Identification of Bacillus subtilis Genes for Septum Placement and Shape Determination

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The Bacillus subtilis divIVB1 mutation causes aberrant positioning of the septum during cell division, resulting in the formation of small, anucleate cells known as minicells. We report the cloning of the wild-type allele of divIVB1 and show that the mutation lies within a stretch of DNA containing two open reading frames whose predicted products are in part homologous to the products of the Escherichia coli minicell genes minC and minD. Just upstream of minC and minD, and in the same orientation, are three genes whose products are homologous to the products of the E. coli shape-determining genes mreB, mreC, and mreD. The B. subtilis mreB, mreC, and mreD genes are the site of a conditional mutation (rodB1) that causes the production of aberrantly shaped cells under restrictive conditions. Northern (RNA) hybridization experiments and disruption experiments based on the use of integrational plasmids indicate that the mre and min genes constitute a five-cistron operon. The possible involvement of min gene products in the switch from medial to polar placement of the septum during sporulation is discussed.

Cells of the gram-positive soil bacterium Bacillus subtilis are capable of entering an alternative developmental pathway that is characterized by the formation of a transverse septum. During vegetative growth, the formation of a septum at the center of the cell partitions the bacterium into identical daughter cells which separate and undergo further cycles of binary fission. The hallmark of the process of sporulation, in contrast, is the formation of a septum that is sited near one pole of the cell. This asymmetrically positioned septum partitions the bacterium into unequal-sized cellular compartments, of which one, the forespore, undergoes metamorphosis into a spore and the other, the mother cell, participates in the formation of the spore but is eventually discarded by lysis. The binary fission septum and the sporulation septum are produced by similar processes (17), involving in both cases the action of B. subtilis homologs to the Escherichia coli septation genes ftsA and ftsZ (2, 3). However, little is known about the mechanisms that govern the alternative placement of the septa at medial or polar positions within the cell.

In the non-spore-forming bacterium E. coli, placement of the septum is governed by genes at the minB locus. Cells of E. coli grow by binary fission and are normally capable of producing only medially sited septa. However, certain mutations at the minB locus allow septa to form at a polar position, thereby generating small, anucleate cells with intact cell walls and membranes which except for their lack of DNA appear to be metabolically normal (1). Minicell production occurs as an alternative to normal division, and consequently, the sister cell is filamentous and carries two or more copies of the chromosome. Because the minicell division process appears to be identical to that of the wild type, the defect is apparently with site selection and not with septum formation. As with sporulation in B. subtilis, minicell formation in E. coli involves the asymmetric positioning of the septum. Unlike sporulating B. subtilis cells, minicell mutants are defective in nucleoid segregation, so minicells are devoid of a chromosome.

The minB locus is an operon consisting of genes minC, minD, and minE (8, 10, 11). Under normal conditions, the gene products of minC and minD act in concert to form an inhibitor of cell division at the three potential division sites: midcell and the two poles. The MinCD complex is given its topological specificity by a third protein, MinE, which blocks the activity of the division inhibitor at midcell, thereby ensuring that the division septum is correctly positioned. Placement of septa at polar locations, the minicell phenotype, occurs in the absence of the minC and minD gene products or as a result of the overexpression of the minE gene. In contrast, as a consequence of the overexpression of minCD or a null mutation in minE, cells are filamentous in appearance. Deletion of the entire minB locus results in a minicell phenotype, indicating that the genes are not required for growth. It appears that placement of the septum in minicell mutants is a nonrandom process which relies on the existence of division sites, the positions of which are strictly determined in minB mutants as well as in wild-type cells prior to the beginning of septation (19). The minC gene product has been implicated in a second-division pathway involving the gene dicB (12, 21), and MinCD is also known to interact with FtsZ to antagonize its cell division activity (4). For a review of the models that explain the regulation of cell division, see de Boer et al. (9).

Is the placement of the sporulation septum in B. subtilis governed by genes similar to those of the minB operon? In a manner similar to septation in minicell mutants, septum formation in vegetative or sporulating B. subtilis cells always occurs at the same positions, either at midcell or in a polar location, indicating that septum placement is not a random process and that certain potential division sites must exist prior to septation. It may be that sporulating B. subtilis cells take advantage of the polar division sites to position the asymmetric septum, as pointed out by Beall and Lutkenhaus.
tioning of the anticipated minB-like genes in E. coli. We attempted to isolate and characterize the divIVB operon, which causes asymmetric septation and results in a wild-type (+) or a minicell (−) phenotype.

Materials and Methods

Bacterial strains. The wild-type strain used in this study was PY79, the prototrophic derivative of PY78 (43). Strains 1A292 (divIVB1 metB5 thyA1 thyB1) (29) and 1A485 (rodB1 leuA8) (32) were obtained from the Bacillus Genetics Stock Center (The Ohio State University). The construction of PL9, a divIVB1 mutant strain congenic with PY79, is described below.

General methods. Competent cells were prepared and transformed as described by Dubnau and Davidoff-Abelson (14). Selection for Cm<sup>+</sup> was conducted on Luria-Bertani (LB) agar containing 5 μg of chloramphenicol per ml.

Sporulation was induced in Difco sporulation (DS) medium and on plates with DS agar (28).

DNA manipulations in E. coli were carried out as described by Sambrook et al. (33).

Microscopic analysis of mutant strains was performed by using phase-contrast optics on a Zeiss microscope at ×1,000 magnification. Cells were photographed with bright-field optics at the same magnification.

Construction of strain PL9. Because of a competence defect in the divIVB1 mutant strain (1A292) we obtained from the Bacillus Genetics Stock Center, it was necessary to move divIVB1 into a competence-proficient background. To construct this new strain, we transformed chromosomal DNA from 1A292 into the spo<sup>−</sup> strain SC834 (spoIVFB152) (6), a strain which is congenic to PY79. We chose strain SC834 because of the close proximity between the spoIVF locus and the divIVB locus. The scheme was to use the 1A292 chromosome to correct the sporulation mutation and select for cells which were Spo<sup>+</sup>; Spo<sup>+</sup> transformants were then screened for those which were mutant at the divIVB locus but did not exhibit the original competence defect.

Spo<sup>+</sup> transformants were selected via chloroform resistance (7, 18), and resulting Spo<sup>+</sup> colonies were screened for minicell production and competence. One such Spo<sup>+</sup> Min<sup>−</sup> Com<sup>−</sup> transformant was designated PL9.

Plasmids. Plasmid pSC224 (6) has an insert which extends from a restriction site internal to the spoIVF operon to a PstI site upstream of the spoIVF start codon (Fig. 1). Plasmid pSR3 (31) was constructed by the method of Youngman (42) for cloning B. subtilis DNA adjacent to a transposon insertion. The insert in pSR3 extends from an EcoRI site within the Tn917 transposon insertion in KSI79.
(34) (spolVFA::Tn917QHU179) to the EcoRI site 4.3 kb upstream of the spoIvFA start codon (Fig. 1).

Plasmids pPL1, pPL5, and pPL6 (Fig. 1) were constructed by subcloning DNA from pSR3 into the integrational vector pSGMU2 (15). Plasmid pSGMU2 is a pUC13 derivative which contains the bla and cat genes and an origin of replication for E. coli but lacks an origin of replication for B. subtilis. Plasmid pPL1 was constructed by gel purifying a 3.7-kb BglII fragment from pSR3 by using the GeneClean Kit (Bio 101) and ligating the fragment into the BamHI site in the polylinker of pSGMU2. Plasmids pPL5 and pPL6 were constructed in a similar manner from separate HindIII-PstI fragments (0.9 and 1.1 kb, respectively) of pSR3.

Plasmids pPM53, pPM54, pPM56, pPM57, and pPM68 were constructed in other work (23) as part of an effort to clone spoIIB, which is located just upstream of orfA, by a chromosome-walking procedure. Plasmid pPL7 was constructed by subcloning the EcoRI-BglII fragment of pPM53 into the polylinker of pSGMU2 in the manner described above.

Correction of the divIVB1 and rodB1 mutations. To test the capacities of the plasmids described above to correct the divIVB1 and rodB1 mutations, competent cells from strains PL9 and 1A485 were transformed with the integrational plasmids, and transformants were selected by chloramphenicol resistance. Single crossover events between a wild-type plasmid insert and mutant chromosomal DNA can occur upstream or downstream of the mutation; those which occur upstream of the mutant allele will result in the correction of the mutation by the creation of a full-length wild-type copy of the gene.

We screened transformants of PL9 for correction of the divIVB1 mutation and 1A485 for correction of the rodB1 mutation by using phase-contrast microscopy. In the case of 1A485, transformants were examined following growth at the nonpermissive temperature of 45°C.

FIG. 2. Bright-field micrographs of B. subtilis divIVB1 and rodB1 mutants, showing corrected and uncorrected phenotypes. All cells were grown overnight on LB agar. Strain 1A485 cells and transformants were grown under nonpermissive conditions at 45°C. (A) Cells from strain PL9, which carries the original divIVB1 mutation. Arrowheads indicate free and attached minicells. (B) Strain PL9 transformed with the integrational plasmid pSC224. Minicell phenotype is corrected. (C) Strain 1A485 exhibiting the classical rodB1 mutation. (D) Strain 1A485 transformed with plasmid pPL1. Although the rod phenotype is corrected, integration of pPL1 results in a slight minicell defect. Arrowhead indicates free minicell. Magnification, ×6,000. Bar = 2 μm.
Nucleotide sequencing. Nucleotide sequencing was carried out according to the method described by Sanger et al. (35) with the Sequenase kit (USB) with modifications for double-stranded sequencing. The strategy used is outlined schematically at the top of Fig. 1.

Heat killing procedure. To check PL9 for sporulation efficiency, we employed a modification of the assay for heat resistance outlined by Nicholson and Setlow (28). Following 2 days of growth in DS medium, cultures of PY79 and PL9 were serially diluted into 1 × T base (7) and plated both prior to张扬 started.
to and after a 15-min incubation at 80°C to determine the proportion of heat-resistant spores as a function of viable cell count.

Isolation of RNA. RNA for Northern (RNA) hybridization analysis was prepared from sporulating cells harvested just after and 2 h after the end of exponential growth in DS medium. The method of RNA isolation was as described by Roels et al. (30) except that we employed a slightly different resuspension buffer (100 mM LiCl, 10 mM EDTA, 10 mM Tris [pH 7.8], 1% sodium dodecyl sulfate) and the precipitated RNA was treated with DNase as a final step in the purification process.
Northern hybridization analysis. Northern hybridization was carried out as a modification of the method presented in Sambrook et al. (33). Samples of RNA (10 and 30 μg) were loaded onto a 1.5% agarose gel containing 2.2 M formaldehyde. Following electrophoresis, the RNA was transferred to a nylon membrane (Hybond-N; Amersham). Prehybridization and hybridization were carried out at 65°C in hybridization buffer (50 mM PIPES [piperazine-N,N'-bis(2-ethanesulfonic acid)] pH 7.0), 50 mM Na2PO4 [pH 7.0], 100 mM NaCl, 10 mM EDTA, 5% sodium dodecyl sulfate). The nick-translated inserts from pPL3 and pPL6 (Fig. 1) and an EcoRI fragment from pPM3 (23), which contains the...
spolIAC gene, were used as probes. A 0.24- to 9.5-kb RNA ladder (BRL) was used for molecular weight standards.

Staining procedure for light microscopy. We employed a modification of the staining procedure described by Miyakawa and Komano (24) to stain Bacillus mutants for light microscopy. Cells were grown overnight on LB agar. The rodB1 strains were grown at the nonpermissive temperature of 45°C, while the divIB1 strains were grown at 37°C. Single colonies were picked from the plates and suspended in a mixture of 50 μL of LB and 50 μL of a 3% formaldehyde solution (3% formaldehyde in 50 mM sodium phosphate buffer [pH 7.0]). Cell suspensions were then smeared on glass microscope slides, flamed to fix the cells, and rinsed under running water for 3 min. The samples were then treated with drops of a 5% tannic acid solution (5% tannic acid in 50 mM sodium phosphate buffer [pH 7.0]) as a mordant, incubated on a heat block at 65°C for 2 min, and rinsed under running water for 3 additional minutes. A drop of crystal violet solution (0.02% crystal violet in 50 mM sodium phosphate buffer [pH 7.0]) was added to the sample, a coverslip was placed on top, and the cells were examined and photographed under bright-field optics at ×1,000 magnification.

Nucleotide sequence accession number. The sequences of the divIB1 operon and two upstream open reading frames (ORFs) have been deposited in the GenBank data base under accession number M96343.

RESULTS

Localization and cloning of the divIB1 mutation. Previous mapping experiments have shown that the divIB1 mutation is located at about 247° on the genetic map of Henner and Hoch (16), the order of loci being leuA, spoIIB, rodB, divIB, and spoIVF (22, 29). The availability of cloned spoIVF DNA (6) made it possible for us in other work (23) to
clone DNA extending from spoIVF to spoIIB via a chromosome-walking procedure. Figure 1 is a physical map of this segment of the chromosome, which was expected to contain the wild-type allele of divIVB. The same chromosomal region of DNA was also cloned by an independent strategy based on the use of a library of random insertions in the B. subtilis chromosome of the integration vector pH101. One such integrant, identified as exhibiting linkage to divIVB by DNA-mediated transformation, was used to walk to the divIVB locus.

To localize divIVB, we used integrational plasmids and various segments of cloned DNA from the region between spoIVF and spoIIB to correct the divIVB1 mutation of the minicell-producing strain PL9, which is congenic to the divIVB+ strain PY79. Integration into the chromosome of PL9 of pSC224 (6), whose insert extends from the downstream end of the spoIVF operon to a PsrI site that is approximately 1.3 kb upstream of the spoIVF start codon (Fig. 1), was found to correct the divIVB1 mutation (Fig. 2A and B). Similarly, integration of pPL1, whose insert extends from the BglIII site just upstream of spoIVF to a second BglIII site located approximately 4 kb further upstream, also corrected the divIVB1 mutation, although the minicell phenotype of the resulting transformants was not fully wild type. (The reason that integration of pPL1 only partially corrected the divIVB phenotype is discussed below.) In contrast, the integration of pPM53 and pPM57 did not correct the mutation (Fig. 1). We conclude that the wild-type allele of the divIVB1 mutation is located between the upstream boundary of the pSC224 plasmid and the downstream boundary of the insert in pPL1, that is, between the PsrI and BglIII sites indicated in Fig. 1.

**Nucleotide sequencing of the divIVB region of the chromosome.** Figure 3 shows the nucleotide sequence of DNA in the region from spoIVB to spoIVF. Sequencing was accomplished according to the strategy outlined in Materials and Methods and as indicated by the arrows at the top of Fig. 1. Starting from the left side of Fig. 1, nucleotide sequencing revealed the existence of seven major ORFs, all in a left-to-right orientation and all with preceding sequences that could serve as ribosome-binding sites according to Moran et al. (25). The first ORF (orfA) is expected to encode a 21-kDa polypeptide that is 189 amino acids in length. A GenBank search indicates that this polypeptide is approximately 70% similar at the amino acid level to an ORF (orfE) located immediately downstream of E. coli mreD (40), an observation to which we were alerted by G. Stewart (37). Intriguingly, orfA is located less than 1 kb upstream of an operon that includes the B. subtilis homologs to E. coli mreB, mreC, and mreD (see below).

The second ORF (orfB) encodes a 26-kDa polypeptide of 231 amino acids. The product of orfB shares 78% amino acid similarity with the product of a Staphylococcus aureus gene that was identified as the insertion site for the transposon Tn554 (27). The function of the S. aureus gene is unknown. This ORF is also 69% similar at the amino acid level to the E. coli radC gene product (37). Southern blotting experiments using chromosomal DNA from wild-type cells and from φ105 lysogens indicate that the φ105 attachment site is located between the SmalI and EcoRI sites in orfB (14a, 23, 23a). These results confirm mapping experiments that position the phage attachment site between rodB1 and spoIIB on the B. subtilis chromosome (16).

Evidence presented below will show that the five remaining ORFs are part of a five-cistron operon. The first three ORFs in this operon have predicted sizes of 337 (36,159 Da), 290 (32,155 Da), and 173 (19,783 Da) codons and show various degrees of similarity to the E. coli shape-determining genes mreB, mreC, and mreD. Figures 4 and 5 show alignments of the E. coli and B. subtilis mre gene products. The product of the gene designated mreB in Fig. 1 shares 57.8% identity with E. coli MreB (Fig. 4) and an identity with the B. cereus mreB product that approaches 66% in certain stretches of the amino acid sequences (not shown). Although the B. subtilis mreC-like gene product does not exhibit high overall similarity to E. coli MreC, regions of significant similarity exist between the predicted product of the mreC-like gene and the corresponding E. coli protein (Fig. 5A). As a result of its position following B. subtilis mreB and mreC and its size, we presume that the product of the third gene of the operon, mreD, is the homolog to E. coli MreD, although it has only a 21% identity with the E. coli protein according to a Best-fit (12a) analysis (not shown).

The last two genes in the operon encode proteins with lengths of 227 (24,965 Da) and 269 (29,338 Da) amino acids. The product of the penultimate gene, minC, shares regions of similarity with the E. coli MinC protein (Fig. 5B). Likewise, the protein encoded by the last gene in the operon, designated minD, is 44.3% identical to the E. coli minD gene product. A Best-fit (12a) alignment of the predicted B. subtilis MinD and E. coli MinD is shown in Fig. 6. As noted in Fig. 2 of Cutting et al. (6), B. subtilis minD is followed by a region of dyad symmetry that may serve as a rho-independent terminator for the operon.

**Localization of rodB1 mutation.** The rodB1 mutation is a conditional mutation that causes the formation of rounded and irregularly shaped cells when mutant cells are grown under restrictive conditions (i.e., low salt concentration or temperatures above 42°C) (32). For an example of the rodB1 phenotype, note the irregularly shaped cells in Fig. 2C. Because rodB1 is located between the spoIVB and divIVB loci (20, 22) and because the rodB1 mutant phenotype is strikingly similar to that of the E. coli mre (envB) mutants (39), we reasoned that rodB1 might be allelic to one of the B. subtilis mre genes. In confirmation of this expectation, integration of pPL1 (Fig. 1) into the chromosome of a strain carrying the rodB1 mutation corrected the rod phenotype of a rodB1 mutant (Fig. 2D) and at the same time created a slight minicell phenotype, the basis for which will be discussed below. In contrast to these results, the integration of pSC224 into the rodB1 strain did not correct the rodB1 mutation. We conclude, then, that the rodB1 mutation lies between the BglIII site at the upstream boundary of the insert in pPL1 and the PsrI site at the upstream end of the insert in pSC224. Thus, the wild-type allele of rodB1 is most likely located within the region of the three B. subtilis mre genes. Varley and Stewart (37a) have reported further localization of the rodB1 allele to the mreD cistron.

**Determining the boundaries of the operon.** To define the functional boundaries of the transcriptional unit(s) encompassing minC and minD, we used plasmids containing DNA internal to the min genes and the mre genes as well as plasmids with inserts that overlapped the ends of the open reading frames of these genes. Since the integrational plasmids did not contain origins of replication for B. subtilis, transformants obtained by drug selection (Cm') for the vector-specific chloramphenical resistance gene (cat) arise from single reciprocal (Campbell-like) recombination between the plasmid insert and the corresponding region of DNA in the chromosome (43, 44). Fragments internal to the operon will separate the chromosomal region homologous to
FIG. 4. Best-fit (12a) amino acid sequence alignment of the E. coli (Ec) and B. subtilis (Bs) mreB gene products. The E. coli MinD sequence is from Doi et al. (13). Lines denote identical amino acids, and dots denote amino acids which are conserved to a greater (+) or lesser (-) degree between the two sequences.

cause a minicell phenotype (Fig. 1). These results indicate that the mre genes and the min genes are part of the same transcriptional unit whose upstream boundary precedes mreB in the region corresponding to the insert in pPM57 and whose downstream boundary is between minD and spoIVF in the region corresponding to the insert in pPL1.

The integration into the chromosome of PY79 of plasmids pPL7 and pPM53, whose inserts extend from within mreB to an EcoRI site located 325 bp upstream of the mreB start codon, did not cause the severe minicell phenotype exhibited by integration of pPL5 and pPL6 (Fig. 1). Nevertheless, the transformants did exhibit a slight minicell phenotype. This is in contrast to the wild-type phenotype observed following integration of pPM56 and pPM45, whose inserts overlap mreB but extend more than 1 kb upstream of the EcoRI site (Fig. 1). A possible interpretation of these results is that transcription for the mre and min operon is generated from at least two promoters, one of which is downstream of the EcoRI site. We infer that the downstream promoter is inadequate to direct the synthesis of sufficient MinC and MinD to fully suppress the minicell phenotype.

Finally, we consider the case of pPL1, whose insert extends from within mreB to the stop codon at the end of minD. Integration of pPL1 into the chromosome of PY79 results in a slight minicell phenotype, similar to the partial minicell phenotype that had been observed following the integration of pPL5 into the chromosome of the rodB1 and divIB1 strains (see above). From the examination of vector sequence and the sequence at the downstream boundary of its insert, we infer that integration of pPL1 was expected to eliminate the minD stop codon and thereby extend the minD ORF an additional 18 codons into vector DNA. Evidently, the resulting elongated MinD protein is only partially functional.

Determining transcript length via Northern hybridization. To confirm that mreBCD and minCD constitute a single operon, we carried out Northern hybridization with RNA from cells harvested at the end of 2 h after the end of exponential growth in DS medium. The results show that both an mre-specific probe (pPL5) and a min-specific probe (pPL6) hybridized to a 4- to 5-kb RNA species, creating the smears shown in Fig. 7. The darker bands within the smear in Fig. 7A correspond to 16S and 23S rRNAs. The level of transcript decreased 2 h after the end of exponential growth (not shown). The smearing is most likely due to the degra-
formation of rounded and irregularly shaped cells under restrictive conditions. In confirmation and extension of these results, Varley and Stewart have further localized rodB1 to the mreD cistron (37a). Thus, unlike the case in *E. coli*, shape-determining and minicell genes in *B. subtilis* are part of the same transcriptional unit.

While disruption of the divIVB operon with integrational plasmids causes a mild to severe minicell phenotype in transformed cells, it does not cause a rod phenotype. This is an unexpected result, as null mutations in any of the three *E. coli* mre genes result in a rod phenotype (39, 41). A possible explanation for the lack of apparent phenotype following disruption of mreC and mreD may lie in the existence of a second copy of the mre genes at a separate locus. In fact, a second operon with significant homology to *E. coli* mreb as well as to the *B. subtilis* mreb isolated in this study has been found at a position downstream of the sporulation locus spoIIID (5). It is not yet known whether this second copy of mreb is part of an operon that also encodes copies of mreC and mreD. If it is the case that a second mre locus exists, our results imply that the original rodB1 mutation was a gain-of-function mutation. An alternative explanation for the lack of phenotype following disruption of the mre genes may be the existence of a cryptic promoter in the integrational vector pSGMU2 that is strong enough to drive sufficient transcription of mreC and mreD to prevent a rod phenotype but is not strong enough to transcribe minC and minD sufficiently to overcome the minicell phenotype. To determine which of the two explanations is correct, it will be necessary to construct true null mutations of each of the mre genes.

Although disruption of the divIVB operon resulted in a minicell phenotype, the sporulation efficiencies of these disruptional mutants as well as that of the classical divIVB1 mutant were similar to that of PY79, the wild-type strain (22a). This result is not unexpected, given that the role of the *E. coli* minC and minD gene products is to block both the polar and nonpolar division sites and that it is the third gene in the minB operon, minE, which conveys topological specificity. If the *B. subtilis* min homologs are involved in sporulation, *B. subtilis* minCD mutants might be expected to form asymmetric septa two out of three times and would thus have an expected sporulation efficiency of 66%, a deviation from wild-type efficiency which we would not be able to measure in a meaningful manner by using the assay for heat-resistant spores. Such a result would be consistent with the fact that min mutations appear to have little effect on the growth rate of vegetative cells, although they septate correctly only one out of three times.

During cell division in *E. coli*, the minE gene product functions to prevent the MinCD complex from inactivating the central site while allowing it to block septation at polar sites (11). We speculate that a homolog or pair of homologs to *E. coli* minE, the *min* gene that was not found at the divIVB locus, is responsible for determining the topological specificity of the *B. subtilis* minC and minD gene products. In one model, a single MinE homolog inactivates the MinCD complex at midcell during vegetative growth in a manner similar to that of *E. coli* MinE. During sporulation, this MinE homolog undergoes some kind of modification that causes it to inactivate the MinCD complex at polar sites and not at midcell, thereby allowing the formation of an asymmetrically positioned septum. In an alternative model, *B. subtilis* possesses two minE-like genes, one expressed during growth and one expressed early during sporulation. The vegetatively expressed minE would allow the formation of media
septa, whereas the sporulation-specific gene would cause the appearance of asymmetrically positioned septa.

In summary, we report here the cloning of a five-cistron operon in *B. subtilis* whose predicted proteins are similar to the products of the *E. coli* shape-determining genes *mreB*, *mreC*, and *mreD* and the *E. coli* cell division genes *minC* and *minD*. The *mre* genes are the site of the cell shape-altering mutation *rodB1*, whereas the *min* genes are the site of the minicell mutation *divIVB1*. A homolog or homologs of the *E. coli minE* gene, which was absent from the *divIVB* operon, could govern the position of septum placement during growth and sporulation.

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