule out this possibility, we transformed JKD7-1(pKD3) with plasmids that lacked *envA* and were resistant to spectinomycin. Plasmids pBS58 and pKD4, the latter containing only an intact *ftsZ* gene, could readily transform this strain and displace pKD3 (Table 2), whereas vector pGB2 could not. JKD7-1(pKD4) grew normally without chain formation and was not sensitive to rifampin, two phenotypes associated with decreased *envA* function (3). In addition, λ16-2 and λ16-25K (*ftsZ*::*kan*) (carrying the same *ftsZ* allele as plasmid pJW5.2K [*ftsZ*::*kan*]) complemented the null allele of *envA* that was constructed previously (3). This result confirmed that expression of *envA* from the phage was sufficient for complementation of a null allele, in contrast to the results obtained with *ftsZ*.

Previous studies have shown that overproduction of FtsZ increased the frequency of cell division, resulting in a minicell phenotype (32). This led to the suggestion that the level of FtsZ is critical and may be limiting for cell division. If this is true, then decreasing the level of FtsZ below its normal physiological level may lead to rapid inhibition of cell division. By using JKD7-1(pKD3), we were able to test this directly. The level of FtsZ in this strain is the same as in a wild-type strain, indicating that expression of *ftsZ* from this low-copy plasmid is quantitatively similar to that of the chromosomal *ftsZ* locus. A culture of JKD7-1(pKD3) growing exponentially at 30°C was shifted to 42°C to inhibit replication of the plasmid. Samples were taken at 10- to 30-min intervals, and cells were examined for average cell length and FtsZ content. FtsZ content, along with OmpA content as an internal control, was determined by immunoblot analysis. Figure 3 shows that the FtsZ level decreased following the temperature shift, with a lag of about 75 min, whereas the OmpA level remained constant. The FtsZ level was quantitated by using radioactive protein A in immunoblots, excising the bands, determining the amount of radioactivity, and normalizing to OmpA. The results of this determination, along with the average cell length, are plotted in Fig. 4. This figure shows that the FtsZ level increased 50% immediately after the temperature shift before decreasing. This increase in FtsZ is due to the presence of the *ftsZ* gene on the plasmid, since it was not seen with a strain containing just the chromosomal gene (data not shown). The explanation for this is unknown. More importantly, this plot shows that the average cell length started to increase about 75 min after the temperature shift, when the FtsZ level had decreased by 30 to 40% from its preshift level. The shifting experiment was repeated, except that the culture was shifted to 37°C. This intermediate temperature is also nonpermissive but does not block replication of the plasmid as quickly. The results of this experiment were similar, except that the parameters average cell length and FtsZ level started changing 120 min after the shift (data not shown). These results demonstrated that cell division is indeed quite sensitive to the level of FtsZ.

Monitoring of cells throughout these shifting experiments revealed that they became extremely filamentous and eventually lysed at the nonpermissive temperature. Filamentous cells were removed near the end of the experiment and examined for nucleoid segregation by staining of the DNA. This analysis (Fig. 5) revealed that the nucleoids were distributed throughout the length of the filament, indicating that nucleoid segregation appears normal even as the level of FtsZ decreases.

JKD7-1 cannot be complemented by λ16-2. Our initial attempts to inactivate the *ftsZ* gene in the presence of λ16-2 were unsuccessful, indicating that λ16-2 could not provide sufficient FtsZ for cell viability. To test this further, JKD7-1(pKD3) was lysogenized with λ16-2 at 30°C and then tested for growth at 42°C. In a streak test, no individual colonies were formed but some growth was observed at the site of inoculation. Microscopic examination of cells from this area revealed that they were extremely filamentous and undergo-

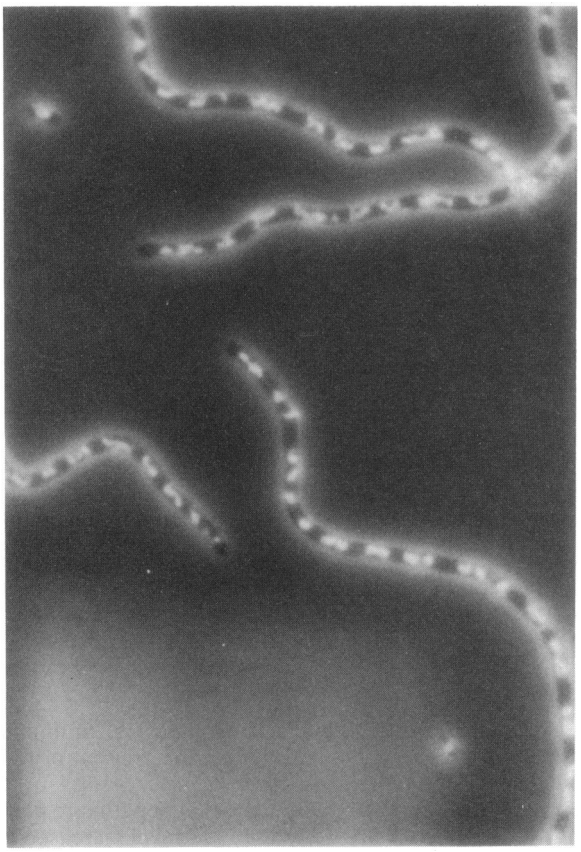

FIG. 5. Nucleoid distribution in FtsZ-depleted filaments. Cells taken 3 h after the temperature shift were examined for nucleoid distribution by DNA staining and fluorescence photomicroscopy.

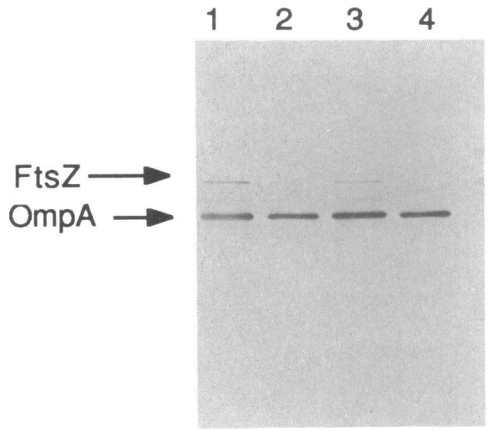

FIG. 6. Comparison of the amount of FtsZ produced from the chromosomal *ftsZ* locus and λ16-2. BEF4(pKD3) and JKD7-1 (λ16-2)(pKD3) growing exponentially at 30°C were shifted to 42°C. At 4 h after the shift, the FtsZ content was determined by loading equal amounts of samples (normalized for optical density at 600 nm). Lanes: 1 and 3, BEF4(pKD3) and JKD7-1 (λ16-2)(pKD3), respectively; 2 and 4, one-half of the sample loaded.

ing lysis. The plating efficiency of this lysogenic strain at 42°C was 4.7×10^{-4}, about a 250-fold increased over the nonlysogen (Table 2). Two of these temperature-resistant colonies were examined further, and we were able to demonstrate that the mutation to temperature resistance was located on the chromosome and not on λ16-2 by exchanging the resident λ16-2 with a fresh λ16-2. Preliminary analysis demonstrated that these mutations enhanced the expression of *ftsZ* from λ16-2 (data not shown).

The change in FtsZ content and kinetics of cell division were monitored by shifting the lysogen to the nonpermissive temperature. The end result was similar to that obtained with the nonlysogen in that extremely filamentous cells that eventually lysed were formed (data not shown). The major difference was that there was a longer delay before the average cell length started to increase. This delay correlated with a delay in the onset of a decrease in the FtsZ level. This correlation further demonstrated that cell division is sensitive to the FtsZ level.

To compare the amount of *ftsZ* expressed from λ16-2 with that expressed from the chromosomal locus, we compared the FtsZ levels of BEF4(pKD3) and JKD7-1 (λ16-2)(pKD3) at 4 h after a shift to 42°C. At this time, expression from the plasmid is negligible, so in the first strain *ftsZ* expression is from the chromosomal locus while in the second strain *ftsZ* expression is from the phage. Figure 6 is an immunoblot that compares the levels of FtsZ in these two strains 4 h after a temperature shift (compare lanes 1 and 3 and 2 and 4; the latter pair are a 1:2 dilution of lanes 1 and 3, respectively). Quantitative determination of the level of FtsZ expressed from λ16-2 revealed that it is 60 to 70% of that of the chromosomal locus. That this level is insufficient for cell division is consistent with the result obtained with the nonlysogen, in which cell division ceased when FtsZ decreased by 30 to 40%. Also, it should be noted that the same phenotype is observed whether the FtsZ level is allowed to decrease completely (nonlysogen) or by only 30 to 40%.

One possible explanation why λ16-2 cannot provide sufficient FtsZ to complement JKD7-1(pKD3) is that promoters further upstream of those included in λ16-2 are required for full expression of *ftsZ*. It is known that genes upstream of *ftsZ* extending to *ftsI* (Fig. 1) are all in the same orientation and all tightly clustered such that transcription initiating anywhere within the cluster might continue to the only known terminator beyond *envA*. To test whether additional upstream DNA would allow complementation, F'104, which contains a chromosomal insert extending from 98 to 7 min and therefore includes the entire 2-min cluster, was transferred from KL723(F'104) to JKD3-1(pKD3). Exconjugants were obtained by selecting for Tet^r and complementation of *leu* at 30°C. The plating efficiency of one of these exconjugants was 0.83 at 42°C (Table 2). Since these survivors were Amp^s, it indicated that F'104 complemented the interrupted *ftsZ* allele. Immunoblot analysis of one of these exconjugants showed a level of FtsZ indistinguishable from that of a control strain (BEF4). The cellular morphology of these exconjugants appeared normal, although occasional filamentous cells (<1%) were observed. This result is consistent with the notion that promoters upstream of those found in λ16-2 are required for full *ftsZ* expression.

The *ftsZ* gene is not autoregulated at adjacent promoters. Studies on autoregulation of *ftsZ* have given conflicting results (14, 27). In two separate studies, *ftsZ* autoregulation was examined by monitoring an *ftsZ-lacZ* fusion carried on a λ transducing phage in the *fts84*(Ts) mutant after a shift to the nonpermissive temperature. Since the mutant protein retained some function at the nonpermissive temperature, the JKD7-1(pKD3) strain provided a better system to monitor possible autoregulation. JKD7-1(pKD3) lysogenized

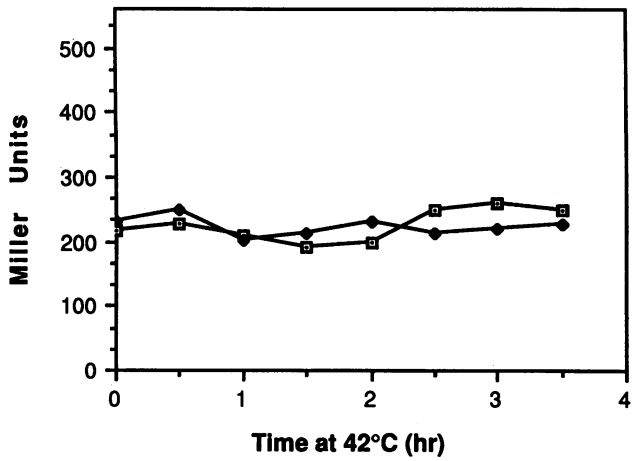

FIG. 7. Expression of *ftsZ* in response to FtsZ depletion. JKD9 λSR124(pKD3) (⊡) and a control, JKD9 λSR124(pBEF0) (●) growing exponentially at 30°C were shifted to 42°C at zero time. Samples were removed, and β-galactosidase activity was determined at the times indicated.

with λSR124 (*ftsZ-lacZ*) was shifted to 42°C and β-galactosidase activity was monitored (Fig. 7). The results show that even at 2 h after the shift, when the FtsZ level was reduced by more than 80%, there was no change in the expression of *ftsZ* as monitored with this fusion. This indicates that the promoters for *ftsZ* located within this 2.3-kb *Eco*RI fragment are not subject to autoregulation, positive or negative. It does not exclude the possibility that promoters located further upstream are affected.

DISCUSSION

In this study, we disrupted the chromosomal *ftsZ* gene in the presence of a cloned copy of the *ftsZ* gene on a temperature-sensitive replicon. The resultant strain was temperature sensitive for cell division and viability, directly demonstrating that *ftsZ* is an essential cell division gene. This study also confirmed that cell division is quite sensitive to the level of FtsZ.

When JKD7-1(pKD3) was shifted to the nonpermissive temperature for plasmid replication (42°C), the culture continued to increase in optical density for 2 to 3 h; however, after 75 min cell division ceased and the cells started to filament, eventually reaching many times their original length. Quantitation of the FtsZ level by immunoblot analysis revealed that the level of FtsZ per cell mass first increased but then started to decline because of dilution by continued cell growth. The time at which cells started to filament coincided with the time at which the level of FtsZ fell below 30% of the preshift level, indicating that the cell division process was quite sensitive to the level of FtsZ. A simultaneous but delayed shift in these two parameters was also observed upon a shift to an intermediate temperature or with JKD7-1 λ16-2(pKD3). In these cases, the parameters were both shifted to a later time. Thus, when we manipulated the experimental conditions, these two parameters shifted simultaneously. This response of cells to depletion of FtsZ is not consistent with the notion that FtsZ is an inhibitor of division, as has been proposed (25).

The failure of λ16-2 to complement the interrupted strain was unexpected, since it can complement the *ftsZ84*(Ts) mutation under the most stringent test conditions (22, 23). This failure must be due to insufficient expression of *ftsZ* from this phage, since plasmids that contain just *ftsZ* (such as pKD4) complement very efficiently. Such plasmids are known to produce more FtsZ than λ16-2 because they induce minicell formation, which is directly correlated with the FtsZ level, and by direct immunoblot measurement (20). This result also implies that the FtsZ84 protein has residual activity at the nonpermissive temperature which is insufficient to support cell growth and that the activity of the FtsZ84 protein can be rescued by the wild-type protein. This could occur through formation of mixed multimers, since it has been proposed that the functional form of FtsZ is a multimer (7).

Previously, we have shown that *ftsZ* is expressed from promoters located within the *ftsA* and *ddl* genes and that these were sufficient for expression of *ftsZ* from a single-copy vector to complement *ftsZ84*(Ts) (22, 23, 34). The failure of λ16-2 to provide sufficient FtsZ for complementation of the null allele could be due to several possibilities. A likely possibility is that a portion of *ftsZ* expression is due to a promoter(s) that lies upstream of DNA in λ16-2. This explanation would be consistent with the F'104 complementation of the interrupted allele and with the sequence data which showed that the upstream genes are in the same orientation and tightly clustered (19). Transcription starting at any point within the cluster—and there appear to be many promoters within the cluster—should continue to the terminator just downstream of *envA*. The experiments presented here indicate that about 30 to 40% of *ftsZ* expression comes from promoters upstream of that found in λ16-2.

Another possible explanation for the lack of complementation by λ16-2 is that the DNA structure, such as local supercoiling, at the *att*λ locus is different from that at the normal *ftsZ* locus and this affects expression. Another possible contributing factor is that different chromosomal locations lead to differences in gene dosage. In an exponential culture in rich medium, the dosage of a gene at the 2-min region would exceed that of a gene located at the *att*λ site, which is nearer the terminus of replication. However, doing the complementation test on medium supporting a slower growth rate, which would minimize this difference, did not yield positive results. Further work will be required to differentiate among these possibilities, but whichever of these is right, it is clear from the work presented here that *envA* expression is not subject to this requirement. The *envA* null allele can be fully complemented by a transducing phage, even a phage in which the *ftsZ::kan* allele on the phage might block any upstream transcription from reaching *envA*.

These and earlier results (7, 32), show that the level of FtsZ is an important factor for cell division. However, the actual role of FtsZ in this process is unclear. FtsZ is a cytoplasmic protein (31) that appears to interact with other cytoplasmic proteins, SulA and MinCD, which can inhibit division (7, 8, 12). Thus, FtsZ could either be a positive regulator of division or play a structural role. We favor the explanation that FtsZ has a structural role because of its abundance and the inhibitory effects on cell division when FtsZ is overproduced more than 10-fold. One could envision FtsZ forming a transient structure at the site at which the septum is to form. Such a structure may act to activate septal peptidoglycan synthetic activity.

ACKNOWLEDGMENTS

We thank D. Oliver, M. Singer, and B. Bachmann for sending strains.

This work was supported by Public Health Service grant GM29764 from the National Institutes of Health.

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