Chromosome partitioning is an accurate and efficient process in bacteria. Several genes that play roles in chromosome partitioning in *Bacillus subtilis* have been characterized, including *smc*, *spoIIJ*, and *spoIIIE* (1, 4, 9, 20, 28). SMC proteins are found in prokaryotes, eukaryotes, and archaea and are involved in a wide range of processes that affect chromosomes, including partitioning, sister chromatid cohesion, dosage compensation, condensation, supercoiling, and recombination (1, 3, 5, 8, 11, 12, 14, 19, 20, 27) (for reviews, see references 7, 10, and 26). SMC proteins have an N-terminal nucleoside triphosphate-binding domain, two long coiled-coil regions separated by a hinge, and a C-terminal signature DA-box motif (13). SMC proteins were first identified in eukaryotes but have now been found to be encoded by most prokaryotic genomes sequenced to date (1, 18).

The *B. subtilis* SMC protein is involved in chromosome structure and partitioning (1, 20), and its function may be analogous to that of MukB in *Escherichia coli* (21). *smc* null mutants are temperature sensitive for growth in rich medium. Under permissive conditions, *smc* null mutants have abnormal nucleoids and approximately 10% of the cells are anucleate (1, 20). A recent biochemical characterization indicates that *B. subtilis* SMC is an antiparallel homodimer (18) that has the ability to aggregate and/or reenate single-stranded DNA in ATP-dependent reactions in vitro (6). The mode of involvement of these SMC activities in chromosome partitioning and nucleoid structure is unknown.

*spoIIJ* of *B. subtilis* is required for faithful chromosome partitioning (and sporulation). *spoIIJ* is a member of the ParB family of partition proteins (22), and *spoIIJ* null mutant cells are ~1 to 2% anucleate, a frequency ~100-fold higher than that of the wild type (9). *spoIIJ* binds to multiple sites in the origin region of the chromosome (15) and forms a large nucleoprotein complex that is visible by the use of immunofluorescence microscopy or a *SpoIIJ*-green fluorescent protein fusion (4, 16). This complex may play a role in pairing newly replicated sister origin regions or in the structural organization of the origin region (1, 15). Consistent with the notion that both SMC and SpotJ are involved in chromosome organization and structure, an *smc spoIIJ* double mutant has a synthetic lethal phenotype in rich medium (1).

*spoIIIE* is involved in postseptational chromosome segregation during sporulation (28, 30). *spoIIIE* mutants are unable to sporulate, and development is arrested with the forespore chromosome bisected by the asymmetric division septum. *SpoIIIE* localizes to the sporulation septum (29), has similarity to DNA translocases, and has been proposed to pump the chromosome destined for the forespore across the polar septum. *SpoIIIE* is also required for efficient segregation during vegetative growth when normal cell division or chromosome partitioning has been perturbed (25). Thus, during vegetative growth, *SpoIIIE* may be a backup mechanism for chromosome partitioning when normal partitioning is defective.

*smc spoIIIE* double mutants appear to have a synthetic lethal phenotype. We attempted to construct an *smc spoIIIE* double mutant by combining an *smc* null mutation (*Δsmc::kan*) with one of two different *spoIIIE* mutations, *spoIIIE36* (29) and *spoIIIE* null (23). *spoIIIE36* contains three missense mutations clustered near a region of *SpoIIIE* that shows similarity to Tra proteins (30). The *spoIIIE* null mutation is a deletion-insertion, with a deletion of codons 86 to 667 (of 787) and an insertion of a spectinomycin resistance cassette (23). Interestingly, the two *spoIIIE* mutations cause different phenotypes with respect to cell-type-specific gene expression (28).

Competent cells of the *spoIIIE36* (PL656) and Δ*spoIIIE* (PL422) mutants were transformed with chromosomal DNA from a Δ*smc::kan* strain (RB35) and plated at 24°C on a glucose-supplemented defined minimal medium (S75b) and on Luria-Bertani (LB) medium. We were unable to isolate stable *spoIIIE36 Δsmc* transformants on either type of medium, suggesting that combining Δ*smc* and *spoIIIE*36 caused a synthetic lethal phenotype. We were able to isolate a transformant containing both *smc* and *spoIIIE* null mutations on minimal medium at 24°C, but this strain was stable only in the presence of spectinomycin (used to select for Δ*spoIIIE::spc*). The Δ*smc* Δ*spoIIIE* double mutant was extremely sick; colonies did not become visible until after 4 days at 24°C and were very small. Culturing of the strain without spectinomycin or at higher temperatures resulted in poorer growth and the accumulation of suppressors.

A conditional allele of *smc*. To test *smc spoIIIE* double mutants, we constructed a conditional allele of *smc*. *smc* was placed under the control of the LacI-repressible, isopropyl-β-
The Pspac-smc spoIIIE mutant strain (RB68) was grown in LB liquid medium at 37°C in both the presence (induced) and the absence (repressed) of IPTG (Fig. 1 legend). The phenotype in the absence of IPTG was similar to though less severe than that of an smc null mutant: the nucleoid structure was abnormal, and anucleate cells accumulated (Fig. 2B). However, in contrast to the Δsmc::kan mutant, the Pspac-smc mutant (in the absence of IPTG) was able to form colonies at 37°C on LB medium. Thus, there appears to be low-level expression of smc in the Pspac-smc mutant in the absence of IPTG.

Because smc is the second gene of a three-gene operon, we also depleted Srb, which is encoded by the gene immediately downstream of smc, and found that Srb depletion was not involved in the phenotypes associated with smc depletion (data not shown). A previous study investigating the effects of depleting SMC and Srb obtained similar results (20).

Depletion of SMC from cells containing spoIIIE mutations.

We constructed Pspac-smc spoIIIE+ double mutants and characterized the phenotypes caused by depletion of SMC following removal of IPTG. When strains harboring Pspac-smc and either spoIIIE36 (RB69) or the spoIIIE null mutation (RB82) were depleted of SMC (following removal of IPTG), growth slowed and eventually ceased (Fig. 1B). When depleted of SMC, the two spoIIIE mutants behaved somewhat differently; the spoIIIE36 mutant had a more severe phenotype than the spoIIIE null mutant. For approximately four generations after removal of IPTG, the Pspac-smc spoIIIE36 strain (RB69) grew similarly to the Pspac-smc spoIIIE+ strain (RB68). After this time, growth of the double mutant slowed and then ceased after approximately six or seven generations (Fig. 1B). The Pspac-smc Δspo0J strain (RB82) had a longer period of slow growth before all growth ceased at approximately 9 to 11 generations after removal of the IPTG.
growth was not observed when SMC was depleted from spo0J mutant cells (see below) or when Srb was depleted in either of the spoIIIE mutant backgrounds (Fig. 1B and data not shown). Also, no difference in growth was observed when these strains were grown in the presence of IPTG (smc<sup>-</sup>) (Fig. 1A), indicating that neither spoIIIE mutation significantly affects growth. These results demonstrate that spoIIIE is required for growth of smc mutant cells.

We measured the viability (plating efficiency) of the Pspac-smc spoIIIE mutant cells after growth in the absence of IPTG. Cells were grown in LB medium, and samples were taken at the time growth ceased (approximately six and nine generations after removal of IPTG for Pspac-smc spoIIIE<sup>36</sup> and Pspac-smc ΔspoIIIE, respectively). Samples were plated under permissive conditions (LB medium in the presence of 1 mM IPTG). Depleting SMC in an otherwise wild-type background (RB68) resulted in ~50% of the cells still being viable compared to strains grown in the presence of IPTG. Pspac-smc

FIG. 2. Chromosome partitioning phenotypes of Pspac-smc strains. Cells were grown as described in the legend to Fig. 1 and in the text. Cells were fixed with methanol and visualized by a combination of fluorescence and Nomarski microscopy as described elsewhere (1). The DNA was stained with the DNA-specific dye 4',6-diamidino-2-phenylindole (DAPI). (A) RB82 (Pspac-smc ΔspoIIIE) grown in the presence of IPTG; (B) Pspac-smc spoIIIE<sup>+</sup> (RB68) grown without IPTG for six generations; (C) Pspac-smc spoIIIE<sup>+</sup> (RB68) grown without IPTG for nine generations; (D) Pspac-smc spoIIIE<sup>36</sup> (RB69) grown without IPTG (cells taken approximately one generation before the cessation of growth); (E) Pspac-smc ΔspoIIIE (RB82) grown without IPTG (cells taken approximately one generation before the cessation of growth); (F to I) Examples of the CUT phenotype: combination Nomarski and fluorescence microscopy of smc spoIIIE double-mutant cells (F and H), and Nomarski images only of the same cells (G and I). Arrows indicate examples of a nucleoid bisected by a septum.
spollIE36 (RB69) and Pspac-smc ΔspollIE (RB82) exhibited ~97% and ~85% loss of viability, respectively. Neither spollIE mutation alone had any detectable effect on cell viability. The difference in the viabilities of Pspac-smc spollIE36 (RB69) and Pspac-smc ΔspollIE (RB82) correlates with the growth phenotypes (Fig. 1B).

**smc spollIE double mutants have a CUT phenotype.** We analyzed the Pspac-smc spollIE double mutants for chromosome-partitioning defects. Samples were taken for analysis approximately one generation before cessation of growth (approximately five and eight generations without IPTG for Pspac-smc spollIE36 [RB69] and Pspac-smc ΔspollIE [RB82], respectively). Pspac-smc spollIE+ (RB68) was grown for six and nine generations without IPTG for comparison. In all three cases, cells displayed a typical smc phenotype (Fig. 2). Nucleoids had a decondensed appearance, and anucleate cells were present in all strains. The frequency of anucleate cells did not differ significantly between the three strains and was ~10%.

In addition to anucleate cells, the Pspac-smc spollIE mutants had many cells with a chromosome bisected by a division septum (Fig. 2F to I). This phenotype is similar to that of the CUT mutants of Schizosaccharomyces pombe (interestingly, two of these S. pombe CUT mutants turned out to have mutations affecting smc genes) (24).

Examples of CUT chromosomes are shown in Fig. 2F to I. In the Pspac-smc single mutant, ~10% of septa were observed to bisect a chromosome (Table 1). In contrast, in the Pspac-smc spollIE36 double mutant, ~38% of septa bisected a chromosome after five generations in the absence of IPTG (Table 1). Similarly, after eight generations without smc expression, the Pspac-smc ΔspollIE double mutant (RB82) had an increase in CUT chromosomes over the number exhibited by the Pspac-smc spollIE+ single mutant (RB68) (~30% versus ~20%), but the overall difference was less pronounced. These results are consistent with the spollIE36 mutation causing a more severe phenotype than the ΔspollIE mutation in combination with Pspac-smc.

We suspect that the CUT phenotype might be contributing to the loss of viability of the Pspac-smc spollIE double mutants. Presumably, in the spollIE+ cells, some of these CUT chromosomes are resolved by the action of SpollIE pumping the chromosome through the septum, whereas in the spollIE mutants a CUT chromosome is likely to be a terminal event. We have not detected induction of the SOS response, as measured by induction of a dinC-lacZ fusion (2), in the Pspac-smc spollIE double mutants (data not shown). The SOS response has been detected in ftspK mutants of E. coli (17); the C-terminal domain of FtspK is similar to that of SpollIE.

**Why does spollIE36 cause a more severe defect?** The major distinction between the two mutations is that spollIE36 encodes a protein that correctly localizes to the septum (29) whereas the null mutation likely results in a complete lack of protein (23). One possibility is that when a chromosome is bisected by a septum, the chromosome is then grabbed by SpollIE and pumped through the septum. We suspect that the defective spollIE36 gene product still makes contact with the chromosome but cannot complete translocation, in effect holding the chromosome in place. Any chromosome caught in the way of the septum in spollIE36 cells would be trapped, and a full complement of the genome would not be received by the daughter cells. In a spollIE null mutant, the chromosome would not be held in place and might be able to move out of the way of the invaginating septum, causing an increase in the number of viable cells compared to spollIE36 mutants. However, the outcome is eventually the same: in the absence of SMC, the ΔspollIE mutant cells do not survive. The loss of viability is likely due to a combination of the CUT phenotype and other, uncharacterized effects on the chromosome.

**Exacerbation of the smc spollIE mutant phenotype by a spo0J null mutation.** A Δspo0J Δsmc double mutation gives rise to more anucleate cells and causes a greater disruption of nucleoid structure than the single Δsmc mutation (1). In addition, the double mutation results in synthetic lethality on LB medium. We hypothesized that the more severe the segregation defect, the more important the backup partitioning function of SpoIIIE becomes. Therefore, we depleted SMC from a Δspo0J spollIE36 mutant.

Depleting SMC from cells harboring both Δspo0J and spollIE36 mutations resulted in cessation of growth earlier than for cells with spollIE36 alone. Strains containing Pspac-smc spo0J+ spo0J (RB68), Pspac-smc Δspo0J spo0J (RB74), or Pspac-smc Δspo0J spollIE36 (RB75) were grown in LB medium at 37°C with (Fig. 1C) or without (Fig. 1D) IPTG. Although the Δsmc Δspo0J double mutant is not viable on LB medium, depletion of SMC from spo0J cells (RB74) did not result in cessation of growth, even after 10 generations (~20% of the cells were anucleate, versus ~10% for RB68, consistent with previous results [1]). In contrast, the triple-mutant (Pspac-smc Δspo0J spollIE36) strain ceased growth approximately four or five generations after removal of IPTG, a full two generations before the Pspac-smc spollIE36 (RB69) mutant. The Δspo0J spollIE36 double mutant had no growth defect.

**Summary.** We have demonstrated that SpollIE, a putative DNA translocase capable of pumping DNA through a septum, is required for the viability of smc mutant cells. Our results suggest that in smc mutants the bulk of the chromosome is not being properly partitioned out of the way of the invaginating septum and that the SpollIE protein provides a critical backup partitioning mechanism to pump DNA through the septum when normal partitioning is disrupted. These results support and extend earlier work demonstrating a postseptational partitioning role for SpollIE during vegetative growth (25). Our results suggest that nucleoid structure is extremely important, if not essential, for proper nucleoid partitioning and that the postseptational partitioning provided by SpollIE helps to resolve defects. Clearly, spollIE does not substitute completely for the lack of smc function, but it can help the cell overcome the partitioning defect in enough cases to yield viable cells.

Mutations in *B. subtilis* smc and *E. coli* mukB cause strikingly similar phenotypes (1, 20, 21). Interestingly, it was recently reported that an *E. coli* mukB and ftspK double mutant, which has a postseptational partitioning function, could not be isolated (31). This further suggests that SMC and MukB play similar roles in nucleoid structure and chromosome partitioning.

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**TABLE 1. Effect of SMC depletion on CUT chromosomes**

<table>
<thead>
<tr>
<th>Strain*</th>
<th>Relevant genotype</th>
<th>% CUT*</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB68 (6)</td>
<td>Pspac-smc spollIE+</td>
<td>10.6 (34/320)</td>
</tr>
<tr>
<td>RB69</td>
<td>Pspac-smc spollIE36</td>
<td>38.0 (274/721)</td>
</tr>
<tr>
<td>RB68 (9)</td>
<td>Pspac-smc spollIE+</td>
<td>19.6 (115/588)</td>
</tr>
<tr>
<td>RB82</td>
<td>Pspac-smc ΔspollIE</td>
<td>29.9 (191/638)</td>
</tr>
</tbody>
</table>

* Strains were grown in LB medium at 37°C in the presence or absence of IPTG. For strain RB68, the number of generations in the absence of IPTG is indicated in parentheses.

* The percentage of septa that bisect a chromosome. RB69 and RB82 were grown in the absence of IPTG for approximately five and eight generations, respectively. Data are cumulative from several experiments. The number of cells with a chromosome bisected by a division septum is likely to be a terminal event. The chromosome through the septum, whereas in the spollIE mutants a CUT chromosome is likely to be a terminal event. We have not detected induction of the SOS response, as measured by induction of a dinC-lacZ fusion (2), in the Pspac-smc spollIE double mutants (data not shown). The SOS response has been detected in ftspK mutants of *E. coli* (17); the C-terminal domain of FtspK is similar to that of SpollIE.
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