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Identification and Characterization of the *Bacillus subtilis spoIIP* Locus

NIELS FRANDSEN AND PATRICK STRAGIER*

Institut de Biologie Physico-Chimique, 75005 Paris, France

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We have identified an additional sporulation gene, named *spoIIP*, in the region of the *Bacillus subtilis* chromosome located immediately downstream of the *gpr* gene (227° on the genetic map). A null mutation of *spoIIP* arrests sporulation at an early stage of engulfment (stage II_{ii}), a phenotype similar to that already described for *spoIID* and *spoIIM* mutants. This gene encodes a 401-residue polypeptide, which is predicted to be anchored in the membrane, most of the protein being localized outside the cytoplasm. The *spoIIP* gene is transcribed from a promoter located in the interval between the *gpr* and the *spoIIP* reading frames. This promoter has the structural and genetic characteristics of a σ^E-dependent promoter. Transcription of *spoIIP* is abolished by a mutation in *spoIIGB*, the gene encoding σ^E, and can be induced during exponential growth in cells engineered to produce an active form of σ^E. Plasmid integration-excision experiments leading to the formation of genetic mosaics during sporulation indicate that as with SpoIID and SpoIIM, SpoIIP is required only in the mother cell. Disruption of *spoIIP* had little or no effect on the expression of σ^F- and σ^E-controlled regulons but inhibited transcription from σ^G-dependent promoters and abolished transcription from promoters under the control of σ^K. We propose that, together with SpoIID and SpoIIM, the SpoIIP protein is involved in the dissolution of the peptidoglycan located in the sporulation septum.

Sporulation in *Bacillus subtilis* involves the production of two cell types, the forespore and the mother cell. These two cells are generated by the formation of a septum in an asymmetric position, followed by engulfment of the smaller forespore by the mother cell (7). Differential transcription occurs in the two cells shortly after septation because of the selective activation of σ^F in the forespore and σ^E in the mother cell (37). Mutations in *spoIIAC* (the gene encoding σ^F) and in *spoIIGB* (the gene encoding σ^E) completely block engulfment (12). Mutations in *spoIID* and *spoIIM*, two genes under the control of σ^E, allow partial degradation of the cell wall in the central part of the septum but prevent further progression of the engulfing membrane (3, 30). Some missense mutations in *spoIIAC* lead to a more advanced phenotype, although engulfment is not complete, which suggests the existence of some gene(s) under the control of σ^F required in the late stages of the engulfment process (12). Only three genes have been identified as belonging to the σ^F regulon, *spoIIIG* (38), *gpr* (41), and *dacF* (28), and only mutations in *spoIIIG* (encoding σ^G, the late-acting forespore sigma factor) block sporulation (15). The *dacF* gene (which encodes a penicillin-binding protein) is located immediately upstream of the *spoIIA* operon (45), and transcription from the *dacF* promoter reads into the *spoIIA* locus (28). The *gpr* gene (which encodes a protease degrading some spore proteins at germination) does not appear to be followed by a rho-independent transcription termination signal (41). As part of our effort to identify additional members of the σ^F regulon, we have cloned and characterized the chromosomal region located immediately downstream of *gpr*. We were encouraged by unpublished observations from Ruth Schmidt and Richard Losick suggesting that in some cases disruption of the *gpr* locus appears to interfere with the normal sporulation process. We report here the identification of a new *spo* locus, *spoIIP*, which is controlled by its own (σ^E-dependent) promoter and which is required for engulfment.

* Corresponding author. Mailing address: Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France. Phone: 33 1 43298599. Fax: 33 1 40468331. Electronic mail address: stragier@ibpc.fr.

MATERIALS AND METHODS

Bacterial strains and media. Strain BEST4079 was kindly provided by M. Itaya (14). All other *B. subtilis* strains were derivatives of JH642 *trpC2 pheA1*. The *spoIIAC*, *spoIIGB*, *spoIIID*, and *spoIIIG* mutants are from our laboratory collection and were constructed by inserting an erythromycin resistance cassette into the corresponding coding sequence. Transformation of *B. subtilis* was carried out as previously described (1). For sporulation, *B. subtilis* was grown in DS medium (27) with addition of chloramphenicol (5 μg/ml), tetracycline (10 μg/ml), or a mixture of erythromycin (0.5 μg/ml) and lincomycin (12.5 μg/ml) when appropriate. For induction of σ^F, σ^E, or σ^G activity during vegetative growth, *B. subtilis* was grown in 2×YT medium (24) with 5 μg of kanamycin per ml.

Plasmid constructions were done in *Escherichia coli* TG1. *E. coli* was grown in Luria-Bertani and 2×YT media (24) containing ampicillin (50 μg/ml) when appropriate.

Cloning of the *spoIIP* locus. The *Eco*RI-*Bam*HI fragment from plasmid pPS1027 (kindly provided by R. Schmidt) containing a 317-bp fragment internal to the *gpr* gene (41) was cloned in the pDG641 vector (11). The resulting plasmid was transformed into strain JH642, and selection for erythromycin resistance led to its integration at the *gpr* locus by Campbell-type recombination. Chromosomal DNA was purified from one such clone, digested separately with *Cla*I and *Bcl*I, ligated at a low DNA concentration, and transformed into *E. coli*. Plasmids which contained, respectively, 2.5 and 3.5 kb of DNA adjacent to the original insert were obtained (see Fig. 1). For *trans* complementation of the *spoIIP* mutation, the 1,382-bp *Bsp*HI-*Sph*I fragment present in these two plasmids was converted into an *Eco*RI-*Bgl*II fragment through a subcloning step in a polylinker. This fragment was then inserted between the *Eco*RI and *Bam*HI sites of pDG364, a vector which allows marker exchange at the *amyE* locus (16).

Construction of *lacZ* fusions. The *csfE-lacZ* fusion is a translational fusion controlled exclusively by σ^F, integrated by a double recombination event at the *amyE* locus (29). The *spoIID-lacZ* fusion is a transcriptional fusion containing a 290-bp *Hin*dIII-*Pvu*II fragment (carrying the σ^E-controlled *spoIID* promoter) (17) fused to a promoterless *E. coli lacZ* gene (using the translation initiation signals of the *B. subtilis spoVG* gene) and integrated at the *amyE* locus (36). The *sspE-lacZ* fusion is a translational fusion containing a 162-bp *Hin*cII-*Pvu*II fragment (carrying the σ^G-controlled *sspE* promoter and the first 22 codons of *sspE*) fused in frame to *lacZ* and integrated at the *amyE* locus (8). The *cotA-lacZ* fusion is a transcriptional fusion containing a 400-bp *Nco*I fragment (carrying the σ^K-controlled *cotA* promoter) fused to a promoterless *E. coli lacZ* gene (using the translation initiation signals of the *B. subtilis spoVG* gene) and integrated at the *amyE* locus (22).

A 315-bp *Bsp*HI-*Pst*I fragment carrying the last 14 codons of *gpr*, the 62-bp interval between the *gpr* and the *spoIIP* reading frames, and the first 71 codons of *spoIIP* was converted into an *Eco*RI-*Bgl*II fragment through a subcloning step

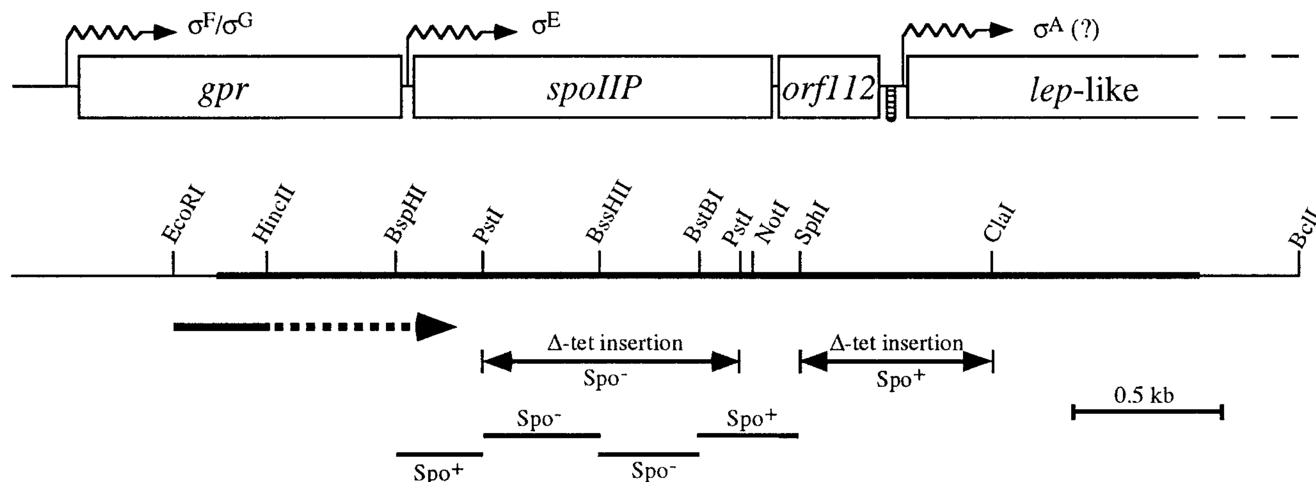

FIG. 1. Genetic organization of the *spoIIP* region. (Top) Open reading frames (boxes) and transcription starts with the sigma factor involved (wavy arrows) are indicated. The hairpin shows a putative transcription termination signal after the *spoIIP-orf112* operon. (Middle) The physical map shows only the restriction sites used for cloning. The thicker segment indicates the region sequenced in the frame of the *Bacillus* genome project (42), our own sequence extending from the *Bsp*HI site to the *Sph*I site, while data from the leftmost part have been published previously (41). (Bottom) The interrupted arrow starts from the fragment that was used to walk up to the *Cla*I and *Bcl*I sites. The fragments that were deleted and replaced by a tetracyclin resistance cassette are shown between arrowheads, with the associated Spo phenotypes indicated. The DNA fragments that were cloned in an integrative vector in order to define the borders of the *spoIIP* locus (bars) and the Spo phenotypes produced by integration of these plasmids into strain JH642 are indicated.

in a polylinker. This fragment was then inserted upstream of a promoterless *E. coli lacZ* gene (using the translation initiation signals of the *B. subtilis spoVG* gene) between the *Eco*RI and *Bam*HI sites of the pJM783 and pDG268 vectors (2, 20). In the former case, the fusion was integrated by a single Campbell-type recombination event at the *spoIIP* locus, while in the latter case the fusion was integrated by a double recombination event at the ectopic *amyE* locus.

β-Galactosidase activity in extracts obtained by sonication or glass bead disruption was measured and was assayed with an automatic microplate reader which was also used for determining protein concentration with the Pierce reagent (33). The activity is expressed as nanomoles of 2-nitrophenyl-β-D-galactopyranoside hydrolyzed per minute per milligram of protein.

Construction of genetic mosaics. The replication-thermosensitive plasmid pKSV7 was used to disrupt *spo* genes at 42°C and to create genetic mosaics by shifting down the cultures at 30°C shortly before the onset of sporulation (31). The number of heat-resistant spores was measured 24 h after the onset of sporulation by treating a culture sample for 10 min at 80°C and incubating several dilutions on DS agar plates at 42°C.

The *spoIID* gene was reversibly inactivated by a 476-bp *Hae*III-*Hin*dIII fragment internal to the *spoIID* reading frame (17). The *spoIIP* gene was reversibly inactivated by a 411-bp *Pst*I-*Bss*HII fragment internal to the *spoIIP* reading frame. The *spoIIIG* gene was reversibly inactivated by a 242-bp *Dde*I-*Bam*HI fragment internal to the *spoIIIG* open reading frame (15).

The *spoIIM* locus was cloned from a strain containing a Tn*917* transposon inserted into the *spoIIM* gene (kindly provided by A. Grossman), using the walking technique devised by Youngman et al. (46). The *spoIIM* gene was then reversibly inactivated by a 353-bp *Hinc*II-*Dra*I fragment internal to the *spoIIM* open reading frame (30).

Promoter mapping. RNA isolation and primer extension analysis of *spoIIP* transcripts were performed by the method of Moran (19). RNA was prepared from a strain containing a *spoIIP-lacZ* fusion inserted at the *amyE* locus. Cells were grown in DS medium and harvested at 1.5 h after the onset of sporulation ($t_{1.5}$). Two primers that could hybridize both to the mRNA originating from the *spoIIP* locus and to the mRNA originating from the *spoIIP-lacZ* fusion were used. Primer P1 corresponds to positions 66 to 48 in the *spoIIP* coding sequence (5′-GGCTTTTACCGCTTTCCCT), and primer P2 corresponds to positions 96 to 78 in the *spoIIP* coding sequence (5′-CACGATAAGGCTTACGATA).

RESULTS

Cloning and sequence of the *spoIIP* gene. A DNA fragment internal to the *gpr* gene was used to integrate a plasmid into that region of the chromosome and subsequently to clone about 3 kb of DNA located immediately downstream of *gpr* (see Fig. 1 and Materials and Methods for details). Replacement of a 0.9-kb *Pst*I fragment (located about 0.3 kb from the 3′ end of the *gpr* reading frame) with a tetracycline resistance cassette led to a Spo$^-$ phenotype, which indicated that a gene essential for sporulation had been inactivated by the *Pst*I deletion. Conversely, replacement of a 0.65-kb *Sph*I-*Cla*I fragment (located about 1.4 kb from the 3′ end of the *gpr* reading frame) with a tetracycline resistance cassette did not interfere with sporulation. To further delineate the borders of the sporulation-essential region, a series of integrative plasmids was constructed, as shown in Fig. 1 (21). The phenotypes of the transformants obtained indicate that the *spo* locus is completely internal to a 1.4-kb *Bsp*HI-*Sph*I fragment and that its expression is not prevented when this locus is separated from the *gpr* gene.

The nucleotide sequence of this region was determined and appeared later in the DDBJ data library as part of the *B. subtilis* genome project (42). Since the two sequences are perfectly identical, only the genetic organization of this region is reported here (Fig. 1). The *spo* locus is located only 65 bp downstream of the *gpr* gene and consists of an open reading frame encoding a 401-residue protein. It is immediately followed by a 112-codon open reading frame which appears to be cotranscribed with the upstream *spo* locus but whose product is not essential for sporulation, as shown by our previous disruption experiments. Interestingly, the available sequences indicate that the *orf112* gene is located immediately downstream of *gpr* in the closely related bacterium *Bacillus megaterium* (41). It is not known whether the order of the two genes (*orf401* and *orf112*) is reversed in *B. megaterium* or whether *orf401* is absent from that region of the *B. megaterium* chromosome. The *B. subtilis* operon is followed by a gene, apparently transcribed from a σ^A-like promoter, encoding a protein which is highly similar to the *E. coli* signal peptidase LepA. Disruption of this gene had no obvious effect on the growth of *B. subtilis*, which is known to contain another gene encoding a signal peptidase (43).

Analysis of the nucleotide sequence of this region revealed the presence of a *Not*I restriction site in *orf401*. The distribution of the *Not*I sites on the *B. subtilis* chromosome has been determined (14), and the *gpr* gene has been reported to be at around 210° (39). It was intriguing to find that insertion of a

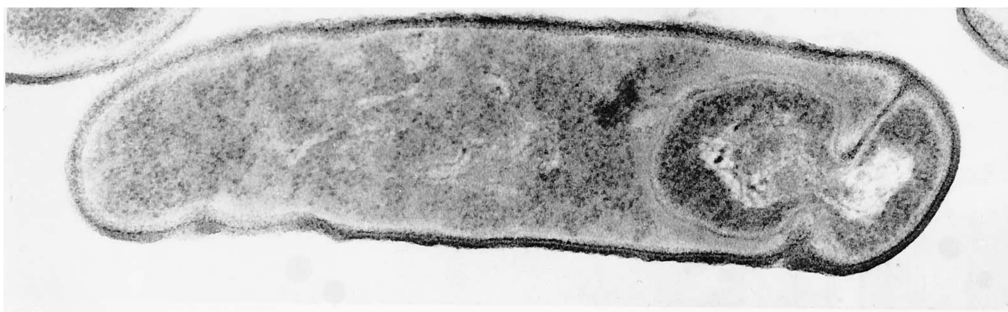

FIG. 2. Electron micrograph of a *spoIIP* mutant. The *spoIIP*Δ::*tet* strain was grown in DS medium, and cells were harvested 4 h after the end of exponential growth. This picture is representative of the most advanced morphological stage reached by the mutant (5). The bleb formed by the septum appears to frequently pinch off to form a membrane protoplast (5), although this could be an artifact due to the section plan.

neomycin resistance cassette in a *Not*I site located at 2627 kb (approximately 227°) led to a Spo$^-$ phenotype, which was not obviously related to any known *spo* gene (14). We have confirmed that this uncharacterized locus is the same as the one described here and that a DNA fragment carrying only *orf401* is able to correct the Spo defect of the original strain, BEST4079 (10).

The predicted product of *orf401* contains a 22-residue hydrophobic domain, between positions 22 and 44, which might anchor the protein in the membrane. The amino-terminal part of the protein contains seven positively charged residues which should block that segment on the inner side of the membrane (44), suggesting that most of the protein (the region from residues 45 to 401) is located outside the cytoplasm. No significant similarity with other proteins could be detected, leaving the function of this protein obscure. However, analysis by electron microscopy of the morphological blockage resulting from inactivation of *orf401* indicated that this mutant is blocked at stage II_{ii} (Fig. 2), with a phenotype very similar to that described for *spoIID* or *spoIIM* mutants (3, 5, 12, 30). Consequently, this gene has been named *spoIIP*. It is absolutely essential for sporulation, since the *spoIIP* mutant does not make any heat-resistant spores.

***spoIIP* belongs to the σ^E regulon.** Since integration of a plasmid containing the *Bsp*HI-*Pst*I fragment overlapping the end of the *gpr* gene and the beginning of the *spoIIP* locus does not block sporulation, it follows that a promoter allowing expression of *spoIIP* must be present in that interval (21). This fragment was cloned upstream of a promoterless *E. coli lacZ* gene in two different vectors (see Materials and Methods), and synthesis of β-galactosidase was monitored, either at the *spoIIP* locus (after Campbell-type integration by a single recombination event) or at the *amyE* locus (after stable integration resulting from a double recombination event). In the former case, expression of the *lacZ* gene reflects transcription both from the *spoIIP* promoter and from the upstream *gpr* promoter, while only transcription from the *spoIIP* promoter is measured at *amyE*. Results shown in Fig. 3 indicate that there is no significant difference between the two fusions and that *spoIIP* transcription starts around t_1 and peaks about 1 h later. To further demonstrate that the *Bsp*HI-*Pst*I fragment contains all the essential determinants for *spoIIP* expression, the 1.4-kb *Bsp*HI-*Sph*I fragment was cloned at the *amyE* locus and was found to fully complement the sporulation defect created by the deletion-insertion in *spoIIP* (10).

The *spoIIP* transcript was mapped by primer extension, and the apparent 5′ end was found to be localized 23 bp upstream of the translation initiation codon (Fig. 4). This allows the identification of a potential promoter sequence [GTTCTAC TT-(13 bp)-CATAGAGT-(7 bp)-transcription start] which matches well the σ^E promoter consensus (32). Expression of the *spoIIP-lacZ* fusion integrated at the *spoIIP* locus was analyzed in various mutant backgrounds (Fig. 5). It was completely abolished by mutations in *spoIIAC* and *spoIIGB*, which prevent synthesis of σ^F and σ^E, respectively. It was not affected by mutations in *spoIIIG*, which encodes σ^G, or *spoIIID* (10), which encodes a DNA-binding protein activating a subset of σ^E-dependent promoters (35). These genetic dependencies as well as the timing of expression of a *spoIIP-lacZ* fusion strongly suggest that *spoIIP* is controlled by σ^E. This was confirmed by monitoring *spoIIP* transcription in various strains in which synthesis of σ^F, σ^E, or σ^G was artificially induced during exponential growth. As shown in Fig. 6, the *spoIIP-lacZ* fusion at *amyE* was strongly turned on by an active form of σ^E but was unresponsive to σ^F and σ^G. When the fusion was inserted at the *spoIIP* locus, a weak level of induction by σ^F and σ^G was observed, indicating that some transcriptional readthrough from the upstream *gpr* gene can take place (10). The physiological significance of this additional mode of transcription is uncertain, since sporulation is normal when expression of *spoIIP* is controlled exclusively by its own σ^E-dependent promoter at the *amyE* locus.

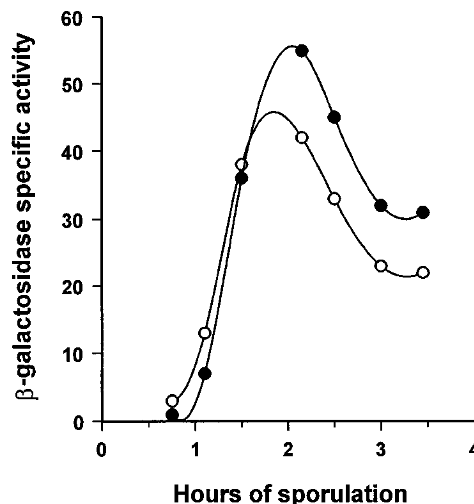

FIG. 3. Expression of *spoIIP-lacZ*. The specific activity of β-galactosidase was monitored in a wild-type strain carrying a *spoIIP-lacZ* fusion, either at the *amyE* locus (○) or at the *spoIIP* locus (●), and induced to sporulate by exhaustion in DS medium.

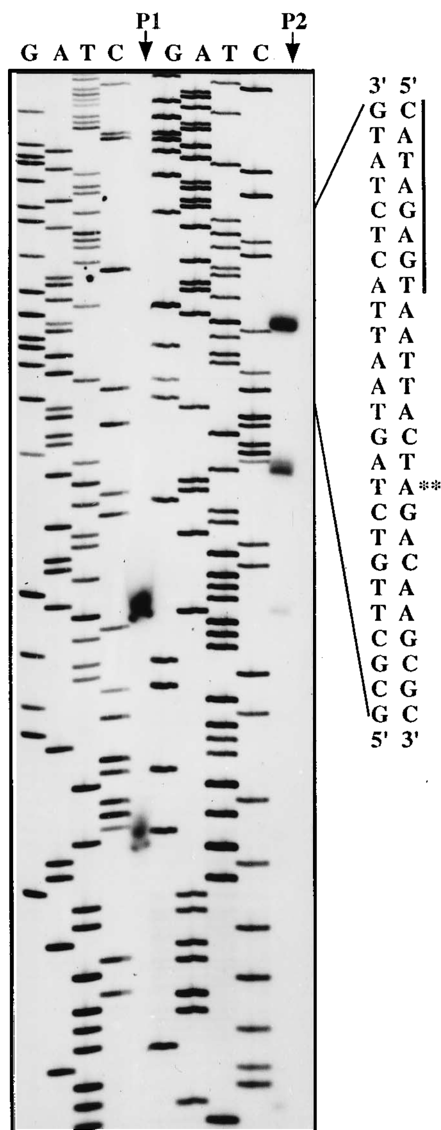

FIG. 4. Determination of the *spoIIP* transcription start. The result of primer extension analysis using the P1 and P2 oligonucleotides (see Materials and Methods) to prime cDNA synthesis from RNA extracted from a wild-type strain at $t_{1.5}$ is shown. The extension products were run in parallel with sequencing ladders (GATC lanes) generated by the chain termination method (25) using the same primers and single-stranded DNA templates containing that region of the *spoIIP* gene. The two sets of reaction mixtures were loaded on the gel simultaneously and are shifted by 30 nucleotides, as expected from the design of the two primers. The transcription start site defined by the major and longer extension product (asterisks) is indicated. Minor shorter products are probably due to premature termination by reverse transcriptase, since they define positions located downstream of the ribosome binding site or in the *spoIIP* coding sequence. The −10 promoter motif (vertical line) is shown.

***spoIIP*, like *spoIID* and *spoIIM*, is required only in the mother cell.** σ^E activity is generally believed to be confined to the mother cell (6, 7, 37). Therefore, the presence of an intact *spoIIP* gene in the forespore should not be required for sporulation. To address this question, we made genetic mosaics, using the strategy devised by Illing et al. (13) and refined by Smith and Youngman (31). A DNA fragment internal to the *spoIIP* open reading frame was cloned in a plasmid carrying a thermosensitive replicon. When the cells were grown at 42°C, selection for the chloramphenicol resistance marker carried by

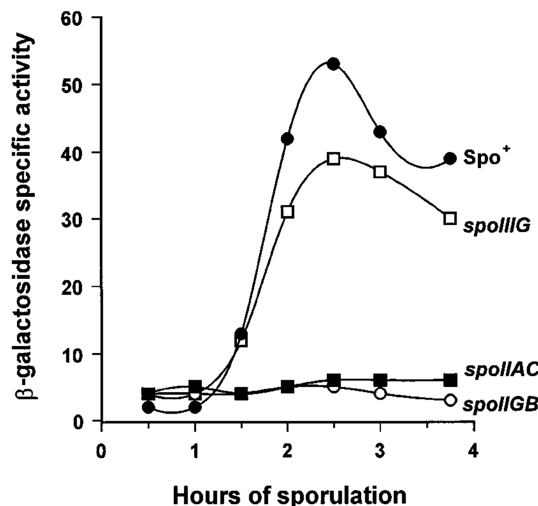

FIG. 5. Effects of various *spo* mutations on *spoIIP-lacZ* expression. β-Galactosidase synthesis from a *spoIIP-lacZ* fusion integrated at the *spoIIP* locus was monitored in Spo$^+$, *spoIIAC*, *spoIIGB*, and *spoIIIG* strains. Cells were induced to sporulate by exhaustion in DS medium.

the plasmid led to its integration into the chromosome and inactivation of the *spoIIP* locus. Shifting the cells at 30°C just before the onset of sporulation activated plasmid replication, which stimulated recombinational excision of the integrated plasmid and increased the presence, in some sporulating cells, of an intact *spoIIP* gene in only one of the two chromosomes. If the *spoIIP* product is required only in the mother cell, it will be possible to obtain a significant amount of heat-resistant spores containing a disrupted *spoIIP* gene which will have been rescued by the intact mother cell copy. These spores will give

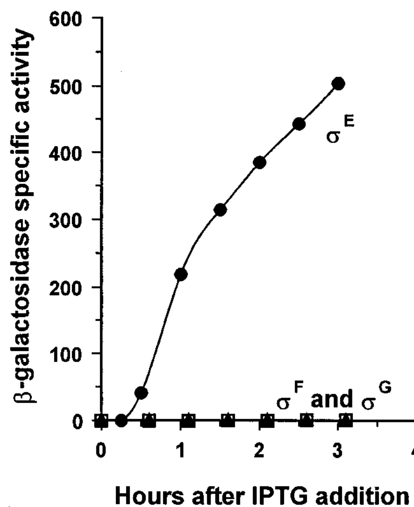

FIG. 6. Induction of *spoIIP-lacZ* during vegetative growth. A strain carrying a *spoIIP-lacZ* fusion integrated at the *amyE* locus and pUB110 derivative pSDA4 (*spoIIAC* under *spac* promoter control) (29) (▲), pDG180 (a truncated version of *spoIIGB* under *spac* promoter control) (22) (●), or pDG298 (*spoIIIG* under *spac* promoter control) (40) (□) was grown in 2×YT medium. The strains with pSDA4 and pDG298 carried a mutation inactivating the *spoIIIG* chromosomal gene. When the optical density at 570 nm reached 0.3, the culture was split and 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside) was added to one part. β-Galactosidase specific activity in the cultures with IPTG is shown as a function of time after addition of IPTG. No β-galactosidase activity was found in the absence of IPTG.

TABLE 1. Plasmid excision from disrupted *spo* genes

Locus disrupted	Temp (°C)[a]	No. of heat-resistant spores/ml of culture[b]	% Cm^r Spo^-
spoIID	42	1.0×10^2	ND[c]
	30	1.5×10^4	28.8
spoIIM	42	3.6×10^4	1.7
	30	1.1×10^5	5.7
spoIIP	42	6.0×10^3	2.2
	30	6.5×10^4	6.6
spoIIIG	42	4.8×10^3	0.4
	30	2.2×10^4	0.5

[a] Growth was always at 42°C. Part of each culture was shifted to 30°C 30 min before the end of exponential growth while the other part was left for sporulation at 42°C. Cultures were grown in DS medium in the presence of chloramphenicol (5 μg/ml).

[b] Samples were taken at t_{24} and heat treated for 10 min at 80°C. Serial dilutions were plated on DS agar plates without chloramphenicol and incubated at 42°C.

[c] ND, not determined.

rise to a progeny of chloramphenicol-resistant, asporogenous colonies. Results of such an experiment are reported in Table 1. When the cells harboring a plasmid integrated in the *spoIIP* gene were shifted to 30°C, sporulation-defective spores were recovered 13 times more often than when the *spoIIIG* gene, which is absolutely required in the forespore (13), was similarly inactivated. The *spoIID* gene, known to be required only in the mother cell (13), was included in that experiment as a control. In that case, a very low level of excision takes place at 42°C (which would occur several generations before the onset of sporulation and would not give rise to mosaics), and the large majority of excisions occurs after the shiftdown at 30°C, which ensures a high proportion of genetic mosaics after asymmetric septation. Comparison of the results obtained with *spoIID*, *spoIIP*, and *spoIIIG* clearly indicates that an intact copy of *spoIIP* in the forespore is dispensable.

spoIIM is the only σ^E-dependent gene that has been reported to be required in the forespore (32). We have reinvestigated the expression of this locus. An integrative plasmid was constructed in order to inactivate the *spoIIM* gene and to create genetic mosaics as described above (see Materials and Methods for details). The results obtained with the *spoIIM*-disrupted strain are shown in Table 1 and indicate that *spoIIM* behaves very similarly to *spoIIP*. Because this conclusion contradicts what was previously published (32), we have duplicated these experiments with an integrative plasmid without any replication origin. The percentages of chloramphenicol-resistant spores were very similar when the *spoIID*, *spoIIM*, and *spoIIP* genes were disrupted (3.3, 5.8, and 4.4%, respectively). Therefore, we conclude that the *spoIIM* product, like the products of all the other σ^E-dependent genes described so far (including *spoIIP*), is required only in the mother cell.

Effect of a *spoIIP* mutation on gene expression during sporulation. We have compared the expression of various *lacZ* fusions in the presence and in the absence of an intact *spoIIP* gene. Since the morphological stage of blockage of a *spoIIP* mutant is more advanced than that observed for *spoIIAC* and *spoIIGB* mutants, the *spoIIP* mutation was not expected to interfere with expression of a σ^F- or σ^E-dependent *lacZ* fusion. This is indeed what was observed, as shown in Fig. 7 (*csfE-lacZ* and *spoIID-lacZ*). Moreover, transcription of a *spoIVCA-lacZ* fusion, which requires both σ^E and the SpoIIID activating protein (26), was also found to be normally induced in the *spoIIP* mutant (10). Conversely, transcription of several σ^G-dependent *lacZ* fusions was severely affected by the *spoIIP* mutation (see *sspE-lacZ* results in Fig. 7 for an example). The amount of β-galactosidase accumulated between $t_{2.5}$ and $t_{4.5}$, compared with that of the wild type, varied from 14 to 46%, depending on the gene fused to *lacZ* (*sspB*, *sspA*, *sspE*, and *spoIVB*, in increasing order of residual activity) (10). This activity appears to be actually due to σ^G, since it is completely abolished in a *spoIIP spoIIIG* double mutant (Fig. 7, *sspE-lacZ*). Finally, no σ^K activity could be detected in a *spoIIP* mutant (Fig. 7, *cotA-lacZ*).

DISCUSSION

We have identified a new *spo* locus, *spoIIP*, located immediately downstream of the *gpr* gene. Although our initial expectation was to find a gene cotranscribed with *gpr* and belonging to the σ^F regulon, *spoIIP* is expressed from its own promoter located in the short interval between the two reading frames. Some transcription from *gpr* appears to read through the *spoIIP* gene, but it does not seem to be physiologically significant, since a copy of the *spoIIP* gene located at the ectopic locus *amyE* (and uniquely controlled by its own promoter) is able to fully complement a *spoIIP* mutation.

All the existing data indicate that *spoIIP* is under the control of σ^E. Expression of a *spoIIP-lacZ* fusion is abolished in a *spoIIGB* mutant, while it is strongly induced by an active form of σ^E during exponential growth. The *spoIIP* promoter contains sequence motifs highly conserved in other σ^E-controlled promoters. The *spoIIP* product is required only in the mother cell, in which σ^E has been reported to be uniquely active (6). Even if some expression of *spoIIP* from the *gpr* promoter occurs in the forespore, it is probably of no physiological significance, as discussed above.

A null mutation in *spoIIP* creates the phenotype that has already been described for *spoIID* and *spoIIM* (3, 30). Hydrolysis of the central region of the septal peptidoglycan occurs but does not extend to the edges, which leads to bulging of the forespore compartment into the mother cell chamber and prevents engulfment from proceeding. Interestingly, these three genes are under the control of σ^E (23, 32) and are required only in the mother cell (we have no clear explanation for our discrepancy with the results previously reported for *spoIIM* [32], the only experimental difference being the presence of chloramphenicol throughout our cultures). Their products are all surmised to be associated with the membrane (by a single transmembrane domain for SpoIID [17] and SpoIIP, with most of the protein located outside the cytoplasm, and as an integral membrane protein in the case of SpoIIM [30]). It is tempting to speculate that they act in a concerted fashion to allow complete degradation of the septal peptidoglycan, although it is not clear why such a reaction should be carried out only from the mother cell side of the septum.

Inactivation of *spoIIP* has no effect on expression of the σ^F and σ^E regulons. It partially prevents transcription from σ^G-dependent promoters. Because of its morphological defect, the *spoIIP* mutant was expected to be completely devoid of late forespore gene expression, since σ^G transcriptional activity is believed to require complete isolation of the forespore within the mother cell (18, 34). We have reanalyzed several stage II and III mutants, among them *spoIID* and *spoIIM* mutants, and we have found similar partial σ^G activity, which therefore is not a unique property of the *spoIIP* mutation (9). The implications of this finding for our current model of regulation of σ^G transcriptional activity will be discussed elsewhere.

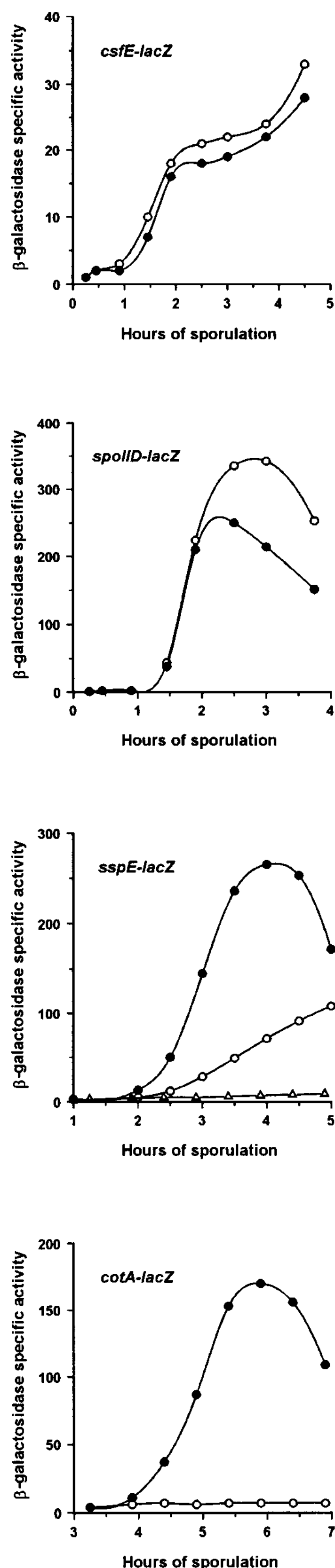

FIG. 7. Expression of various sporulation genes in a *spoIIP* mutant. β-Galactosidase synthesis from the indicated fusions was measured in a Spo$^+$ strain (●) and in a *spoIIP* mutant (○) or a *spoIIP spoIIIG* double mutant (△). Cells were induced to sporulate by exhaustion in DS medium.

The *spoIIP* mutant does not display any σ^K activity, presumably because of the lack of pro-σ^K processing. Since all the genes involved in σ^K production appear to be expressed in the *spoIIP* mutant (as deduced from the expression of a representative member of the σ^E-controlled regulon and as inferred from our assays with *spoIVCA* and *spoIVB* [10]), some specific feature of the developing forespore must be missing. A similar conclusion can be drawn from the fact that the *bofA* and *bofB* mutations do not bypass a *spoIID* mutation (4), suggesting that even when the signal transduction pathway from the forespore is not needed, the pro-σ^K processing machinery requires some morphological state of the forespore envelope to become active. Presumably, the SpoIID, SpoIIM, and SpoIIP proteins play a critical role in achieving that developmental step.

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