FtsZ in *Bacillus subtilis* is required for vegetative septation and for asymmetric septation during sporulation

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A *Bacillus subtilis* strain was constructed in which the cell division gene, *ftsZ*, was placed under control of the isopropyl-β-D-thiogalactoside (IPTG)-inducible *spac* promoter. This strain was dependent upon the presence of IPTG for cell division and colony formation indicating that *ftsZ* is an essential cell division gene in this organism. In sporulation medium this strain increased in mass and reached stationary phase in the presence or absence of IPTG, but only sporulated in the presence of IPTG. The expression of the sporulation genes *spolIG*, *spolIA*, and *spolIE* occurred normally in the absence of IPTG as monitored by *spo−lacZ* fusions. However, expression of *lacZ* fusions to genes normally induced later in the developmental pathway, and that required processed *pro−αE* for expression, was inhibited. Immunoblot analysis revealed that *pro−αE* was not processed to its active form (*αE*) under these experimental conditions. Electron microscopy revealed that these FtsZ-depleted cells did not initiate asymmetric septation, suggesting that FtsZ has a common role in the initiation of both the vegetative and sporulation septa.

**Key Words:** Sporulation; septation; *ftsZ*; cell division; σ-factors

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In *Escherichia coli*, the *ftsZ* (filamentation temperature-sensitive) gene plays a pivotal and essential role in cell division. The *ftsZ*\(^84\) temperature-sensitive mutation is defective in the initiation of septation at the nonpermissive temperature although macromolecular synthesis and chromosome segregation occur normally (Ricard and Hirota 1973; Lutkenhaus et al. 1980). Genetic evidence has implicated *ftsZ* as the target of two different cell division inhibitors. One, *sulA* (*sfiA*), is a component of the SOS response and prevents formation of anucleate cells following an interruption in DNA replication (Jaffe and D’Ari 1985); the second, *minCD*, is a component of the *min* system that is involved in prevention of division at polar sites (de Boer et al. 1989). These inhibitors can be suppressed by either mutational alteration of *ftsZ* (Bi and Lutkenhaus 1990a,c) or overexpression of *ftsZ* (Bi and Lutkenhaus 1990c; de Boer et al. 1990). The antagonism of MinCD by increased FtsZ in wild-type cells is seen as a minicell phenotype that is characterized by polar divisions and more than one division event per cell doubling. This result suggested that the level of FtsZ regulates the frequency of septation (Ward and Lutkenhaus 1985; Bi and Lutkenhaus, 1990b).

Previously we cloned and characterized the *Bacillus subtilis* *ftsZ* gene and found it had 50% amino acid sequence identity with the *E. coli* homolog (Beall et al. 1988). Despite these sequence similarities the *B. subtilis* homolog did not complement an *E. coli* *ftsZ* mutation and, in fact, inhibited division by apparently interfering with the function of the *E. coli* *ftsZ*. Nonetheless, the *ftsZ* gene of *B. subtilis* appears to function in cell division because *ts1*, a well-characterized cell division mutation, has been mapped to this gene (Beall et al. 1988; Harry and Wake 1989).

The life cycle of *B. subtilis* is characterized by two similar, but distinct, septation events. During vegetative growth, septation occurs at the midpoint of the cell whereas during sporulation a septum is located near the cell pole. This asymmetric septum is characterized by the lack of cell wall peptidoglycan. At present it is not known to what extent components of the vegetative septation apparatus function in forming the asymmetric septum. Thus, it was of interest to determine if FtsZ could have a role in initiation of the asymmetric septum.

According to a model proposed by Stragier et al. (1988) formation of the asymmetric septum exerts a morphologic control over development through regulation of the processing of *pro−αE*. The processing of *pro−αE* (*P\(^{31}\)*), the *spolIG* gene product, to its active form, *αE* (*σ\(^E\)*), occurs through cleavage of 29 amino-terminal amino acids early...
in the differentiation process at a time that is coincident with formation of the asymmetric septum (Trempe et al. 1985, LaBell et al. 1987, Jonas et al. 1988). This proteolytic processing of pro-αF is necessary for sporulation and requires the products of several stage II sporulation genes (Kenney and Moran, Jr. 1987; Jonas and Haldenwang 1988; Jonas et al. 1990), including the spoIIGA product, which is thought to be a membrane-spanning protease that is activated by insertion into the unique asymmetric septum (Stragier et al. 1988).

In this report we demonstrate that ftsZ is an essential gene that is required for formation of both the vegetative and asymmetric septa, and that a reduced level of FtsZ prevents proteolytic activation of pro-αF.

Results

The effect of varying FtsZ levels on cell division and growth

B. subtilis strain IA610 carrying the shuttle plasmid pSI-Z [Fig. 1], which contains the ftsZ gene downstream of the inducible spac promoter, grew at low inducer isoproply-β-D-thiogalactoside (IPTG) concentrations (0.01–0.05 mM) with normal cellular physiology. This plasmid could efficiently complement the temperature-sensitive ftsZ mutation, ts 1, at these same low IPTG concentrations but not in the absence of IPTG (data not shown). At IPTG concentrations >0.25 mM, cells of ts 1 or IA610 containing pSI-Z were filamentous and unable to form colonies (data not shown). This inhibitory effect of overexpression of ftsZ on cell division and colony formation is similar to that observed in E. coli when the expression of ftsZec is increased over 10-fold. In contrast, moderate overproduction (two- to sevenfold) of FtsZec results in a dramatic minicell phenotype (Ward and Lutkenhaus 1985), but this phenomenon was not significantly detectable in B. subtilis strains carrying pSI-Z in the presence of IPTG concentrations between 0–2 mM (data not shown).

To examine the effect of decreasing the FtsZ level, the single chromosomal copy of ftsZ was placed under spac promoter control. To do this, a fragment containing the 5’ end of the ftsZ gene [Fig. 1] was cloned along with the spac promoter and the lacI promoter into an integrational plasmid vector to give pJSIZΔp or pJSIZΔpbl (differing only in antibiotic resistance). Transformation of B. subtilis strain IA610 with either of these plasmids only yielded transformants in the presence of IPTG (designated BB1 and BB11, respectively). The integration of either of these plasmids by a Campbell insertion placed the single intact copy of ftsZ downstream of the spac promoter [Fig. 2]. In contrast with the inhibition of colony formation seen when placing the ftsZ gene on a multicopy plasmid under spac promoter control, colony formation of strains BB1 and BB11 was not inhibited by the addition of IPTG up to 2 mM. These strains exhibited identical growth curves in the presence or absence of IPTG when initially inoculated to an OD600 of >0.15. If inoculated at a lower initial OD600, the cells, in the absence of IPTG, lysed before reaching stationary phase.

To determine if the effect of IPTG on the level of FtsZ and the resultant cellular morphology, strain BB1 was inoculated into media containing varying amounts of IPTG. After incubation for several hours, the cultures were examined for FtsZ content and cellular morphology. Immunoblot analysis revealed that the level of FtsZ increased with the IPTG concentration up to 0.2 mM, but further increases in IPTG did not further increase FtsZ, indicating that maximal induction was achieved at 0.2 mM IPTG (data not shown). The level of FtsZ observed at high IPTG concentrations (>0.2 mM) was comparable with the wild-type level, and the cellular morphology appeared normal. Below 0.2 mM IPTG, the FtsZ level decreased and cells became extremely filamentous [Fig. 3]. These results demonstrated that FtsZ was required for cell division, and the fully induced spac promoter could provide sufficient FtsZ for normal growth and morphology.

To determine if the IPTG-dependent phenotypes of BB1 and BB11 were caused by insufficient expression of genes downstream of ftsZ, IA610 was transformed with the integrational plasmids pJSI-41 and pJSI-42 [Fig. 1]. These integrational insertions separate ORF4 from all downstream copy of ftsZ, designated a, was obtained by Ball digestion of pBZ1, which was described earlier (Beall and Lutkenhaus 1988).

Figure 1. Plasmids used in this study. Plasmids pSI-A and pSI-Z are both derivatives of the shuttle vector pSI-1 containing the ftsA and ftsZ genes, respectively. The other plasmids are derivatives of the integrative plasmid JH101. Each of these plasmid derivatives contains the indicated DNA fragments cloned downstream of the spac promoter. Each plasmid expresses the lacI gene from the pen promoter with the exception of pJSI-42, which is deleted for the lacI gene. The arrows indicate the orientation of the four open reading frames. With the exception of pJSI-42, the open reading frames contained in each plasmid are in the same orientation as the spac promoter. The endpoint upstream of ftsZ, designated a, was obtained by Ball digestion of pBZ1, which was described earlier (Beall and Lutkenhaus 1988).
ftsZ and septation in *B. subtilis*

![Diagram](image)

**Figure 2.** Construction of IPTG-dependent *B. subtilis* strains, BB1 and BB11, with *ftsZ* under *spac* promoter control. Plasmid pJSIZΔp, which contains the ribosome-binding site and first 147 codons of the *ftsZ* gene downstream of the *spac* promoter, was used to transform wild-type strain IA610 to CmR in the presence of 0.5 mM IPTG to give strain BB1. Campbell insertion of this plasmid places the *ftsZ* gene under *spac* promoter control. A similar strain, BB11, was constructed using plasmid pJSIZΔpble, which contains resistance to phleomycin instead of chloramphenicol.

(data not shown). This result indicated that *ftsZ* expression normally required promoter(s) upstream of *ftsA* and that *ftsA* and *ftsZ* are in an operon.

We also positioned the *spac* promoter upstream of the *ftsA* and *ftsZ* genes. This was done by Campbell insertion of pJSIAΔpV, which contains the *spac* promoter just upstream of the 5' end of the *ftsA* gene (Fig. 1). In contrast with the other strains constructed (BB6, BB1, and BB11) this strain (BB7) grew slowly and the cells were filamentous. It would appear that the *spac* promoter, all-

**Figure 3.** Cell division in a strain with the *ftsZ* gene under *spac* promoter control is IPTG dependent. An exponential culture of strain BB1 growing in IPTG-supplemented 2XYT was harvested, washed free of IPTG, and resuspended in fresh 2XYT with or without 1 mM IPTG. After incubation at 37°C for approximately four generations (as determined by the increase in optical density) the cells were fixed in 10% formaldehyde and photographed. [A] + IPTG, [B] − IPTG.
though sufficient for \(ftsZ\) expression, cannot provide ample \(ftsA\) expression for normal growth and morphology.

**Effect of a decrease of FtsZ on sporulation and expression of various spo genes**

When inoculated at an initial \(OD_{600}\) of 0.2–0.4 into DS sporulation medium with or without IPTG, strains BB1 and BB11 increased in mass for about three doublings before reaching stationary phase (Fig. 4). Typically, 10 hr after reaching stationary phase in the absence of IPTG, strain BB11 had a viable count of \(\sim 3 \times 10^7\)/ml and produced \(10^9\) spores/ml. In contrast, in the presence of IPTG strain BB11 had a viable count of \(5 \times 10^8\)/ml and produced about \(2 \times 10^8\) spores/ml. Monitoring the FtsZ level in this experiment revealed that in the presence of IPTG, FtsZ was constant and in the absence of IPTG, FtsZ decreased two- to threefold during the first 2 hr but then remained constant (data not shown). As a result of these findings we wanted to determine at what point sporulation was blocked in the absence of IPTG. To do this we examined expression of several \(spo\)--\(lacZ\) fusions.

Figure 5 shows the results of monitoring six different \(lacZ\) fusions introduced into strain BB11. In the presence of IPTG, the expression of \(spo\)I--\(lacZ\), \(spo\)I\(E\)--\(lacZ\), \(spo\)IG--\(lacZ\), \(spo\)ID--\(lacZ\), \(sspE--lacZ\), and \(ger\)E--\(lacZ\) occurred normally following induction of sporulation as compared with the parental strain (not shown). In the absence of IPTG, only the expression of the \(spo\)IIA--\(lacZ\), \(spo\)IE--\(lacZ\), and \(spo\)IG--\(lacZ\) fusions occurred normally at about \(t_{0.5}\) and initially at the same levels as in the presence of inducer (Fig. 5A–C). At later times the levels of B-galactosidase activity expressed from the \(spo\)IG--\(lacZ\) and \(spo\)IE--\(lacZ\) fusions, but not the \(spo\)IIA--\(lacZ\) fusion, were significantly higher in the absence of IPTG (Fig. 5B,C).

In contrast with the results observed with these \(spo\)II--\(lacZ\) fusions, expression of fusions to genes normally expressed later in development, \(spo\)ID--\(lacZ\), \(sspE--lacZ\), and \(ger\)E--\(lacZ\), was prevented by removal of IPTG (Fig. 5D–F). This result suggested that the block to sporulation was very specific and that the earliest step blocked was expression of \(spo\)ID. To determine if reduction in \(ftsZ\) expression led to an irreversible block to sporulation, IPTG was added back at various times to the strain containing the \(sspE--lacZ\) fusion. Expression of this forespore-specific fusion was recovered by the addition of IPTG when the IPTG was added back before \(t_0\) (data not shown).

Expression of the \(spo\)ID gene and other sporulation genes expressed later in the differentiation process is known to require processing of pro-\(\sigma^E\), the product of the \(spo\)ILGB gene. Because \(spo\)IG was expressed efficiently in \(ftsZ\)-depleted cells following \(t_0\) but \(spo\)ID expression was not induced, it appeared likely that pro-\(\sigma^E\) was not being processed. Figure 6 shows that in the absence of IPTG pro-\(\sigma^E\) accumulated (lanes 2 and 3), but was not processed to its active 29-kD form (cf. lane 1 with lanes 2 and 3). This result also ruled out the possibility that the pro-\(\sigma^E\) precursor was unstable due to a reduction in the level of FtsZ.

A role for \(ftsZ\) in sporulation was also supported by examination of the \(ftsZ1\) mutation (formerly \(ts\) 1). This mutation is known to affect vegetative division, but an effect on sporulation has not been reported \(\text{[Nukushina and Ikeda 1969]}\). To determine if this mutation affected sporulation, the \(ts\) 1 mutation was inoculated into DS medium at 30°C. At various times, samples from this culture were shifted to 48°C, and the number of spores was measured after 22 hr (Fig. 7). Shifting to 48°C at any time before \(t_1\) resulted in a significant inhibition of sporulation; only \(\sim 10^5\) spores/ml were produced. Shifting at times later than \(t_2\) resulted in a level of spores that was similar to the culture maintained at 30°C. Thus, sporulation no longer required FtsZ after \(t_2\), the time when asymmetric septation should be completed.

**Effect of FtsZ depletion on formation of the asymmetric septum**

At approximately 5.5 hr after reaching stationary phase in DS medium, cells of strain BB11 were examined by electron microscopy. In the control culture (+IPTG) a large percentage (>75%) of the cells displayed asymmetric septa or spores characteristic of later developmental stages (Fig. 8A). In the culture lacking IPTG, the cells displayed no sign of asymmetric septum formation (Fig. 8B). In addition, the nuclear regions in these cells seemed morphologically distinct from the control cells. Whereas in the control cells the nuclear regions appeared diffuse, in cells from the culture lacking IPTG the nuclear re-
regions appeared condensed as though development was arrested at the stage of axial filament formation (stage I; Fitz-James and Young 1969). These results indicated that the level of \( ftsZ \) gene product must be maintained at the normal physiological level for formation of the asymmetric septum.

**Discussion**

In this paper, we demonstrate that the \( B. subtilis \) \( ftsZ \) gene is an essential gene. Placing the \( ftsZ \) gene under \( spac \) promoter control led to an inducer-dependent phenotype. We observed that reducing the level of FtsZ or shifting the \( ts \) mutant to the nonpermissive temperature resulted in extensive filamentation of vegetative...
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Figure 8. FtsZ is required for formation of the asymmetric septum. Strain BB11 was grown to mid-log phase in the presence of IPTG and washed before inoculating into PS medium to an OD<sub>600</sub> of 0.35 in the presence of 1 mM IPTG (A) and in the absence of IPTG (B). Cells were harvested 5.5 hr after the end of exponential growth and processed for electron microscopy.

cells and the failure of sporulating cells to form an asymmetric septum. Thus, FtsZ is required for both vegetative septation and asymmetric septation during sporulation and appears to be required for initiation of these events.

FtsZ and septation

The septation process in <i>B. subtilis</i>, both during vegetative growth and sporulation, was sensitive to the level of FtsZ. A two- to threefold reduction in the level of FtsZ was sufficient to completely block the initiation of septation. In <i>E. coli</i> where we have monitored this more precisely, less than a 30% decrease in the level of FtsZ was sufficient to block the initiation of septation (Dai and J. Lutkenhaus, in prep.). In <i>E. coli</i> a 2-7-fold increase in the level of FtsZ stimulated cell division in the form of a minicell phenotype. In <i>B. subtilis</i> we did not observe a significant minicell phenotype, although we used a multicopy plasmid containing the <i>ftsZ</i> gene under <i>spac</i> promoter control and varied the inducer concentration over a wide range. However, we did observe inhibition of cell division with overexpression of FtsZ similar to that observed with overexpression of <i>ftsZ</i> in <i>E. coli</i>. The failure to observe a demonstrable minicell phenotype in <i>B. subtilis</i> could be due to any one of several possibilities including a difference in the mechanism by which division sites are determined, or if polar division is governed by a <i>min</i> system, it may have a higher intrinsic resistance to FtsZ. Another possibility is that, as in <i>E. coli</i>, there may be a narrow range between the increase in the amount of FtsZ required for minicell formation and the amount that will induce filamentation; we were not able to achieve the proper steady-state level with <i>ftsZ</i> expressed from the <i>spac</i> promoter on a multicopy plasmid.

It was possible that FtsZ would not be required for asymmetric septation and that this septation event would be independent of the vegetative septation machinery. Our results demonstrating that FtsZ was required indicated that these two morphologically distinct septation events share at least some of the septation machinery. It is possible that the mechanism for localizing the septation event may be related to the <i>min</i> system of <i>E. coli</i>. In <i>E. coli</i> the poles appear to be potential division sites (remnants of a previous division) that are masked by the <i>min</i> system (de Boer et al. 1989). Either loss of the <i>min</i> system or an increase in the level of FtsZ can cause a minicell phenotype (Ward and Lutkenhaus 1985), and there is genetic evidence that <i>ftsZ</i> and <i>min</i> interact (Bi and Lutkenhaus 1990b). Asymmetric septation during sporulation in <i>B. subtilis</i> may involve the unmasking of polar sites through inactivation of a <i>min</i>-like system. We favor this possibility because we did not see overproduction of FtsZ during sporulation, and overproduction of FtsZ in vegetative cells of <i>B. subtilis</i> was not sufficient to induce minicells. During sporulation, unmasking of polar sites would allow asymmetric septation to occur at either pole with one of them chosen at random. The availability of both cell poles is evident in many <i>spoII</i> mutants which display a terminal disporic phenotype (Piggot and Coote 1976).

Based upon these observations we propose the following model for asymmetric septation in which we assume that the poles are potential division sites masked by a <i>min</i>-like system: (1) Initiation of sporulation inactivates the <i>min</i> system freeing the poles for septation; (2) the normal septation machinery operates at one of the poles forming a septum, and (3) <i>spoII</i> gene products modify the septum (removing peptidoglycan) into a mature asymmetric septum.

Asymmetric septation and processing of pro-σ<sup>E</sup>

Our results are consistent with the model for regulation of pro-σ<sup>E</sup> processing proposed by Stragier et al. (1988), namely that the presence of the asymmetric septum is required for processing. We observed that reducing the level of FtsZ blocked formation of the asymmetric septum, processing of pro-σ<sup>E</sup> (Figs. 5C, 8), and expression of <i>spoIID</i> and other genes normally expressed later in the
sporulation pathway [Fig. 5D–F]. Although we propose that the block to processing arises from the lack of septum formation, a more direct role for FtsZ in processing cannot be ruled out.

The complexity of pro-σE synthesis and processing is apparent by the many genes that are required for these events [Jonas and Haldenwang 1989]. Mutations in spoIIA and spoIIE operons are characterized by normal spoIIIG expression, the absence of pro-σE processing, and the presence of abnormal asymmetric septa that often contain cell wall material. These genes may function to convert a nascent septum to the morphological distinct asymmetric septum as suggested above. It is clear from our results that a decreased level of FtsZ did not act by inhibiting the expression of the spoIIA and spoIIE operons [Fig. 5A,B].

Jonas et al. [1990] used penicillin treatment of sporulating cells to examine the coupling of asymmetric septum formation to the processing of pro-σE. They concluded that significant pro-σE processing was occurring at penicillin concentrations that significantly inhibited asymmetric septation. Their experiments were unexpectedly complicated by the observation that penicillin inhibited spoIIIG expression. Therefore, penicillin had to be added after significant pro-σE had accumulated and processing was already in progress. Our experiments were technically more straightforward in that decreasing FtsZ completely blocked septation under the conditions used without interfering with spoIIIG expression.

ftsZ and ftsA gene organization

The organization of the ftsA and the ftsZ genes in B. subtilis appears less complex than in E. coli. In E. coli the ftsA and ftsZ genes are located near the 3' end of a large cluster of closely packed essential genes, all of which appear to be involved in cell wall physiology or cell division [Lutkenhaus 1990]. In contrast, in B. subtilis, the genes immediately downstream of ftsZ [ORF4, Fig. 1] and upstream of the sbp gene are not essential [Beall et al. 1988, Beall and Lutkenhaus 1989]. Our results indicate that the ftsA and ftsZ genes of B. subtilis are arranged in an operon with a vegetative promoter upstream of the ftsA gene. Thus, expression of ftsA and ftsZ in B. subtilis may be uncoupled from surrounding genes, whereas in E. coli this is not the case. The E. coli ftsZ gene is expressed from a number of promoters located within the ftsA gene, and along with the ftsQ and ftsA genes from promoters even farther upstream [Yi et al. 1985].

The ftsA gene is thought to be an essential cell division gene in E. coli [Lutkenhaus and Donachie 1979]. Attempts to replace the ftsA gene in strain BB1 with a disrupted ftsA allele in the presence of IPTG [to express ftsZ] have been unsuccessful, indicating that the ftsA gene of B. subtilis is essential for viability [B. Beall and J. Lutkenhaus, unpubl.]. A previously isolated conditional stage II mutation, spoIIN279 [Young 1976], maps in the ftsA gene indicating that this cell division gene may also be required for sporulation [T. Leighton and P. Stragier, pers. comm.]. This mutation blocked sporulation by preventing spoIIIG expression at the nonpermissive temperature [Jonas and Haldenwang 1989].

Inspection of the DNA sequence upstream of the ftsA vegetative promoter in B. subtilis revealed a consensus promoter for σE [Beall and Lutkenhaus 1988]. This raises the possibility that the expression of ftsA and ftsZ is increased at the end of exponential growth. In the strains we have constructed, which place ftsZ downstream of the spac promoter, the σE promoter was not required for ftsZ expression to achieve sporulation [+IPTG cultures]. The fully induced spac promoter must provide sufficient FtsZ for sporulation.

Materials and methods

Bacterial strains

The parental bacterial strain used was B. subtilis 1A610 [Yande- zar and Zahler 1986]. Strains carrying various integrated plasmids that introduce the spac promoter at various locations within the ftsZ chromosomal region were obtained by transformation as described [Dubnau and Davidoff-Abelson 1971]. Derivatives of BB11 [containing an integrated pSI-ZΔpble that places the ftsZ gene downstream of the spac promoter] containing various lac fusions to sporulation genes were constructed by several methods. BB11 was lysogenized with SBSspoIIIG217-lacZ [Kenney and Moran, Jr 1987] and SBSspoIIE-lacZ [Piggot and Curtis 1987] by selecting chloramphenicol resistance. These phage were obtained from C. Moran, Jr. A chromosomal spoE-lacZ translational fusion from strain EUR9030 [Mason et al. 1988] and a chromosomal gcr-lacZ translational fusion from strain EUR9040 [obtained from S. Cutting] were introduced into BB11 by transformation through selection for a linked cat gene. The shuttle plasmid pKT90 carrying a spoIID-lacZ fusion [Rong et al. 1986] and the integrational plasmid pPP81 [Guzman et al. 1988] containing a spoIIA-lacZ translational fusion were introduced into BB11 by selection for a linked cat gene. Strain ts1 (Callister et al. 1983) contains the ftsZ1 [Ts] mutation.

Plasmids

Plasmid pSI-1, which contains the pUB110 and pBR322 replication origins, the spac promoter, and the lacZ gene expressed from the pen promoter [Yamura and Henner 1984], was obtained from D.J. Henner at Genentech. A derivative of pSI-1 containing the ble gene and lacking the cat gene was constructed in two steps. First, the 1.5-kb BamHI-BglII fragment from pUB110 containing the ble gene [Semon et al. 1987], was cloned into the BamHI site of pSI-1 and used to transform E. coli strain JM101 to bleomycin and chloramphenicol resistance. Subsequently, the cat gene was deleted from this plasmid by cleavage with Eco 0109 and Stul, treatment with Klenow fragment plus the four dXTPs, and ligation. This treated DNA was used to transform strain JM101 to bleomycin resistance. The resultant plasmid was designated pSI-1b.

Plasmid pSI-Z, which contains the ftsZ gene under spac promoter control, was constructed by first ligating the promoter-less ftsZ gene contained on a 1.6-kb Smal fragment from pKZ1-1 [Beall et al. 1988] into plasmid M13mp19 [Messing 1983]. One plasmid derivative with the ftsZ gene in the proper orientation was cleaved with XbaI and Spbl, and the 1.6-kb fragment was cloned into the same sites in pSI-1 downstream of the spac promoter. A derivative of pSI-Z, pSI-ZΔp, in which the 3' end of
the *ftsZ* gene was deleted but the first 147 codons from the 5' end of *ftsZ* complete with its ribosome-binding site was retained, was obtained by deleting a 1.1-kb *PstI* fragment (Fig. 1). *plSI-ZΔ* was used to construct two plasmids differing in antibiotic resistance, which could be subsequently used to put the *ftsZ* gene under spac promoter control by Campbell insertion. The first of these plasmids, *plSI-ZΔ*, is a plH101 derivative (Ferrari et al. 1983) that carries the 2.1-kb EcoRI–BamHI fragment from *plSI-ZΔ* with the 5' end of *ftsZ* downstream of the spac promoter. The second plasmid, a derivative of the first, was constructed by cloning the 1.5-kb BamHI–BglII bleomycin–phleomycin resistance fragment from pUB110 into the BamHI site of *plSI-ZΔ*. This *plSI-ZΔ* derivative was recovered and deleted of the *cat* gene by cleavage with Sall and Stul, followed by blunting with Klenow treatment, ligation, and transformation of JM101 to bleomycin resistance. This *plSI-ZΔ* derivative was designated *plSI-ZΔble*.

Plasmid *plSI-A* contains the *ftsA* gene lacking its promoter downstream of the spac promoter control and was obtained by cloning the 1.4-kb *XbaI–PstI* fragment from pUC18A (Beall et al. 1988) into the same sites in plSI-1 (Fig. 1). The 2.8-kb EcoRI–BamHI fragment from *plSI-A* was cloned into plH101 to obtain an integrable plasmid designated plSIA, which contained the entire *ftsA* gene under spac promoter control and *lacI* under pen promoter control. Plasmid plSIAV was obtained by deleting the PvuII fragment of plSIA, which resulted in deletion of the 3' end of *ftsA* and the *lacI* gene to yield an integrable plasmid containing only the first 122 codons of *ftsA* under spac promoter control. Plasmids plSIAI and plSI-A2 contain opposite orientations of a *PstI* fragment downstream of the spac promoter and the associated *lacI* gene. This *PstI* fragment contains the 3' 540 nucleotides of *ftsZ* and the 5' end of ORF4, which is in the same orientation and directly downstream of *ftsZ* (Fig. 1).

**Growth and sporulation media**

*B. subtilis* and *E. coli* strains were propagated in 2XYT (Messing 1983). For *B. subtilis* carrying the *cat* and *ble* genes, chloramphenicol and phleomycin were used at 6 μg/ml and 2 μg/ml respectively. The *ble* gene in *E. coli* was selected with bleomycin at 1 μg/ml.

Sporulation was induced by nutrient depletion in DS medium (Sheffler et al. 1965). *B. subtilis* cultures were grown to mid-log phase in 2XYT plus 1 mM IPTG containing the appropriate antibiotic to select for the various plasmids and chromosomal insertions. Cultures were harvested and washed in DS medium before resuspension in DS with the appropriate antibiotics in the presence or absence of IPTG. Chloroform-resistant spores were quantitated on L agar plates containing 1 mM IPTG.

**β-Galactosidase assay**

Aliquots of the cultures were pelleted periodically and assayed for β-galactosidase as described by Miller (1972).

**Immunoblot analysis**

Aliquots of *B. subtilis* cultures were pelleted and resuspended in TEST buffer (Messing 1983) containing 0.5 μg/ml lysozyme. Cell extracts from *B. subtilis* were prepared by three cycles of freeze–thawing cell suspensions followed by the addition of 3 volumes of SDS–sample buffer. Following SDS–polyacrylamide electrophoresis and transfer to nitrocellulose, the immunodetection of the *spoIIGB* gene products was done as described with anti-a monoclonal antibody obtained from W.G. Haldenwang [Jonas et al. 1988]. Immunodetection of the *ftsZ~sa* gene product with polyclonal anti-FtsZ serum was as described previously [Beall et al. 1988] except that the secondary antibody was an anti-rabbit IgG–alkaline phosphatase conjugate.

**Electron microscopy**

Cells were pelleted and fixed in 2% glutaraldehyde overnight at 4°C, postfixed in 1% osmium tetroxide for 1 hr at room temperature, and stained for 2 hr at 4°C in 2% uranyl acetate. Samples were dehydrated in a graded ethanol series followed by infiltration and embedding in L. R. resin (White, LTD, London) prior to thin sectioning. Thin sections were stained in uranyl acetate and Reynolds lead citrate prior to examination.

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