JOURNAL OF BACTERIOLOGY, Mar. 1991, p. 1911–1919
0021-9193/91/061911-09$02.00/0

Vol. 173, No. 6

The *Bacillus subtilis spo0J* Gene: Evidence for Involvement in Catabolite Repression of Sporulation

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Received 10 August 1990/Accepted 8 January 1991

Previous observations concerning the ability of the *Bacillus subtilis* bacteriophages SP10 and PMB12 to suppress mutations in *spo0J* and to make wild-type sporulation catabolite resistant suggested that *spo0J* had a role in catabolite repression of sporulation. This suggestion was supported in the present report by the ability of the catabolite-resistant sporulation mutation *crsF4* to suppress a Tn*917* insertion mutation of the *B. subtilis spo0J* locus (*spo0J*::Tn*917*ΩHU261) in medium without glucose. Although *crsF4* and SP10 made wild-type *B. subtilis* sporulation catabolite resistant, neither *crsF4* nor SP10 caused a mutant with *spo0J*::Tn*917*ΩHU261 to sporulate in medium with glucose. Sequencing the *spo0J* locus revealed an open reading frame that was 179 codons in length. Disruption of the open reading frame resulted in a sporulation-negative (Spo$^-$) phenotype that was similar to those of other *spo0J* mutations. Analysis of the deduced amino acid sequence of the *spo0J* locus indicated that the *spo0J* gene product contains an α-helix–turn–α-helix unit similar to the motif found in λ Cro-like DNA-binding proteins.

Bacillus subtilis sporulation is a model system that is used to study procaryotic gene expression and cellular differentiation as responses to environmental stimuli. Sporulation is subject to catabolite repression; i.e., the presence of glucose or other readily metabolized carbon sources inhibits sporulation by wild-type cells (33). Initiation of sporulation is controlled by at least seven genes, *spo0A*, *spo0B*, *spo0E*, *spo0F*, *spo0H*, *spo0J*, and *spo0K* (23). Glucose represses transcription of *spo0A* and *spo0F* (3, 43). However, it is not known how availability of nutrients regulates initiation of sporulation. Several *spo0* genes have been sequenced (5, 9, 14, 15, 30, 41). It is evident from this work that the *spo0H* gene codes for a sigma subunit of RNA polymerase and that some *spo0* genes are responsible for sensing environmental conditions. The *spo0A* and *spo0F* gene products are homologous to the effector molecules of the two-component response regulator systems that have been described for a variety of bacteria (15, 41). The effector molecule of a two-component system is phosphorylated by a cognate histidine kinase in response to an appropriate environmental stimulus, such as the presence of an important nutrient (36). It is possible that the *spoIIJ* gene product (KinA) is the cognate kinase of *spo0A* or *spo0F* or both (1, 29). Although recent data indicate that the *spo0A* gene product is a DNA-binding protein, it is not known how the *spo0A* gene product acts to regulate initiation of sporulation (37).

Although sporulation by *B. subtilis* is normally repressed by glucose, wild-type cells infected by bacteriophage PMB12 or SP10 initiate sporulation in medium containing enough glucose to repress sporulation by uninfected bacteria (34). Essentially, bacteria infected by PMB12 or SP10 have a catabolite-resistant sporulation (Crs) phenotype. The Crs phenotype of PMB12- and SP10-infected bacteria is similar to the phenotype of the *crs* mutations that have been reported to occur in several different genes (38, 39). In addition to conferring a Crs phenotype upon host bacteria, PMB12 and SP10 are also able to suppress the Spo$^-$ and oligosporogenic phenotypes of various *spo0J* mutations (6, 20, 34). As a result, both PMB12-infected and SP10-infected *spo0J* mutants sporulate at a significantly higher frequency than uninfected *spo0J* mutants. The observations that PMB12- and SP10-infected bacteria display catabolite-resistant sporulation and that these bacteriophages suppress *spo0J* mutations suggest that *spo0J* has a role in catabolite repression of sporulation.

The *spo0J* locus was originally thought to be represented by two mutations, *spo0J87* and *spo0J93* (17). However, *spoIIA* and *spoIID* are expressed in a strain with *spo0J87*, whereas *spo0J93* blocks expression of these genes (7, 13). The wild-type alleles of both mutations have been cloned into bacteriophage ϕ105 vectors (10, 12). The cloned wild-type allele of *spo0J87* does not complement *spo0J93*, and the wild-type allele of *spo0J93* does not complement *spo0J87*. Hence, *spo0J87* and *spo0J93* are in different genes. The *spo0J93* mutation marks the locus referred to as *spo0J* in this report. Unfortunately, little information concerning *spo0J* is available in the literature, because many studies concerning the activity of *spo0* genes have utilized *spo0J87* as a representative of the *spo0J* locus rather than *spo0J93*. Nevertheless, it is clear that *spo0J* behaves differently than the other *spo0* genes, as evidenced by the effects of *spo0J93* on expression of *spoIIA* and *spoVG*. Expression of *spoIIA* is blocked by mutations in any of the *spo0* genes, including *spo0J93* (13). In contrast, mutations in *spo0A*, *spo0B*, *spo0E*, *spo0F*, *spo0H*, and *spo0K* interfere with expression of *spoVG*, but expression of *spoVG* is not significantly affected by *spo0J93* (45).

The data presented in this report provide additional evidence for the involvement of *spo0J* in catabolite repression of sporulation. This contention was supported by the observation that two independently isolated *spo0J* mutations were suppressed by a *crs* mutation. Determination of the nucleotide sequence of *spo0J* indicated that the *spo0J* gene product may be a DNA-binding protein.

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TABLE 1. *B. subtilis* strains

Strain	Description[a]	Source or reference
Derivatives of strain 168		
168	Wild type	
PY79	Wild type	31
BR151	*lys-3 metB10 trpC2*	44
CM-1	*lys-3 metB10 trpC2 spoCM-1*	6
93.2	*trpC2 spo0J93*	12
KS261	*spo0J*::Tn*917*ΩHU261	31
1A579	*crsA1*	*Bacillus* Genetic Stock Center
1A582	*crsB40*	*Bacillus* Genetic Stock Center
1A583	*crsC1*	*Bacillus* Genetic Stock Center
1A584	*crsC2*	*Bacillus* Genetic Stock Center
1A585	*crsD1*	*Bacillus* Genetic Stock Center
1A586	*crsE1*	*Bacillus* Genetic Stock Center
1A587	*crsF4*	*Bacillus* Genetic Stock Center
MGB3006	Erm^r transformant of 1A579 that contains *spo0J*::Tn*917*ΩHU261	This study
MGB3007	Erm^r transformant of 1A587 that contains *spo0J*::Tn*917*ΩHU261	This study
MGB3008	Chl^r transformant of BR151 that has pSMGU32 inserted into *spo0J*	This study
MGB3009	Chl^r transformant of 168 that has pSMGU32 inserted into *spo0J*	This study
MGB3010	Chl^r transformant of 1A587 that has pSMGU32 inserted into *spo0J*	This study
MGB3013	Erm^r transformant of BR151 that contains *spo0J*::Tn*917*ΩHU261	This study
Derivatives of ATCC 7003		
3-13	His$^-$	24
MGB3004	His$^-$, *spo0J*::Tn*917*ΩHU261	34

[a] His$^-$, Auxotrophic requirement for histidine; Chl^r, resistance to chloramphenicol; Erm^r, erythromycin-lincomycin resistance encoded by Tn*917*.

MATERIALS AND METHODS

Bacterial strains, culture conditions, and sporulation assays. The bacterial strains used in this study are described in Table 1.

All liquid culture experiments were performed in phosphate-buffered 2× SG, which was the same as 2× SG (22) except that $FeCl_2$ was omitted and the medium contained 14 g of K_2HPO_4 and 6 g of KH_2PO_4 per liter (pH 7.0). The effect of glucose on sporulation was tested by supplementing phosphate-buffered 2× SG with glucose to a final concentration of 2% from a 50% stock solution at the time of inoculation. All liquid cultures were incubated at 37°C with gyratory shaking at approximately 100 rpm. Sporulation was obtained by inoculating 10 ml of culture medium with a single colony from an overnight tryptose blood agar plate (Difco Laboratories) and allowing the bacteria to enter the stationary phase of growth and exhaust the medium. After the indicated time of incubation (16 to 24 h after inoculation), the frequency of sporulation was determined as the percentage of the total viable count that was heat resistant (70°C for 20 min).

The cross-streak assay for PMB12-enhanced sporulation of *spo0J* mutants has been described previously (20, 25, 34) and was performed as follows. PMB12 lysate was applied as a narrow band across the center of a petri plate containing 2× SG solidified with 1.5% agar that was no more than 24 h old. Strains with *spo0J* mutations were streaked across the dried lysate. After incubation at 37°C for the specified time, bacteria from the uninfected and bacteriophage-infected regions of the cross-streaks were viewed in wet mounts under a phase-contrast microscope. The frequency of sporulation was determined by calculating the mean percentage of phase-bright bodies in samples containing at least 100 cells that were counted in five different fields of the same wet mount.

The procedures concerning SP10-mediated sporulation and measurement of α-amylase activity in overnight cultures of phosphate-buffered 2× SG were described previously (34).

All bacteria were made competent for transformation by standard procedures (4). Erythromycin-lincomycin-resistant (Erm^r) transformants were selected on tryptose blood agar plates containing erythromycin (1 μg/ml) and lincomycin (25 μg/ml). Chloramphenicol-resistant (Chl^r) transformants were selected on tryptose blood agar plates containing 5 μg of the antibiotic per ml.

Construction of ϕ105J113 deletion mutants. All methods for propagation and manipulation of ϕ105J113 and its derivatives have been described previously (10, 12, 18). ϕ105J106, the vector from which ϕ105J113 was derived, has no *Bgl*II restriction sites (10). Therefore, the 0.7-kb *Bgl*II fragment was deleted from ϕ105J113 (Fig. 1) to produce ϕ105J113ΔV by cutting ϕ105J113 with *Bgl*II and ligating the DNA to rejoin the right and left arms of ϕ105J113 without the 0.7-kb *Bgl*II fragment. Restriction endonucleases and T4 DNA ligase were used in accordance with the directions of the manufacturer (IBI, Inc.). Strain 93.2 protoplasts were transfected with the ligated DNA, and lysogenic bacteria from the

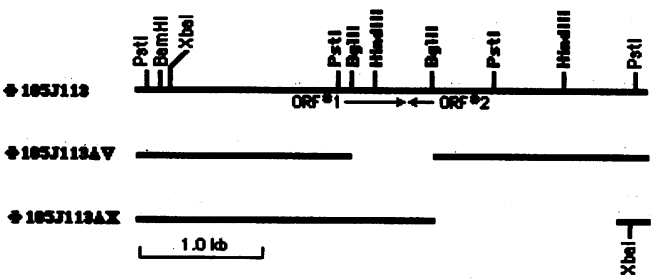

FIG. 1. Restriction map of ϕ105J113 and deletion mutants. Bold letters indicate endonuclease restriction sites within the cloned DNA. Plain letters indicate the endonuclease restriction sites of the vector's polylinker region.

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5'.....CACTTTGATGACGAGGCATTAGTGAACTAAAAGAATCTGAGTCTGCAGCATGGCATTCTTCAGCCGCTTATCGTC

AGAAAATCTTTAAAAGGCTATGATATTGTTGCGGGTGAACGGCGTTTTCGAGCGGCAAAGCTGGCAGGTTTAGATACAGTTCC

GGCCATTGTCCGTGAATTATCAGAGGCGTTA ATG AGG GAA ATT GCT TTA TTA GAA AAC CTT CAG CGT GAA
                                met arg glu ile ala leu leu glu asn leu gln arg glu

GAT TTA TCT CCG CTT GAA GAG GCT CAG GCA TAT GAC TCC CTT TTG AAA CAC TTA GAT CTC ACA
asp leu ser pro leu glu glu ala gln ala tyr asp ser leu leu lys his leu asp leu thr

CAA GAG CAG CTT GCC AAA CGT CTT GGG AAA AGC AGA CCG CAT ATT GCG AAT CAT TTA AGA CTG
gln glu gln leu ala lys arg leu gly lys ser arg pro his ile ala asn his leu arg leu

CTG ACA CTG CCA GAA AAT ATT CAA CAG CTT ATT GCC GAA GGC ACG CTT TCT ATG GGA CAT GGA
leu thr leu pro glu asn ile gln gln leu ile ala glu gly thr leu ser met gly his gly

CGC ACG CTT CTT GGC TTA AAA AAC AAA AAT AAG CTT GAA CCG CTG GTA CAA AAA GTG ATT GCG
arg thr leu leu gly leu lys asn lys asn lys leu glu pro leu val gln lys met asn ala

GAG CAG CTC AAT GTT CGC CAA CTT GAG CAG CTG ATT CAG CAG TTG AAT CAG AAT GTT CCA CGT
glu gln leu asn val arg gln leu glu gln leu ile gln gln leu asn gln asn val pro arg

GAA ACA AAG AAA AAA GAA CCT GTG AAA GAT GCG GTT CTA AAA GAA CGG GAA TCC TAT CTC CAA
glu thr lys lys lys glu pro val lys asp ala val leu lys glu arg glu ser tyr leu gln

AAT TAT TTT GGA ACA ACA GTT AAT ATT AAA AGA CAG AAG AAA AAA GGC AAA ATC GAA ATT GAA
asn tyr phe gly thr thr val asn ile lys arg gln lys lys lys gly lys ile glu asn glu

TTT TTC TCT AAT GAA GAC CTT GAC CGG ATT TTA GAG CTT TTG TCT GAA CGA GAA TCA TAA ATG
phe phe ser asn glu asp leu asp arg ile leu glu leu leu ser glu arg glu ser

AAAAAACCATCTTTCAAACGAAGATGGTTTTTTATTTTATATGCGCCCAGTGAGCCTTTGCAACGGTTAAGGCGTTCTTGCCTC
CACTCTGTCCGATCTGTTAAACTCAAACCTTCCGCTAGAACATTAGCCATATTCATGACCAACTTGAGTCTAGTATTTTGCAA
TACAAAATATTCCATAAAACCGCTCACATTCACGATTCCGTTAATATGAAGATC.....3'
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FIG. 2. Sequence of the *spo0J* locus. A single underline marks the position of *Pst*I, *Bgl*II, and *Hin*dIII restriction sites that are located on the left end of the cloned insert of ϕ105J113. A double underline designates the conserved portion of a putative Shine-Dalgarno sequence. The stop codon that terminates the *spo0J* ORF is in bold lettering. The stem-loop structure that may terminate transcription of the *spo0J* ORF is designated by a dotted underline. The amino acid residues constituting an α-helix–turn–α-helix unit are italicized.

centers of the resulting plaques were patched onto 2× SG solidified with 1.5% agar and examined for sporulation. A Spo$^-$ lysogen was selected for further characterization. The bacteriophage produced by that lysogen was designated ϕ105J113ΔV.

ϕ105J113ΔX, which lacked the DNA to the right of the 0.7-kb *Bgl*II restriction fragment of ϕ105J113, was constructed by replacing the right arm of ϕ105J113 with the right arm of ϕ105J106 as follows. ϕ105J113 was cut with *Bgl*II. The left arm of ϕ105J113 (which contained the leftmost portion of the cloned DNA) and the 0.7-kb *Bgl*II restriction fragment of ϕ105J113 (which contained the center portion of the cloned DNA) were separated from the right arm of ϕ105J113 (which contained the right portion of the cloned DNA) by extraction from a 0.8% low-melting-point agarose gel (2). ϕ105J106 DNA was cut with *Bam*HI, and the right arm of the vector was extracted from a 0.8% low-melting-point agarose gel. The left arm of ϕ105J113, the 0.7-kb *Bgl*II restriction fragment, and the right arm of ϕ105J106 were mixed and ligated. Strain 93.2 protoplasts were transfected with the ligated DNA, and the resulting bacteriophages were harvested from the overlays of the transfection plates. ϕ105J113ΔX was identified by screening the lysates for a bacteriophage that complemented *spo0J*::Tn*917*ΩHU261 in strain KS261.

Sequencing of DNA. The 0.7-kb *Bgl*II fragment of ϕ105J113 was subcloned in both orientations into the *Bam*HI site of M13mp18 (2). The 1.6-kb *Xba*I-*Hin*dIII restriction fragment of ϕ105J113ΔX was subcloned into pBluescript KS(+) (Stratagene, La Jolla, Calif.). Dideoxy-chain termination sequencing reactions (2, 32) were initiated with universal primer (2) and custom synthesized oligonucleotides. The sequencing reactions were performed with Sequenase according to the manufacturer's instructions (United States Biochemicals, Inc.), using the single-stranded method for the M13mp18 subclones and the double-stranded method for the pBluescript KS(+) subclone.

Insertion of pSGMU32 into ORF1. Plasmid pSGMU32 does not replicate in *B. subtilis* (11). This plasmid contains a chloramphenicol resistance (*cat*) gene that is expressed in *B. subtilis* and a promoterless *lacZ* gene. The *cat* and *lacZ* genes make up a *lac-cat* cartridge that is flanked by restriction sites for *Bam*HI and a variety of other restriction endonucleases. Several attempts to fuse the promoterless *lacZ* to open reading frame 1 (ORF1) were unsuccessful. However, a mutant in which pSGMU32 had integrated into ORF1 was identified as follows. Since the *Bgl*II restriction site located near the beginning of ORF1 was the only *Bgl*II restriction site of ϕ105J113ΔX (Fig. 1 and 2), ϕ105J113ΔX DNA was cut with *Bgl*II and ligated to pSGMU32 DNA that had been cut with *Bam*HI. The ligated DNA was transformed into BR151(ϕ105J113ΔX) with selection for chloramphenicol resistance. BR151(ϕ105J113ΔX) was used in this procedure to minimize loss of transformants due to zygotic induction of ϕ105J113ΔX genomes contained in the transforming DNA. Since pSGMU32 could not replicate in *B. subtilis*, Chl^r transformants were expected to result from integration of the *cat* gene into the *B. subtilis* chromosome because of homologous recombination between the chromosome and the ϕ105J113ΔX sequences flanking the *cat* gene. MGB3008 was a Spo$^-$ Chl^r transformant that resulted from transferring the *cat* gene by transformation from a Spo$^+$ Chl^r

TABLE 2. Suppression of *spo0J*::Tn*917*ΩHU261 by a catabolite-resistant sporulation mutation

Strain	Glucose	% Sporulation
168	−	100.0
1A579	−	24.7
MGB3006	−	0.1
1A587	−	100.0
MGB3007	−	72.0
168	+	0.001
1A587	+	100.0
MGB3007	+	0.02

TABLE 3. Sporulation by SP10-infected MGB3004 in medium with glucose[a]

Strain	Glucose	SP10	% Sporulation
3-13	−	−	100.0
3-13	+	−	0.3
3-13	+	+	100.0
MGB3004	−	−	22.6
MGB3004	−	+	81.8
MGB3004	+	−	0.0004
MGB3004	+	+	0.007

[a] SP10-infected cultures were inoculated with SP10-infected carrier colonies.

BR151(ϕ105J113ΔX) transformant to BR151. The 0.7-kb *Bgl*II restriction fragment of ϕ105J113 was end labeled with ^{32}P and hybridized to MGB3008 DNA by the Southern technique (2, 35) to verify that ORF1 had been disrupted.

Nucleotide sequence accession number. The GenBank accession number of the sequence in Fig. 2 is M59938.

RESULTS

Suppression of *spo0J*::Tn*917*ΩHU261 by *crsF4* in *B. subtilis* 168. The observation that bacteriophages PMB12 and SP10 suppressed *spo0J* mutations and made wild-type *B. subtilis* catabolite resistant for sporulation suggested the possibility that *spo0J* was involved in catabolite repression of sporulation (34). Therefore, several *crs* mutations were tested for the ability to suppress *spo0J*::Tn*917*ΩHU261. The strain 168 *crs* mutants 1A579, 1A582, 1A583, 1A584, 1A585, 1A586, and 1A587 were tested for glucose-resistant sporulation under the conditions in which PMB12 and SP10 induce catabolite-resistant sporulation; i.e., the *crs* mutants were grown to stationary phase in phosphate-buffered 2× SG containing 2% glucose. After 24 h, only 1A579 and 1A587 produced significant levels of heat-resistant spores under these conditions (data not shown). The transposon insertion mutation *spo0J*::Tn*917*ΩHU261 was transferred from KS261 to 1A579, 1A584, 1A585, 1A586, and 1A587 by transformation. None of the 1A579, 1A584, 1A585, or 1A586 transformants examined sporulated at a level detectable by phase-contrast microscopy, whereas all of the 1A587 transformants examined sporulated at a high level. The sporulation frequencies of a typical Erm^r 1A579 transformant (MGB3006) and a typical Erm^r 1A587 transformant (MGB3007) were determined in phosphate-buffered 2× SG (Table 2). After 24 h, the sporulation frequency of MGB3006 was <0.5% of the sporulation frequency of 1A579. In contrast, MGB3007 sporulated nearly as well as wild-type 168 and 1A587. Hence, *spo0J*::Tn*917*ΩHU261 was suppressed by *crsF4* but not by *crsA1*.

Although sporulation by wild-type 168 was severely inhibited in phosphate-buffered 2× SG with 2% glucose, sporulation by 1A587 was not significantly affected by addition of glucose to the medium (Table 2). However, in spite of the ability of the *crsF4* mutation to make sporulation glucose resistant and to suppress *spo0J*::Tn*917*ΩHU261, MGB3007 sporulation was severely inhibited by glucose. Since the sporulation phenotype of MGB3007 was similar to wild type, the presence of *spo0J*::Tn*917*ΩHU261 in MGB3007 was verified as follows. Erm^r was transferred to strain BR151 by transformation with DNA from MGB3007. Twenty Erm^r Spo$^-$ tranformants with the auxotrophic requirements of BR151 were tested for complementation by ϕ105J113ΔX. ϕ105J113ΔX was a deletion mutant of ϕ105J113 that complemented *spo0J93*, *spoCM-1*, and *spo0J*::Tn*917*ΩHU261 (Fig. 1; see Table 5). The mutation causing the Spo$^-$ phenotype of all 20 Erm^r transformants was complemented by ϕ105J113ΔX. This observation indicated that MGB3007 contained *spo0J*::Tn*917*ΩHU261. Therefore, the *spo0J* gene product was not necessary for sporulation in the presence of the *crsF4* mutation but was necessary for *crsF4* to make sporulation catabolite resistant.

Necessity of wild-type *spo0J* for bacteriophage-induced catabolite-resistant sporulation in a derivative of *B. subtilis* ATCC 7003. Suppression of *spo0J*::Tn*917*ΩHU261 by *crsF4* in MGB3007 in phosphate-buffered 2× SG was similar to the previously described effect of PMB12 and SP10 on the oligosporogenic mutant MGB3004 in the same culture medium (34). The observation that MGB3007 sporulation was not catabolite resistant (Table 2) suggested the possibility that bacteriophage-induced MGB3004 sporulation might also be sensitive to catabolite repression. Since SP10-infected cells generally sporulate at higher frequencies than PMB12-infected cells (34), the effect of glucose on SP10-induced MGB3004 sporulation was examined (Table 3). After 19 h of incubation, sporulation by strain 3-13 was repressed in phosphate-buffered 2× SG with 2% glucose, and SP10-infected 3-13 cells displayed catabolite-resistant sporulation in this medium. MGB3004 had an oligosporogenic phenotype in phosphate-buffered 2× SG, and SP10-infected MGB3004 cells sporulated at a frequency that was nearly fourfold higher than uninfected cells. These observations were essentially the same as reported previously (34). The data from this experiment indicated that MGB3004 sporulation was considerably more sensitive to catabolite repression than 3-13 sporulation. The sporulation frequency of MGB3004 cultured in phosphate-buffered 2× SG was more than 4 orders of magnitude higher than the sporulation frequency of MGB3004 cultured in phosphate-buffered 2× SG with 2% glucose, whereas 3-13 sporulation in phosphate-buffered 2× SG was only about 300-fold higher than 3-13 sporulation in phosphate-buffered 2× SG with 2% glucose. SP10-infected 3-13 cells sporulated in phosphate-buffered 2× SG with 2% glucose at the same level as uninfected 3-13 in phosphate-buffered 2× SG. However, SP10 had little effect on MGB3004 sporulation in phosphate-buffered 2× SG with 2% glucose. All of the colonies on the viable count assay plates for the SP10-infected MGB3004 cultures in this experiment had the smooth morphology of SP10-infected carrier colonies (34), indicating that SP10-infected MGB3004 had not sporulated in phosphate-buffered 2× SG with 2% glucose

TABLE 4. Production of α-amylase by MGB3004

Strain	Glucose	α-Amylase activity (U/mg of cells)
3-13	−	14.5
3-13	+	4.0
MGB3004	−	41.1
MGB3004	+	1.1

even though all of the cells in the culture were infected with SP10. These data indicated that infection with SP10 was similar to the activity of *crsF4*; i.e., SP10 and *crsF4* made sporulation by wild-type bacteria catabolite resistant and effectively suppressed *spo0J*::Tn*917*ΩHU261 in phosphate-buffered 2× SG, but suppression of *spo0J*::Tn*917*ΩHU261 was sensitive to catabolite repression.

Suppression of *spo0J*::Tn*917*ΩHU261 by *crsF4* and SP10 was consistent with the conclusion that *spo0J* was involved in catabolite-mediated regulation of sporulation and suggested that sporulation was inappropriately catabolite repressed in *spo0J* mutants. If *spo0J*::Tn*917*ΩHU261 caused a generalized repression of catabolite-regulated systems, then production of a catabolite-repressible enzyme by MGB3004 would be diminished in comparison to a wild-type strain. We demonstrated previously that, as in other strains of *B. subtilis*, production of α-amylase by strain 3-13 is repressed by glucose (34). The α-amylase activity in a typical overnight culture of MGB3004 lacking glucose was severalfold higher than for a culture of strain 3-13, and production of α-amylase by MGB3004 was repressed in medium containing glucose (Table 4). The cause of increased α-amylase activity in cultures of MGB3004 was not investigated. Nevertheless, these data demonstrated that *spo0J*::Tn*917*ΩHU261 did not cause generalized repression of catabolite-regulated systems in MGB3004.

Localization of *spo0J* on ϕ105J113. Since *spo0J* apparently had an important role in catabolite-mediated regulation of sporulation, characterization of *spo0J* and the deduced *spo0J* gene product was initiated. Cloning of *spo0J* and isolation of the nondefective bacteriophage ϕ105J113 from a ϕ105 library was described previously (10). ϕ105J113 contains cloned *B. subtilis* 168 DNA that complements *spo0J93*. Lysogenic derivatives of 93.2, CM-1, and KS261 were constructed by infecting these sporulation mutants with ϕ105J113. The data in Table 5 confirmed that ϕ105J113 complemented *spo0J93* and *spo0J*::Tn*917*ΩHU261 as reported previously (10, 34). In addition, ϕ105J113 complemented *spoCM-1*. ϕ105J113 did not complement *spo0J87* (data not shown). This observation was consistent with the previous report that the cloned wild-type allele of *spo0J87* does not complement *spo0J93* (12). The restriction map of the cloned DNA in ϕ105J113 is illustrated in Fig. 1. The cloned DNA was approximately 3.9 kb in size. The *Bgl*II restriction sites were unique to the cloned DNA. The following endonucleases did not cut within the cloned DNA: *Apa*I, *Bal*I, *Cla*I, *Eco*RI, *Eco*RV, *Hpa*I, *Kpn*I, *Sal*I, *Sma*I, *Sst*I, *Xba*I, and *Xho*I. The ϕ105J113 restriction map did not overlap the restriction map described by Moriya et al. (26) for the 10-kb region surrounding the chromosomal origin of replication.

The *spo0J* gene was localized on ϕ105J113 by isolating two deletion mutants. The first deletion mutant, ϕ105J113ΔV, lacked the 0.7-kb *Bgl*II fragment of ϕ105J113 (Fig. 1). Strains 93.2(ϕ105J113ΔV), CM-1(ϕ105J113ΔV), and KS261(ϕ105J113ΔV) were Spo$^-$ (Table 5). This observation indicated that the 0.7-kb *Bgl*II restriction fragment of ϕ105J113 was essential for complementation of *spoCM-1*, *spo0J93*, and *spo0J*::Tn*917*ΩHU261. The second deletion mutant, ϕ105J113ΔX, lacked the DNA between the right *Bgl*II site and the fourth *Pst*I site of ϕ105J113 in Fig. 1. Since ϕ105J113ΔX was constructed with the right arm of ϕ105J106 which contained a portion of the vector's polylinker region, ϕ105J113ΔX had two *Xba*I sites flanking the cloned DNA. The sporulation frequencies of 93.2(ϕ105J113ΔX), CM-1(ϕ105J113ΔX), and KS261(ϕ105J113ΔX) were virtually the same as the lysogens containing ϕ105J113 (Table 5). Hence, the cloned DNA located to the right of the 0.7-kb *Bgl*II fragment of ϕ105J113 was not necessary for complementation of *spo0J93*, *spoCM-1*, and *spo0J*::Tn*917*ΩHU261.

TABLE 5. Complementation of *spo0J* mutations

Strain	% Sporulation
168	82.1
PY79	76.3
93.2	0.3
93.2(ϕ105J113)	76.0
93.2(ϕ105J113ΔX)	88.8
93.2(ϕ105J113ΔV)	0.01
CM-1	0.10
CM-1(ϕ105J113)	54.2
CM-1(ϕ105J113ΔX)	68.4
CM-1(ϕ105J113ΔV)	0.01
KS261	1.1
KS261(ϕ105J113)	79.1
KS261(ϕ105J113ΔX)	80.6
KS261(ϕ105J113ΔV)	0.01

Sequence of *spo0J*. Since the 0.7-kb *Bgl*II fragment was essential for complementation of *spo0J* mutations, this restriction fragment was sequenced first (Fig. 2). The *Bgl*II fragment contained the 3′ portions of two long ORFs. Relative to the ϕ105J113 restriction map, ORF1 would be transcribed from left to right across the left *Bgl*II site, and ORF2 would be transcribed from right to left across the right *Bgl*II restriction site (Fig. 1). ORF1 was separated by 7 nucleotides from a putative transcription termination sequence that had 12 nucleotides in the stem and 7 unpaired bases in the loop. ORF2 terminated within this stem-loop structure. A similar arrangement has been reported for *spoIIJ* (*kinA*) and ORF Y (29). Since ϕ105J113ΔX had a deletion that would remove the 5′ portion of ORF2 and ϕ105J113ΔX complemented *spo0J93*, *spoCM-1*, and *spo0J*::Tn*917*ΩHU261 (Table 5), it was likely that ORF1 was the 3′ portion of the *spo0J* sequence. ORF1 was extended leftward beyond the left *Bgl*II restriction site by sequencing part of the 1.6-kb *Xba*I-*Hin*dIII restriction fragment of ϕ105J113ΔX. A possible ATG start codon was identified 89 nucleotides upstream from the left *Bgl*II restriction site. The putative start codon was separated by 3 nucleotides from a likely Shine-Dalgarno sequence. Sequences that were similar to the −10 or −35 portions of consensus sequences for various sigma factors were found upstream of ORF1, but an obvious promoter sequence for ORF1 was not identifiable.

Disruption of ORF1. MGB3008 was originally isolated in an attempt to fuse ORF1 to a promoterless *lacZ* gene. MGB3008 was resistant to chloramphenicol and produced

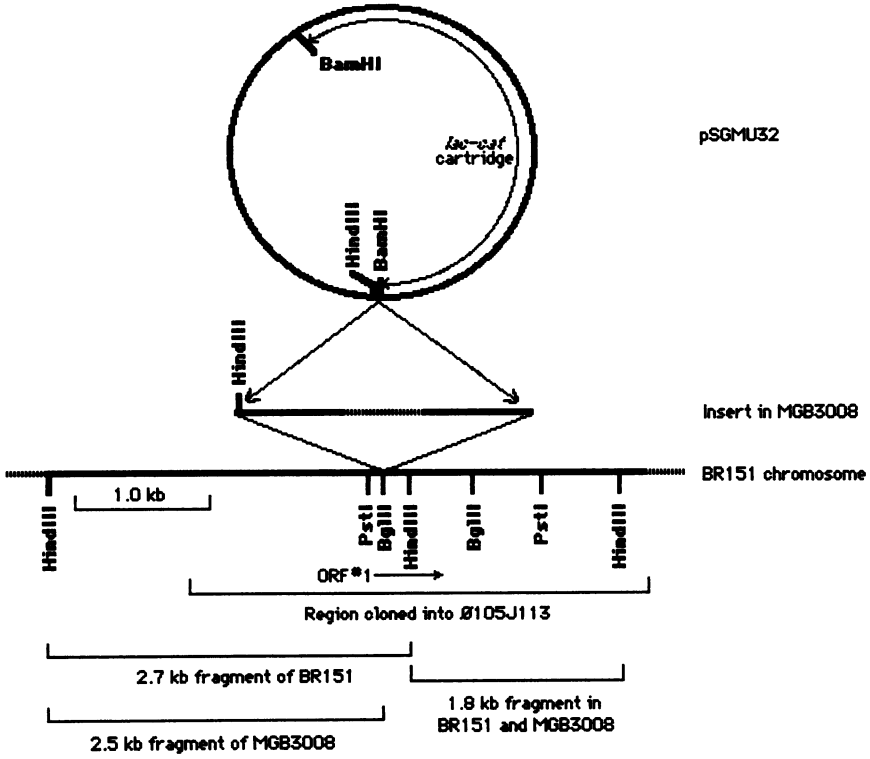

FIG. 3. Insertion of pSGMU32 into *spo0J*.

low levels of β-galactosidase (data not shown). Hence, the phenotype of MGB3008 indicated that the *lac-cat* cartridge of pSGMU32 was present in this strain. However, the results of Southern hybridization analysis were consistent with the vector portion of pSGMU32 being located between the promoter region of ORF1 and *lacZ* in MGB3008. MGB3008 DNA and BR151 DNA had two *Hin*dIII fragments that hybridized with the 0.7-kb *Bgl*II restriction fragment of ϕ105J113. As expected, a small 1.8-kb restriction fragment was detected in both strains. This restriction fragment corresponded to the *Hin*dIII restriction fragment that was contained entirely within the cloned insert of ϕ105J113. BR151 had a large *Hin*dIII restriction fragment that was 2.7 kb and corresponded to the chromosomal fragment that would include the left portion of the cloned insert of ϕ105J113. MGB3008 had a large *Hin*dIII restriction fragment that was approximately 0.2 kb smaller than the large *Hin*dIII fragment of BR151. Since the left *Bgl*II restriction site of ϕ105J113 was 160 bp from the left *Hin*dIII restriction site (Fig. 1 and 2) and pSGMU32 had a *Hin*dIII restriction site immediately adjacent to one of the *Bam*HI restriction sites, it was most likely that pSGMU32 was integrated in the MGB3008 chromosome at the *Bgl*II restriction site located within ORF1. These results are summarized in Fig. 3.

Since *lacZ* was not directly fused to ORF1 in MGB3008, it was not possible to use this mutant to study expression of ORF1. However, it was possible to demonstrate that disruption of ORF1 in MGB3008 resulted in a Spo$^-$ phenotype. The sporulation frequency of MGB3008 was 0.04%. MGB3008(ϕ105J113ΔX) sporulated as well as the Spo$^+$ parental strain BR151. These two strains sporulated at frequencies of 61.4 and 50.0%, respectively. Therefore, ϕ105J113ΔX complemented the insertion mutation that resulted from disrupting ORF1.

Suppression by the spore-converting bacteriophage PMB12 distinguishes mutations in *spo0J* from mutations in most other stage 0 sporulation genes (6, 20). In addition, *spo0J*::Tn*917*ΩHU261 was suppressed by *crsF4* (Table 2). Therefore, the effects of PMB12 and *crsF4* on the Spo$^-$ phenotype of MGB3008 were examined. Suppression by PMB12 of the Spo$^-$ mutation caused by disrupting ORF1 was compared with PMB12-mediated suppression of two *spo0J* mutations in a cross-streak assay (Table 6). MGB3008 and two *spo0J* mutants derived from BR151 (CM-1 and MGB3013) failed to sporulate at a detectable frequency after 20 h of incubation at 37°C on 2× SG agar. However, the sporulation frequencies of PMB12-infected MGB3008, PMB12-infected CM-1, and PMB12-infected MGB3013 were similar to each other and at least 10-fold higher than the sporulation frequencies of uninfected cells. Therefore, these data indicated that the Spo$^-$ mutation caused by disruption of ORF1 was suppressed by PMB12 as is characteristic of other *spo0J* mutations. The ability of *crsF4* to suppress the Spo$^-$ mutation caused by disruption of ORF1 was tested by transferring the insertion mutation by transformation from

TABLE 6. Cross-streak assay for PMB12-enhanced sporulation by MGB3008

Strain	Genotype	% Sporulation	
		Uninfected	Infected
BR151	Wild type	22.3	35.0
CM-1	*spoCM-1*	<0.2	4.3
MGB3013	*spo0J*::Tn*917*ΩHU261	<0.2	2.3
MGB3008	ORF1 disrupted by pSMGU32	<0.2	3.0

TABLE 7. Suppression of an insertion mutation in ORF1 by *crsF4*

Strain	Glucose	% Sporulation
168	−	100.0
168	+	0.3
MGB3009	−	0.3
MGB3009	+	0.02
1A587	−	93.8
1A587	+	57.4
MGB3010	−	95.8
MGB3010	+	0.3

MGB3008 to wild-type strain 168 and the catabolite-resistant sporulation mutant 1A587. The sporulation frequencies of a typical Chl^r 168 transformant (MGB3009) and a typical Chl^r 1A587 transformant (MGB3010) were determined in phosphate-buffered 2× SG alone and with 2% glucose (Table 7). After 24 h, MGB3009 had sporulated at a frequency of 0.3% in medium lacking glucose, whereas MGB3010 had sporulated at essentially the same frequency as wild-type 168 and 1A587 in the same medium. Hence, *crsF4* improved the ability of a strain with an insertion mutation of ORF1 to sporulate by >300-fold in medium lacking glucose. Although the sporulation frequency of 1A587 was nearly 200-fold higher than that of wild-type strain 168 in medium that initially contained 2% glucose, sporulation by MGB3010 was repressed to the same extent as sporulation by strain 168 in medium containing glucose. This observation was similar to the observation that was already described concerning suppression of *spo0J*::Tn*917*ΩHU261 by *crsF4* (Table 2).

The data above and in Tables 6 and 7 supported the conclusion that ORF1 encoded the *spo0J* gene product.

Potential DNA-binding domain in Spo0J. Beginning with the putative ATG start codon, translation of ORF1 yielded a predicted polypeptide (Spo0J) that was 179 amino acids in length with a molecular weight of approximately 21,000. Spo0J had an estimated pI of 9.47, indicating that the predicted polypeptide had a net positive charge. A FASTA search (28) of protein sequence data bases was conducted to determine whether Spo0J shared homology with any previously characterized proteins. None of the proteins selected in the search contained long amino acid sequences that were highly homologous to Spo0J. However, 17 of the 20 amino acids in the region of amino acids 35 to 54 of Spo0J were identical to or conservative changes of KorB amino acid residues 171 to 190 (Fig. 4). KorB represses transcription of the *trfA* operon of the broad-host-range plasmid RK2 (21, 40). Analysis of KorB by the method of Dodd and Egan indicated that amino acid residues 171 to 190 have a greater likelihood of being a DNA-binding domain than other regions of KorB (8, 40).

Dodd and Egan used a master set of λ Cro-like DNA-binding proteins to determine the likelihood of each amino acid appearing at each position in the α-helix–turn–α-helix motif frequently found in DNA-binding proteins (8, 27). Comparison of each amino acid in a potential DNA-binding site to the matrix developed by Dodd and Egan yields a score that reflects the probability of a protein being a λ Cro-like DNA-binding protein. Dodd and Egan judged all of the proteins examined that had scores of >1,699 to be λ Cro-like. Application of the Dodd and Egan method to Spo0J amino acid residues 35 to 54 yielded a score of 2,101 and indicated that highly favored amino acids occupied 16 of the 20 positions in the putative DNA-binding domain of Spo0J (Fig. 4). Furthermore, highly conserved alanine, glycine, and isoleucine residues occupied positions 5, 9, and 15, respectively, of the Spo0J α-helix–turn–α-helix unit as has been observed for many DNA-binding proteins (27). Hence, this analysis indicated that the sequence of amino acids 35 to 54 of Spo0J strongly resembled the α-helix–turn–α-helix motif found in λ Cro-like DNA-binding proteins.

DISCUSSION

Involvement of *spo0J* in catabolite repression of sporulation was first suggested by the ability of bacteriophages PMB12 and SP10 to make wild-type sporulation catabolite resistant and to suppress mutations in *spo0J* (34). It is possible that mutants with defects in *spo0J* are Spo$^-$ due to inability to escape catabolite repression of sporulation; i.e., sporulation is repressed in the mutants even when catabolites such as glucose are not present. The idea that *spo0J* is involved in catabolite repression of sporulation was supported by the observation that *spo0J* mutations were suppressed by the catabolite-resistant sporulation mutation *crsF4*.

The nature of *crsF4* is not known. This mutation is located in the same region of the chromosome as *spo0E* and *spoIIJ* (38), suggesting the possibility that *crsF4* is an allele of one of these two sporulation genes. Although *crsF4* allowed *spo0J* mutants to sporulate at wild-type levels in medium without glucose, *crsF4* did not enable these mutants to sporulate in medium with glucose. Kawamura et al. (19) previously described a similar situation in which *crsA47* (an allele of *rpoD*, the structural gene of the sigma factor σ^{43}) and *spo0K141* suppressed each other, i.e., a mutant with *crsA47* and *spo0K141* sporulated nearly as well as wild type, but sporulation of the double mutant was repressed by glucose. Mutations in *spo0J* and *spo0K* are similar in that both are suppressed by PMB12 (6, 20). However, the inability of *crsA1* (an allele of *rpoD* that is identical to *crsA47* [19])

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Spo0J	gln	glu	gln	leu	ala	lys	arg	leu	gly	lys	ser	arg	pro	his	ile	ala	asn	his	leu	arg
	.	.	.	.	:	:		.	:	:	:	.	.		:	.	.	:	.	
KorB	lys	gly	asp	ile	ala	lys	glu	ile	gly	lys	ser	pro	ala	phe	ile	thr	gln	his	val	thr
λ Cro	gln	thr	lys	thr	ala	lys	asp	leu	gly	val	tyr	gln	ser	ala	ile	asn	lys	ala	ile	his

FIG. 4. Comparison of the Spo0J α-helix–turn–α-helix unit and the KorB α-helix–turn–α-helix unit. Double dots indicate identical amino acids, and single dots indicate conservative differences. In the Spo0J sequence, a single underline designates amino acid residues that are highly favored and a double underline designates amino acid residues that are highly conserved in DNA-binding proteins that have an α-helix–turn–α-helix motif (8, 27). The sequence of the λ Cro α-helix–turn–α-helix unit is included for comparison.

to suppress *spo0J*::Tn*917*ΩHU261 suggests that the function of *spo0J* differs from that of *spo0K* in stationary-phase cells.

The *spo0A* gene product has a central role in controlling a variety of stationary-phase functions in *B. subtilis*, including sporulation (16, 23, 37). Initiation of sporulation apparently involves conversion of the *spo0A* gene product from an inactive form to an active form through reversible phosphorylation (16, 29, 37). It is possible to imagine several models in which *spo0J* and *crsF* interact with *spo0A* to regulate catabolite repression of sporulation. However, the simplest model may be one in which *crsF* and *spo0J* affect phosphorylation of the *spo0A* gene product in response to the availability of carbon sources such as glucose. If *crsF* influenced phosphorylation of the *spo0A* gene product to repress sporulation in the presence of glucose, then a *crsF* mutant might display catabolite-resistant sporulation because there would be no *crsF* gene product to exert negative control. The presence of an α-helix–turn–α-helix motif in the putative *spo0J* gene product suggests that the *spo0J* gene product could be a transcriptional repressor. If transcription of *crsF4* were negatively regulated by the *spo0J* gene product in response to low catabolite levels, then a *spo0J* mutant might be Spo$^-$ because *crsF* could not be inactivated as catabolites became depleted. According to this model, double mutants with defects in *spo0J* and *crsF* would be Spo$^+$ because it would be unnecessary for *spo0J* to repress transcription of *crsF* to derepress sporulation. In addition, this model suggests the possibility that PMB12 and SP10 suppress *spo0J* mutations and make wild-type sporulation catabolite resistant by inactivating *crsF*.

The observation that sporulation by *spo0J crsF* double mutants was repressed by glucose suggests that catabolite repression of sporulation is controlled at more than one level. Transcription of *spo0A* and *spo0F* is repressed by glucose (3, 43). A conserved catabolite repression operator sequence has been found to precede *spo0A* (42). Therefore, it is possible that, if one level of catabolite repression of sporulation is removed by mutations in *spo0J* and *crsF*, catabolite repression of sporulation may still be exerted at another level by repressing transcription of key sporulation genes such as *spo0A* and *spo0F*.

ACKNOWLEDGMENTS

We thank the *Bacillus* Genetic Stock Center for providing several strains.

Work in the laboratory of M.G.B. was supported by Biomedical Research Support grant 1 S07RR07241. Work in the laboratory of J.E. was supported by the Science and Engineering Research Council.

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