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# Postseptational chromosome partitioning in bacteria

**(*Bacillus subtilis*/*spoIIIE* gene/antibiotic resistance/cell division)**

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**ABSTRACT** **Mutations in the *spoIIIE* gene prevent proper partitioning of one chromosome into the developing prespore during sporulation but have no overt effect on partitioning in vegetatively dividing cells. However, the expression of *spoIIIE* in vegetative cells and the occurrence of genes closely related to *spoIIIE* in a range of nonsporulating eubacteria suggested a more general function for the protein. Here we show that SpoIIIE protein is needed for optimal chromosome partitioning in vegetative cells of *Bacillus subtilis* when the normal tight coordination between septation and nucleoid partitioning is perturbed or when septum positioning is altered. A functional SpoIIIE protein allows cells to recover from a state in which their chromosome has been trapped by a closing septum. By analogy to its function during sporulation, we suggest that SpoIIIE facilitates partitioning by actively translocating the chromosome out of the septum. In addition to enhancing the fidelity of nucleoid partitioning, SpoIIIE also seems to be required for maximal resistance to antibiotics that interfere with DNA metabolism. The results have important implications for our understanding of the functions of genes involved in the primary partitioning machinery in bacteria and of how septum placement is controlled.**

Bacterial cell division involves the formation of a septum that separates the cytoplasm of the parent cell into two compartments. This process must be tightly coordinated with chromosome replication. Every time a cell divides, the two products of a round of DNA replication must be positioned so that one is inherited by each of the daughter cells, a process called "partitioning" (1). Despite the complexity of this overall process, it occurs with high fidelity; anucleate cells are formed in <0.03% of divisions in *Escherichia coli* (2). Microscopic examination of bacterial cells has failed to reveal the existence of an equivalent to the eukaryotic mitotic apparatus, and recent observations of nucleoid partitioning suggest that nascent sister nucleoids move apart gradually, in concert with cell growth, rather than moving abruptly (3).

There have been several attempts to identify components of the partitioning mechanism by isolation of mutants. Mutations in various genes designated *par* have all turned out to lie in genes required for DNA replication or for the decatenation of newly formed sister chromosomes, rather than for chromosome movement (4). Hiraga and colleagues (5) devised an elegant genetic screen for mutants producing an increased proportion of anucleate cells and identified several loci designated *muk*, one of which (*mukB*) is now thought to be a motor protein involved in nucleoid movement. The screen also yielded mutations in the *minB* locus. The three genes in this locus, *minC*, *minD*, and *minE*, are involved in septum positioning, and some *min* mutants produce anucleate minicells by aberrant septum placement close to the cell pole (6). However, mutations in *minD*, at least, also seem to impair nucleoid partitioning (7), and MinD shows sequence similarity to a family of proteins involved in plasmid partitioning (8). Mutations in the *Bacillus subtilis spo0J* gene also result in increased anucleate cell production during vegetative growth, and the protein product of the gene again shows sequence similarity to proteins involved in plasmid partitioning (9). Several genes have thus been implicated in nucleoid partitioning, but the precise functions of their products are not known.

During sporulation, *B. subtilis* undergoes a modified asymmetric cell division in which the septum forms close to one pole of the cell. This modified division is morphologically similar to vegetative division and the two forms of division require common gene products (10–12). The extreme movement needed for one of the chromosomes to reach the prespore pole of the cell specifically requires the product of the *spoIIIE* gene (13). Recent work suggests that during sporulation, the prespore septum forms before the prespore chromosome has partitioned and that the role of the SpoIIIE protein is to translocate the major part of the chromosome through the nascent septum (14). This process is quite different from the partitioning of chromosomes in vegetative cells, which normally precedes septation (4). Nonetheless, two lines of evidence suggested that the function of SpoIIIE might not be restricted to the asymmetric division of sporulation. First, the gene seems to be transcribed in vegetative cells (15) and the protein can be detected in vegetative cells by Western blot analysis (13). Second, genes encoding proteins closely related to SpoIIIE have been found in a diverse range of nonsporulating bacteria, such as *Coxiella burnetti* (16), *Campylobacter jejuni* (17), *E. coli* (K. J. Begg, S. J. Dewar, and W. D. Donachie, personal communication), and *Neisseria gonherriae* (J. S. Gunn, personal communication). Here we show that SpoIIIE provides an important and previously unsuspected postseptational partitioning function, which operates when the normal mechanisms of nucleoid separation or septum positioning are perturbed, and a septum closes around the nucleoid. This mechanism has important implications for our understanding of several aspects of nucleoid partitioning and septum positioning.

## MATERIALS AND METHODS

**Bacterial Strains and Media.** *B. subtilis* strains used were SG38 *trpC2* (18), 647 *trpC2* Ω(*spoIIIE647*::*aph-A3*)647 (contains a null allele of *spoIIIE*; ref. 13), 36.3 *trpC2 spoIIIE36* (a missense allele of *spoIIIE*; ref. 13), and SG10 *purA16 minD1* (previously *divIVB1*; ref. 19). Strain 647.5 *purA16 minD1 spoIIIE647* was constructed by transformation (20, 21) of SG10 with chromosomal DNA from 647, with selection for kanamycin resistance. PAB was Difco antibiotic medium 3. BHIYE was Oxoid (Basingstoke, U.K.) brain heart infusion broth containing 0.5% Oxoid yeast extract. SMMG was the pretransformation medium of Jenkinson (21) except that the glucose concentration was increased to 2% (wt/vol). Medium 2×SG

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Abbreviations: HPUra, 6-(*p*-hydroxyphenylazo)uracil; MIC, minimum inhibitory concentration.

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was as described by Ireton *et al.* (9). NA was Oxoid nutrient agar. Kanamycin was used at a final concentration of 5 μg/ml.

**Effect of 6-(*p*-hydroxyphenylazo)uracil (HPUra) on Nucleoid Segregation.** Single colonies of SG38 and *spoIIIE* point and null mutants were inoculated into PAB and grown overnight at 30°C. Cultures were diluted into PAB to an $OD_{600}$ of 0.1 and growth was followed until an $OD_{600}$ of 0.5 was reached. Each culture was divided into two equal portions, one of which was treated with HPUra (50 μg/ml). After 1 hr, the HPUra was removed by centrifuging the culture and resuspending the cells in fresh warm medium. The growth of the culture was followed at $OD_{600}$ until the cultures reached stationary phase.

**Growth and Observations of Minicell-Producing Strains.** The minicell strain SG10 and its *spoIIIE minD* derivative were grown to stationary phase, essentially as described in the previous section, except that the cultures were not treated with HPUra.

**Visualization of Vegetative Septa and DNA.** Samples of PAB cultures (0.1 ml) were prepared for visualization of the septa by an ethanol fixation procedures (14). Cell length was determined by noting the positions of the septa on a digital image and measured in pixels with IMAGE software (version 1.49; W. Rasband, National Institutes of Health). Conversion to length in micrometers was done by comparison with measurements of a standard preparation of *B. subtilis* spores for which the mean length (1.18 ± 0.1 μm) was known (22). DNA was visualized by staining with 4′,6-diamidino-2-phenylindole (DAPI; Sigma) as described (14).

**Measurement of Antibiotic Resistance.** Strains SG38 and 647 were grown in PAB to early stationary phase ($OD_{600} \approx 1.5$). A sloppy agar overlay (one-third concentration Oxoid trypticase blood agar base) was seeded with 0.1 ml of culture and poured onto an NA plate. When the overlays were set, Oxoid antibiotic disks were placed on them and the overlays were dried briefly in a laminar-flow cabinet. Inhibition of growth was assessed after 18 hr at 30°C, in terms of the width of the zone of inhibition. The following antibiotic disks were used: ampicillin (AMP 10), chloramphenicol (C 30), nalidixic acid (NA 30), novobiocin (NV 30), polymixin B (PB 300), rifampicin (RD 5), and sulfonamides (S3 300).

To determine the minimum inhibitory concentration (MIC) for an antibiotic quantitatively, a series of NA plates were prepared, containing 2-fold dilutions of antibiotic. Midexponential-phase cultures (as above) were diluted in PAB to the point where about 20 separated single colonies would be produced by a 20-μl drop of culture. Wild-type and *spoIIIE* mutant cultures were placed side by side on plates containing the range of antibiotic concentrations and growth was assessed after 24 hr at 37°C. The MIC was defined as that concentration of antibiotic which just prevented the appearance of single colonies.

## RESULTS

***spoIIIE* Mutations Have No Effect on Chromosome Partitioning in Unperturbed Vegetative Cells.** To test whether *spoIIIE* mutations had any effect on chromosome partitioning during vegetative growth, strains SG38 (wild type), 647 (*spoIIIE* null mutant), and 36.3 (with a *spoIIIE* point mutation) were cultured in several different media, BHIYE, PAB, SMMG and 2×SG, giving growth rates (doubling times) ranging from 25 to 60 min. No significant differences were observed between the growth rates of the wild type and either of the mutant strains. Samples of each of the cultures taken during exponential growth, and at several time points thereafter, were examined by fluorescence microscopy. The nucleoid distributions of wild-type and *spoIIIE* mutant cells were indistinguishable in all samples examined (1000–2000 cells counted; results not shown). We conclude that *spoIIIE* is not required for nucleoid partitioning in unperturbed vegetatively growing cells, even though the gene is absolutely required for partitioning of the prespore chromosome during sporulation.

**Effect of HPUra on Septation and Chromosome Partitioning During Vegetative Growth.** The absence of any overt defect in chromosome partitioning during vegetative growth might be due to the fact that the nucleoids normally move apart before septation. We reasoned that SpoIIIE might come into play in vegetative cells if the chromosomes failed to separate before septation. The septum should then close around the nucleoid, creating a structure similar to the one on which SpoIIIE seems to act during sporulation. To test this hypothesis, we transiently treated isogenic wild-type and *spoIIIE* mutant cells with HPUra, a specific inhibitor of DNA replication (23). Inhibition for 1 hr should ensure that in a large proportion of the cells the completion of DNA replication would be delayed until well after the septum would normally have formed.

Exponential cultures of SG38 and 647 were treated with HPUra for 1 hr and then returned to fresh warm medium devoid of the inhibitor. The delay in DNA replication had little effect on growth of the wild-type strain, but in some experiments the *spoIIIE* mutant culture tended to lyse, accounting for the dip in $OD_{600}$ at 160 and 190 min (Fig. 1*A*).

To determine what effect the HPUra treatment had on septum formation and on nucleoid structure and partitioning, samples of both cultures were examined by phase-contrast and fluorescence microscopy immediately after removal of the inhibitor ($t_0$) and 1 hr later ($t_1$). The average cell length was greater in the treated cultures than in the controls, presumably because formation of some septa had been inhibited by the block in DNA replication. However, all of the samples contained a high proportion (about 10%) of anucleate cells (Fig. 1; Table 1), indicating that the HPUra treatment had caused a suitable delay in DNA replication and that formation of some septa took place despite the block in DNA replication. (Note that *B. subtilis* differs from *E. coli* in this respect; ref. 24.) In addition to the formation of anucleate cells, in the $t_0$ samples of both cultures about 10% of the septa appeared to have bisected a nucleoid (Fig. 1 *C* and *E*; Table 1). Such cells would be expected if septation occurred before its cognate round of DNA replication had been completed. At this time the nucleoids that had been bisected generally consisted of two large, roughly equally fluorescent bodies lying on either side of the septum and close to it (Fig. 1 *C* and *E*). Presumably, replication of these nucleoids had been near to completion when septation occurred; McGinness and Wake (25) have shown that in germinating spores of *B. subtilis*, septation can be initiated if DNA replication is inhibited after the first round is about 70% complete.

At $t_1$ there was little change in the proportion of anucleate cells; again there were about 10% in both cultures (Table 1). There was also little change in the proportion of septa that bisected nucleoids in the *spoIIIE* culture. However, the appearance of the bisected nucleoids had changed. Generally, they were now highly asymmetric, with only a relatively small fragment of DNA having been lost to the sister cell (Fig. 1 *H* and *I*). This change in appearance was consistent with DNA replication having been completed: one of the daughter nucleoids might then have been freed and could move away from the septum, whereas the other remained partly trapped in the septum. Similar results were obtained in experiments with strain 36.3, containing the *spoIIIE36* missense allele (results not shown). In contrast, in the wild-type culture the proportion of cells with bisected nucleoids had fallen by a factor of about 5 at $t_1$ (Table 1). The disappearance of such cells in the wild-type culture was consistent with the predicted action of SpoIIIE in allowing or promoting the partitioning of chromosomes after septation (as seems to occur in the case of the prespore chromosome).

**Effect of a *spoIIIE* Mutation on Partitioning in a Minicell Mutant.** If SpoIIIE did provide an alternative postseptational

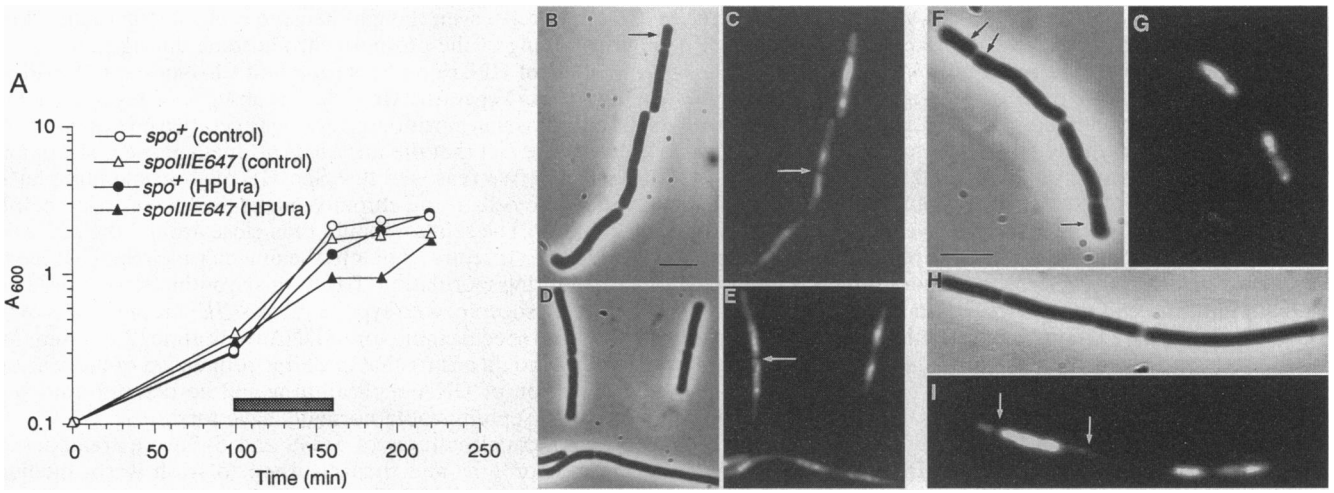


FIG. 1. A *spoIIIE* mutation prevents complete recovery from transient inhibition of DNA replication by HPUra. Strains SG38 (wild type; circles) and 647 (*spoIIIE647*; triangles) were inoculated into PAB, and culture growth was followed by measurement of $OD_{600}$ (*A*). Each culture was divided into two portions, one of which (filled symbols) was treated with HPUra for the period indicated by the bar. The other, untreated portions (open symbols) served as controls. Phase-contrast (*B*, *D*, *F*, and *H*) and fluorescence (*C*, *E*, *G*, and *I*) images of typical cells are shown, taken immediately after removal of the inhibitor ($t_0$) (*B–E*) and 1 hr later ($t_1$) (*F–I*). The upper panels (*B*, *C*, *F*, and *G*) show cells from the SG38 culture and those in the lower panels (*D*, *E*, *H*, and *I*) are from the 647 culture. The arrows in the phase-contrast images indicate anucleate cells. The background fluorescence evident in the images from the $t_0$ samples is probably due to residual HPUra. The arrows in the fluorescence images point to septa that appear to have bisected a nucleoid. In *E*, for example, large nucleoidal masses lie on either side of, and close to, the septum. This contrasts with the more normal nucleoid placement, near the midpoint of the cell, as exhibited by the other cell pair in the panel. At $t_1$, most of the nucleoids in the wild-type culture had moved to central positions (*G*). In the 647 culture, the nucleoids that were trapped by septa tended to be elongated, with only a small portion of the nucleoid lying on the wrong side of the septum (*I*). (Bars = 2 μm.)

mechanism of partitioning, *spoIIIE* mutations might enhance the effects of mutations acting on the primary partitioning machinery. Of the partitioning genes that have been characterized in *E. coli* (4), only one, *minD* (previously *divIVB*), has so far been identified in *B. subtilis* (26–28). Although *min* mutations are best known for their effects on septum positioning, mutations in *minD* also seem to affect partitioning (7), and MinD shares sequence similarity with several plasmid partitioning factors (8). We therefore constructed a *minD spoIIIE* double mutant to determine whether the latter mutation would enhance any nucleoid partitioning defect resulting from the *minD* mutation.

Cultures of the double mutant (647.5) and the *minD* parent strain (SG10) were grown in PAB. No difference was detected between the growth rates of the cultures (results not shown). Samples of the two cultures were taken at intervals for examination by fluorescence and phase-contrast microscopy. During exponential growth (30- and 90-min time points) the cultures were indistinguishable microscopically. The majority of cells possessed relatively compact nucleoids and the nucleoid distribution appeared normal in all but a small proportion of the cells. As expected, a substantial proportion of minicells was present in both cultures (Table 2). As described previously (6), the minicells of the *minD* mutant (and the double mutant) were anucleate and relatively heterogeneous in length.

Table 1. Effect of the *spoIIIE647* null mutation on nucleoid partitioning and septation, following transient inhibition of DNA replication

| Strain | Time,* min | % anucleate cells† | % bisected nucleoids† | Cell length, μm (mean ± SD) | |
|---|---|---|---|---|---|
| | | | | Untreated | HPUra |
| SG38 | 0 | 9.2 | 8.7 | 2.7 ± 0.6 | 3.8 ± 1.3 |
| | 60 | 9.4 | 1.9 | 2.5 ± 0.5 | 3.1 ± 1.1 |
| 647 | 0 | 9.6 | 10.6 | 2.9 ± 0.7 | 4.2 ± 1.5 |
| | 60 | 11.3 | 12.4 | 2.6 ± 0.6 | 3.8 ± 1.7 |

Cultures of strains SG38 (control) and 647 (*spoIIIE647*) were grown in PAB and treated with HPUra as described in the legend to Fig. 1.
*After removal of HPUra.
†HPUra-treated cultures only; 100–500 cells representing complete fields were scored. Anucleate cells were not detected in untreated cultures (<1 per 1000 cells).

Following entry into stationary phase (150- and 330-min time points), the chromosomes of both cultures became more diffuse, eventually filling the entire cell (as reported previously; ref. 29). In the *minD* culture there were no other distinct morphological changes and both the abundance of the minicells and their microscopic appearance were almost unchanged (Fig. 2; Table 2). However, in the *minD spoIIIE* culture some of the minicells now appeared to contain DNA (Fig. 2). After 1 hr in stationary phase, DNA was distinctly visible in ≈18% of minicells (Table 2). The amount of DNA in these cells was much less than that of an entire chromosome. Because cell separation is delayed relative to septation in *B. subtilis* (in contrast to *E. coli*), a substantial proportion of the minicells, in both cultures and at both time points, were still attached to their sister cells. All of the DNA-containing minicells were of this type, presumably because the presence of the trapped DNA prevented cell separation.

To our knowledge, this is the first report of the presence of chromosomal DNA in minicells. The existence of such cells is consistent with the notion that in the *minD spoIIIE*$^+$ background, polar (minicell) septa begin to bisect nucleoids in stationary phase, when the nucleoid tends to fill the cell, and that the SpoIIIE protein normally participates in the removal of this DNA from the small compartment.

**SpoIIIE and Antibiotic Resistance.** On the basis of the above results it seemed that the postseptational mechanism of partitioning provided by SpoIIIE might play an important role under conditions in which the normal partitioning machinery fails. In the natural environment, partitioning might often be impaired by DNA damage or the action of antibiotic inhibitors of DNA replication. If so, *spoIIIE* mutants might exhibit increased sensitivity to certain antibiotics. Antibiotic discs were used to compare the antibiotic-sensitivity profiles of the *spoIIIE* deletion mutant (strain 647) and its isogenic wild-type strain. These preliminary qualitative results suggested that the *spoIIIE* mutant was more sensitive to nalidixic acid and novobiocin (inhibitors of DNA gyrase) than the parent strain.

Table 2. Effect of the *spoIIIE647* null mutation on nucleoid partitioning in minicells

| Strain | % minicells | | | | % minicells containing DNA at time | | | |
|---|---|---|---|---|---|---|---|---|
| | 30 min | 90 min | 150 min | 330 min | 30 min | 90 min | 150 min | 330 min |
| SG10 (*minD1*) | 20 (132) | 16 (185) | 15 (144) | 14 (190) | ND (27) | ND (30) | ND (21) | 0.5 (270) |
| 647.5 (*minD1 spoIIIE647*) | 19 (165) | 18 (190) | 16 (172) | 12 (320) | 3 (36) | 3 (34) | 7 (27) | 18 (239) |

Cultures of strains SG10 (*minD1*) and 647.5 (*minD1 spoIIIE647*) were grown in PAB. At the time intervals shown, samples were prepared for examination by phase-contrast and fluorescence microscopy. Complete fields of cells were scored for the proportion of minicells, or minicells were counted and scored for the presence or absence of DNA.
*No. of cells counted is in parentheses. ND, not detected.

No differences were detected in sensitivity to several other antibiotics affecting the synthesis of peptidoglycan (ampicillin), protein (chloramphenicol), or RNA (rifampicin) or affecting membrane integrity (polymyxin B) (data not shown). To confirm quantitatively the difference between the wild-type and mutant strains, we determined the MICs for nalidixic acid and found a reproducible 2-fold difference between the two strains (Fig. 3). Similar results were obtained for mitomycin C, which causes DNA damage (Fig. 3). It thus appears that the *spoIIIE* gene is required for maximal resistance to some inhibitors of DNA synthesis.

## DISCUSSION

The constitutive expression of *spoIIIE* in vegetatively growing cells (15) and the identification of genes closely related to *spoIIIE* in a range of Gram-positive and Gram-negative organisms (see above) suggested that the role of SpoIIIE was not specific to sporulation. Unperturbed vegetatively growing cultures of wild-type and *spoIIIE* mutant strains were indistinguishable in growth rate, septation, nucleoid conformation, or partitioning. However, when chromosome partitioning was perturbed, a distinction between the strains became apparent. Transient inhibition of DNA replication was used to force some of the cells to initiate septation before DNA replication was complete. Cells with nucleoids that had been bisected by septa were formed in both the wild-type and *spoIIIE* mutant strains, in similar proportions (Table 1). The majority of the cells in this class seemed to have been close to the completion of a round of DNA replication, as observed previously (25). Presumably the two large nucleoid lobes lying either side of the septum (Fig. 1 *C* and *E*) represent the origin-proximal segments of the nascent sister chromosomes. If the unreplicated, origin-distal part of the parent chromosome lay predominantly on one side or other of the septum, only one of the chromosomes might be trapped by the septum when replication was completed. Accordingly, we found that 1 hr after treatment of the *spoIIIE* mutant culture was lifted, the trapped nucleoids were generally asymmetric, with only a small segment of DNA trapped on the "wrong" side of the septum (Fig. 1*I*). We assume that the near absence of such cells in the wild-type culture and the overall reduction in the proportions of trapped nucleoids were due to the action of the SpoIIIE protein. In principle, SpoIIIE could act by preventing the DNA from being trapped by the septum and by allowing the DNA to be drawn through the septum by some other partitioning function. However, two lines of evidence are consistent with SpoIIIE being involved in active transport of DNA through the septum: (*i*) the SpoIIIE-dependent partitioning of the prespore chromosome during sporulation requires formation of the septum (14) and (*ii*) SpoIIIE exhibits significant sequence similarity to a group of proteins that mediate conjugative transfer of plasmid DNA in *Streptomyces* (14).

As an independent means of demonstrating a vegetative role for SpoIIIE, we examined the properties of a *spoIIIE minD* double mutant, since *minD* mutations are thought to affect partitioning in *E. coli* (7). Surprisingly, we found little evidence for a partitioning defect in the *minD1* mutant of *B. subtilis*. The nucleoid distribution in the mutant looked more or less normal when we took into account the slightly abnormal distribution of septa. However, when the cells entered stationary phase a striking difference between the *minD* mutant and the *spoIIIE minD* double mutant was observed. In the presence of the *spoIIIE* mutation, a substantial proportion of the minicells contained DNA. The reason why nucleated minicells were not

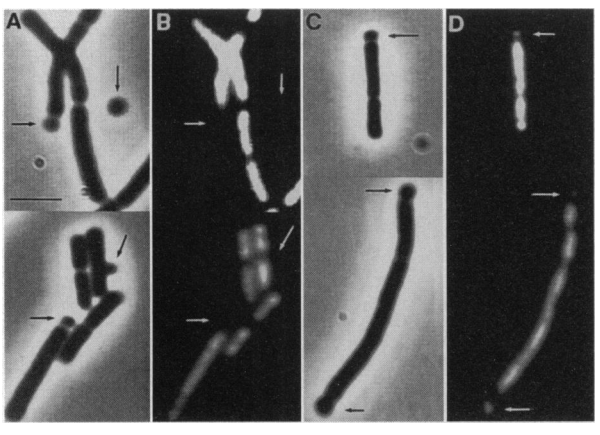


FIG. 2. Effect of the *spoIIIE647* mutation on partitioning in the presence of a *minD* mutation. Strains SG10 (*minD1*) (*A* and *B*) and 647.5 (*minD1 spoIIIE647*) (*C* and *D*) were grown in PAB to stationary phase. Phase-contrast (*A* and *C*) and fluorescence (*B* and *D*) images of typical cell chains containing minicells are shown. The minicells are indicated by arrows in both sets of images. The upper panels of the fluorescence images have been deliberately overexposed to confirm the presence of DNA in the minicells of the 647.5 and its absence from those of SG10. (Bar = 2 μm.)

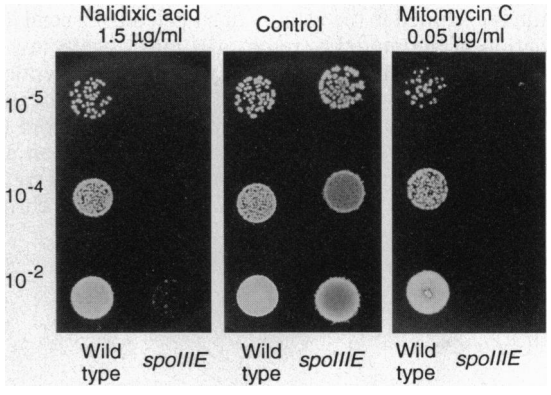


FIG. 3. The *spoIIIE647* mutation increases sensitivity to certain antibiotics. Cultures of SG38 (control) and 647 (*spoIIIE647*) were grown in PAB to midexponential phase. Each culture was diluted by the amount indicated, and 20-μl samples of each dilution were plated on a series of NA plates containing antibiotics. The central panel with no antibiotic shows that the viabilities of both cultures were equivalent and that the cultures were of equivalent density. For strain 647, the MICs for nalidixic acid and mitomycin C were 1.5 and 0.05 μg/ml, respectively. As shown in the right and left panels, respectively, the mutant was not able to grow at these concentrations of inhibitor, but the wild-type remained viable.

seen in exponential growth is probably as follows. In exponential growth the nucleoids occupy a fairly descrete region of the cytoplasm, located near the midpoint of the long axis of the cell. Consequently, the polar regions, in which the septa that produce minicells are formed, are devoid of DNA. However, after entry into stationary phase, the cells become shorter and the nucleoid begins to occupy a much larger proportion of the cell (29). Bisection of a nucleoid would be much more likely at this stage. It seems likely that septa also bisect nucleoids in both of the strains we examined but that in the *spoIIIE*$^+$ background the SpoIIIE protein then drives the DNA, or allows it to be pulled, out of the minicell. Again, the observations are compatible with the existence of a postseptational chromosome partitioning system which requires SpoIIIE.

The existence of a postseptational partitioning mechanism has important implications for interpretation of the effects of mutations that perturb the preseptational partitioning machinery. In particular, it may in part explain why only a relatively small proportion of anucleate cells are produced by mutations in genes such as *mukB* (5). In *mukB* mutants, nucleoid positioning is perturbed, with sister chromosomes tending to stay closer together than normal (2). Since septum positioning is more or less normal in the mutant, nucleoids should be bisected by septa at a much higher rate than normal. However, only a small proportion of such cells were detected (2). In most cells the nucleoids were partitioned correctly, on either side of the septum. Most of the remaining divisions produce one anucleate cell and a sister with two chromosomes. The near absence of cells between these two extremes argues strongly for a postseptational mechanism that allows the trapped nucleoid to be removed from the septum. The finding of a *spoIIIE*-like gene in *E. coli* (K. Begg, personal communication) supports this explanation and provides a means of testing this argument. Clearly, the provision of two distinct mechanisms for chromosome partitioning would greatly increase the fidelity of the process, perhaps accounting for the great accuracy of partitioning in wild-type cells (2).

The existence of a postseptational mechanism of partitioning also has implications for the study of septation itself. In several previous studies of cell division, the effects of perturbing DNA replication or chromosome decatenation on septum positioning have been investigated. The possibility that formation of a septum could result in repositioning of the nucleoid has generally not been considered. The nucleoid occlusion model (30), for example, assumes that formation of a septum is excluded in the vicinity of a nucleoid. Crucial in the formulation of the model were observations of cells in which partitioning was perturbed in a variety of ways. The septa that formed under these conditions were shown to tend to lie to one side of the nucleoid, rather than to bisect the nucleoid (31). The postseptational mechanism could well provide an alternative explanation for this effect, by moving otherwise trapped nucleoids away from the septum. Future studies of chromosome partitioning and septation will need to take account of the postseptational partitioning mechanism.

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1. Schaechter, M. & Yarmolinsky, M. (1993) *ASM News* **59,** 378.
2. Hiraga, S., Niki, H., Ogura, T., Ichinose, C., Mori, H., Ezaki, B. & Jaffe, A. (1989) *J. Bacteriol.* **171,** 1496–1505.
3. van Helvoort, J. M. L. M. & Woldringh, C. L. (1994) *Mol. Microbiol.* **13,** 577–583.
4. Hiraga, S. (1992) *Annu. Rev. Biochem.* **61,** 283–306.
5. Niki, H., Jaffe, A., Imamura, R., Ogura, T. & Hiraga, S. (1991) *EMBO J.* **10,** 183–193.
6. de Boer, P. A. J., Crossley, R. E. & Rothfield, L. I. (1992) *J. Bacteriol.* **174,** 63–70.
7. Akerlund, T., Bernander, R. & Nordstrom, K. (1992) *Mol. Microbiol.* **6,** 2073–2083.
8. Motallebi-Veshareh, M., Rouch, D. A. & Thomas, C. M. (1990) *Mol. Microbiol.* **4,** 1455–1463.
9. Ireton, K., Gunther, N. W., IV, & Grossman, A. D. (1994) *J. Bacteriol.* **176,** 5320–5329.
10. Beall, B. & Lutkenhaus, J. (1992) *J. Bacteriol.* **174,** 2398–2403.
11. Yanouri, A., Daniel, R. A., Errington, J. & Buchanan, C. E. (1993) *J. Bacteriol.* **175,** 7604–7616.
12. Levin, P. A. & Losick, R. (1994) *J. Bacteriol.* **176,** 1451–1459.
13. Wu, L. J. & Errington, J. (1994) *Science* **264,** 572–575.
14. Wu, L. J., Lewis, P. J., Allmansberger, R., Hauser, P. & Errington, J. (1995) *Genes Dev.* **9,** 1316–1326.
15. Foulger, D. & Errington, J. (1989) *Mol. Microbiol.* **3,** 1247–1255.
16. Oswald, W. & Thiele, D. (1993) *J. Vet. Med.* **40,** 366–370.
17. Miller, S., Pesci, E. C. & Pickett, C. L. (1994) *Gene* **146,** 31–38.
18. Errington, J. & Mandelstam, J. (1986) *J. Gen. Microbiol.* **132,** 2967–2976.
19. Reeve, J. M., Mendelson, N. H., Coyne, S., Hallock, L. L. & Cole, R. M. (1973) *J. Bacteriol.* **114,** 860–873.
20. Anagnostopoulos, C. & Spizizen, J. (1961) *J. Bacteriol.* **81,** 741–746.
21. Jenkinson, H. F. (1983) *J. Gen. Microbiol.* **127,** 81–91.
22. Hauser, P. A. & Errington, J. (1995) *J. Bacteriol.* **177,** 3923–3931.
23. Brown, N. C. (1970) *Proc. Natl. Acad. Sci. USA* **67,** 1454–1461.
24. Donachie, W. D., Martin, D. T. M. & Begg, K. J. (1971) *Nature (London)* **231,** 274–276.
25. McGinness, T. & Wake, R. G. (1979) *J. Mol. Biol.* **134,** 251–264.
26. Varley, A. W. & Stewart, G. C. (1992) *J. Bacteriol.* **174,** 6729–6742.
27. Lee, S. & Price, C. W. (1993) *Mol. Microbiol.* **7,** 601–610.
28. Levin, P. A., Margolis, P. S., Setlow, P., Losick, R. & Sun, D. (1992) *J. Bacteriol.* **174,** 6717–6728.
29. Bylund, J. E., Haines, M. A., Piggot, P. J. & Higgins, M. L. (1993) *J. Bacteriol.* **175,** 1886–1890.
30. Woldringh, C. L., Mulder, E., Huls, P. G. & Vischer, N. (1991) *Res. Microbiol.* **142,** 309–320.
31. Mulder, E. & Woldringh, C. L. (1989) *J. Bacteriol.* **171,** 4303–4314.