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spo0J Is Required for Normal Chromosome Segregation as well as the Initiation of Sporulation in *Bacillus subtilis*

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The *spo0J* gene of *Bacillus subtilis* is required for the initiation of sporulation. We show that the sporulation defect caused by null mutations in *spo0J* is suppressed by a null mutation in the gene located directly upstream from *spo0J*, *soj* (suppressor of *spo0J*). These results indicate that Soj inhibits the initiation of sporulation and that Spo0J antagonizes that inhibition. Further genetic experiments indicated that Soj ultimately affects sporulation by inhibiting the activation (phosphorylation) of the developmental transcription factor encoded by *spo0A*. In addition, the temperature-sensitive sporulation phenotype caused by the *ftsA279* (*spoIIN279*) mutation was partly suppressed by the *soj* null mutation, indicating that FtsA might also affect the activity of Soj. Soj and Spo0J are known to be similar in sequence to a family of proteins involved in plasmid partitioning, including ParA and ParB of prophage P1, SopA and SopB of F, and IncC and KorB of RK2. *spo0J* was found to be required for normal chromosome partitioning as well as for sporulation. *spo0J* null mutants produced a significant proportion of anucleate cells during vegetative growth. The dual functions of Spo0J could provide a mechanism for regulating the initiation of sporulation in response to activity of the chromosome partition machinery.

The generation of new cells during growth or development requires proper replication, repair, and segregation of chromosomes. Conditions that perturb chromosome replication or damage DNA often inhibit cell cycle progression or development through checkpoint mechanisms. Such regulatory mechanisms are present in organisms as diverse as bacteria, yeasts, and mammals and act to prevent the unproductive formation of cells lacking intact chromosomes.

Spore formation by the gram-positive bacterium *Bacillus subtilis* is a developmental process requiring two different cell types. Each cell type has an intact chromosome and a characteristic and distinct pattern of gene expression. The two cell types are created by formation of an asymmetric division septum early during development. The smaller cell, known as the forespore, develops into the mature spore while enclosed in the larger mother cell (30).

One of the most important early events necessary for the initiation of sporulation is the activation of the developmental transcription factor encoded by *spo0A*. Spo0A is activated by phosphorylation, and Spo0A~P induces expression of several genes, including *spoIIA*, *spoIIE*, and *spoIIG* (4, 6, 46, 47, 55, 58) that are essential for sporulation and the establishment of cell type-specific gene expression (30). Phosphorylation of Spo0A requires histidine protein kinases, i.e., KinA, -B, and -C (2, 27, 38, 56), and two phospho-transfer proteins, Spo0F and Spo0B (7). The histidine protein kinases autophosphorylate on a histidine residue, and phosphate is transferred to Spo0F, and then from Spo0F to Spo0B, and finally from Spo0B to Spo0A (7).

At least two different mechanisms couple the activation of Spo0A to the ability to provide intact chromosomes for the cell types needed for spore development. One mechanism inhibits phosphorylation of Spo0A in response to DNA damage and induction of a *recA*-dependent SOS response (17). The other mechanism affects phosphorylation of Spo0A in response to alterations in the initiation of DNA replication (19). In this article, we describe a regulatory system that controls the initiation of sporulation, perhaps in response to a third DNA-related signal. Genetic experiments indicate that phosphorylation of Spo0A is regulated, in part, by the two products of the *spo0J* operon and that the activities of these products might respond to signals related to chromosome segregation.

spo0J is located in the origin region of the chromosome, and its gene product is similar (~50%) to members of the ParB family of proteins (15, 36) involved in plasmid partitioning. In addition, the product of the gene directly upstream of *spo0J*, *orf253* (*soj*), is similar (~50%) to members of the ParA family of ATPases (15, 36). ParA and ParB proteins function as a pair to direct partitioning of several stably maintained plasmids. Members of the ParA and ParB families include IncC and KorB from the broad-host-range plasmid RK2, SopA and SopB from F, and ParA and ParB from P1 prophage (reviewed in reference 13). In addition, two genes from the origin region of *Pseudomonas putida* encode products homologous to the *orf253* (*soj*) and *spo0J* gene products (36) and perhaps have a role in chromosome partitioning in that organism. Soj is ~51% identical (~76% similar) to Orf263 of *P. putida*, and Spo0J is ~43% identical (~64% similar) to Orf290 of *P. putida*.

In this article, we demonstrate that *spo0J* is required for normal chromosome partitioning during vegetative growth. Interestingly, in contrast to ParA-like counterparts in the plasmid segregation systems, *soj* (*orf253*) is not required for chromosome partitioning. Rather, Soj plays a role in signal transduction during development, inhibiting the activation of Spo0A in the absence of *spo0J*. These results suggest that the products of the *spo0J* operon may function as a checkpoint mechanism that couples the onset of development to the potential to segregate chromosomes.

MATERIALS AND METHODS

Strains. The *B. subtilis* strains used are listed in Table 1 and are derived from strain JH642 (41). Strains were constructed

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TABLE 1. *B. subtilis* strains used

Strain	Genotype
JH642	*trpC2 pheA1*
AG1468	JH642 Δ*spo0J*::*spc*
AG1505	JH642 Δ(*soj-spo0J*)::*spc*
KI1113	JH642 *spoIIA*⁺::pPP81 (*spoIIA-lacZ*)
KI1114	JH642 *spoIIE*⁺::pZΔ326-GV39 (*spoIIE-lacZ*)
KI1771	JH642 *spoVG*⁺::pZL207 (*spoVG-lacZ*)
KI1773	JH642 Δ*spo0J*::*spc sof-1-cat*
KI1774	JH642 Δ*spo0J*::*spc spo0A*⁺*-cat*
KI1775	JH642 Δ*spo0J*::*spc rvtA11-cat*
KI1778	JH642 *spoIIA-lacZ* Δ*spo0J*::*spc*
KI1779	JH642 *spoIIE-lacZ* Δ*spo0J*::*spc*
KI1780	JH642 *spoVG-lacZ* Δ*spo0J*::*spc*
KI1798	JH642 *spoIIA-lacZ* Δ(*soj-spo0J*)::*spc*
KI1799	JH642 *spoIIE-lacZ* Δ(*soj-spo0J*)::*spc*
KI1800	JH642 *spoVG-lacZ* Δ(*soj-spo0J*)::*spc*
KI1872	JH642 *ftsA279*(Ts)*-cat*
KI1885	JH642 *ftsA279*(Ts)*-cat* Δ(*soj-spo0J*)::*spc*
KI1888	JH642 *ftsA279*(Ts)*-cat rvtA11-spc*
KI1889	JH642 *ftsA279*(Ts)*-cat* Δ*spo0J*::*spc*
KI1944	JH642 Δ(*soj-spo0J*)::*spc thr*::(Δ*soj spo0J*⁺ *erm*)
KI1874	JH642 *thrC*::(*soj-lacZ erm*)
KI1917	JH642 *thrC*::(*soj-lacZ erm*) Δ(*soj-spo0J*)::*spc*
KI1918	JH642 *thrC*::(*soj-lacZ erm*) Δ*spo0J*::*spc*

by transformation with plasmid or chromosomal DNA by using standard procedures (11). The Δ*spo0J*::*spc*, Δ(*soj-spo0J*)::*spc*, and Δ*soj* mutations are indicated in Fig. 1, and construction of these mutations is described below. *spoIIA-lacZ* (*spoIIA*⁺::pPP81) (57) and *spoIIE-lacZ* (*spoIIE*⁺::pZΔ326-GV39) (10) are transcriptional fusions made by integrating the indicated plasmids (containing the *lacZ* fusion) by single crossover into the *spoIIA* and *spoIIE* loci, respectively. The constructs have been modified to confer resistance to neomycin (19). *spoVG-lacZ* (*spoVG*⁺::pZL207) is a translational fusion integrated by

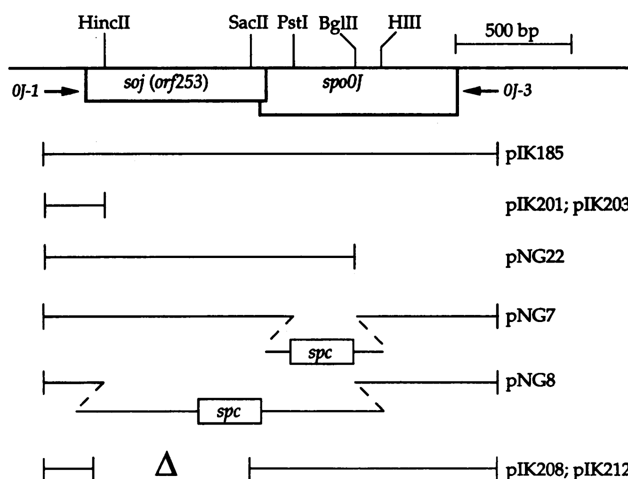


FIG. 1. Map of the *spo0J* operon and plasmids. Plasmids are also indicated in Table 2. The location of the primers *0J-1* and *0J-3* used to clone the *spo0J* operon by PCR amplification are indicated. The PCR product was cloned into pGEM*cat* (59) to create pIK185. pNG7 contains a deletion-insertion mutation in *spo0J* (Δ*spo0J*::*spc*). pNG8 contains a deletion-insertion mutation in both *soj* and *spo0J*[Δ(*soj-spo0J*)::*spc*]. pIK212 contains an in-frame deletion in *soj* (see Materials and Methods). The map is based on the DNA sequence of the origin region (accession number X62539) (36).

a single crossover into the *spoVG* locus (60). *rvtA11* (50) and *sof-1* (16) are missense mutations in *spo0A* that suppress, or partly suppress, the sporulation defect caused by mutations in *spo0F* and *spo0B*. Strains containing *rvtA11* and *sof-1* were constructed by transformation and selection for a linked antibiotic resistance gene (*cat* or *spc*) as described previously (9, 19). The *ftsA279*(Ts) [*spoIIN279*(Ts)] mutation is a missense mutation in the essential cell division gene *ftsA* (23) that causes a temperature-sensitive defect in sporulation. A linked *cat* insertion (kindly provided by P. Stragier) was used to transfer *ftsA279* into our strain background by selecting chloramphenicol-resistant transformants and then testing for temperature-sensitive sporulation.

Standard *Escherichia coli* strains were used for cloning and maintaining plasmids as described previously (18). *E. coli* CJ236 (*dut-1 ung-1 thi-1 rel-1*; pCJ105) (26) was used for preparing uracil-containing single-stranded DNA for site-directed mutagenesis of *soj*.

Media. Routine growth and maintenance of *E. coli* and *B. subtilis* were done in Luria-Bertani (LB) medium (31). 2×SG medium (29) was used as the nutrient sporulation medium in spore assays and gene expression studies. It was also used for experiments involving DNA staining of exponentially growing cultures. Media in plates were solidified with 15 g of agar (Difco Laboratories) per liter. Sporulation proficiency was visualized on DS (48) or 2×SG plates. Antibiotics were used at the following concentrations: ampicillin, 100 μg/ml; chloramphenicol, 5 μg/ml; spectinomycin, 100 μg/ml; neomycin, 5 μg/ml; erythromycin and lincomycin together (MLS) at 0.5 and 12.5 μg/ml, respectively, to select for the *erm* gene.

Materials. Molecular biological reagents (enzymes and PCR and DNA sequencing reagents) were purchased from commercial suppliers and used as described in standard procedures (3, 44) or the supplier's instructions. Synthetic oligonucleotides for PCR and site-directed mutagenesis were purchased from the Biopolymers Laboratory of the MIT Center for Cancer Research. DAPI (4′,6-diamidino-2-phenylindole) for DNA staining was purchased from Sigma.

Spore assays. Cells were grown in 2×SG medium at 37°C unless otherwise indicated, and spores were assayed at least 12 h after the end of exponential growth. The number of viable cells per milliliter of culture was determined as the total number of CFU on Luria-Bertani medium plates. The number of spores per milliliter of culture was determined as the number of CFU after heat treatment (80°C for 20 min). Percent sporulation is the ratio of spores per milliliter to viable cells per milliliter times 100.

β-Galactosidase assays. Cells were grown in 2×SG medium, and samples were taken at the indicated times for determination of β-galactosidase specific activity. Prior to the enzyme assay, cells were removed by centrifugation and resuspended in Spizizen salts (53). β-Galactosidase specific activity is expressed as: (ΔA_{420} per minute per milliliter of culture per unit of optical density at 600 nm) × 1,000 (31).

Clones and plasmid constructions. The plasmids used are indicated in Table 2 and Fig. 1. pIK185 contains the *spo0J* operon cloned into the integrative vector pGEM3ZF+ *cat-1* (pGEM*cat*) (59). The operon was amplified from the chromosome of *B. subtilis* JH642 by the PCR using oligonucleotide primers *0J-1* (5′-CCGAATTCCTGTACTGTGACTTCTTC TT) and *0J-3* (5′-CCGGATCCGTGGAGGCAAGAACGC CTTA). The first 8 nucleotides of *0J-1* and *0J-3* contain restriction sites (*Eco*RI or *Bam*HI, respectively) for cloning, while the last 20 nucleotides are complementary to sequences located ~225 bp 5′ to the start of *soj* (*orf253*) and ~100 bp 3′ to the end of *spo0J*, respectively (36).

TABLE 2. Plasmids used

Plasmid	Description[a]
pGEM3ZF+ *cat-1* (pGEM*cat*)	Ap Cm; integrative vector (59)
pJL74	Ap Sp; *spc* cassette from pUS19 (5) cloned into pBluescript II SK$^+$ (Stratagene) (27); used as source of *spc* cassettes for construction of Δ*spo0J*::*spc* and Δ(*soj-spo0J*)::*spc* mutations
pDR66	Ap Cm; plasmid used to recombine gene of interest at *amyE* locus of *B. subtilis* chromosome; used to construct pIK187 and pNG22 and to place an intact copy of *soj* in strain containing Δ(*soj-spo0J*)::*spc* mutation
pDG793	Ap MLS; vector used to construct transcriptional fusions to *lacZ* and recombine into the chromosome at the *thrC* locus (provided by P. Stragier); used to construct pIK203, containing the *soj-lacZ* fusion
pDG795	Ap MLS; vector used to recombine gene of interest at *thrC* locus of *B. subtilis* chromosome (provided by P. Stragier). Used for construction of pIK212 and a strain containing an in-frame deletion in *soj* [*thrC*::(Δ*soj spo0J*)]
pIK185	Ap Cm; PCR product from 0J-1 and 0J-3 containing *soj* (*orf253*) and *spo0J* cloned into pGEM*cat* (*Eco*RI-*Bam*HI)
pIK187	Ap Cm; *Eco*RI(blunt)-*Sal*I fragment from pIK185 containing *soj* and *spo0J* cloned into pDR66 [*Hin*dIII(blunt)-*Sal*I] [*amyE*::(*soj*$^+$ *spo0J*$^+$)]; used to construct pNG22
pIK192	Ap Cm; made by deleting between *Sph*I and *Bam*HI sites of pIK185; removes several restriction sites in polylinker, including *Hin*cII, *Pst*I, and *Sph*I (*Bam*HI site is retained); used in construction of pNG7
pIK201	Ap Cm; made from pIK185 by deleting from the *Hin*cII site in the vector to the *Hin*cII site in *soj* (Fig. 1); used to demonstrate, by integrational mapping, that the promoter was contained in the remaining ~300 bp fragment; also used as a source of the promoter fragment in the construction of the *soj-lacZ* transcriptional fusion (pIK203)
pIK203	Ap MLS; plasmid containing the operon promoter fused to *lacZ* (*soj-lacZ*); made by cloning the ~300-bp *Eco*RI-*Hin*dIII fragment from pIK201 into the *Eco*RI-to-*Hin*dIII sites of pDG793
pIK208	Ap Cm; made by site-directed mutagenesis using single-stranded DNA from pIK185 and primer sojΔ (see Materials and Methods); removes codons 6 to 235 of *soj* and was used in construction of pIK212
pIK212	Ap MLS; *Eco*RI-*Bam*HI fragment from pIK208 containing Δ*soj* and entire *spo0J*$^+$ gene subcloned into pDG795 (*Eco*RI-*Bam*HI); used to construct strain containing in-frame deletion in *soj* (KI1944)
pNG7	Ap Sp; *Pst*I-*Bam*HI fragment containing *spc* cassette from pJL74 subcloned between *Pst*I and *Bgl*II sites in pIK192; used to construct strain with Δ*spo0J*::*spc* mutation (AG1468)
pNG8	Ap Sp; made by deleting between *Hin*cII and *Pst*I sites of pNG7; used to construct strain containing Δ(*soj-spo0J*)::*spc* mutation (AG1505)
pNG22	Ap Cm; made by deleting between *Bgl*II and *Sal*I sites of pIK187, which removes 3′ half of *spo0J*; used to place copy of *soj*$^+$ (*amyE*::*soj*$^+$) in chromosome of strain with Δ(*soj-spo0J*)::*spc* mutation

[a] Ap, Cm, Sp, and MLS refer to resistance to ampicillin, chloramphenicol, spectinomycin, and erythromycin plus lincomycin, respectively.

The Δ*spo0J*::*spc* mutation is contained in pNG7 and was constructed by inserting the *spc* cassette (from pJL74) between the *Pst*I and *Bgl*II sites in *spo0J* (Fig. 1). The Δ(*soj-spo0J*)::*spc* mutation is contained in pNG8 and was constructed by inserting the *spc* cassette between the *Hin*cII site in *soj* and the *Bgl*II site in *spo0J* (Fig. 1). These mutations were introduced into the *B. subtilis* chromosome by transformation of strain JH642 with linearized plasmid (pNG7 or pNG8) and selection for spectinomycin resistance. The recombinants chosen [strain AG1468, Δ*spo0J*::*spc*; strain AG1505, Δ(*soj-spo0J*)::*spc*] were sensitive to chloramphenicol, indicating that they did in fact result from replacement of the *spo0J* region by double crossover and not by integration of the entire plasmid (with the pGEM*cat* backbone) by single crossover.

An in-frame deletion from codon 6 to codon 235 in *soj* was constructed by site-directed mutagenesis (26) using single-stranded DNA from plasmid pIK185 and the primer sojΔ [5′-AAAGTAGGTGACATCGTGGGAAAAATCATA-(Δ)-TCAAGAGGTGCGGAAGTATATTTAGATTTA], where the (Δ) indicates the junction from the deleted DNA. Candidate plasmids containing the Δ*soj* mutation were identified by DNA sequencing, and one such plasmid, pIK208 (Table 2), was used to introduce this mutation into the *B. subtilis* chromosome. The Δ*soj* mutation in pIK208 was subcloned along with an unaltered copy of *spo0J*$^+$ into the *thrC* replacement vector pDG795 (provided by A.-M. Guerout-Fleury and P. Stragier) to produce pIK212. *thrC*::(Δ*soj spo0J*$^+$) was recombined into the chromosomal *thrC* locus by transformation of AG1505 [Δ(*soj-spo0J*)::*spc*] with linearized pIK212 and selection for MLS^r to give strain KI1944 (Table 1).

pNG22 contains *soj*$^+$ cloned into the *amyE* vector pDR66 (21) and was used to recombine *soj*$^+$ into the chromosome at the nonessential *amyE* locus for complementation tests with the *soj* mutations.

pIK201 contains the 5′ end of the operon extending ~225 bp upstream of the beginning of the *soj* open reading frame and was made from pIK185 by deleting from the *Hin*cII site in the vector to the *Hin*cII site in the beginning of the *soj* structural gene. pIK203 contains the 5′ regulatory region of the *spo0J* operon fused to *lacZ* (*soj-lacZ*) and was made by cloning the *Eco*RI-*Hin*dIII fragment from pIK201 into the *Eco*RI-*Hin*dIII sites of the *lacZ* transcriptional fusion vector pDG793. The *soj-lacZ* fusion was recombined into the *thrC* locus of the chromosome by transforming cells with linearized pIK203 and selecting for MLS^r transformants.

DNA staining, photography, and quantitation of anucleate cells. Cells were grown in 2×SG medium (29) for seven to eight generations, and samples were taken while cultures were

in exponential growth (at an optical density at 600 nm of ~0.5). Samples were fixed with glutaraldehyde, treated with the DNA stain DAPI (0.2 or 0.4 μg/ml), and mounted onto microscope slides essentially as described previously (49). Cells were viewed with a Zeiss microscope by using combined Nomarski and fluorescence microscopies. The Nomarski and fluorescence systems were used simultaneously simply by reducing the light from the halogen lamp to a level at which both fluorescent nucleoids and cell shape were easily visualized. This technique is essentially the same as the combined phase and fluorescence microscopy technique described by Hiraga et al. (14), except that Nomarski optics are used in place of phase-contrast optics. Photographs (Fig. 3) were taken with a 35-mm camera and TMAX400 film (Kodak).

The frequency of anucleate cells present in a given sample was determined by counting the total number of cells and number of anucleate cells present in several fields of view. In general, each field of view contained 50 to 150 cells, and approximately 5,000 cells (~50 fields of view) were counted for each sample. After the fields were viewed, they were illuminated until photobleached to avoid recounting the same cells. A cell was considered anucleate if no blue fluorescence was detected within the cell contour. A cell body present in a filament was counted as a cell only if a visible septum separated it from the rest of the filament. Cultures of the Δ*spo0J*::*spc*, Δ(*soj-spo0J*)::*spc*, and Δ*soj* mutants did not appear to have a significantly different proportion of cells present in filaments compared with cultures of the isogenic wild type.

RESULTS

The sporulation defect caused by a null mutation in *spo0J* is suppressed by a null mutation in *soj* (*orf253*). Sequence information indicated that *spo0J* is in an operon with an upstream gene called *orf253* or *soj* (36). To test the role of *soj* in sporulation and to make a defined null mutation in *spo0J*, we cloned the *spo0J* operon by PCR (Materials and Methods) based on published DNA sequences (34, 36). We made two different deletion-insertion mutations: one in *spo0J* alone (Δ*spo0J*::*spc*), and the other in both *soj* and *spo0J* [Δ(*soj-spo0J*)::*spc*]. These mutations were recombined into the *B. subtilis* chromosome by double crossover (Fig. 1; see Materials and Methods). As expected, the Δ*spo0J*::*spc* mutation caused a sporulation defect (Table 3) that was very similar to the defect caused by previously described *spo0J* mutations (34, 42, 45). In addition, like the other *spo0J* mutations, this mutation prevented the initiation of sporulation and the expression of *spoIIA* and *spoIIE* (10) but had no significant effect on expression of *spoVG* (60) (Fig. 2).

Surprisingly, disruption of *soj* (suppressor of *spo0J*) suppressed the sporulation defect normally caused by a null mutation in *spo0J*. The Δ(*soj-spo0J*)::*spc* strain sporulated at an efficiency similar to that of the isogenic wild-type strain (Table 3), indicating that in the absence of *spo0J*, the product of *soj* normally acts to inhibit sporulation. In addition, expression of *spoVG*, *spoIIA*, and *spoIIE* was normal in the Δ(*soj-spo0J*)::*spc* double mutant (Fig. 2), indicating that this strain is not significantly altered in the kinetics of the initiation of sporulation and early sporulation gene expression. On the basis of these results, *orf253* is called *soj* for suppressor of *spo0J*.

Preliminary results indicated that the presence of soj^+ on a multicopy plasmid inhibited sporulation of otherwise wild-type cells and greatly reduced the sporulation frequency of *spo0J* single mutants (data not shown). These results are consistent with the notion that Soj inhibits sporulation.

We did a complementation test to verify that the Spo^+ phenotype of the Δ(*soj-spo0J*)::*spc* mutant was in fact due to inactivation of both genes. An intact wild-type copy of *soj* was recombined into the chromosome at the *amyE* locus (Materials and Methods). *amyE*::soj^+ was able to complement the Δ*soj* mutation in the Δ(*soj-spo0J*)::*spc* double mutant and resulted in a Spo^- phenotype because of the remaining *spo0J* mutation. In fact, the phenotype of the Δ(*soj-spo0J*)::*spc* mutant containing *amyE*::soj^+ was indistinguishable from the phenotype of *spo0J* single mutants (data not shown).

TABLE 3. Altered function mutations in *spo0A* and a null mutation in *soj* suppress the sporulation defect caused by a null mutation in *spo0J*

Strain	Relevant genotype	No. of spores/ml	% Sporulation
JH642	Wild type	1.5×10^8	52
KI1774	Δ*spo0J*::*spc*	1.3×10^6	0.64
AG1505	Δ(*soj-spo0J*)::*spc*	1.5×10^8	73
KI1944	Δ*soj*	2.1×10^8	93
KI1773	Δ*spo0J*::*spc sof-1*	4.9×10^7	31
KI1775	Δ*spo0J*::*spc rvtA11*	3.2×10^7	47

soj is not required for sporulation in the presence of $spo0J^+$. A strain containing an in-frame deletion that removes codons 6 through 235 of *soj* (Δ*soj*) was constructed by site-directed mutagenesis (see Materials and Methods). This strain sporulated at a frequency that was similar to that of the isogenic wild type (Table 3). The Δ*soj* mutation was not polar on *spo0J* since a sporulation defect did not result when *amyE*::soj^+ was introduced into the Δ*soj* mutant.

The null mutation in *soj* did not suppress the sporulation defects caused by mutations that cause a partial block in the initiation of sporulation, such as *kinA*, *spo0K*, or *spo0E11* (data not shown). These results indicate that Soj is not a general inhibitor of sporulation which, when removed, can compensate for any mutation that inhibits the initiation of sporulation. Rather, Soj appears to inhibit sporulation in response to the status of Spo0J. We suspect that Spo0J senses a physiological signal and controls the activity of Soj in response to that signal.

Spo0J and Soj affect activation of the Spo0A transcription factor. Altered function mutations in *spo0A* can bypass the requirement for *spo0F* or *spo0B* in sporulation (16, 24, 50–52). At least two of these *spo0A* mutations, *sof-1* and *rvtA11*, allow significant sporulation in the absence of the phosphorelay by creating a form of Spo0A that can be phosphorylated by an alternate kinase, KinC (27). The *sof-1* and *rvtA11* mutations in *spo0A* also suppressed the sporulation defect normally caused by null mutations in *spo0J* (Table 3). Taken together, the results with *spo0J*, *soj*, *sof*, and *rvt* mutants indicate that Soj inhibits production or accumulation of Spo0A~P and that Spo0J antagonizes the action of Soj.

Another protein that inhibits production of Spo0A~P has recently been characterized. *spo0E* encodes a phosphatase that seems to act directly to convert Spo0A~P to Spo0A (37). Null mutations in *spo0E* increase sporulation proficiency, while an altered form of Spo0E protein (encoded by *spo0E11*) encodes a more active phosphatase and inhibits the initiation of sporulation (37, 39, 40). Although both Soj and Spo0E inhibit production of Spo0A~P, these proteins seem to act separately, since the sporulation defect of a *spo0J spo0E11* double mutant is significantly more severe than that of either single mutant (data not shown). In addition, a null mutation in *spo0E* does not suppress the sporulation defect caused by *spo0J* mutations (data not shown), indicating that Spo0E is not part of the Spo0J-Soj regulatory pathway.

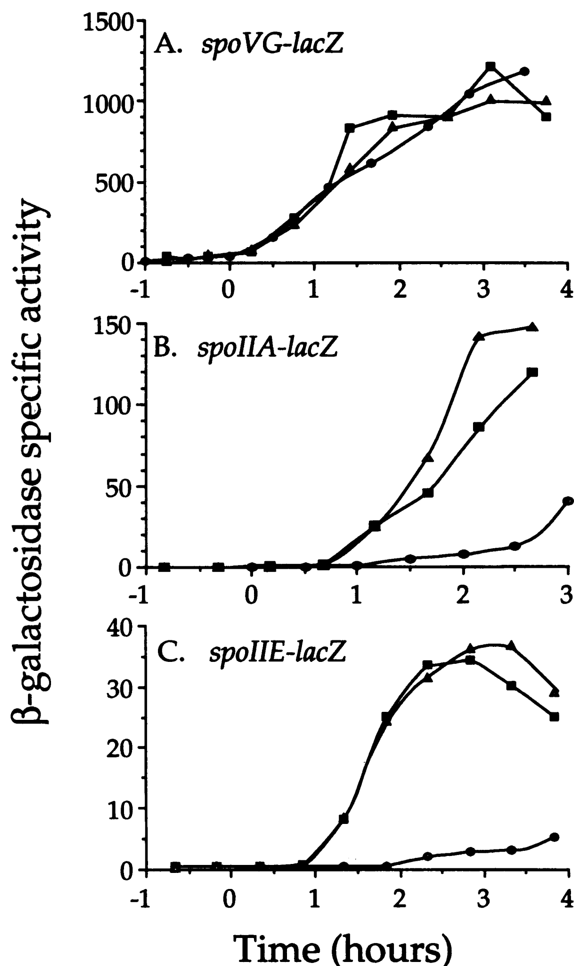


FIG. 2. Expression of *spoVG*, *spoIIA*, and *spoIIE* in strains containing mutations in *spo0J* and *soj*. The indicated strains were grown in 2× SG medium, and samples were taken for determination of β-galactosidase specific activity. Time zero indicates the time at which the culture departed from exponential growth. (A) Expression of *spoVG-lacZ*. Symbols: △, KI1771 (wild type); ○, KI1780 (Δ*spo0J*::*spc*); ■, KI1800 [Δ(*soj-spo0J*)::*spc*]. (B) Expression of *spoIIA-lacZ*. Symbols: △, KI1113 (wild type); ○, KI1778 (Δ*spo0J*::*spc*); ■, KI1798 [Δ(*soj-spo0J*)::*spc*]. (C) Expression of *spoIIE-lacZ*. Symbols: △, KI1114 (wild type); ○, KI1779 (Δ*spo0J*::*spc*); ■, KI1799 [Δ(*soj-spo0J*)::*spc*].

There are other mutations or conditions that also bypass the need for *spo0J* in sporulation. Infection of cells with phage PMB12 or SP10 partly suppresses the sporulation defect caused by mutations in *spo0J* and causes a catabolite (glucose)-resistant sporulation (Crs) phenotype in wild-type cells (34). The catabolite-resistant sporulation mutation *crsF* also partly bypasses the need for *spo0J* (34). In addition, we have found that overexpression of *kinA* (*spoIIJ*) causes a Crs phenotype and suppresses a *spo0J* mutant (data not shown). Since *crsF* maps near *kinA* (54) and causes phenotypes similar to overexpression of *kinA*, it seems possible that the *crsF* might be an allele of *kinA* and/or cause increased production or activity of the *kinA* gene product. The *soj spo0J* double mutant did not seem to cause a Crs phenotype (data not shown), and we suspect that *spo0J* normally has little if anything to do with the glucose inhibition of sporulation.

A null mutation in *soj* partly suppresses the sporulation defect caused by a mutation in the cell division gene *ftsA*. The *ftsA279*(Ts) [*spoIIN279*(Ts)] mutation is an allele of the cell division gene *ftsA* that causes a temperature-sensitive defect in sporulation (23, 28). This mutation affects sporulation, at least in part, by causing a defect in the activation of Spo0A. Expression of several genes normally induced early during sporulation by Spo0A~P, including *spoIIA*, *spoIIE*, and *spoIIG*, is reduced or delayed in the *ftsA279*(Ts) mutant (28). Moreover, the sporulation defect caused by *ftsA279* is partly suppressed by the *rvtA11* mutation in *spo0A* (28), indicating that the *ftsA279* mutation directly or indirectly affects transfer of phosphate through the phosphorelay.

TABLE 4. A null mutation in *soj* partly suppresses the sporulation defect caused by the temperature-sensitive sporulation mutation *ftsA279* at a nonpermissive temperature

Strain	Relevant genotype	No. of spores/ml	% Sporulation[a]
JH642	Wild type	1.1×10^8	100
KI1872	*ftsA279*(Ts)	1.1×10^5	0.17
KI1885	*ftsA279*(Ts) Δ(*soj-spo0J*)::*spc*	9.5×10^6	16
KI1888	*ftsA279*(Ts) *rvtA11*	2.2×10^6	3.4
AG1468	Δ*spo0J*::*spc*	7.2×10^5	0.70
KI1889	*ftsA279*(Ts) Δ*spo0J*::*spc*	1.8×10^3	0.0044

[a] For each strain, cells were grown in 2×SG medium at the permissive temperature of 32°C until early exponential growth. At this point, each culture was split in half, and one part was grown at 32°C and the other part was shifted to the restrictive temperature of 45°C. In this experiment, the percent sporulation was determined as the number of the spores per milliliter approximately 20 h after the end of exponential growth as a fraction of the total number of viable cells 1 to 2 h after the end of exponential growth. This was done because of the decrease in cell viability in strains containing the *ftsA279*(Ts) mutation at times well after time zero (i.e., 20 h later). During the first 2 h after the end of exponential growth, cell viability in these strains was not significantly different from that of the wild type. The range of viable cells per milliliter was 5.8×10^7 to 1.2×10^8. For the sake of simplicity, only data for cells grown at high temperature are shown. Strains containing the *ftsA279*(Ts) mutation did not show a significant defect in spore production at 32°C.

We found that the Δ(*soj-spo0J*) mutation significantly suppressed the sporulation defect caused by *ftsA279*(Ts) (Table 4). At the restrictive temperature of 45°C, the Δ(*soj-spo0J*)::*spc* *ftsA279*(Ts) strain consistently produced more spores than the *ftsA279*(Ts) mutant, sporulating as well as or better than the *rvtA11 ftsA279*(Ts) mutant. Suppression by Δ(*soj-spo0J*)::*spc* was partial, however, since spore production by the Δ(*soj-spo0J*)::*spc ftsA279*(Ts) strain was always lower than that of the wild-type strain. These results suggest that the *ftsA279*(Ts) mutation inhibits the activation of Spo0A through Soj.

Interestingly, a double mutant containing both the Δ(*spo0J*::*spc* and *ftsA279*(Ts) mutations sporulated worse than strains containing either mutation alone (Table 4). The additive effect of the *spo0J* and *ftsA279*(Ts) mutations on sporulation suggests that the products of these genes separately affect Soj and sporulation.

***spo0J* and *soj* are not involved in regulating activation of Spo0A in response to DNA damage or replication signals.** Activation of Spo0A is prevented by separate mechanisms in response to inhibition of initiation of DNA replication (19) or induction of the SOS response (17). Our initial interest in the *spo0J* operon was to test for a role in regulating the phosphorelay in response to the DNA replication and DNA damage (SOS) signals. Activation of Spo0A, as indicated by expression of a Spo0A-controlled target gene, was still inhibited in the Δ(*soj-spo0J*) double mutant when initiation of replication was inhibited with the *dnaB19*(Ts) mutation or when the SOS response was induced by treating cells with 6-(*p*-hydroxyphe-

nylazo)-uracil, an inhibitor of elongation of DNA synthesis (data not shown). These results indicate that *soj* and *spo0J* do not play a significant role in regulating the phosphorelay in response to the previously described DNA replication and DNA damage signals.

***spo0J* is required for normal chromosome segregation during growth.** Given the similarity of the products of the *spo0J* operon to plasmid partitioning proteins, we wondered whether *soj* and *spo0J* play a role in chromosome segregation. The Δ*spo0J::spc* mutation caused a defect in chromosome segregation, as determined by DAPI staining of DNA in cells of an exponentially growing culture (Fig. 3). Approximately 1 to 2% of the cells contained in a culture of the Δ*spo0J::spc* mutant were anucleate, while the frequency of anucleate cells in a culture of the otherwise isogenic wild-type strain was approximately 0.02% (Table 5). A similar proportion of anucleate cells was observed in cultures of the *spo0J93* mutant (data not shown). The magnitude of the segregation defect caused by the *spo0J* mutations is similar to that caused by the *muk* mutations in *E. coli* (13, 14, 35). In addition, the frequency of anucleate cells in wild-type *B. subtilis* cultures was similar to the frequency observed for wild-type *E. coli* (14), indicating that these two organisms have similar efficiencies of chromosome transmission.

Surprisingly, *soj* is not required for chromosome segregation. Cultures of the Δ*soj* mutant contained a frequency of anucleate cells that was similar to that found in cultures of the wild-type strain (Table 5). In addition, the *soj spo0J* double mutant had a chromosome segregation defect indistinguishable from that of the *spo0J* single mutant (Table 5). Thus, while the *soj* mutation suppressed the sporulation defect of the *spo0J* mutant, it did not suppress the chromosome segregation defect.

Transcription of the *spo0J* operon is not autoregulated. Several of the operons encoding plasmid partition proteins are subject to transcriptional autoregulation in which transcription increases in the absence of one or both operon products (reference 12 and references therein). To test if the *spo0J* operon is also autoregulated, we monitored transcription from the operon promoter (by using an *soj-lacZ* fusion) during growth and sporulation in the wild type and several mutants.

Integrational mapping was used to show that the 5′ end of the transcription unit was contained in the DNA fragment in pIK201. pIK201 (as well as pIK203 and pIK185) contains DNA extending from the 5′ PCR primer used to amplify the operon (approximately 225 bp upstream from the beginning of the *soj* open reading frame) to the *Hin*cII site in the *soj* coding region (Fig. 1; Table 2). When pIK201 was integrated by single crossover into the Δ*spo0J::spc* mutant, the resulting transformants were Spo$^-$, like the Δ*spo0J::spc* parent, indicating that *soj* was not disrupted and that pIK201 contains the 5′ regulatory sequences. If the fragment of DNA in pIK201 did not contain the 5′ sequences necessary for expression, then integrating pIK201 should have disrupted the transcription unit and caused a Spo$^+$ phenotype.

A transcriptional fusion to *lacZ* (*soj-lacZ*, contained in pIK203) was constructed by using the fragment contained in pIK201 and recombined into the chromosome at the *thrC* locus (see Materials and Methods). When grown in 2×SG medium, wild-type cells containing the fusion had significant accumulation of β-galactosidase during exponential growth (Fig. 4). Expression increased during the stationary phase, and β-galactosidase specific activity accumulated to approximately fivefold of that during growth (Fig. 4). Expression was normal or slightly higher in otherwise isogenic strains containing null mutations in *spo0H* or *spo0A* (data not shown). In addition, the Δ*spo0J::spc* mutation and the Δ(*soj-spo0J*)::*spc* mutation had little or no effect on expression of the *soj-lacZ* fusion (Fig. 4), indicating that there is not transcriptional autoregulation of the operon. This is in marked contrast to regulation of the *parAB* operon of bacteriophage P1, where ParA protein causes partial repression of the operon (approximately 5-fold) and ParA and ParB together cause full repression (approximately 50-fold) (12).

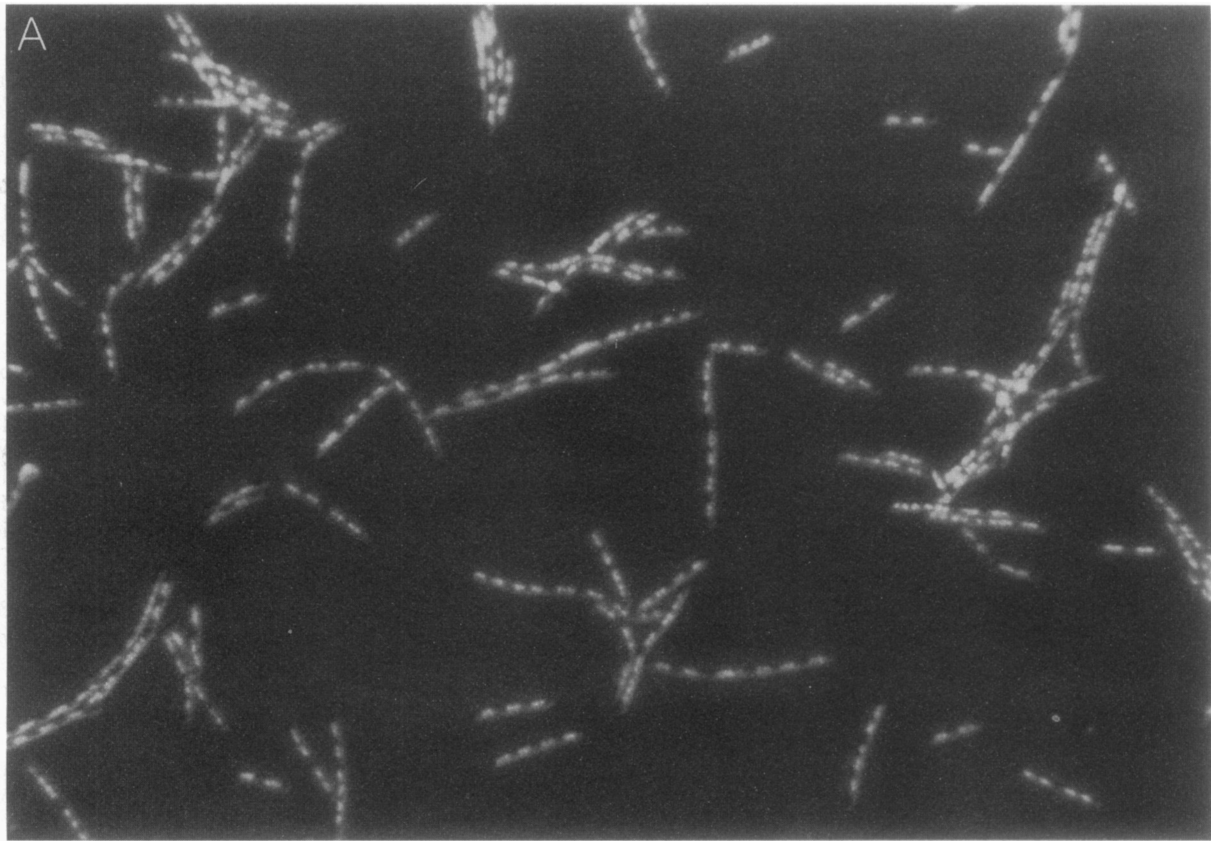
DISCUSSION

spo0J was originally defined as a gene required for the initiation of sporulation (42). Sequence information revealed that the product of this gene is similar to members of a class of proteins (the ParB family) that control partitioning of several bacterial plasmids, including F, P1 prophage, and RK2 (15, 34, 36). In addition, the product of the gene located directly upstream of *spo0J* (now called *soj*) is similar to partitioning proteins of the ParA class (15, 36). These proteins are ATPases (8, 13, 25, 32) and appear to work in conjunction with a ParB partner to direct partitioning of the cognate plasmid (13, 32). A similar pair of genes is found in the origin region of *P. putida* (36).

***spo0J* and chromosome partitioning.** In addition to its role in sporulation, *spo0J* is required for normal chromosome partitioning or segregation during vegetative growth. Null mutations in *spo0J* cause a defect in chromosome partitioning, resulting in production of 1 to 2% anucleate cells in a growing culture. On the basis of the similarity of Spo0J to plasmid partitioning proteins, it seems likely that Spo0J plays a direct role in chromosome partitioning, perhaps serving as one of the mechanisms or machines driving segregation in the cell. However, there are probably other mechanisms for chromosome partitioning, as the *spo0J* null mutation does not cause a severe partitioning defect. The defect caused by the *spo0J* null mutation is similar in magnitude to those caused by partitioning mutants (*muk*) in *E. coli* (14, 35). We suspect that the protein homologous to Spo0J that is encoded by *orf290* in the origin region of *P. putida* (36) is also involved in chromosome segregation.

In plasmid systems like the P1 prophage, it appears that both ParA and ParB proteins are required for partitioning (32). In contrast, *soj*, which encodes a protein similar to ParA, does not appear to be required for chromosome partitioning in *B. subtilis*, as a null mutation in this gene does not cause a significant segregation defect. It is possible that the chromosome system has evolved to allow Spo0J to direct partitioning in the absence of a ParA partner. Alternatively, a different chromosomally encoded ParA-like protein might work with Spo0J to direct partitioning. One such protein could be MinD, which has sequence similarity to ParA ATPases (25, 32) and appears to have some effect on chromosome segregation in *E. coli* (1, 22, 33).

It is not known how Par proteins direct partitioning of chromosomes or plasmids. It seems likely, however, that translocation of a chromosome or plasmid through the cell requires that ParA or ParB proteins interact with some cellular structure or protein that is membrane bound. One protein that may play such a role, at least for chromosome segregation in *B. subtilis*, is FtsA. *B. subtilis* FtsA is likely to be a membrane protein, given its homology to *E. coli* FtsA, which has been localized to the inner membrane (43). Our experiments with the *ftsA279*(Ts) mutation in *B. subtilis* suggest that, like Spo0J, FtsA also affects production of Spo0A~P during sporulation through Soj (Table 4). In addition, the presence of some anucleate cells in cultures of the *ftsA279*(Ts) mutant (data not



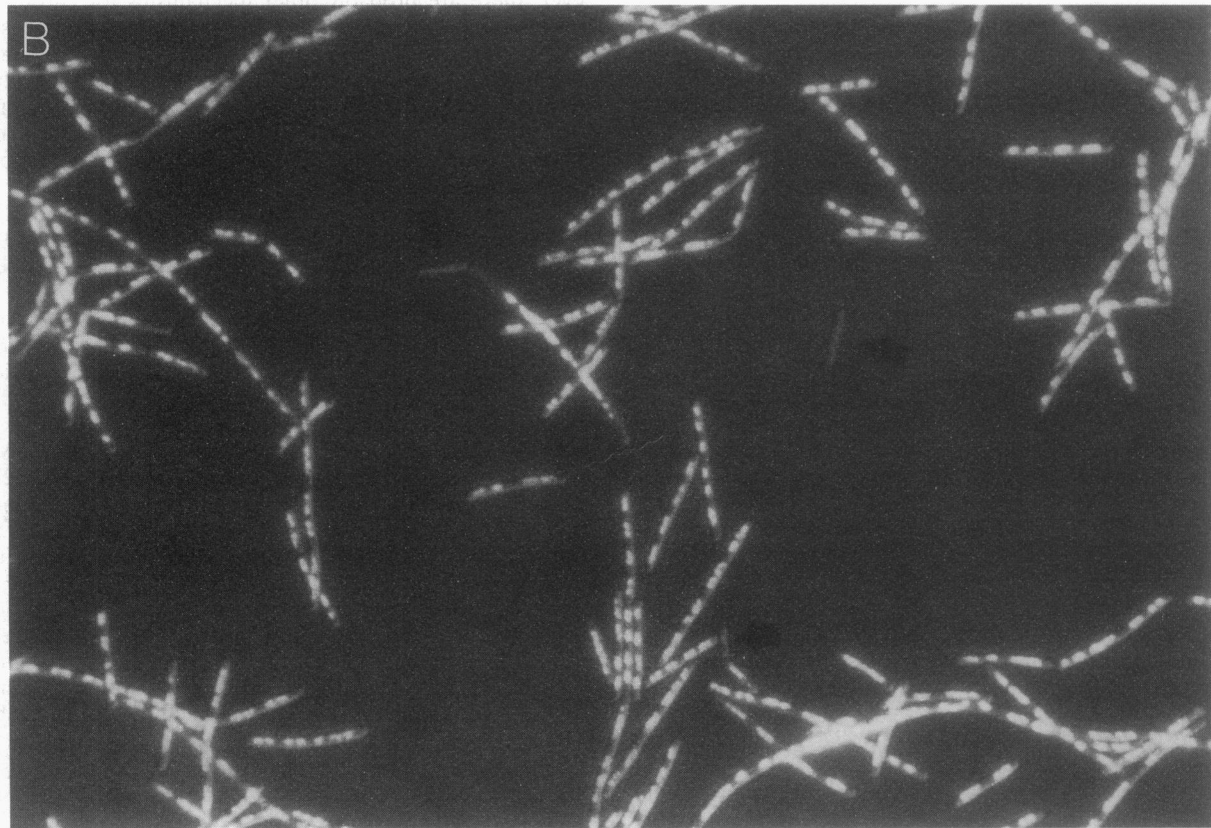


FIG. 3. *spo0J* null mutants produce anucleate cells. Cells were grown in 2× SG medium, and samples were taken for staining with DAPI while cultures were in exponential growth (see Materials and Methods). (A) JH642 (wild type); (B) AG1468 (Δ*spo0J*::*spc*). Arrows indicate positions of some of the anucleate cells.

TABLE 5. A null mutation in *spo0J* results in the production of anucleate cells[a]

Strain	Genotype	Total no. of cells counted	No. of anucleate cells	% Anucleate cells
JH642	Wild type	23,250	4	0.017
AG1468	Δ*spo0J*::*spc*	22,945	322	1.40
AG1505	Δ(*soj-spo0J*)::*spc*	24,687	353	1.43
KI1944	Δ*soj*	23,695	14	0.059

[a] The indicated strains were grown in 2×SG medium at 37°C, and samples were taken during exponential growth for DAPI staining as described in Materials and Methods. Data shown are from four separate experiments, each of which contained all four strains. Between 3,500 and 7,500 cells were counted for each strain in each experiment. The percentages of anucleate cells present in a culture for a given strain were similar from experiment to experiment.

shown) suggests that FtsA might also have a function in chromosome partitioning that is similar to that of Spo0J and that these two proteins might interact to affect partitioning.

***soj* and sporulation.** Our results indicate that the requirement for *spo0J* in spore formation is solely through *soj*. The sporulation defect caused by a null mutation in *spo0J* is suppressed by a null mutation in *soj*, indicating that Soj inhibits spore formation in the absence of *spo0J* or Spo0J activity. The fact that the sporulation defect of *spo0J* mutants is also suppressed by missense mutations in *spo0A* that bypass the phosphorylation pathway required for activation of the Spo0A transcription factor suggests that Soj inhibits the production or accumulation of Spo0A~P. Soj could act to inhibit activity or expression of Spo0A itself or of a component of the phosphorylation pathway.

The genetic results indicate that Spo0J antagonizes or prevents the action of Soj under normal sporulation conditions. Spo0J does not appear to inhibit transcription of *soj*, since expression of this gene is not affected by mutations in *spo0J* (or in *soj* and *spo0J*) (Fig. 4). On the basis of the sequence similarities of Soj and Spo0J to plasmid partitioning proteins, it seems likely that these proteins might interact

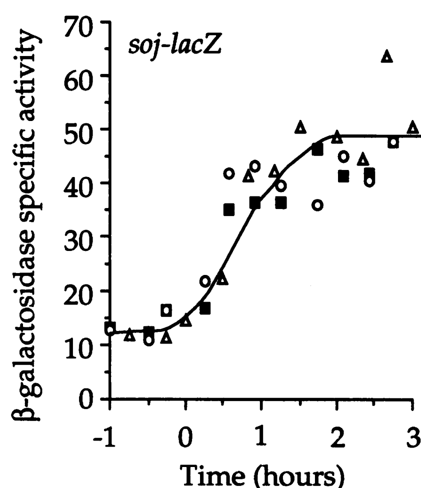


FIG. 4. Expression of *soj-lacZ*. The indicated strains were grown in 2×SG medium, and samples were taken at the indicated times for determination of β-galactosidase specific activity (see Materials and Methods). Time zero indicates the time at which the culture left exponential growth. *soj-lacZ* expression is shown for strains KI1874 (wild type) (△), KI1918 (Δ*spo0J*::*spc*) (○), and KI1917 [Δ(*soj-spo0J*)::*spc*] (■).

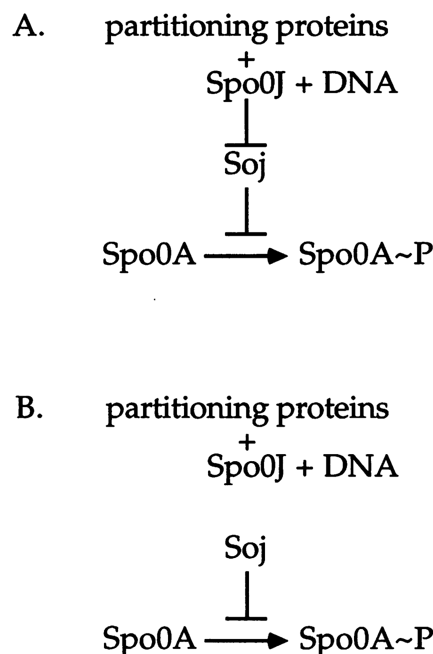


FIG. 5. Model for the role of Spo0J and Soj in signal transduction and the production or accumulation of Spo0A~P. Assembly of the partition complex could either activate Spo0J to antagonize Soj, allowing production of Spo0A~P (A), or sequester Spo0J, allowing Soj to inhibit production of Spo0A~P (B).

directly. The ParA and ParB proteins of phage P1 seem to interact as ParB stimulates the ATPase activity of ParA in vitro (8).

Checkpoint mechanisms controlling development. While it is possible that the dual functions of *spo0J* in chromosome segregation and the initiation of sporulation are coincidental, we suspect that incorporating these two functions into a single protein serves an important biological purpose. Coupling the initiation of development to the activity of part of the partitioning machinery (Spo0J) would provide an effective way of ensuring that cells do not attempt to sporulate unless efficient and proper chromosome segregation can occur. We suspect that Spo0J interacts with a specific DNA site and with other proteins involved in partitioning and cell division. The activity or integrity of this complex might regulate activity of Soj and control production or accumulation of Spo0A~P in response to cell cycle events related to chromosome partitioning (Fig. 5). At least two types of regulation are possible. In the first model (Fig. 5A), a complex between the DNA site and the partition machinery, including Spo0J, activates Spo0J to antagonize Soj. In this way, production of Spo0A~P is possible when the chromosome segregation machinery is intact and assembled, and the initiation of sporulation would depend on the potential to segregate chromosomes. Alternatively, assembly of the partitioning machinery could sequester Spo0J, leaving Soj active to inhibit the initiation of sporulation (Fig. 5B). In this case, the initiation of sporulation would be inhibited during a specific stage of the cell cycle related to chromosome segregation. In either case, we suspect that Spo0J and Soj respond to a cell cycle-related signal to control production of Spo0A~P.

We have identified, by DNA sequence comparisons, a possible Spo0J binding site, containing 7-bp inverted repeats located downstream of the *spo0J* operon. Proteins of the ParB

family generally bind to a specific plasmid partition site which contains 7-bp inverted repeats and are usually located downstream of the structural gene for the ParB protein (13). Spo0J is approximately 40% identical (~64% similar) to the Spo0J homolog (Orf290) from *P. putida* (36), and it seemed possible that these two proteins might recognize similar sequences. We compared the DNA sequences downstream of *spo0J* with those downstream of the *P. putida orf290* and found conserved regions that contain similar 7-bp repeat sequences (20). It is tempting to speculate that these sequences might be binding sites for Spo0J.

The initiation of sporulation is controlled by multiple signals that respond to the potential to provide functional chromosomes for the forespore and mother cell. In addition to the response mediated by Spo0J and Soj, other signals include a response to DNA damage (17) and a response affected by the initiation of DNA replication (19). All of these signals or conditions, as well as nutrient deprivation and high cell density (21), control accumulation of Spo0A~P, which appears to be the convergence point for the multiple developmental signals regulating the initiation of sporulation.

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