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Expression of *spoIIIJ* in the Prespore Is Sufficient for Activation of σ^G and for Sporulation in *Bacillus subtilis*

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During sporulation in *Bacillus subtilis*, the prespore-specific developmental program is initiated soon after asymmetric division of the sporangium by the compartment-specific activation of RNA polymerase sigma factor σ^F. σ^F directs transcription of *spoIIIG*, encoding the late forespore-specific regulator σ^G. Following synthesis, σ^G is initially kept in an inactive form, presumably because it is bound to the SpoIIAB anti-sigma factor. Activation of σ^G occurs only after the complete engulfment of the prespore by the mother cell. Mutations in *spoIIIJ* arrest sporulation soon after conclusion of the engulfment process and prevent activation of σ^G. Here we show that σ^G accumulates but is mostly inactive in a *spoIIIJ* mutant. We also show that expression of the *spoIIIGE155K* allele, encoding a form of σ^G that is not efficiently bound by SpoIIAB in vitro, restores σ^G-directed gene expression to a *spoIIIJ* mutant. Expression of *spoIIIJ* occurs during vegetative growth. However, we show that expression of *spoIIIJ* in the prespore is sufficient for σ^G activation and for sporulation. Mutations in the mother cell-specific *spoIIIA* locus are known to arrest sporulation just after completion of the engulfment process. Previous work has also shown that σ^G accumulates in an inactive form in *spoIIIA* mutants and that the need for *spoIIIA* expression for σ^G activation can be circumvented by the *spoIIIGE155K* allele. However, in contrast to the case for *spoIIIJ*, we show that expression of *spoIIIA* in the prespore does not support efficient sporulation. The results suggest that the activation of σ^G at the end of the engulfment process involves the action of *spoIIIA* from the mother cell and of *spoIIIJ* from the prespore.

During the early stages of endospore development in *Bacillus subtilis*, the rod-shaped bacterial cell is divided into a smaller prespore and a larger mother cell. The prespore is engulfed by the mother cell, which lyses at the end of the sporulation process to liberate the mature spore into the environment. Each cell type receives a copy of the bacterial chromosome, and each deploys specific but interdependent genetic programs controlled by the successive appearance of the σ^F, σ^E, σ^G, and σ^K subunits of RNA polymerase (29, 32, 42, 46). The σ^F and σ^G regulators control gene expression during the early and late stages of prespore development, respectively, whereas σ^E controls the expression of early mother cell genes and is replaced at later stages by σ^K. The activation of the first compartment-specific sigma factor, σ^F, involves the action of three regulatory proteins, SpoIIAA, SpoIIAB, and SpoIIE (33, 48). SpoIIAB is an anti-sigma factor that binds to σ^F as a dimer and inhibits its transcriptional activity (6, 7, 14, 36), whereas SpoIIAA is an anti-anti-sigma factor that in an unphosphorylated state interacts with SpoIIAB and releases σ^F from the SpoIIAB-σ^F complex (1, 12, 13). SpoIIE, a septum-bound phosphatase, promotes the preferential dephosphorylation of SpoIIAA-P in the prespore, its binding to SpoIIAB, and consequently the activation of σ^F (3, 4, 12, 15). Transcription of the *spoIIIG* gene, encoding the late prespore regulator σ^G, is driven by the σ^F form of RNA polymerase (55). However, transcription of *spoIIIG* is delayed towards the end of the engulfment process relative to transcription of a first class of σ^F-dependent genes (41). In addition, it requires both the activity of σ^E in the mother cell and expression of the σ^F-controlled gene *spoIIQ* (41, 56). Following synthesis, σ^G does not become active until engulfment of the prespore by the mother cell is complete (32, 53; reviewed in references 42 and 46). Once active, σ^G directs expression of its own gene, allowing a rapid increase in the cellular levels of σ^G (25, 55). Activation of σ^G at the end of the engulfment sequence demands the expression of the *spoIIIA* operon (26, 43), which is transcribed in the mother cell by σ^E RNA polymerase (23). The *spoIIIA* locus encodes eight proteins predicted to be associated with the prespore outer membrane, all of which are required for the activation of σ^G (23, 53). σ^G accumulates in a *spoIIIA* mutant but is unable to activate transcription from its target promoters (26). Genetic, biochemical, and structural evidence suggests that the anti-sigma factor SpoIIAB negatively regulates σ^G (7, 8, 17, 26, 28, 44). In particular, during sporulation SpoIIAB tends to disappear from the forespore soon after completion of the engulfment sequence in a *spoIIIA*-dependent mode (28), and expression of an allele of *spoIIIG* (*spoIIIGE155K*) encoding a form of σ^G that is not efficiently bound by SpoIIAB in vitro permits activation of σ^G in vivo in the absence of an intact *spoIIIA* locus (26). These results suggest that the *spoIIIA* products are required soon after conclusion of the engulfment process to inactivate SpoIIAB, thereby releasing active σ^G (26).

Mutations in the *spoIIIJ locus* were also shown to prevent expression of σ^G-controlled genes but not transcription of the *spoIIIG* gene (16). *spoIIIJ* is the first gene of a bicistronic

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TABLE 1. *B. subtilis* strains

Strain	Relevant genotype	Origin (reference)
MB24	*trpC2 metC3*	Laboratory stock
AH45	*trpC2 metC3 spoIIIGΔ1*	Laboratory stock
AH62	*trpC2 metC3 spoIIIA*::Tn*917ΩHU24*	Laboratory stock (26)
EUE9537	*trpC2 metC3* SPβ::*sspE-lacZ spoIIIG*::pEK15 (wild-type *spoIIIG*)	Laboratory stock (26)
EUE9538	*trpC2 metC3* SPβ::*sspE-lacZ spoIIIG*::pEK16 (*spoIIIGE155K*)	Laboratory stock (26)
AH1042	*trpC2 metC3* Δ*sspE*::*sspE-lacZ*	Laboratory stock
AH2350	*trpC2 metC3*(pMK3)	This work
AH5008	*trpC2 metC3* Δ*amyE*::*spoIIIG-lacZ*	This work
AH5009	*trpC2 metC3* Δ*spoIIIJ*::*km* Δ*amyE*::*spoIIIG-lacZ*	This work
AH5011	*trpC2 metC3* Δ*spoIIIJ*::*km* Δ*amyE*::$P_{spoIIIJ}$*spoIIIJ*	This work
AH5012	*trpC2 metC3* Δ*spoIIIJ*::*km* Δ*amyE*::P_{spoIIQ}-*spoIIIJ*	This work
AH5013	*trpC2 metC3* Δ*spoIIIJ*::*km* Δ*amyE*::P_{spoIID}-*spoIIIJ*	This work
AH5014	*trpC2 metC3* Δ*spoIIIJ*::*km* Δ*amyE*::P_{spoIIJ}-*spoIIIJ* Δ*sspE*::*sspE-lacZ*	This work
AH5015	*trpC2 metC3* Δ*spoIIIJ*::*km* Δ*amyE*::P_{spoIIQ}-*spoIIIJ* Δ*sspE*::*sspE-lacZ*	This work
AH5016	*trpC2 metC3* Δ*spoIIIJ*::*km* Δ*amyE*::P_{spoIID}-*spoIIIJ* Δ*sspE*::*sspE-lacZ*	This work
AH5017	*trpC2 metC3* Δ*spoIIIJ*::*km* Δ*amyE*::P_{spoIIJ}-*spoIIIJ-gfp*	This work
AH5018	*trpC2 metC3* Δ*spoIIIJ*::*km* Δ*amyE*::P_{spoIIQ}-*spoIIIJ-gfp*	This work
AH5019	*trpC2 metC3* Δ*spoIIIJ*::*km* Δ*amyE*::P_{spoIID}-*spoIIIJ-gfp*	This work
AH5020	*trpC2 metC3 spoIIIA*::P_{spoIIQ}-*spoIIIA*	This work
AH5024	*trpC2 metC3* Δ*spoIIIJ*::*km* Δ*sspE*::*sspE-lacZ spoIIIG*::pEK15	This work
AH5025	*trpC2 metC3* Δ*spoIIIJ*::*km* Δ*sspE*::*sspE-lacZ spoIIIG*::pEK16	This work
AH5029	*trpC2 metC3 spoIIIA*::P_{spoIID}-*spoIIIA*	This work
AH5030	*trpC2 metC3* Δ*sspE*::*sspE-lacZ spoIIIG*::pEK15	This work
AH5031	*trpC2 metC3* Δ*sspE*::*sspE-lacZ spoIIIG*::pEK16	This work
AH5035	*trpC2 metC3 spoIIIJ*::*sp*(pMK3)	This work
AH5036	*trpC2 metC3 spoIIIJ*::*sp*(pMS210)	This work
AH5037	*trpC2 metC3*(pMS210)	This work
JOB20	*trpC2 metC3* Δ*spoIIIJ*::*sp*	This work
JOB34	*trpC2 metC3* Δ*spoIIIJ*::*km* Δ*sspE*::*sspE-lacZ*	This work
JOB44	*trpC2 metC3* Δ*spoIIIJ*::*km*	This work

operon transcribed during vegetative growth (16, 39). It encodes a putative lipoprotein that localizes to the cell membrane and septal regions during vegetative growth and sporulation but also to the membranes that surround the developing spore (39). SpoIIIJ is related to the YidC Sec-independent and Sec-dependent membrane protein translocase of *Escherichia coli (*about 33% identity and 51% conserved residues for a region of overlap between the two proteins of 226 amino acids) (47, 49, 57). SpoIIIJ is also related to the CcfA protein of *Enterococcus faecalis*, with about 65% similarity and 39% identity between the two proteins (2). Like *spoIIIJ*, *ccfA* codes for a putative lipoprotein precursor, but it also encodes the cCF10 peptide pheromone within the signal peptide (2). The cCF10 peptide pheromone is required for the conjugative transfer of the *E. faecalis* plasmid pCF10. CcfA, like YidC in *E. coli*, seems to be required for normal growth of *E. faecalis* (2).

Here we have analyzed the role of *spoIIIJ* in the activation of σ^G. We show that σ^G accumulates in a *spoIIIJ* mutant but is mostly inactive. We also show that expression of the *spoIIIGE155K* allele restores transcription of the σ^G-dependent gene *sspE* to *spoIIIJ* mutant cells. Expression of *spoIIIJ* from a prespore-specific promoter produces a sporulation-proficient strain, whereas its expression from a mother cell-specific promoter results in a block soon after completion of the engulfment process. Conversely, expression of *spoIIIA* in the prespore does not support efficient sporulation. These results suggest that SpoIIIJ from the prespore and the *spoIIIA*-encoded products from the mother cell compartment are cell type-specific elements of a signaling pathway that antagonizes the inhibitory action of SpoIIAB and promotes the activation of σ^G soon after conclusion of the engulfment process.

MATERIALS AND METHODS

Bacterial strains and general methods. The *B. subtilis* strains used in this work, most of which are congenic derivatives of the wild-type strain MB24 (*trpC2 metC3*), are listed in Table 1. Luria-Bertani medium was used for routine growth of *E. coli* DH5α (BRL) and of *B. subtilis* strains. Sporulation was induced by exhaustion in Difco sporulation medium (DSM), and the extent of sporulation (expressed as the percentage of heat resistant CFU relative to the total cell count) was assessed as described previously (20, 21). Antibiotics were used as previously described (20, 21).

Construction of a *spoIIIJ* nonpolar null mutant. To create a nonpolar insertion into the *spoIIIJ* gene, we used PCR with *Taq* DNA polymerase and primers RNPA-75 and JAGD-235R (Table 2) to produce a DNA fragment extending from nucleotide 421 upstream of the *spoIIIJ* start codon to nucleotide 240 downstream of the *jag* stop codon. This DNA fragment was cloned into the pCR2.1 TOPO vector (Invitrogen) to create plasmid pJO34. An *Eco*RI fragment containing the *spoIIIJ* operon was released from pJO34 and inserted into the *Eco*RI site of pLitmus38 (New England Biolabs) to create pJO57. Inverse PCR with primers JXMA-1 and JXMA-2 (Table 2) was performed on pJO57 to create a linear fragment that deleted nucleotides +41 to +707 of the *spoIIIJ* gene (with respect to the transcription start site). This PCR product was ligated with an *Xma*I restriction fragment containing a promoterless kanamycin resistance (Km^r) determinant resulting from the digestion of pUC18-K (35). The DNA resulting from this ligation was a recircularized plasmid in which the *spoIIIJ* gene was replaced with the *km* gene, which was now under control of the *spoIIIJ* promoter. This plasmid was named pJO60. Strains JOB34 and JOB44 were created by transformation of AH1042 (*sspE-lacZ*) and MB24 (wild type) (Table 1), respectively, with *Sca*I-linearized pJO60, selecting for Km^r. Disruption of the *spoIIIJ* gene by a double-crossover event (marker replacement) was verified by PCR. The resulting strains, JOB34 and JOB44 (Table 1), were tested by reverse transcription-PCR analysis to confirm that the insertion was nonpolar on *jag* (not shown). Strains JOB34 and AH1042 were transformed with chromosomal DNA

TABLE 2. Primers

Primer	Sequence (5′→3′)
RNPA-75	CCGCCAGTTTGTCTTATATACGC
JAGD-235R	CGAAGTGGAAGATTTGTTATCCACA
JXMA-1	CCTTTTCAACAACATTCCCGGGTATAATTAATCTTTACACTC
JXMA-2	GCGATCAACTTCCCGGGGGCTCTTTCTCTTTATTGGG
spoIIIJ-460D	CGAAAGCGATGCAGGCTTTACAGCCGG
gfp-spoIIIJ-R	CTCCAGTGAAAAGTTCTTCTCCTTTACTCTTTTTCTTTCCTCCGGCTTTTTGCGGC
gfp-30D	AGTAAAGGAGAACTTTTCACTGGA
gfp-R	GGCGAATTCTTATTTGTATAGTTCATCCAT
spoIIIJ-150R	CCAGCTTTACGATCGCAGGC
spoIIIJ-spoIIQ	GTTGCTGAGGTGATGAAACAATGTTGTTGAAAAGGAGAATAGGG
spoIIIJ-spoIID	CGAGCAGGAGGCAGCTGAATATGTTGTTGAAAAGGAGAATAGGG
spoIIIJ-1039R	CAATCCGGATCCTGTACTGCTTCATCGACATTTCGCCC
spoIIQ-152D	GTTTCAAAGCTGAATTCCAGGCAGCG
spoIIQ-500R	TGTTTCATCACCTCAGCAACATTCTG
spoIID-1D	CGGAAGAATTCCGCCGTATGAATGG
spoIID-500R	ATTCAGCTGCCTCCTGCTCGGG
spoIIA-spoIIQ	GTTGCTGAGGTGATGAAACATTGAATGAAATCGCTGAGG
spoIIA-spoIID	CGAGCAGGAGGCAGCTGAATTTGAATGAAATCGCTGAGG
spoIIA-5004D	GGAAGCAGCGGATCCGCAATCCCC
spoIIIG-392D	GGGAAAAAAGATCTCGAGAAATAAAGTCG
spoIIIG-1217R	GCCTTTAAAACAACTCGAGTTAAAGGC

from EUE9538 (*spoIIIGE155K* allele) or EUE9537 (wild-type *spoIIIG* allele) with selection for spectinomycin resistance (Sp^r), and appropriate transformants were identified as described previously (Table 1) (26). During the course of this work we also used a *spoIIIJ* mutant created by insertion of an Sp^r cassette. pJO34 (see above) was digested with *Eco*RI, and the resulting fragment was cloned into the *Eco*RI site of pUC19 to create pJO36. An Sp^r cassette was then liberated from pAH250 (20) with *Sma*I and *Xho*I and cloned between the *Eco*RV and *Xho*I sites of pJO36 to create pJO41. *Sca*I-digested pJO41 was used to transform MB24 to Sp^r, yielding strain JOB20 (Table 1; Fig. 1).

Construction of a *spoIIIG-lacZ* fusion. To construct a *spoIIIG-lacZ* translational fusion, we first isolated a 448-bp *Eco*RI-to-*Pvu*II fragment from pTK4 encompassing the *spoIIGB-spoIIIG* intergenic region and including the first 30 codons of *spoIIIG* (27). The 448-bp fragment was introduced between the *Eco*RI and *Sma*I sites of pNM480 (37), yielding pMS140. pMS140 was digested with *Eco*RI and *Sac*I, and the 2,448-bp fragment encompassing the *spoIIGB-spoIIIG* intergenic region and 2,000 bp of the *lacZ* gene was cloned between the same sites of the *amyE* integrational vector pSN32 (38), yielding pMS141. MB24 and JOB44 were both transformed with pMS141 to chloramphenicol resistance (Cm^r), and Cm^r $AmyE^-$ transformants were identified and named AH5008 and AH5009, respectively (Table 1).

Fusions of *spoIIIJ* to different sporulation promoters. To insert a copy of the *spoIIIJ* gene under the control of its promoter at the nonessential *amyE* locus, we first used PCR amplification with *Taq* DNA polymerase and primers RNPA-75 and *spoIIIJ*-150R (Table 2) to produce a DNA fragment extending from nucleotide 421 upstream of the *spoIIIJ* translational start codon to nucleotide 144 downstream of the end of the *spoIIIJ* gene. This fragment was cloned into pCR2.1 TOPO (Invitrogen), creating plasmid pJO46. An *Eco*RI restriction fragment containing the *spoIIIJ* gene from pJO46 was subcloned into the *Eco*RI restriction site of pDG364 (10) to create pJO49. Fusions of *spoIIIJ* to the *spoIIQ* and *spoIID* promoters were constructed by the method of splicing by overlay extension (SOE) (22). The *spoIIQ* and *spoIID* promoters included regions from −322 to +14 and −464 to +31 relative to the transcriptional start site, respectively. Initially, the *spoIIIJ* and promoter sequences were amplified separately from chromosomal DNA of MB24 (Table 1). The following primers were used (Table 2): for *spoIIIJ*, *spoIIIJ-spoIIQ* or *spoIIIJ-spoIID* and *spoIIIJ*-1039R; for *spoIIQ*, *spoIIQ*-152D and *spoIIQ*-500R; and for *spoIID*, *spoIID*-1D and *spoIID*-

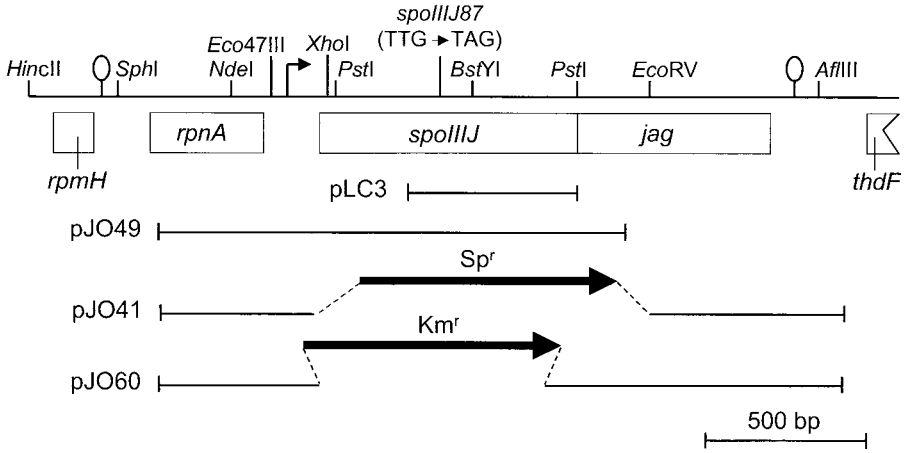

FIG. 1. Genetic organization of the *spoIIIJ-jag* locus of *B. subtilis.* A partial restriction map and the genetic organization of the *spoIIIJ* region are shown. The boxes below the line representing a partial restriction map of the region indicate the extents of the different cistrons in the region (30), all of which are transcribed from left to right. The stem-and-loop structures upstream of *rpnA* and downstream of *jag* indicate the position of possible transcription terminators. The position of the *spoIIIJ* promoter is shown by a horizontal arrow. The position of the mutation present in the *spoIIIJ87* allele (L138STOP) is indicated. The lines below the restriction map represent DNA fragments present in the indicated plasmids.

500R. The 839-bp *spoIIIJ* fragment was mixed with the 350-bp *spoIIQ* fragment or with the 500-bp *spoIID* fragment, and the resulting fragments of 1,189 or 1,339 bp were amplified with primers *spoIIQ*-152D and *spoIIIJ*-1039R, to create a P_{spoIIQ}-*spoIIIJ* fusion, or with primers *spoIID*-1D and *spoIIIJ*-1039R, to create a P_{spoIID}-*spoIIIJ* fusion. The P_{spoIIQ}-*spoIIIJ* and P_{spoIID}-*spoIIIJ* fragments were digested with *Eco*RI and *Bam*HI and ligated to pDG364 (10) digested with the same enzymes, to yield pMS189 and pMS190, respectively. JOB44 was transformed with pJO49, pMS189, and pMS190 to produce the Cm^r $AmyE^-$ strains AH5011, AH5012, and AH5013, respectively (Table 1). Derivatives of these strains bearing an *sspE-lacZ* fusion were obtained by transformation with chromosomal DNA from AH1042 (Table 1). To construct a P_{spoIID}-*spoIIIJ* fusion in a multicopy vector, we isolated a 1,339-bp *Eco*RI-to-*Bam*HI fragment from pMS190 encompassing the P_{spoIID}-*spoIIIJ* fusion. The 1,339-bp fragment was introduced between the same sites of pMK3 (54), yielding pMS210. Plasmids pMK3 and pMS210 were introduced in both MB24 and JOB20 (Table 1).

Construction of a *spoIIIJ-gfp* fusion. We used the SOE technique (see above) to construct a fusion of *spoIIIJ* to *gfp*. Initially, the *spoIIIJ* and *gfp* sequences were amplified separately, from chromosomal DNA of a wild-type *B. subtilis* strain and from pEA18 (a gift from Alan Grossman), respectively. The following primer pairs were used (Table 2): for *spoIIIJ*, *spoIIIJ*-460D and *gfp-spoIIIJ*-R; and for *gfp*, *gfp*-30D and *gfp*-R. The 551-bp *spoIIIJ* fragment and the 772-bp *gfp* fragment were purified, mixed together, and amplified with primers *spoIIIJ*-460D and *gfp*-R (see above). This produced a 1,245-bp *spoIIIJ-gfp* fragment which was cloned into pCR2.1 TOPO (Invitrogen) to yield pLC1. The presence and orientation of the insert were confirmed by restriction analysis. An Sp^r cassette was released from pAH256 (20) with *Eco*RV and *Xho*I and cloned in pLC1 that had been cut with the same restriction enzymes. The resulting plasmid, pLC3, was used to transform strains AH5011, AH5012, and AH5013; Sp^r transformants that were the result of a single reciprocal crossover event (Campbell-type mechanism) were identified and designated AH5017, AH5018, and AH5019, respectively (Table 1).

Fusions of *spoIIIA* to different sporulation promoters. Fusions of *spoIIIA* to the *spoIIQ* and *spoIID* promoters were constructed by SOE (see above). Initially, the *spoIIIA* and promoter sequences were amplified separately from chromosomal DNA of a wild-type *B. subtilis* strain. The following primers were used (Table 2): for *spoIIIA*, *spoIIIA-spoIIQ*, *spoIIIA-spoIID*, and *spoIIIA*-5004D; for *spoIIQ*, *spoIIQ*-152D and *spoIIQ*-500R; and for *spoIID*, *spoIID*-1D, and *spoIID*-500R. The 386-bp *spoIIIA* fragment was mixed with the 350-bp *spoIIQ* fragment or with the 500-bp *spoIID* fragment, and the mixture was subjected to PCR amplification with primers *spoIIQ*-152D and *spoIIIA*-5004D for *spoIIQ* or primers *spoIID*-1D and *spoIIIA*-5004D for *spoIID*. The resulting P_{spoIIQ}-*spoIIIA* and P_{spoIID}-*spoIIIA* fragments were digested with *Bam*HI and introduced into *Bam*HI- and *Sma*I-digested pUS19 (5), to yield pMS155 and pMS198. Transformation of MB24 with pMS155 or pMS198 (with selection for Sp^r cells) produced strains AH5020 and AH5029, respectively (Table 1). In both strains the Campbell-type integration of pMS155 or pMS198 separated *spoIIIAA* from its native promoter and placed the *spoIIIA* operon under the control of the *spoIIQ* or *spoIID* promoter, respectively. Correct integration was confirmed by PCR.

Overproduction and purification of σ^G. *The spoIIIG* coding region was PCR amplified with primers *spoIIIG*-392D and *spoIIIG*-1217R (Table 2). The PCR product was digested with *Bgl*II and *Xho*I and inserted between the *Bam*HI and *Xho*I sites of pET30c(+) (Novagen), creating pMS112, in which a His_6 tag was introduced between the first and second codons of *spoIIIG*. Plasmid pMS112 was introduced into competent cells of BL21(DE3)/pLysS (Novagen). Growth, induction, and lysate preparation were essentially as described previously (50). The His_{6}-σ^G fusion protein was partially purified on Hi-Trap chelating columns in the presence of 8 M urea as described by the manufacturer (Amersham Pharmacia Biotech) and used to raise a polyclonal anti-σ^G antibody in rabbits (Eurogentec, Herstal, Belgium).

Immunoblotting. Preparation of *B. subtilis* whole-cell lysates and Western blot analysis were essentially as described previously (50). The anti-σ^G polyclonal antiserum was incubated with the membranes at a dilution of 1:1,000 in phosphate-buffered saline (8 mM sodium phosphate [pH 7.5], 150 mM NaCl) containing 0.5% low-fat milk. Incubation with an anti-rabbit secondary antibody conjugated to horseradish peroxidase (from Sigma) was for 30 min at a 1:5,000 dilution. A rabbit anti-green fluorescent protein (anti-GFP) antiserum (Clontech) was used according to the manufacturer's protocol.

β-Galactosidase assays. β-Galactosidase activity was determined with the substrate *o*-nitrophenol-β-D-galactopyranoside, and enzyme activity was expressed in Miller units as described previously (20, 21).

Transmission electron microscopy. Samples (5 ml) of DSM cultures of various strains were collected 12 h after the onset of sporulation. The cells were fixed for 2 h with 2.5% glutaraldehyde in 0.1 M cocadylate buffer (pH 7.4) on ice. Samples were washed twice with 0.1 M cocadylate buffer (pH 7.4) and then were treated with 1% osmium tetroxide solution [1.5% $K_4Fe(CN)_6$, 1% OsO_4, 0.1 M cocadylate buffer]. The cells were dehydrated at room temperature by washing with increasing concentrations of ethanol (up to 100%), followed by a wash with acetone. The cells were embedded in EM bed-812 resin (Electron Microscopy Sciences). Ultrathin sections were processed for observation as described previously (21). Transmission electron microscopy was performed on a Hitachi H7500 transmission electron microscope operated at 75 keV.

Light microscopy and image processing. Samples (0.5 ml) of DSM cultures were collected throughout growth and sporulation and resuspended in the same volume of phosphate-buffered saline supplemented with DAPI (4′,6′-diamidino-2-phenylindole) (10 μg/ml). Microscope slides were prepared by using an adaptation of the method developed by van Helvoort and Woldringh (58). Images were acquired on a scientific-grade cooled charge-coupled device (Cooke Co.) on a multiwavelength wide-field three-dimensional microscopy system (63x/1.4 OIL Plan Apochromat objective, Zeiss 100 M; Intelligent Imaging Innovation). Samples were imaged in successive 0.2-μm focal planes, and out-of-focus light was removed with a constrained iterative deconvolution algorithm. Standard filters for fluorescein isothiocyanate (for GFP) and DAPI were used. The pattern of nucleoid staining with DAPI was used to as an indication of the sporulation stage. Just after formation of the asymmetric septum, the prespore nucleoid appears highly condensed, whereas soon after conclusion of the engulfment process, the mother cell and prespore nucleoids appear equally condensed (19, 51).

RESULTS

σ^G accumulates but is mostly inactive in a *spoIIIJ* mutant. *spoIIIJ* is the first gene of a bicistronic operon (16) (Fig. 1). Mutations in the *spoIIIJ* locus block sporulation after completion of prespore engulfment and abolish σ^G activity (16). Errington et al. have examined the effect on sporulation of the *spoIIIJ87* allele (16), which we found to be a nonsense mutation at codon 138 of SpoIIIJ (Fig. 1). Those authors have also studied the effect of a *spoIIIJ* insertional mutation on sporulation-specific gene expression and found that neither allele impaired transcription of the σ^E-dependent *phoAIII* gene or of the σ^F-dependent *spoIIIG* gene (16). However, both *spoIIIJ87* and the insertional mutation prevented expression of the σ^G-dependent *spoVA* operon (16). Disruption of the second gene of the *spoIIIJ* operon, *jag*, did not affect either growth or sporulation (16). However, because both types of *spoIIIJ* mutants could have a polar effect on the expression of the *jag* gene, the sporulation phenotype could be caused by the lack of expression of the two genes. Here, we have used a nonpolar mutation in *spoIIIJ* (Δ*spoIIIJ*::*km*; strain JOB44) (Table 1), which permits expression of the *jag* gene, as verified by reverse transcription-PCR (not shown), to reexamine the individual contribution of this gene to sporulation. In agreement with the results of Errington and coauthors (16), we found that inactivation of *spoIIIJ* has little effect on the transcription of genes expressed in the mother cell (such as *spoIID*) or in the prespore (such as *spoIIQ*) prior to the completion of engulfment (not shown). In contrast, and as also found by Errington and coauthors (16), the Δ*spoIIIJ*::*km* mutation reduced the rate of *spoIIIG-lacZ* expression (Fig. 2A [Fig. 2A is included to allow direct comparison with the immunoblot analysis of σ^G accumulation; see below]). The Δ*spoIIIJ*::*km* mutation also greatly reduced transcription of the σ^G-dependent gene *sspE* (34) (data not shown). The Δ*spoIIIJ*::*sp* mutant JOB20 (Table 1) behaved like JOB44 (Δ*spoIIIJ*::*km*) with respect to *spoIIIG*- and *sspE-lacZ* expression (not shown).

Since substantial expression of a *spoIIIG-lacZ* fusion occurs in a *spoIIIJ* mutant (16) (Fig. 2A), it is likely that SpoIIIJ acts

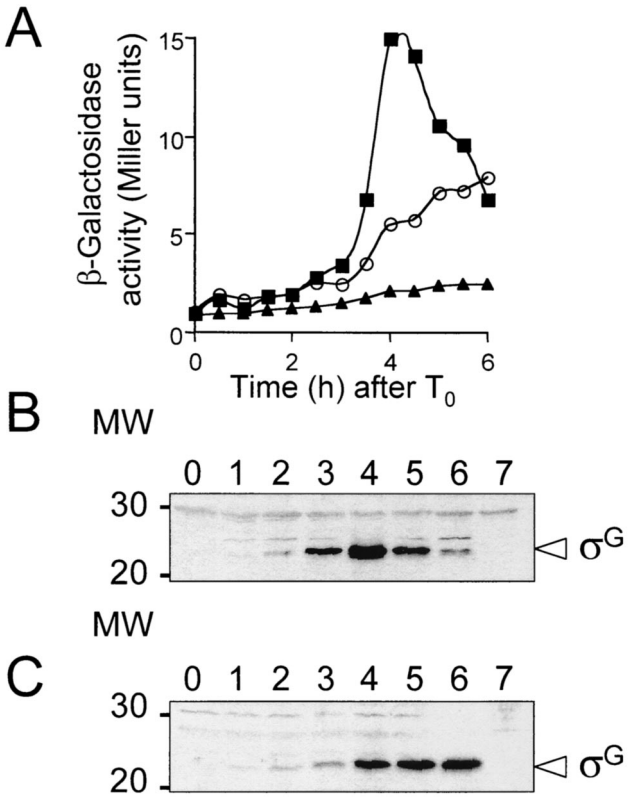

FIG. 2. Effect of a nonpolar *spoIIIJ* mutation on the expression of *spoIIIG-lacZ* and immunoblot analysis of σ^G accumulation in the *spoIIIJ* mutant. (A) Expression of a *spoIIIG-lacZ* fusion was monitored at the indicated times after the onset of sporulation in the *spoIIIJ* mutant, JOB44 (circles) or in the wild-type strain, MB24 (squares). Endogeneous levels of β-galactosidase activity were determined in the wild-type strain MB24 (triangles). Enzyme activity was measured in Miller units. T_0 indicates the end of the exponential phase of growth, defined as the onset of sporulation. (B and C) Immunoblot analysis of σ^G accumulation in DSM in the wild-type strain MB24 (B) or in the *spoIIIJ::km* mutant JOB44 (C). Samples from sporulating cultures were collected at the onset of sporulation in DSM (lanes 0) and at hourly intervals thereafter, as indicated. Proteins (30 μg) in each sample were subjected to immunoblot analysis with an anti-σ^G rabbit polyclonal antibody (see Materials and Methods). Lanes 7, 30 μg of an extract prepared from a culture of a *spoIIIG* deletion mutant (AH45 [Table 1]) at h 4 of sporulation. The arrowhead indicates the position of σ^G; other bands represent nonspecific cross-reactive material. The positions of molecular weight (MW) markers are shown on the left.

at the level of σ^G accumulation or activity. To distinguish between these two possibilities, we used an antiserum directed against σ^G to monitor its accumulation during sporulation in the wild type and in the Δ*spoIIIJ*::*km* mutant (JOB44) (Table 1). This σ^G antiserum detected a species of 35 kDa in immunoblot analysis of extracts prepared from sporulating wild-type cultures of *B. subtilis* but not from cultures of a *spoIIIG* deletion mutant (Fig. 2B and C, lanes 7) (26). In the wild-type strain, MB24, σ^G was first detected at 2 h after the onset of sporulation, and its levels increased during h 3 and 4 (Fig. 2B). In the *spoIIIJ* mutant, σ^G accumulated to nearly wild-type levels, although at h 3 the level of σ^G was reduced about threefold compared to that in the wild type (Fig. 2C). The pattern of σ^G accumulation in the *spoIIIJ* mutant probably reflects the decreased rate of *spoIIIG* transcription (Fig. 2). The somewhat reduced levels of *spoIIIG* transcription and σ^G accumulation in the *spoIIIJ* mutant are probably a result of the autoregulatory effect that σ^G exerts on transcription of its encoding gene (55). We conclude that the failure to detect expression of σ^G-dependent genes in the *spoIIIJ* mutant (16; this work) results from the inactivity of σ^G.

The E155K form of σ^G no longer requires SpoIIIJ for activation. In a *spoIIIA* mutant, σ^G also accumulates in an inactive form (26). Activity of σ^G can be restored to *spoIIIA* mutant cells by the production of a form of σ^G (σ^{GE155K}) that is not efficiently bound by SpoIIAB in vitro (26). To test whether σ^{GE155K} could also bypass the need for *spoIIIJ* expression for σ^G activity, we introduced the wild-type or *spoIIIGE155K* allele of *spoIIIG* (the later encoding σ^{GE155K}) linked to a Sp^r marker into a strain bearing an *sspE-lacZ* fusion and either Δ*spoIIIJ*::*km* (JOB34 [Table 1]) or the wild-type *spoIIIJ* allele (AH1042 [Table 1]). Strains EUE9537 and EUE9538 carry Campbell-type insertions at the 3′ end of the *spoIIIG* gene that recreate either the wild-type *spoIIIG* sequence or the *spoIIIGE155K* allele, respectively, and have been described before (26). JOB34 or AH1042 was transformed with chromosomal DNA from EUE9537 or EUE9538, and appropriate transformants were designated AH5030 (*spoIIIG* wild type), AH5031 (*spoIIIGE155K*), AH5024 (*spoIIIG* wild type/Δ*spoIIIJ*::*km*), and AH5025 (*spoIIIGE155K*/Δ*spoIIIJ*::*km*) (Table 1). Strains AH5030 and AH5024 were constructed in parallel to control for any expression differences due to strain construction. The activity of σ^G was then monitored during sporulation by using the *sspE-lacZ* reporter fusion. We found the profile of *sspE-lacZ* expression in AH5024 (*spoIIIG* wild type/Δ*spoIIIJ*::*km*) to be very similar to that of the same fusion in the Δ*spoIIIJ*::*km* mutant JOB34) (Fig. 3). Strains AH5030 (*spoIIIG* wild type) and AH5031 (*spoIIIGE155K*) showed almost identical profiles of *sspE-lacZ* expression, which seemed to attain a maximum (about 40 Miller units) between h 10 and 12 of sporulation (26) (Fig. 3). Expression of *sspE-lacZ* in strain AH5025 (*spoIIIGE155K*/Δ*spoIIIJ*::*km*) commenced at the same time as in AH5030 and AH5031 and again seemed to reach a maximum between h 10 and 12 of sporulation (Fig. 3). Expression of *sspE-lacZ* in AH5025 was greatly reduced compared to that in a wild-type strain such as AH1042 (Table 1; see Fig. 5). However, maximum levels of β-galactosidase production in AH5025 showed a reduction of only about 50% compared to strain AH5030 or AH5031 (Fig. 3), both of which sporulate with wild-type efficiency (26) (Table 3). We also found that the *spoIIIGE155K* allele permitted expression of a *gerE-lacZ* fusion in the Δ*spoIIIJ*::*km* mutant or in cells carrying the *spoIIIA*::Tn*917*Ω*HU24* allele, the same used in the study of Kellner et al. (26) (data not shown). *gerE-lacZ* served as a reporter for σ^K-dependent transcriptional activity (9), which requires σ^G function (reviewed in references 29, 32, 42, and 46). We conclude that the absence of σ^G activity in the *spoIIIJ* mutant is partially relieved by the *spoIIIGE155K* allele (Fig. 3). Because the E155K substitution in σ^G, which reduces its affinity for SpoIIAB, partially bypasses the requirement for the *spoIIIA*-encoded products (26) and for *spoIIIJ* for σ^G activity, we suggest that both loci are required after completion of the engulfment process to antagonize the inhibition exerted by SpoIIAB upon σ^G. However, even though the *spoIIIGE155K* allele restores activity of σ^G and of σ^K to *spoIIIJ* mutant cells,

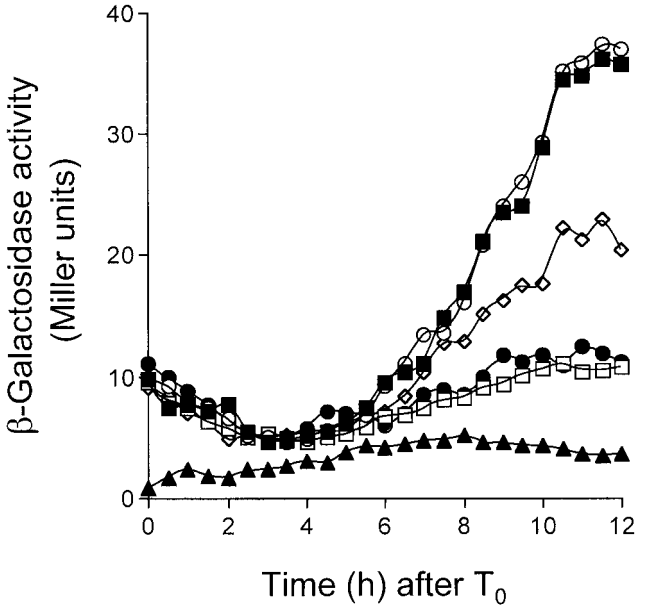

FIG. 3. The *spoIIIGE155K* allele partially bypasses the need for *spoIIIJ* expression for σ^G activity. Expression of *sspE-lacZ* was monitored during sporulation in the following strains (Table 1): AH5030 (*spoIIIG*::pEK15) (closed squares), AH5031 (*spoIIIG*::pEK16) (open circles), AH5024 (*spoIIIG*::pEK15 *spoIIIJ*::*km*) (open squares), AH5025 (*spoIIIG*::pEK16 *spoIIIJ*::*km*) (open diamonds), and JOB34 (*spoIIIJ*::*km*) (closed circles). Note that integration of pEK15 into the *spoIIIG* locus recreates a wild-type *spoIIIG* sequence, whereas integration of pEK16 results in expression of the *spoIIIGE155K* allele (see Materials and Methods). Strains were grown in DSM, and samples were taken at the indicated times after the onset of sporulation (T_0) and assayed for β-galactosidase activity. The endogenous levels of β-galactosidase production were determined in the wild-type strain MB24 (closed triangles). Enzyme activity is indicated in Miller units (see Materials and Methods).

the *spoIIIGE155K*/Δ*spoIIIJ*::*km* double mutant is still unable to sporulate (Table 3). Moreover, electron microscopy observations reveal that, as previously reported for the *spoIIIJ87* mutant (16), in both JOB44 (Δ*spoIIIJ*::*km*) (Table 1) and AH5025 (*spoIIIGE155K*/Δ*spoIIIJ*::*km*) (Table 1) sporulation is arrested soon after completion of the engulfment process (Fig. 4, compare panels B and C with panel A, which depicts a Spo^+ strain). Possibly, the level of activity of σ^G allowed by the *spoIIIGE155K* allele is not sufficient to restore sporulation to a *spoIIIJ* mutant. Alternatively, in addition to promoting activation of σ^G, SpoIIIJ is required in some other way for sporulation.

SpoIIIJ does not appear to encode a pheromone peptide. We also tested whether the SpoIIIJ protein could yield a peptide similar to the one encoded by the *ccfA* locus of *E. faecalis* (2). We assayed the effects on sporulation of double alanine substitutions at three different locations of the lipoprotein signal sequence. Each of these strains produced wild-type levels of heat-resistant spores (not shown). We also made substitutions in the signal sequence of *spoIIIJ* that would be expected to create a peptide identical to the cCF10 peptide from *E. faecalis*. We tested whether cell-free culture supernatants from this *B. subtilis* strain would induce wild-type *E. faecalis* to aggregate, which is a response consistent with the production of the cCF10 peptide (2); however, these supernatant solutions failed to induce clumping of *E. faecalis* (not shown). Moreover these multiple substitutions in the signal sequence of the SpoIIIJ protein had no effect on sporulation by this *B. subtilis* strain. Therefore, we found no evidence that *spoIIIJ* encodes a secreted peptide required for sporulation.

Expression of *spoIIIJ* in the prespore is sufficient for sporulation. *spoIIIJ* is normally expressed during vegetative growth (16), but induction of *spoIIIJ* transcription from the IPTG (isopropyl-β-D-thiogalactopyranoside)-inducible P_{spac} promoter at the onset of sporulation is sufficient for efficient spore formation (39). Because both *spoIIIJ* and the P_{spac}-*spoIIIJ* allele are expressed prior to the asymmetric division of the sporangium, SpoIIIJ could accumulate in the prespore and in the mother cell. To investigate whether expression of *spoIIIJ* is explicitly required in either the mother cell or the prespore, we fused the coding region of *spoIIIJ* to the prespore-specific σ^F-recognized *spoIIQ* promoter (31) or to the mother cell-specific σ^E-dependent *spoIID* promoter (45). The promoter fusions were introduced at the nonessential *amyE* locus, producing strains AH5012 (P_{spoIIQ}-*spoIIIJ*) and AH5013 (P_{spoIID}-*spoIIIJ*) (Table 1). A strain carrying *spoIIIJ* under the control of its native promoter at *amyE* (AH5011 [Table 1]) was also constructed in parallel to control for any differences due to ectopic expression of *spoIIIJ* at *amyE*. Strain AH5011, which expresses *spoIIIJ* at *amyE* under the control of its native promoter, and strain AH5012 (P_{spoIIQ}-*spoIIIJ* at *amyE*) both formed spores with the same efficiency as the wild-type strain MB24 (Table 3). In contrast, although expression of *spoIIIJ* from the *spoIID* promoter significantly increased the frequency of sporulation of the Δ*spoIIIJ*::*km* mutant (JOB44), from 1×10^2 to 5.9×10^6 (Table 3), it did not support wild-type levels of sporulation (AH5013) (Table 3). Electron microscopy revealed that strains AH5011 and AH5012 completed morphogenesis of the spore protective layers (Fig. 4A and D), which relies on the activities of σ^G and σ^K (29, 32). In contrast, strain AH5013 was

TABLE 3. Influence of various mutations on efficiency of sporulation

Strain	Genotype	Sporulation (CFU/ml)[a]		% Sporulation
		Viable cells	Heat-resistant cells	
MB24	Wild type	5.9×10^8	3.5×10^8	59.0
JOB44	*spoIIIJ*::*km*	1.8×10^8	1.0×10^2	<0.001
JOB20	*spoIIIJ*::*sp*	3.0×10^8	1.9×10^3	<0.001
AH5030	*spoIIIGwt*[b]	5.4×10^8	1.6×10^8	30.0
AH5031	*spoIIIGE155K*	9.0×10^8	2.9×10^8	32.0
AH5024	*spoIIIJ spoIIIGwt*	3.5×10^8	1.8×10^3	<0.001
AH5025	*spoIIIJ spoIIIGE155K*	3.4×10^8	2.9×10^3	<0.001
AH5011	*spoIIIJ* $P_{spoIIIJ}$-*spoIIIJ*	5.5×10^8	2.2×10^8	40.0
AH5012	*spoIIIJ* P_{spoIIQ}-*spoIIIJ*	4.3×10^8	2.6×10^8	61.0
AH5013	*spoIIIJ* P_{spoIID}-*spoIIIJ*	1.2×10^8	5.9×10^6	5.0
AH2550	Wild type(pMK3)	7.9×10^8	4.5×10^8	57.0
AH5037	Wild type(pMS210)	1.5×10^8	6.0×10^6	4.0
AH5035	*spoIIIJ*(pMK3)	1.8×10^8	5.0×10^3	0.003
AH5036	*spoIIIJ*(pMS210)	1.5×10^8	5.8×10^6	3.9
AH62	*spoIIIAΩHU24*	2.3×10^7	9.0×10^2	<0.001
AH5020	P_{spoIIQ}-*spoIIIA*	3.2×10^8	9.0×10^3	0.003
AH5029	P_{spoIID}-*spoIIIA*	5.5×10^8	3.1×10^8	56.0

[a] Sporulation was measured 24 h after the onset of the process in liquid sporulation medium as described in Materials and Methods.
[b] *spoIIIGwt*, wild-type *spoIIIG*.

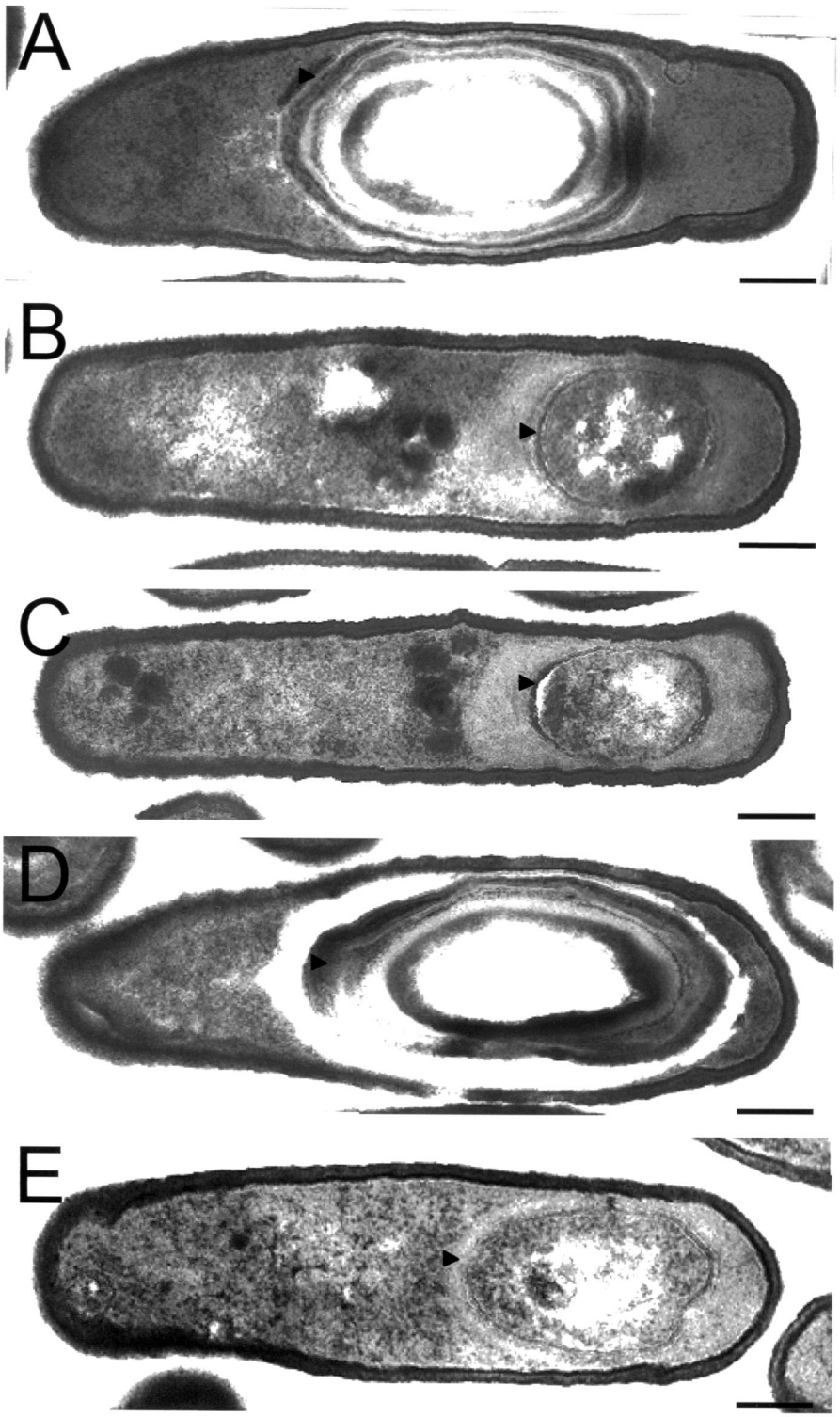

FIG. 4. Electron microscopy of the wild type and the *spoIIIJ* mutants. The strains were grown in DSM, and samples were taken 12 h after the onset of sporulation and processed for electron microscopy analysis as described in Materials and Methods. (A) Strain AH5011 (*spoIIIJ* expressed under the control of the *spoIIIJ* promoter at *amyE*); (B) JOB44 (*spoIIIJ*::*km*); (C) AH5025 (*spoIIIJ*::*km*/*spoIIIGE155K*); (D) AH5012 (*spoIIIJ* expressed under the control of the *spoIIQ* promoter at *amyE*); (E) AH5013 (*spoIIIJ* expressed under the control of the *spoIID* promoter at *amyE*). Strain AH5011 ($P_{spoIIIJ}$-*spoIIIJ*) is shown instead of the wild-type MB24 to allow comparison with strains expressing *spoIIIJ* from the *spoIID* or *spoIIIJ* promoter. AH5011 sporulates with wild-type incidence (Table 3) and is indistinguishable from MB24 by electron microscopy (not shown). Both AH5011 (A) and AH5012 (D) form free mature spores like the wild-type MB24 but are represented at a stage prior to lysis of the mother cell to facilitate comparison with the sporulation mutants in panels B, C, and E. Strain JOB44 (B) is blocked just after completion of the engulfment process, as previously reported for the *spoIIIJ87* mutant (16). AH5025 (C) and AH5013 (E) are also blocked at the engulfment stage of sporulation. The arrowheads indicate the protective layers that surround the spore (A and D) or the prespore membranes (B, C, and E). Bars, 0.2 μm.

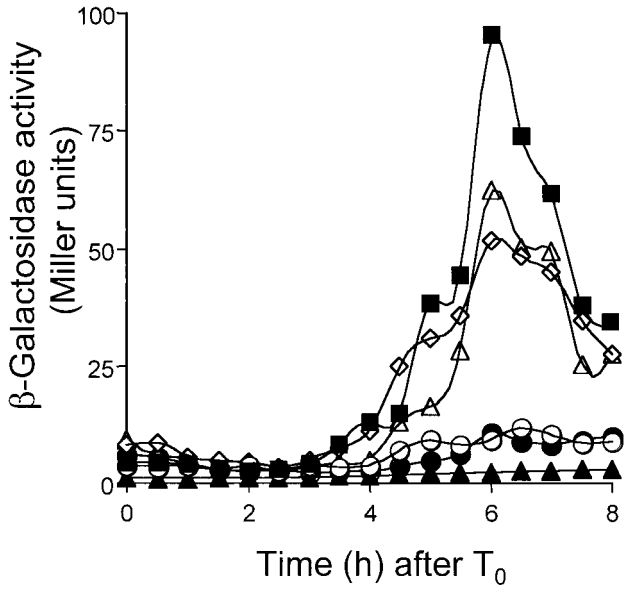

FIG. 5. Effect of the compartmentalization *of spoIIIJ* expression on σ^G-dependent gene expression. An *sspE-lacZ* gene was introduced into the *spoIIIJ* mutant JOB44, into the wild-type strain MB24, and into the strains expressing *spoIIIJ* under the control of its native promoter, the *spoIIQ* promoter, or the *spoIID* promoter. The resulting strains (Table 1; see below) were grown in DSM, and samples were taken at 30-min intervals after the onset of sporulation (T_0) and assayed for β-galactosidase activity. Closed squares, expression in the wild-type strain (AH1042); open circles, expression in the *spoIIIJ::km* mutant (JOB34); open diamonds, expression in the $P_{spoIIIJ}$-*spoIIIJ* strain (AH5014); open triangles, expression in the P_{spoIIQ}-*spoIIIJ* strain (AH5015); closed circles, expression in the P_{spoIID}-*spoIIIJ* strain (AH5016). Endogeneous levels of β-galactosidase production were determined in the wild-type strain MB24 (closed triangles). Enzyme activity is expressed in Miller units (see Materials and Methods).

blocked soon after completion of the engulfment process and was indistinguishable from a *spoIIIJ* null mutant (Fig. 4B and E). In addition, the results in Fig. 5 show that *sspE-lacZ* was expressed in strains bearing the $P_{spoIIIJ}$-*spoIIIJ* allele at *amyE* (AH5014 [Table 1]) or the P_{spoIIQ}-*spoIIIJ* fusion (AH5015 [Table 1]) but not the P_{spoIID}-*spoIIIJ* fusion (AH5016 [Table 1]), indicating that σ^G was not active in this last strain. Failure of the P_{spoIID}-*spoIIIJ* fusion to support efficient sporulation may result from low levels of transcription from the *spoIID* promoter (see below). However, we think that this is unlikely to be the cause of the sporulation phenotype of AH5013. First, translational fusions of the *spoIID* or *spoIIQ* promoters to *lacZ* give rise to similar levels of β-galactosidase activity (52), and even though we used a shorter *spoIID* promoter fragment (see Materials and Methods), it carries all sequences known to be required for expression of *spoIID* (45). In addition, a *spoIID-lacZ* transcriptional fusion is expressed at higher levels than a *spoIIQ-lacZ* transcriptional fusion in either wild-type or *spoIIIJ* mutant cells (not shown). We also introduced a replicative plasmid carrying the P_{spoIID}-*spoIIIJ* fusion (pMS210) into the Δ*spoIIIJ*::*sp* mutant JOB20 (Table 1). JOB20 behaves like the Δ*spoIIIJ*::*km* mutant with respect to expression of *spoIIIJ* at *amyE* from the *spoIIIJ*, *spoIID*, or *spoIIQ* promoter (not shown). The resulting strain (AH5036 [Table 1]) sporulated at the same level as AH5013 (5.9×10^6 spores/ml) (Table 3). The vector used (pMK3 [54]) did not interfere with sporulation or change the spore titer of a *spoIIIJ* mutant (Table 3). However, we found that pMS210 interfered with sporulation of the wild-type strain MB24 (strain AH5037) (Table 3). Strain AH5037 (multicopy P_{spoIID}-*spoIIIJ*) sporulated at the same level as AH5013 (Δ*spoIIIJ::km*/P_{spoIID}-*spoIIIJ* in single copy) (Table 3). It therefore seems that failure of the P_{spoIID}-*spoIIIJ* allele to complement a *spoIIIJ* null mutation is not due to reduced expression of *spoIIIJ*. Possibly, synthesis of SpoIIIJ is not well tolerated in the mother cell. In any case, our results show that expression of *spoIIIJ* in the prespore is sufficient for σ^G activation and for efficient sporulation.

SpoIIIJ-GFP may be degraded in the mother cell. When produced in the mother cell, SpoIIIJ may not localize around the prespore, or if it does, then localization around the prespore is not sufficient for function. To investigate the ability of SpoIIIJ-GFP to localize around the prespore when produced in the mother cell, we fused GFP to the C terminus of SpoIIIJ in cells that express *spoIIIJ* in single copy at *amyE* from the *spoIID* promoter. In addition, we examined the localization of SpoIIIJ-GFP when expressed under the control of its native promoter or from the *spoIIQ* promoter. In parallel we monitored the accumulation of the SpoIIIJ-GFP fusion protein with an anti-GFP antibody (see Materials and Methods). Strains AH5011, AH5012 and AH5013 (Table 1; see above) were transformed with pLC3 (Fig. 1). Because all three strains bear a deletion of the *spoIIIJ* locus, transformants were readily obtained that resulted of Campbell-type integration of pLC3 at the *amyE* region, a recombinational event that fused *spoIIIJ* to *gfp* (see Material and Methods). Introduction of the SpoIIIJ-GFP fusion into AH5011 produced a Spo^+ strain (AH5017 [Table 1]). Thus, as also found by Murakami et al. (39), the activity of SpoIIIJ does not seems to be inhibited or altered by its fusion to GFP. In strain AH5017, SpoIIIJ-GFP was found associated with the cell membrane and the division septum during vegetative growth (data not shown) and at the onset of sporulation (T_0) (Fig. 6A, panel a) (39). Also in confirmation of the observations of Murakami et al. (39), at h 2 of sporulation SpoIIIJ-GFP localized to the asymmetric septum region and around the prespore (Fig. 6A, panel b), and at h 4 the fusion protein was found surrounding the developing spore (Fig. 6A, panel c). Decoration of the cell membrane was also observed at h 2 and persisted at h 4 of sporulation (Fig. 6A, panel c). In AH5017 ($P_{spoIIIJ}$-*spoIIIJ-gfp*), SpoIIIJ-GFP accumulated during growth, and its cellular level seemed to increase at about h 2 of sporulation (Fig. 6B). When synthesized in the prespore from the *spoIIQ* promoter (strain AH5018 [Table 1]), SpoIIIJ-GFP decoration of the asymmetric septum region was first detected 2 h after the onset of sporulation (Fig. 6A, panels d and e), and by h 4 of sporulation SpoIIIJ-GFP was mostly found in a ring-like pattern around the prespore (Fig. 6A, panel f). The results of immunoblot analysis reveal that SpoIIIJ-GFP accumulated from h 2 of sporulation onwards and that its cellular level reached a maximum at around h 4 (Fig. 6B, strain AH5018). This pattern of accumulation coincides with the temporal pattern of expression of a *spoIIQ-lacZ* fusion (31) (data not shown).

When synthesized in the mother cell under the control of the *spoIID* promoter (strain AH5019 [Table 1]), SpoIIIJ-GFP was again first detected 2 h after the onset of sporulation by fluo-

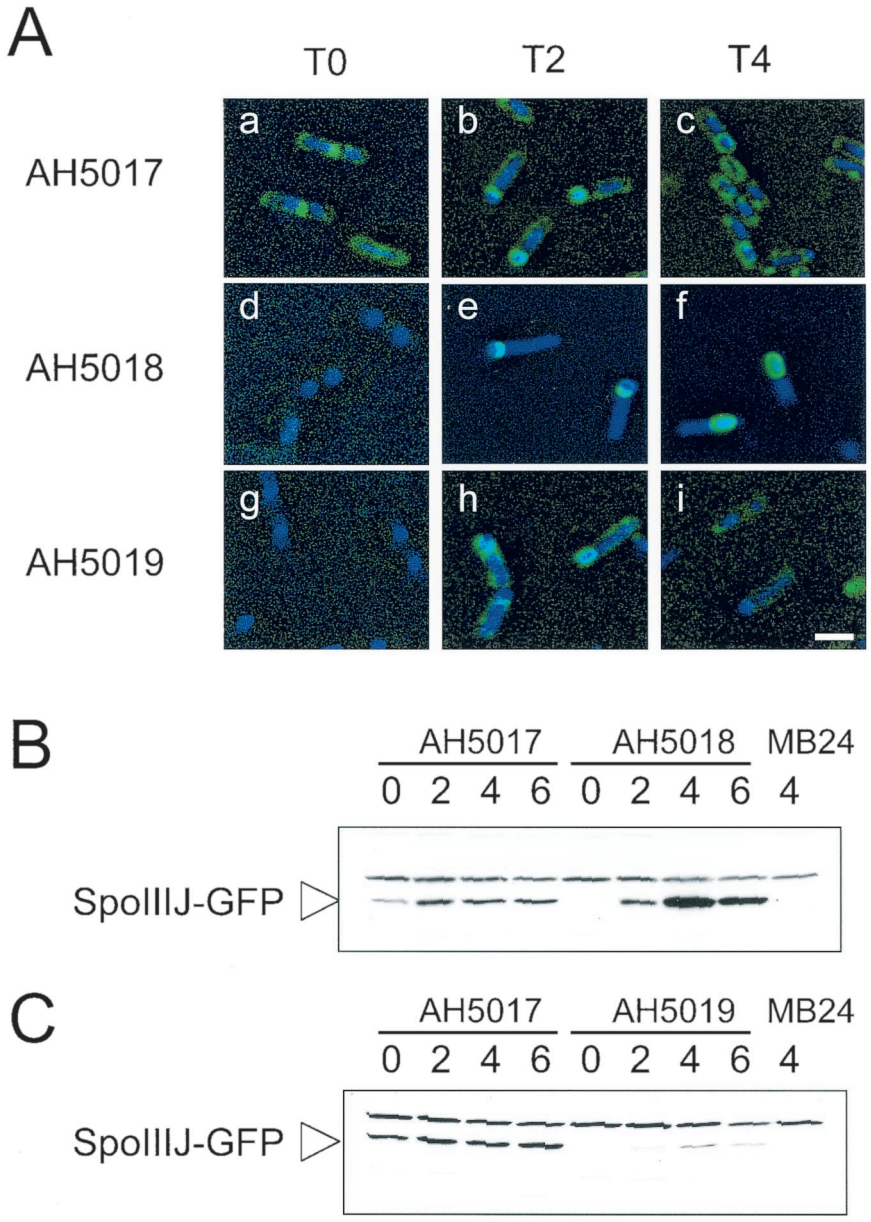

FIG. 6. SpoIIIJ-GFP localization and accumulation. (A) Typical localization patterns of SpoIIIJ-GFP in the various strains at the indicated times. The strains were grown in DSM, and samples were taken throughout sporulation for observation by fluorescence microscopy with the DNA stain DAPI. Strains AH5017 ($P_{spoIIIJ}$-*spoIIIJ-gfp*) (panels a to c), AH5018 (P_{spoIIQ}-*spoIIIJ-gfp*) (panels d to f), and AH5019 (P_{spoIID}-*spoIIIJ-gfp*) (panels g to i) were observed at the onset of sporulation (panels a, d, and g), at T_2 (panels b, e, and h), and at T_4 (panels c, f, and i). Bar, 2 mm. (B and C) Immunoblot analysis of SpoIIIJ-GFP accumulation in DSM in strains AH5017 ($P_{spoIIIJ}$-*spoIIIJ-gfp*) and AH5018 (P_{spoIIQ}-*spoIIIJ-gfp*) (B) and in strains AH5017 ($P_{spoIIIJ}$-*spoIIIJ-gfp*) and AH5019 (P_{spoIID}-*spoIIIJ-gfp*) (C). Results for strain AH5017 are repeated in panels B and C to allow direct comparison. Samples were collected at the end of the exponential phase of growth in DSM, which was defined as the onset of sporulation (lanes 0) and at 2, 4, and 6 h thereafter. Proteins (30 μg) were electrophoretically resolved on sodium dodecyl sulfate-polyacrylamide gels and transferred to nitrocellulose membranes, and GFP was detected with a polyclonal rabbit antibody (see Materials and Methods). The arrowhead indicates the position of the SpoIIIJ-GFP fusion protein. Another band with slower migration that is also found in MB24 represents a cross-reactive species. The last lane in panels B and C contains 30 μg of an extract prepared from a culture of the wild-type strain (MB24) at h 4 of sporulation in DSM.

rescence microscopy (Fig. 6A, panels g and h) and by immunoblot analysis (Fig. 6C). At this time in spore development, the fusion protein decorated the cell membrane region and the septal region, and it also tended to localize around the prespore (Fig. 6A, panels g and h). Although the immunoblot analysis of AH5019 reveals that SpoIIIJ-GFP accumulated to reduced levels compared to the strain (AH5017) expressing the $P_{spoIIIJ}$-*spoIIIJ-gfp* fusion at *amyE* (Fig. 6C), the intensities of the fluorescence signal in the cell membrane region, the septal region, or around the prespore were equivalent in the two strains (Fig. 6A, compare panels b and h). Moreover, by h 2 of sporulation, about 22% of the cells in AH5017 or AH5019

showed condensed prespore chromosomes (>100 cells counted), as judged from the analysis of DAPI-stained images (see Materials and Methods). These cells are presumed to have completed the asymmetric division of the sporangium. Of these, about 80% in either AH5017 ($P_{spoIIIJ}$-*spoIIIJ-gfp*) or AH5019 (P_{spoIID}-*spoIIIJ-gfp*) showed decoration of the septum or prespore regions by SpoIIIJ-GFP (see also below). It appears that the P_{spoIID}-*spoIIIJ-gfp* allele results in sufficient product to decorate the prespore region in most of the sporulating cells at h 2 of sporulation. About 17% of the cells (over 100 cells counted) of both AH5017 or AH5019 appeared to have completed the engulfment process by h 4 of sporulation, based on the analysis of DAPI images (see Materials and Methods). However, at this time in sporulation, SpoIIIJ-GFP decoration of both the cell membrane and the prespore region was considerably reduced in AH5019 compared to AH5017 (Fig. 6A, compare panels c and i). This reduction in the decoration of the prespore was not accompanied by an increase in cytoplasmic fluorescence, even though the accumulation of SpoIIIJ-GFP as monitored by immunoblot analysis appeared to increase (Fig. 6C). The immunoblot analysis did not reveal signs of proteolysis of SpoIIIJ-GFP (Fig. 6C). Nevertheless, even though a *spoIID-lacZ* fusion appears to be expressed at higher levels in both wild-type and *spoIIIJ* mutant cells than is a *spoIIQ-lacZ* fusion (see above), our P_{spoIID}-*spoIIIJ-gfp* fusion resulted in reduced cellular levels of SpoIIIJ-GFP. The observation that a P_{spoIID}-*spoIIIJ* multicopy allele interfered with sporulation of a wild-type strain (see above) suggests that accumulation of SpoIIIJ in the mother cell is not well tolerated. Together, the results suggest that when produced in the mother cell, the SpoIIIJ-GFP protein may be subjected to degradation.

Expression of spoIIIA in the prespore does not permit activation of σ^G. The results described above suggest that SpoIIIJ and the *spoIIIA*-encoded products may function at the end of the engulfment process to antagonize the action exerted by SpoIIAB upon σ^G. However, while expression of *spoIIIJ* is sufficient in the prespore (this work), earlier studies have shown that the *spoIIIA* operon, which is transcribed from a promoter recognized by the σ^E form of RNA polymerase, is specifically required in the mother cell (23, 24). To determine whether expression of *spoIIIA* in the mother cell is an absolute requirement or whether the *spoIIIA*-encoded products could promote σ^G activation and sporulation if produced only in the prespore, we fused the *spoIIIA* operon to the prespore-specific *spoIIQ* promoter (strain AH5020 [Table 1]). The results in Table 3 indicate that expression of the *spoIIIA* operon from the *spoIIQ* promoter resulted in very poor sporulation (about 10^4 spores/ml). We also found that expression of the *spoIIIA* operon solely from the *spoIIQ* promoter (in strain AH5020, bearing the P_{spoIIQ}-*spoIIIA* allele) did not support expression of the σ^G-dependent *sspE-lacZ* fusion (not shown). Moreover, electron microscopy observations revealed that strain AH5020 is blocked soon after completion of the engulfment sequence (data not shown). In contrast, expression of the *spoIIIA* operon from the similarly expressed mother cell-specific *spoIID* promoter (45, 52) resulted in wild-type levels of sporulation (Table 3). We suggest that at the end of the engulfment process, the *spoIIIA*-encoded products from the mother cell and SpoIIIJ from the prespore are both required to antagonize the inhibitory action imposed by SpoIIAB upon σ^G in the prespore (Fig. 7).

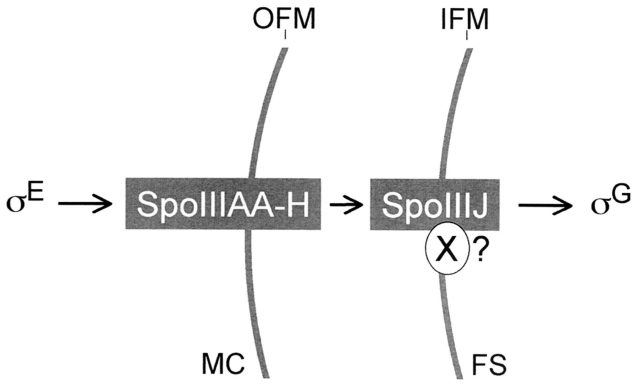

FIG. 7. Model for the signaling pathway that controls σ^G activation. Shown is the proposed sequence of events that originate in the mother cell leading to σ^G activation in the prespore at engulfment. The eight membrane-associated proteins encoded by the *spoIIIA* locus (SpoIIIAA to SpoIIIAH) and SpoIIIJ participate in conveying the signal that activates σ^G. The *spoIIIA*-encoded products act from the mother cell across the space delimited by the outer forespore membrane (OFM) and the inner forespore membrane (IFM). SpoIIIJ in turn acts from the prespore. SpoIIIJ may be required for localizing a prespore-specific component (X) of the *spoIIIA* signaling pathway. The signal conveyed through the *spoIIIA*-encoded proteins and SpoIIIJ results in the activation of σ^G; it appears to serve in part to antagonize the inhibitory action of SpoIIAB upon σ^G, but it may also involve other, as-yet-unidentified factors. MC, mother cell; FS, engulfed prespore.

DISCUSSION

Here we have analyzed the role of *spoIIIJ* in the activation of σ^G. Mutations in *spoIIIJ* arrest development soon after engulfment of the prespore by the mother cell, and greatly diminish the activity of σ^G (16). However, mutations in *spoIIIJ* do not prevent transcription of the *spoIIIG* gene (16; this work), and as shown here they allow the accumulation of σ^G to nearly wild-type levels (Fig. 2). Therefore, most of the σ^G that accumulates in *spoIIIJ* mutant cells is inactive. This is reminiscent of the situation in a *spoIIIA* mutant, in which σ^G also accumulates but is inactive (26). Activation of σ^G at the end of the engulfment process then relies on *spoIIIA*- and *spoIIIJ*-dependent events. The available evidence suggests that the inhibitory action of SpoIIAB is responsible for the absence of σ^G activity in a *spoIIIA* mutant. SpoIIAB tends to disappear from the prespore compartment after conclusion of the engulfment process in a *spoIIIA*-dependent manner (28). More importantly, a single amino acid substitution in σ^G that results in inefficient binding by SpoIIAB in vitro bypasses the requirement for *spoIIIA* expression for σ^G activity (26). This implies that SpoIIIA acts after engulfment of the prespore by the mother cell to antagonize the inhibition of σ^G activity by SpoIIAB (26). We found that expression of the *spoIIIGE155K* allele (encoding the σ^{GE155K} form of σ^G) also allows σ^G activity in the absence of SpoIIIJ (Fig. 3). Therefore, our results suggest that both SpoIIIJ and the *spoIIIA*-encoded products are involved in relieving the inhibition imposed by SpoIIAB upon σ^G in the prespore. The *spoIIIGE155K* mutation does not causes premature expression of *sspE-lacZ* and in that sense does not

uncouple the activity of σ^G from the conclusion of the engulfment sequence (Fig. 3) (26). One possible explanation is that SpoIIAB is important to antagonize σ^G only towards the end of the engulfment sequence and that another, as-yet-unidentified factor contributes to the negative regulation of σ^G activity during the process of engulfment.

We also note that even though the *spoIIIGE155K* allele bypasses the requirement for SpoIIIJ for both σ^G and σ^K activities, it did not restore formation of heat-resistant spores (Fig. 4C; Table 3). Interestingly, the *spoIIIGE155K* allele does not restore sporulation to a *spoIIIA* mutant either (26). There are several possible explanations for these observations. First, because σ^G is autoregulatory (25, 55), a mutation that would make it less susceptible to SpoIIAB could in principle result in increased levels of σ^G activity in the mother cell. However, ectopic activation of σ^G in the mother cell is unlikely to be the cause of the sporulation phenotype of the *spoIIIGE155K*/Δ*spoIIIJ*::*km* double mutant, since a strain carrying the *spoIIIGE155K* allele in an otherwise wild-type background produces heat-resistant spores with wild-type efficiency (26) (not shown). The block in sporulation of the *spoIIIGE155K*/Δ*spoIIIJ*::*km* double mutant could also result from insufficient activity of σ^G, even though peak levels of *sspE-lacZ* expression in the *spoIIIGE155K*/Δ*spoIIIJ*::*km* double mutant are only half of what is found in a strain carrying only the *spoIIIGE155K* allele and which is Spo^+ (Fig. 3). The E155K substitution was introduced in σ^G (26) on the basis of the finding that a glutamic acid-to-lysine substitution was found at an equivalent position (E149K) in a screen for σ^F mutants with reduced affinity for SpoIIAB (11). The structure of a complex between a dimer of the *Bacillus stearothermophilus* SpoIIAB protein and σ^F helps to explain the specificity of SpoIIAB for σ^F and for σ^G (7). In the *B. stearothermophilus* σ^F protein, the residue equivalent to E149 of *B. subtilis* σ^F, as well as three other residues found in genetic screens for mutants resistant to inhibition by SpoIIAB (11), is located within a region that contains 17 amino acids found to interact with SpoIIAB in the crystal structure (7). Of those residues, 15 are either identical or homologous in σ^G and 3 are uniquely conserved between σ^F and σ^G (7). However, the structure also suggests that even though σ^G appears to contact SpoIIAB through the same region that mediates the interaction of σ^F with SpoIIAB, the details of the interaction of σ^G with SpoIIAB may differ (7). It is possible that the E155K substitution makes σ^G less refractory to SpoIIAB than the corresponding mutation (E149K) in σ^F. Lastly, another possibility for the incapacity of *spoIIIGE155K*/*spoIIIA* or *spoIIIGE155K*/Δ*spoIIIJ*::*km* double mutants to sporulate is that in addition to the activation of σ^G, SpoIIIJ and the eight products of the *spoIIIA* operon play other roles in sporulation (26).

In any case, SpoIIIJ and the products of the *spoIIIA* locus function at least in part in the same signaling pathway that is involved in the activation of σ^G in the prespore after completion of the engulfment sequence. This signaling pathway appears to originate in the mother cell with the σ^E-dependent transcription of the *spoIIIA* operon (23). Because their synthesis is restricted to the mother cell compartment, it seems unlikely that the *spoIIIA*-encoded proteins interact directly with SpoIIAB in the prespore (26). However, other than SpoIIAB and σ^G, no prespore-specific components of the σ^E-to-σ^G signaling pathway have been identified. We tested whether the function of SpoIIIJ is specifically required in either the prespore or the mother cell by expressing *spoIIIJ* under the control of a promoter utilized by the σ^F (*spoIIQ*) or σ^E (*spoIID*) form of RNA polymerase. We found that expression of *spoIIIJ* is sufficient in the prespore both for the activation of σ^G and for sporulation (Fig. 6; Table 3). Nevertheless, expression of a P_{spoIID}-*spoIIIJ* fusion in single copy significantly increases the sporulation efficiency of a *spoIIIJ* null mutant, and thus it appears that SpoIIIJ retains some functionality when expressed in the mother cell (Table 3). The lack of full complementation of a *spoIIIJ* null mutant by P_{spoIID}-*spoIIIJ* does not seem to be due to reduced expression of *spoIIIJ*, because the presence of the same fusion in a multicopy plasmid does not further increase the efficiency of sporulation. Since the multicopy P_{spoIID}-*spoIIIJ* allele interferes with sporulation of the wild-type strain, we speculate that SpoIIIJ may promote the incorporation into the mother cell side of the prespore membranes of a protein that interferes with the signaling events that lead to σ^G activation and that the mother cell has mechanisms to prevent SpoIIIJ accumulation to high levels. Preferential degradation of SpoIIIJ in the mother cell could be another example of proteolysis playing a role in the reinforcement of the correct compartmentalization on σ factor activity during sporulation (see, for example, references 18 and 40).

In contrast to the case for *spoIIIJ*, we found that expression of *spoIIIA* from the prespore-specific *spoIIQ* promoter does not support efficient sporulation (Fig. 6; Table 3). Like SpoIIIJ (39; this work), the *spoIIIA*-encoded products also localize around the developing spore (46; W. Blaylock and C. P. Moran, Jr., unpublished results). Together, our results suggest that the *spoIIIA*-encoded products from the mother cell and SpoIIIJ from the prespore act together to convey a signal that results in the activation of σ^G in the prespore after completion of the engulfment process (Fig. 7). We favor the idea that the *spoIIIA*-encoded proteins and SpoIIIJ function in the same pathway as suggested in Fig. 7, because mutations in either loci respond similarly to the *spoIIIGE155K* allele. However, we cannot presently exclude that *spoIIIA* and *spoIIIJ* act independently of each other. SpoIIIJ is related to the YidC membrane protein translocase of *E. coli* (47, 49) and may have a similar function in *B. subtilis* (57). Since its function is sufficient in the prespore, SpoIIIJ may be required for the membrane localization of an as-yet-unknown forespore-specific component in the σ^E to σ^G signaling pathway (Fig. 7). It remains unclear whether SpoIIAB is the only factor responsible for keeping σ^G inactive prior to engulfment and, if this is the case, how σ^F can escape from SpoIIAB while σ^G is held inactive. It is also unknown whether SpoIIAA and SpoIIE, which are required for σ^F activation (1, 3, 4, 12, 13, 15), play a role in the activation of σ^G.

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REFERENCES

1. **Alper, S., L. Duncan, and R. Losick.** 1994. An adenosine nucleotide switch controlling the activity of a cell type-specific transcription factor in *B. subtilis*. Cell **77:**195–205.
2. **Antiporta, M. H., and G. M. Dunny.** 2002. *ccfA*, the genetic determinant for the cCF10 peptide pheromone in *Enterococcus faecalis* OG1RF. J. Bacteriol. **184:**1155–1162.
3. **Arigoni, F., K. Pogliano, C. D. Webb, P. Stragier, and R. Losick.** 1995. Localization of protein implicated in establishment of cell type to sites of asymmetric division. Science **270:**637–640.
4. **Arigoni, F., L. Duncan, S. Alper, R. Losick, and P. Stragier.** 1996. SpoIIE governs the phosphorylation state of a protein regulating transcription factor sigma F during sporulation in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **93:**3238–3242.
5. **Benson, A. K., and W. G. Haldenwang.** 1993. Regulation of σ^B levels and activity in *Bacillus subtilis*. J. Bacteriol. **175:**2347–2356.
6. **Campbell, E. A., and S. A. Darst.** 2000. The anti-σ factor SpoIIAB forms a 2:1 complex with σ^F, contacting multiple conserved regions of the σ factor. J. Mol. Biol. **300:**17–28.
7. **Campbell, E. A., S. Masuda, J. L. Sun, O. Muzzin, C. A. Olson, S. Wang, and S. A. Darst.** 2002. Crystal structure of the *Bacillus stearothermophilus* anti-σ factor SpoIIAB with the sporulation factor σ^F. Cell **108:**795–807.
8. **Coppolechia, R., H. DeGrazia, and C. P. Moran, Jr.** 1991. Deletion of *spoIIAB* blocks endospore formation in *Bacillus subtilis* at an early stage. J. Bacteriol. **173:**6678–6685.
9. **Cutting, S., S. Panzer, and R. Losick.** 1989. Regulatory studies on the promoter for a gene governing synthesis and assembly of the spore coat in *Bacillus subtilis*. J. Mol. Biol. **207:**393–404.
10. **Cutting, S. M., and P. B. V. Horn.** 1990. Genetics analysis, p. 27–74. *In* C. R. Harwood and S. M. Cutting (ed.), Molecular biological methods for *Bacillus*. John Wiley & Sons Ltd., Chichester, England.
11. **Decatur, A., and R. Losick.** 1996. Multiple sites of contact between the *Bacillus subtilis* developmental transcription factor σ^F and its anti-sigma factor SpoIIAB. Genes Dev. **10:**2348–2358.
12. **Diederich, B., J. F. Wilkinson, T. Magnin, M. Najafi, J. Errington, and M. D. Yudkin.** 1994. Role of interactions between SpoIIAA and SpoIIAB in regulating cell-specific transcription factor sigma F of *Bacillus subtilis*. Genes Dev. **8:**2653–2663.
13. **Duncan, L., A. Alper, and R. Losick.** 1996. SpoIIAA governs the release of the cell-type specific transcription factor σ^F from its anti-sigma factor SpoIIAB. J. Mol. Biol. **260:**147–164.
14. **Duncan, L., and R. Losick.** 1993. SpoIIAB is an anti-sigma factor that binds to and inhibits transcription by regulatory protein sigma F from *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **90:**2325–2329.
15. **Duncan, L., S. Alper, F. Arigoni, R. Losick, and P. Stragier.** 1995. Activation of cell-specific transcription by a serine phosphatase at the site of asymmetric division. Science **270:**641–644.
16. **Errington, J., L. Appleby, R. A. Daniel, H. Goodfellow, S. R. Partridge, and M. D. Yudkin.** 1992. Structure and function of the *spoIIIJ* gene of *Bacillus subtilis*: a vegetatively expressed gene that is essential for sigma G activity at an intermediate stage of sporulation. J. Gen. Microbiol. **138:**2609–2618.
17. **Foulger, D., and J. Errington.** 1993. Effects of new mutations in the *spoIIAB* gene of *Bacillus subtilis* on the regulation of σ^F and σ^G activities. J. Gen. Microbiol. **139:**3197–3203.
18. **Fujita, M., and R. Losick.** 2002. An investigation into the compartmentalization of the sporulation transcription factor sigma E in *Bacillus subtilis*. Mol. Microbiol. **43:**27–38.
19. **Harry, E. J., K. Pogliano, and R. Losick.** 1995. Use of immunofluorescence to visualize cell-specific gene expression during sporulation in *Bacillus subtilis.* J. Bacteriol. **177:**3386–3393.
20. **Henriques, A. O., B. W. Beall, and C. P. Moran, Jr.** 1997. CotM of *Bacillus subtilis*, a member of the alpha-crystallin family of stress proteins, is induced during development and participates in spore outer coat formation. J. Bacteriol. **179:**1887–1897.
21. **Henriques, A. O., L. R. Melsen, and C. P. Moran, Jr.** 1998. Involvement of superoxide dismutase in spore coat assembly in *Bacillus subtilis*. J. Bacteriol. **180:**2285–2291.
22. **Horton, R. M., H. D. Hunt, S. N. Ho, J. K. Pullen, and L. R. Pease.** 1989. Engineering hybrid genes without the use of restriction enzymes: gene splicing by overlap extension. Gene **77:**61–68.
23. **Illing, N., and J. Errington.** 1991. The *spoIIIA* operon of *Bacillus subtilis* defines a new temporal class of mother-cell-specific sporulation genes under the control of the sigma E form of RNA polymerase. Mol. Microbiol. **5:** 1927–1940.
24. **Illing, N., M. Young, and J. Errington.** 1990. Use of integrational plasmid excision to identify cellular localization of gene expression during sporulation in *Bacillus subtilis*. J. Bacteriol. **172:**6937–6941.
25. **Karmazyn-Campelli, C., C. Bonamy, B. Savelli, and P. Stragier.** 1989. Tandem genes encoding σ-factors for consecutive steps of development in *Bacillus subtilis.* Genes Dev. **3:**150–157.
26. **Kellner, E. M., A. Decatur, and C. P. Moran, Jr.** 1996. Two-stage regulation of an anti-sigma factor determines developmental fate during bacterial endospore formation. Mol. Microbiol. **21:**913–924.
27. **Kenney, T. J., and C. P. Moran, Jr.** 1987. Organization and regulation of an operon that encodes a sporulation-essential sigma factor in *Bacillus subtilis.* J. Bacteriol. **169:**3329–3339.
28. **Kirchman, P. A., H. DeGrazia, E. M. Kellner, and C. P. Moran, Jr.** 1993. Forespore-specific disappearance of the sigma-factor antagonist spoIIAB: implications for its role in determination of cell fate in *Bacillus subtilis*. Mol. Microbiol. **8:**663–671.
29. **Kroos, L., B. Zhang, H. Ichikawa, and Y.-T. N. Yu.** 1999. Control of σ factor activity during *Bacillus subtilis* sporulation. Mol. Microbiol. **31:**1285–1294.
30. **Kunst, F., et al.** 1997. The complete genome sequence of the Gram-positive bacterium *Bacillus subtilis*. Nature **390:**249–256.
31. **Londoño-Vallejo, J.-A., C. Fréhel, and P. Stragier.** 1997. *spoIIQ*, a forespore-expressed gene required for engulfment in *Bacillus subtilis*. Mol. Microbiol. **24:**29–39.
32. **Losick, R., and P. Stragier.** 1992. Crisscross regulation of cell-type-specific gene expression during development in *Bacillus subtilis*. Nature **355:**601–604.
33. **Margolis, P., A. Driks, and R. Losick.** 1991. Establishment of cell type by compartmentalized activation of a transcription factor. Science **254:**562–565.
34. **Mason, J. M., R. H. Hackett, and P. Setlow.** 1988. Regulation of transcription of genes coding for small, acid-soluble proteins of *Bacillus subtilis* spores: studies using *lacZ* fusions. J. Bacteriol. **170:**239–244.
35. **Ménard, R., P. J. Sansonetti, and C. Parsot.** 1993. Nonpolar mutagenesis of the *ipa* genes defines IpaB, IpaC, and IpaD as effectors of *Shigella flexneri* entry into epithelial cells. J. Bacteriol. **175:**5899–5906.
36. **Min, K.-T., C. M. Hilditch, B. Diederich, J. Errington, and M. D. Yudkin.** 1993. σ^F, the first compartment-specific transcription factor of *B. subtilis*, is regulated by an anti-σ factor that is also a protein kinase. Cell **74:**735–742.
37. **Minton, N. P.** 1984. Improved plasmid vectors for the isolation of translational *lac* gene fusions. Gene **31:**269–273.
38. **Mota, L. J., P. Tavares, and I. Sá-Nogueira.** 1999. Mode of action of AraR, the key regulator of L-arabinose metabolism in *Bacillus subtilis*. Mol. Microbiol. **33:**476–489.
39. **Murakami, T., K. Haga, M. Takeuchi, and T. Sato.** 2002. Analysis of the *Bacillus subtilis spoIIJ* gene and its paralogue gene, *yqjG*. J. Bacteriol. **184:**1998–2004.
40. **Pan, Q., D. A. Garsin, and R. Losick.** 2001. Self-reinforcing activation of a cell-specific transcription factor by proteolysis of an anti-σ factor in *B. subtilis*. Mol. Cell **8:**873–883.
41. **Partridge, S., and J. Errington.** 1993. The importance of morphological events and intercellular interactions in the regulation of prespore-specific gene expression during sporulation in *Bacillus subtilis*. Mol. Microbiol. **8:**945–955.
42. **Piggot, P. J., and R. Losick.** 2001. Sporulation genes and intercompartmental regulation, p. 483–517. *In* A. L. Sonenshein, J. A. Hoch, and R. Losick (ed.), *Bacillus subtilis* and its closest relatives from genes to cells. ASM Press, Washington, D.C.
43. **Rather, P. N., and C. P. Moran, Jr.** 1988. Compartment-specific transcription in *Bacillus subtilis*: identification of the promoter *gdh.* J. Bacteriol. **170:**5086–5092.
44. **Rather, P. N., R. Coppolechia, H. DeGrazia, and C. P. Moran, Jr.** 1990. Negative regulator of σ^G-controlled gene expression in stationary-phase *Bacillus subtilis*. J. Bacteriol. **172:**709–715.
45. **Rong, S., M. S. Rosenkrantz, and A. L. Sonenshein.** 1986. Transcriptional control of the *Bacillus subtilis spoIID* gene. J. Bacteriol. **165:**771–779.
46. **Rudner, D. Z., and R. Losick.** 2001. Morphological coupling in development: lessons from prokaryotes. Dev. Cell **1:**733–742.
47. **Samuelson, J. C., M. Chen, F. Jiang, I. Moller, M. Wiedmann, A. Kuhn, G. J. Phillips, and R. E. Dalbey.** 2000. YidC mediates membrane protein insertion in bacteria. Nature **406:**637–641.
48. **Schmidt, R., P. Margolis, L. Duncan, R. Coppolecchia, C. P. Moran, Jr., and R. Losick.** 1990. Control of developmental transcription factor sigma F by sporulation regulatory proteins SpoIIAA and SpoIIAB in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **87:**9221–9225.
49. **Scotti, P. A., M. L. Urbanus, J. Brunner, J. W. de Gier, G. von Heijne, C. van der Does, A. J. Driessen, B. Oudega, and J. Luirink.** 2000. YidC, the *Escherichia coli* homologue of mitochondrial Oxa1p, is a component of the Sec translocase. EMBO J. **19:**542–549.
50. **Serrano, M., R. Zilhão, A. J. Ozin, E. Ricca, C. P. Moran, Jr., and A. O. Henriques.** 1999. A *Bacillus subtilis* secreted protein with a role in endospore coat assembly and function. J. Bacteriol. **181:**3632–3643.
51. **Setlow, B., N. Magill, P. Febbroriello, L. Nakhimovsky, D. E. Koppel, and P. Setlow.** 1991. Condensation of the forespore nucleoid early in sporulation of *Bacillus* species. J. Bacteriol. **173:**6270–6278.
52. **Sharp, M. D., and K. Pogliano.** 2002. Role of cell-specific SpoIIIE assembly in polarity of DNA transfer. Science **295:**137–139.
53. **Stragier, P.** 1992. Establishment of forespore-specific gene expression during

sporulation of *Bacillus subtilis*, p. 297–310. *In* J. A. Cole, F. Mohan, and C. Dow (ed.), Prokaryotic structure and function. Society for General Microbiology, Cambridge, United Kingdom.

54. **Sullivan, M. A., R. E. Yasbin, and F. E. Young.** 1984. New shuttle vectors for *Bacillus subtilis* and *E. coli* which allow rapid detection of inserted fragments. Gene **29:**21–26.
55. **Sun, D., R. M. Cabrera-Martinez, and P. Setlow.** 1991. Control of transcription of the *Bacillus subtilis spoIIIG* gene, which codes for the forespore-specific transcription factor σ^G. J. Bacteriol. **173:**2977–2984.
56. **Sun, Y.-L., M. D. Sharp, and K. Pogliano.** 2000. A dispensable role for forespore-specific gene expression in engulfment of the forespore during sporulation of *Bacillus subtilis*. J. Bacteriol. **182:**2919–2927.
57. **Tjalsma, H., S. Bron, and J. M. van Dijl.** 2003. Complementary impact of paralogous Oxa1-like proteins of *Bacillus subtilis* on post-translocational stages in protein secretion. J. Biol. Chem., **278:**15622–15632.
58. **van Helvoort, J. M., and C. L. Woldringh.** 1994. Nucleoid partitioning in *Escherichia coli* during steady-state growth and upon recovery from chloramphenicol treatment. Mol. Microbiol. **13:**577–583.