Deletion of spoIIAB Blocks Endospore Formation in Bacillus subtilis at an Early Stage

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During an early stage of endospore formation in Bacillus subtilis, the cell divides asymmetrically into two compartments that follow different developmental paths. The differential expression of genes in these two compartments is controlled in part by the production of compartment-specific transcription factors, σG and σK. It is not known how σG accumulation is restricted to one of the two compartments, the forespore. However, the observations that σK directs transcription of the structural gene for σG and that σK activity can be modified by the product of a gene, spoIIAB, has led us to investigate the role of spoIIAB during sporulation. We have isolated mutants that carry deletion alleles of spoIIAB. Electron microscopic examination of these mutants revealed that these mutations blocked endospore formation at an early stage before septation and caused extensive cell lysis. The spoIIAB deletion alleles caused hyperexpression of genes that are normally expressed exclusively in the forespore compartments of sporulating wild-type cells, whereas these alleles reduced expression of other genes, including spoIIE, which is expressed before septation in wild-type cells. These observations confirm that spoIIAB is essential for sporulation and are consistent with models in which the product of spoIIAB plays a role in regulating the timing and/or compartment specificity of σK- and σG-directed transcription.

Bacillus subtilis can respond to nutrient depletion by undergoing a complex differentiation that culminates in the production of an endospore (reviewed in references 15 and 29). During an early stage of this differentiation, the bacterium divides asymmetrically into two cells that follow different developmental paths. The smaller cell, or forespore, becomes the endospore. The larger mother cell engulfs the forespore and provides the developing spore with several specialized products (e.g., a family of proteins that coat the endospore). The mother cell can be thought of as a terminally differentiated cell since it lyses to release the mature endospore in the final act of this differentiation. During endospore development, a different set of genes is transcribed in each cell type. The regulation of this transcription is controlled, at least in part, by the production of several RNA polymerase sigma factors, which direct RNA polymerase to specific classes of promoters (reviewed in reference 18). Transcription of one set of genes is directed in the forespore by the sigma factor σG, which accumulates predominantly in the forespore (9, 17, 32).

It is not known how σG accumulation is restricted to the forespore, but attention has focused recently on the role of a sigma factor, σK, that is produced during the early stage of sporulation (28). Several lines of evidence indicate that transcription of the structural gene for σG, spoIIIG, is directed by RNA polymerase containing σK. Transcription from the spoIIIG promoter is prevented by mutations in the structural gene for σK, spoIAC (3, 9, 20), and EK can use the spoIIIG promoter in vitro (31). Moreover, use of an inducible promoter to express σK resulted in transcription of spoIIIG in vegetative cells (28). σG is believed to act early during sporulation since mutations in spoIAC block endospore formation at stage II, causing aberrant septum formation and blocking the engulfment of the forespore proplast (6). Furthermore, genetic evidence indicates that the spoIIB operon, which includes spoIAC and two additional genes, spoIIAA and spoIIAB, is transcribed before the asymmetric cell division (5). Since σK is active during the stage at which the cell-type-specific transcription of spoIIIG is established, the discovery that the product of spoIIAB (SpoIIAB) antagonizes σK activity prompted the suggestion that SpoIIAB may play a crucial role in restricting σK-directed transcription of spoIIIG to the forespore (28).

If SpoIIAB plays an important role in establishing cell-type-specific gene expression during sporulation, then mutations in spoIIAB would be expected to prevent endospore formation. However, mutations in spoIIAB were not found during traditional searches for sporulation mutants, although these searches did yield mutations in spoIIAA and spoIAC, the other two genes in the spoIIB operon (2, 36). The first mutation found in spoIIAB was a missense allele that caused increased expression from σK-dependent promoters during stationary phase in cells that were not forming endospores (24). This missense mutation in spoIIAB has little or no effect on sporulation (24). Subsequently, during experiments in which an in-frame deletion in spoIIAB was created in the chromosome in order to examine the effect of spoIIAB on σK activity (28), it was shown that deletion of spoIIAB is toxic in stationary phase and causes extensive cell lysis. Moreover, these mutants acquire second-site mutations in spoIAC (28). Obviously, the acquisition of these second-site mutations in spoIAC complicated the examination of the effects of spoIIAB mutations on sporulation.

To examine the effects of spoIIAB mutations on sporulation, we have taken two approaches. We isolated a mutant with a special mutation in spoIAC that partially suppresses the toxic effect of the spoIIAB deletion but does not itself affect sporulation. We were able to use this strain to construct isogenic strains that differed only at the spoIIAB
TABLE 1. B. subtilis strains

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<th>Strain</th>
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locus. We found that deletion of spoIIAB prevented sporulation and that the sporulation defect in this strain could be complemented in trans by a plasmid-borne copy of spoIIAB. We also showed that a deletion in spoIIAB blocked sporulation in an otherwise wild-type strain. To prevent the acquisition of second-site mutations, this strain was never grown to stationary phase before the experimental culture was subjected to sporulation conditions. We characterized the block in endospore development caused by the spoIIAB mutations by electron microscopy and by measuring the expression of several developmentally regulated genes. The results are consistent with the model that spoIIAB is essential for sporulation and that spoIIAB plays a role in establishing compartment-specific gene expression.

MATERIALS AND METHODS

Bacterial strains. The B. subtilis strains used in this study are listed in Table 1.

Construction of the spoIIAB deletion alleles. The spoIABA1 allele was constructed previously by using oligonucleotide-directed mutagenesis to delete 45 bp from M13AB (24). M13AB replicative form was also used for the construction of a larger deletion (spoIIAB2). To make this construction, the M13AB DNA was cut with NcoI, digested with BAL 31 nuclease, and treated with the Klenow fragment of DNA polymerase to create blunt ends. After religation, the DNA was used to transform Escherichia coli 71.18 as described previously (24). The nucleotide sequence of the spoIIAB region of the resulting phages was determined, and one phage with an in-frame deletion of 309 nucleotides in spoIIAB was identified. This deletion allele of spoIIAB (spoIIABΔ2) was used to replace the wild-type allele in the chromosome by homologous recombination as described previously (24). In the construction experiments, 4 μg of M13ABΔ2 replicative-form DNA that had been cut with PsiI and 60 ng of B. subtilis JH642 chromosomal DNA were used to transform EU2000 to D-alanine prototrophy and Lac+ as described previously (24). Chromosomal DNA was extracted from one of the Dal+ Lac+ strains (EUR9003), and the spoIIAB gene region was amplified by the polymerase chain reaction (PCR) (26), using appropriate primers to check that the deletion allele had replaced the wild-type allele of spoIIAB. The number of spores formed by this and other strains after growth for 24 h in DS medium (27) was determined by assaying survival of CFU after incubation of 1 ml of culture for 10 min at 80°C.

Cloning of spoIIAB in an expression vector. Site-directed mutagenesis of M13ABHD (24) with the oligonucleotide 5'‐CTCGAGAGGAGGATAACATATAGA3'‐3 was used to produce a better ribosome binding site upstream from spoIIAB and a PsiI site as described previously (24). The resulting PsiI fragment containing spoIIAB was cloned into pJ89 (8). In the resulting plasmids, pJABR and pJAB, the PsiI fragment is oriented in opposite directions, spoIIAB being transcribed from the lacBS promoter (12) in pJAB. Western immunoblot analysis with a SpoIIAB‐specific antiserum was used to measure the SpoIIAB expressed from both pJAB and pJABR. pJAB expressed three- to fivefold more SpoIIAB than did pJABR, which expressed essentially the same amount of SpoIIAB as found in wild-type B. subtilis strains (data not shown). Since the increased expression of SpoIIAB from pJAB in an otherwise wild-type strain inhibited sporulation about 10-fold, we used pJABR for complementation studies.

Characterization of spoIIAC from strains bearing the spoIABA1 allele. The region of spoIIAC spanning from the oligonucleotide 5'‐GGCTCAGCTGAAGGATC3'‐3 to 5'-GCC TGCCTCGAGCAGACTAG3' was amplified by PCR (26) from the chromosomal DNA of three independent Dal+ Lac+ strains bearing the spoIIABΔ1 allele. The amplified DNA fragment was cut with PsiI and PvuII and cloned into pJM103, a pUC19 derivative containing the cat gene (kindly provided by M. Perigo, Scripps). In each case, the DNA sequence of the spoIIAC gene was determined from two independently derived clones to ensure that the mutation found was not due to a mutation acquired during amplification.

A strain carrying only the spoIIAC236SA allele was isolated after replacement of the spoIIABΔ1 allele in EUR9002. For this purpose, transformation of EUR9002 with an integrating plasmid, pPP51 (22), which contained spoIAA and spoIIAB, was used to produce a chloramphenicol-resistant strain that was merodiploid for spoIAA and spoIIAB. PCR amplification of the spoIIAB region was used to screen spontaneous chloramphenicol-sensitive derivatives to identify a strain (EUR9020) that had lost the integrated plasmid and had the spoIIABA1 allele replaced with the wild-type spoIIAB allele.

Construction of a strain with a spoIIAC disruption. Plasmid pSDA7, kindly provided by P. Stragier, contains the spoIAC gene interrupted at codon 128 by a 1,200-bp fragment containing the ermC gene from pEI94 (34). pSDA7 was digested with SphiI and EcoRI and then used to transform a sporulation-deficient strain to erythromycin resistance. spoIIAC was shown to be disrupted by ermC in a transformant (EUR930) by Southern blotting and PCR amplification.
Construction of isogenic strains containing promoter-lacZ fusions. Strains EUR9000, EUR9002, and EUR9020 are isogenic strains containing a gdh-lacZ fusion linked to ermc. To analyze other promoter-lacZ fusions in these strains, they were transformed to chloramphenicol resistance with chromosomal DNA from EU88912 (23), which carries a Campbell-type insertion of a pHJ101 derivative located close to the gdh promoter. We selected a Cm³ Erm³ transformant of each and grew them without selection to find derivatives that had lost the Campbell insertion and were therefore chloramphenicol sensitive. These strains, named EUR9100, EUR9102, and EUR9120, were used as hosts for the different fusions. The spoIIG-lacZ fusion was introduced in these strains by transduction, using an SPB lysate from EU8743 (10). The spo1ID-lacZ fusion was carried by pC11 (8), a derivative of pSR5 containing a HindIII-NorI-BamHI polylinker, a deletion of the Sphl-BamHI fragment from the vector, and a 285-bp HindIII-PvuII fragment containing the spo1ID promoter. The spo1IID-lacZ fusion was carried by pKTI1ID (33). The sspE-lacZ fusion was introduced in these strains by transformation with chromosomal DNA from a JH642 derivative in which plasmid pJF751sspE (16) had been integrated. The gerE-lacZ fusion was introduced by integration of pSC147 (1) in the chromosome. The β-galactosidase assays were performed as described previously (23).

RNA isolation and primer extension analysis. RNA was prepared from cells grown in D5 sporulation medium (27) as described previously (24). The primer extension procedure was performed with the following oligonucleotides: 5′-GTC CGCCATTGCGCCG-3′ for spoIID, 5′-GCCAAATTGAC CTGACC-3′ for spoIIE, and 5′-GCTGAATCATCGCCTT TGGAC-3′ for spoIIAB. Twenty micrograms of total RNA was used for each annealing reaction. Extensions were performed with avian myeloblastosis virus reverse transcriptase as described previously (24).

Electron microscopy. Cells from 1 ml of culture were harvested by centrifugation and resuspended in 2% (wt/vol) glutaraldehyde in 0.1 M potassium phosphate buffer, pH 7.2, and incubated overnight at 4°C. These fixed cells were harvested by centrifugation and washed in 0.1 M phosphate buffer containing 7.5% sucrose. Thin sections were stained with 4% uranyl acetate and 0.1% lead citrate on copper grids and viewed with an RCA EMU-4 electron microscope at a magnification of ×10,024.

RESULTS

spoIIAB is essential for sporulation. We previously isolated a nonsporulating strain, EUR8901, in which the wild-type allele of spoIIAB had been replaced with a deletion allele (spoIIABΔ1) constructed in vitro (24). The sporulation defect in EUR8901 was not complemented by transformation of this strain with a plasmid that expressed spoIIAB (24); however, we later found that EUR8901 had acquired a second-site mutation in spoIAC that contributed to the sporulation deficiency (28). The mutation in spoIAC apparently reduced the toxicity caused by increased activity of σF in the absence of spoIIAB (28). The spoIAC mutation in EUR8901 is a T-to-G transversion at position 1859 (numbered according to Yudkin (35)) that causes substitution of glycine for the valine at position 224 in σF.

We suspected that it might be possible to identify other mutations in spoIIAC that reduce the activity of σF, thereby reducing its toxicity in the spoIIAB deletion strain, but retain sufficient σF function to allow sporulation. In such a genetic background we would be able to examine the effects of spoIIAB mutations on sporulation while reducing the risk that the strains would acquire additional mutations. Therefore, we again transferred the spoIIABΔ1 allele into a sporulation-proficient strain carrying a gdh-lacZ fusion (24). The transformants that retained the highest levels of σF function were expected to produce the highest level of β-galactosidase. Two strains that contained the spoIIABΔ1 allele were isolated and characterized (EUR9001 and EUR9002). In addition to the spoIIABΔ1 allele, EUR9001 contained an A-to-T transversion at position 1934 in spoIAC that caused substitution of leucine for glutamine at position 249 of σF (spoIAC249QL). EUR9002 contained a T-to-G transversion at position 1894 that caused substitution of alanine for serine at position 236 of σF (spoIAC236SA). Neither of these strains formed endospores efficiently (<100 spores per ml). To determine whether correction of the spoIIABΔ1 allele in these strains would restore their ability to sporulate, we used an integrative plasmid, pPP51, that contains spoI1A and spoI1B but not spoI1AC to transform these strains. Transformation of strain EUR9002 with pPP51 resulted in a sporulation-proficient strain (>10⁸ spores per ml), whereas the sporulation defect of EUR9001 was not corrected completely (10⁸ spores per ml). The sporulation defect of EUR9002 was also complemented by transformation with a replicative plasmid that expressed only spoIIAB (pJABR). These Spo⁺ transformants reverted to Spo⁻ when cured of pJABR and were shown by PCR amplification to have retained the spoIIABΔ1 allele. Therefore, the sporulation defect of EUR9002 was complemented by pJABR. We chose EUR9002 for further analysis since it appeared that its sporulation defect was due to a mutation in spoI1AB and not in spoI1AC.

To further test the effect of the spoI1AC236SA allele on sporulation, an integrative plasmid containing the wild-type allele of spoIIAB was used to replace the spoIIABΔ1 allele of EUR9002 (as described in Materials and Methods). The resulting strain, EUR9020, was isogenic with EUR9002 except at the spoIIAB locus. EUR9020 formed spores efficiently; therefore, the spoI1AC236SA allele had little effect on sporulation. We concluded that the sporulation defect of EUR9002 was caused by the absence of a functional allele of spoIIAB, since the spoI1AC allele in EUR9002 did not affect sporulation and since the sporulation defect of EUR9002 was complemented by a plasmid-borne copy of spoIIAB.

Strain EUR9002 provided us with one strain in which we could examine the block of spore development caused solely by the absence of spoIIAB. In addition, we isolated another strain (EUR9003) that contained a second deletion allele of spoIIAB (spoIIABΔ2). This in-frame deletion in spoIIAB (isolated as described in Materials and Methods) resulted in a spoIIAB product that is missing the residues from and including the histidine at position 6 to the methionine at position 108. We attempted to prevent this strain (EUR9003) from acquiring a second mutation in spoI1AC by never growing this strain to stationary phase before subjecting the experimental cultures to sporulation conditions. EUR9003 was unable to form spores efficiently (<100 spores per ml), and this sporulation phenotype was complemented by transformation with the spoIIAB expression plasmid pJABR (>10⁸ spores per ml). We concluded that the defect in sporulation formation EUR9003 was caused by the spoIIABΔ2 allele. Therefore, EUR9003 provided us with a second strain in which to examine the block of spore development caused by the absence of spoIIAB.
Deletions in spoIAB cause a block at an early stage of sporulation. We used electron microscopy to examine the morphology of the spoIAB mutants that were grown in sporulation medium. By 2.5 h after the end of exponential growth (T 2.5), strain EUR9020, which contains the spoIAC236SA allele and sporulates efficiently, had formed forespore protoplasts (Fig. 1A). This culture was indistinguishable from wild-type cultures. On the other hand, at T 2.5 there were no normal forespore protoplasts visible in the culture of EUR9002 (spoIABΔ1 spoIAC236SA). No asymmetric septum was visible in most (more than 95%) of these cells. Where the septum was visible, it appeared aberrant, and a few disporic cells (less than 2%) were visible (Fig. 1B). Forespore protoplasts were also not evident in samples of EUR9002 that were harvested 5 h after the end of the exponential growth phase. Evidently, sporulation in EUR9002 was blocked at an early stage, since most of the cells failed to form the asymmetric septum.

The phenotype of EUR9003 (spoIABΔ2) was more severe. By T 2.5 there was extensive cell lysis. Only a few intact cells were visible; none of these cells contained asymmetric septa (Fig. 1C).

Deletion of spoIAB differentially affects forespore- and mother cell-specific promoter activity. Most mutations that block sporulation are pleiotropic; i.e., a mutation that blocks sporulation at an early stage also blocks the expression of genes that would be expressed at later stages of sporulation in wild-type strains. To characterize the block of sporulation caused by the spoIAB mutations, we examined the activities of several promoters that are expressed at different times during sporulation. For this purpose, we constructed spoIAB mutant strains (and the isogenic parental strains) that contained promoter-lacZ fusions and monitored the accumulation of β-galactosidase during sporulation (Fig. 2).

We used the spoIIG promoter as an indicator of early sporulation-specific gene expression. In wild-type strains, the spoIIG promoter is activated during the first hour of sporulation (11). Activity of the spoIIG promoter was slightly reduced by the spoIABΔ1 allele (Fig. 2A). The spoIID promoter is activated later, and its activity is dependent on the spoIIG products (25). In the spoIABΔ1 mutant, spoIID promoter activity was reduced to about 25% of the level observed in the wild-type strain. This reduced level of spoIID promoter activity was expected since most of these cells failed to produce asymmetric septa and because completion of septation has been associated with processing of pro-αE (14, 30), which is required for spoIID transcription (25).

The spoIID and gerE promoters are active exclusively in the mother cell compartment of sporulating wild-type strains (13, 15, 37). We found that spoIID promoter activity was reduced to an undetectable level by the spoIABΔ1 allele (Fig. 2C). As expected, the gerE promoter, which is expressed later and is dependent on spoIID, was also inactive in the spoIABΔ1 mutant (data not shown).

In wild-type strains, the sspE promoter is activated exclusively in the forespore compartment of sporulating cells (4). The spoIABΔ1 allele caused increased expression from the sspE promoter (Fig. 2D). The sspE promoter in the spoIABΔ1 mutant was activated at least an hour earlier after the end of exponential growth than in wild-type cells. Although the spoIABΔ1 allele blocked expression from promoters that are normally active exclusively in the mother cell, expression from a forespore-specific promoter, sspE, was stimulated in these cells that do not form forespores. These results were anticipated from the results of Schmidt et al.

FIG. 1. Electron micrographs showing the sporulation phenotypes of B. subtilis strains carrying various spoIAB and spoIAC alleles. (A) EUR9020 (spoIAC236SA); (B) EUR9002 (spoIABΔ1 spoIAC236SA); (C) EUR9003 (spoIABΔ2). The cultures were grown in DS sporulation medium (27) and harvested at T 2.5. The scale bar in panel A indicates 0.5 μm.
(28) that showed that SpoIAB antagonizes the activity of σ^{G} in sporulating cells. Because SpoIAB is absent in the deletion mutants, the increased activity of σ^{F} probably resulted in increased expression of σ^{G} and therefore in increased transcription of forespore-specific genes, such as sspE. This model predicts that the increased transcription of sspE in the spoIAB deletion strains would be dependent on σ^{F} and σ^{G}.

The effects of spoIAB are mediated through σ^{F} and σ^{G}. We also used primer extension analyses to examine the effects of the spoIAB mutations on transcription from several promoters during sporulation. These results were consistent with those obtained with the promoter-lacZ fusions. The spoIABA1 and the spoIABΔ2 alleles caused increased accumulation of the sspE transcript (Fig. 3A). These alleles also increased accumulation of the spoIIIG transcript, which encodes σ^{G}. However, accumulation of the spoIIIC transcript was greater in EUR9003 (spoIABΔ2) than in EUR9002 (spoIABA1 spoIAC236SA) (Fig. 3A). spoIIIG transcription is directed, at least in part, by σ^{F} (28, 31), and evidently the spoIAC236SA allele results in a less active form of σ^{F}.

sspE transcription is directed by σ^{G} (19), and transcription of the structural gene for σ^{G}, spoIIIG, is directed at least in part by σ^{F} (28, 31). Therefore, we expected and found that the increased transcription of sspE caused by the spoIAB deletion alleles requires both σ^{F} and σ^{G}. This was demonstrated by examining the accumulation of the sspE transcript in spoIABA2 strains in which the structural gene for σ^{F} or σ^{G} had been inactivated (Fig. 3B). These strains were also used to show that the increased accumulation of the spoIIIG transcript caused by spoIAB deletion alleles was dependent on the structural genes for σ^{F} and σ^{G} (Fig. 3B). The increased transcription of spoIIIG and sspE caused by the spoIAB deletion alleles was seen most clearly early after the onset of sporulation (Fig. 4). Evidently, in wild-type sporulating cells the spoIAB product acts to delay σ^{G}-directed transcription of spoIIIG.

The spoIAB deletion alleles had an unanticipated effect on transcription of spoIIE. This transcript, which accumulates early during sporulation in wild-type cells, was almost absent in the spoIAB deletion strains (Fig. 3A, lanes b and c). This reduction of spoIIE transcription is consistent with the electron micrographic observations that showed that the spoIAB mutants were blocked at an early stage of sporulation. The inhibition of spoIIE transcription was suppressed by inactivation of the σ^{G} structural gene and partially suppressed by inactivation of the σ^{G} structural gene (Fig. 3C). We concluded that SpoIAB is not an activator of spoIIE transcription, since SpoIAB is not required for spoIIE transcription when spoIAC is also inactive. We interpreted this result to indicate that the hyperactivity of σ^{F} and increased production of σ^{G} in the spoIAB deletion strains caused premature repression of spoIIE transcription.

FIG. 2. Effects of spoIAB deletion alleles on expression of various promoter-lacZ fusions during sporulation. The graphs show the accumulation of β-galactosidase in each strain at hourly intervals after the end of exponential growth (time 0). The strains are derivatives of EUR9100 (wild type; open squares), EUR9102 (spoIABA1 spoIAC236SA; diamonds), and EUR9120 (spoIAC236SA; closed squares) carrying the fusion indicated in each panel.
DISCUSSION

Schmidt et al. (28) found that backcrosses which separated a deletion allele of spoIIAB from suppressor mutations in spoIIAC resulted in extensive cell lysis and a sporulation defective phenotype. Our results confirm that spoIIAB is essential for sporulation, since we showed that deletion of spoIIAB resulted in a sporulation-defective phenotype and that this phenotype was complemented by a plasmid that expresses only spoIIAB. Schmidt et al. (28) also found that SpoIIAB antagonizes the activity of $\sigma^F$ during sporulation. Therefore, the hyperactive $\sigma^F$ in a spoIIAB mutant would be expected to direct increased transcription of spoIIIG, the product of which, $\sigma^F$, would direct transcription of sspE and gdh. This prediction was also confirmed since we found that in the spoIIAB mutants, forespore-specific gene expression
RNA was total the peptidoglycan layer that repression of the peptidoglycan, repression of additional septation, engulfment of the forespore protoplast, and transcription of spoIIIG.

The production of $\sigma^G$ by $\sigma^E$-directed transcription of spoIIIIG in the forespore may be amplified by transcription of spoIIIG by RNA polymerase containing $\sigma^G$. Karmazyn-Campelli et al. (9) found that transcription of spoIIIIG was reduced by a mutation in spoIIIG. We also found that accumulation of the spoIIIIG transcript was prevented by an insertion in spoIIIG in an otherwise wild-type strain and in spoIIAB mutants. This amplification of spoIIIIG transcription by $\sigma^G$ could play an important role in establishing forespore-specific transcription. It should be noted, however, that Sun et al. (31) reported that inactivation of spoIIIIG had little or no effect on spoIIIG transcription. The discrepancy between their results and ours may be caused by the use of different alleles of spoIIIIG (e.g., the insertion used in their experiments may have decreased the stability of spoIIIIG mRNA).

Regardless of the role of $\sigma^G$ in directing transcription of spoIIIIG, it is clear that $\sigma^G$ directs the transcription of several genes in the forespore (19). Moreover, recent preliminary results (12a) indicate that in addition to its effect on $\sigma^G$, SpoIIAB may antagonize $\sigma^F$ activity. If this is true, transcription of forespore-specific genes in the mother cell may be prevented by a single factor (SpoIIAB) that can act on two sigma factors.

Our results and models raise several important issues. For example, it is not known how SpoIIAB is inactivated to derepress $\sigma^F$ and $\sigma^G$-directed transcription in the forespore or how SpoIIAB antagonizes $\sigma^F$ activity. The resolution of these issues may require reconstruction of SpoIIAB-regulated, $\sigma^F$-directed transcription in vitro.

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REFERENCES


FIG. 4. Primer extension analysis of transcription during sporulation. The experiment is similar to that shown in Fig. 3 except that total RNA was isolated from EUR9000 (wild type [Wt]; lanes a to c), EUR9040 (lanes d to f), or EUR9003 (lanes g and h) at 0, 1, 2, 3, or 4 h after the end of the exponential growth phase, as indicated above each lane.

(e.g., sspE, spoIIIIG, and gdh [data not shown]) is enhanced despite the fact that forespores are not formed.

The unexpected findings of our study are that the deletion of spoIIAB blocked endospore development before septation and blocked transcription from the spoIIE promoter, which is active early during sporulation in wild-type cells. Evidently the inhibition of spoIIE transcription in the spoIIAB mutant is caused by the hyperactivity of $\sigma^F$ in this mutant, since spoIIE transcription was restored in the spoIIAB deletion strain by a second mutation in spoIIAC. The block of septum formation by the spoIIAB deletion is also probably caused by the hyperactivity of $\sigma^F$, since asymmetric septa were seen in a few of the spoIIABΔ1 cells that carried the spoIIAC236SA allele. It appears that the spoIIAC236SA allele results in a less active form of $\sigma^G$. This conclusion is based on the decreased level of transcription from the $\sigma^F$-dependent promoter, spoIIIIG, in the strain carrying spoIIABΔ1 and spoIIAC236SA alleles. The decreased transcription of mother cell-specific genes (e.g., spoIIIID) in the spoIIAB mutants probably results from the decreased transcription of spoIIE, since expression of spoIIIID and other mother cell-specific genes is dependent on $\sigma^F$ (13, 15, 37) and the posttranslational processing of pro-$\sigma^G$ is dependent on spoIIE (7, 14).

Recently Illing and Errington (6) characterized the roles of several stage II sporulation genes in septum formation and engulfment. They found that in wild-type cells the hydrolysis of the peptidoglycan layer of the spore septum follows completion of the septum. In a spoIAC null mutant, this peptidoglycan layer was not hydrolyzed and the cells formed additional septa to produce disporic cells. It seems likely to us that $\sigma^F$ directs the transcription of genes that encode products which directly or indirectly result in hydrolysis of the peptidoglycan and repression of additional septum formation. Moreover, the increased transcription of these genes in the spoIIABΔ2 strain results in its lytic phenotype and the repression of septum formation. (It should be noted however, that Illing and Errington [6] favor an interesting alternative model in which $\sigma^F$ does not direct the transcription of genes needed for septal peptidoglycan hydrolysis, since this hydrolysis was observed in a mutant with a missense allele of spoIIAC. They assume that this missense allele of spoIIAC results in a transcriptionally inactive $\sigma^F$, but this assumption has not been tested directly.) Since $\sigma^G$ is produced before septation (5), it appears that its activity must be repressed by SpoIIAB to allow septation. After septation this repression is relieved, at least in the forespore compartment, resulting in hydrolysis of the septal peptidoglycan, repression of additional septation, engulfment of the forespore protoplast, and transcription of spoIIIG.


