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Genetics

Control of developmental transcription factor σ^F by sporulation regulatory proteins SpoIIAA and SpoIIAB in *Bacillus subtilis*

(RNA polymerase/σ factor/promoter)

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ABSTRACT **The sporulation operon *spoIIA* of *Bacillus subtilis* consists of three cistrons called *spoIIAA*, *spoIIAB*, and *spoIIAC*. Little is known about the function of *spoIIAA* and *spoIIAB*, but *spoIIAC* encodes a σ factor called σ^F, which is capable of directing the transcription *in vitro* of genes that are expressed in the forespore chamber of the developing sporangium. We now report that the products of the *spoIIA* operon constitute a regulatory system in which SpoIIAA is an antagonist of SpoIIAB (or otherwise counteracts the effect of SpoIIAB) and SpoIIAB is, in turn, an antagonist of SpoIIAC (σ^F). This conclusion is based on the observations that (*i*) overexpression of *spoIIAB* inhibits σ^F-directed gene expression, (*ii*) a mutation in *spoIIAB* stimulates σ^F-directed gene expression, (*iii*) a mutation in *spoIIAA* blocks σ^F-directed gene expression, and (*iv*) a mutation in *spoIIAB* relieves the block in σ^F-directed gene expression caused by a mutation in *spoIIAA*. The SpoIIAA/SpoIIAB/SpoIIAC regulatory system could play a role in controlling the timing of σ^F-directed gene expression and/or could be responsible for restricting σ^F-directed gene expression to the forespore chamber of the sporangium.**

Gene expression during the process of endospore formation in the Gram-positive soil bacterium *Bacillus subtilis* is governed in part by five developmentally specific RNA polymerase σ factors, σ^H, σ^F, σ^E, σ^G, and σ^K (1, 2). These factors come into play in an ordered sequence that is temporally and spatially correlated with the morphological stages of spore formation. Thus σ^H controls gene expression at the onset of sporulation. Next, σ^F acts during the stage of septum formation, at which time the sporangium is partitioned into separate mother-cell and forespore compartments. σ^E, which is produced after the septum is formed, is, in turn, active during the engulfment stage of sporulation. Finally, the compartment-specific factors σ^G and σ^K direct gene expression in the forespore and mother-cell chambers of the sporangium, respectively. σ^F is of central importance because it helps to govern the transition from a single-celled sporangium to one that consists of two cellular compartments of divergent developmental fates.

σ^F is encoded by the promoter-distal member of the three-cistron sporulation operon *spoIIA* (3–7). The σ^F-encoding cistron is designated *spoIIAC* and the two upstream cistrons are called *spoIIAA* and *spoIIAB*. Although the product of *spoIIAC* (SpoIIAC or σ^F) has been characterized biochemically (7), little is known about the function of the *spoIIAA* gene product (SpoIIAA) and the *spoIIAB* gene product (SpoIIAB). SpoIIAA and σ^F (SpoIIAC) evidently play similar roles in sporulation, since *spoIIAA* and *spoIIAC* mutations have indistinguishable phenotypic effects (8–10). It has been uncertain whether SpoIIAB is required for sporulation, because no lesions in *spoIIAB* were found in the extensive collection of traditionally isolated mutations in the *spoIIA* operon. Recently, a *spoIIAB* mutation of a special kind was obtained by a screen that did not depend upon a defect in spore formation (11). As we will show here, *spoIIAB* does have an important role in sporulation, but *spoIIAB* mutants were not discovered in traditional searches for *spo* mutants because such mutants lose viability during stationary phase.

Here we present genetic evidence that the products of the *spoIIA* operon constitute a regulatory system in which SpoIIAA is an antagonist of SpoIIAB (or otherwise counteracts the effect of SpoIIAB), and SpoIIAB is, in turn, an antagonist of SpoIIAC (σ^F). σ^F directs the transcription *in vitro* of the forespore regulatory gene *spoIIIG* and of other genes (e.g., *gpr*) that are preferentially expressed in the forespore (refs. 7 and 12; D. Sun, R. M. Cabrera-Martinez, and P. Setlow, personal communication). The use of an isopropyl β-D-thiogalactopyranoside (IPTG)-inducible promoter to cause expression of *spoIIAC* in vegetative cells has enabled us to show that σ^F can drive the transcription of *spoIIIG* (the present paper) and *gpr* (unpublished results) *in vivo*. We discuss the possibility that the SpoIIAA/SpoIIAB/SpoIIAC regulatory system plays a role in the timing of σ^F-directed gene expression and/or is responsible for restricting σ^F-directed gene expression to the forespore chamber of the sporangium.

RESULTS

Use of the P_{spac} Promoter to Induce σ^F-Directed Transcription of *spoIIIG* During Growth. To study the capacity of σ^F to direct transcription of the forespore regulatory gene *spoIIIG in vivo*, we fused *spoIIAC* to the IPTG-inducible promoter P_{spac} (13). Plasmid pRS11 contains a segment of the *spoIIA* operon, extending from the 3′ end of *spoIIAB* into the middle of *spoIIAC*, fused to P_{spac}. Integration of this plasmid by single-reciprocal (Campbell) recombination into the chromosome brings the σ^F-encoding *spoIIAC* under the control of P_{spac} but leaves *spoIIAA* and *spoIIAB* under the control of the *spoIIA* operon's normal promoter (Fig. 1*a*). To monitor *spoIIIG* expression, we used a transcriptional fusion of *spoIIIG* to the *lacZ* gene, inserted into the chromosome at the *amyE* locus (15).

Because *spoIIIG* is subject to autoregulation, we carried out our experiments in cells containing the mutation *spoIIIGΔ1* (15) to ensure that any *spoIIIG–lacZ* expression was not an indirect consequence of the induction of σ^G synthesis. Addition of IPTG to *spoIIIGΔ1* mutant cells bearing the P_{spac}–*spoIIAC* fusion caused a rapid induction of *spoIIIG–lacZ* expression (Fig. 2*a*). β-Galactosidase reached maxi-

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Abbreviation: IPTG, isopropyl β-D-thiogalactopyranoside.

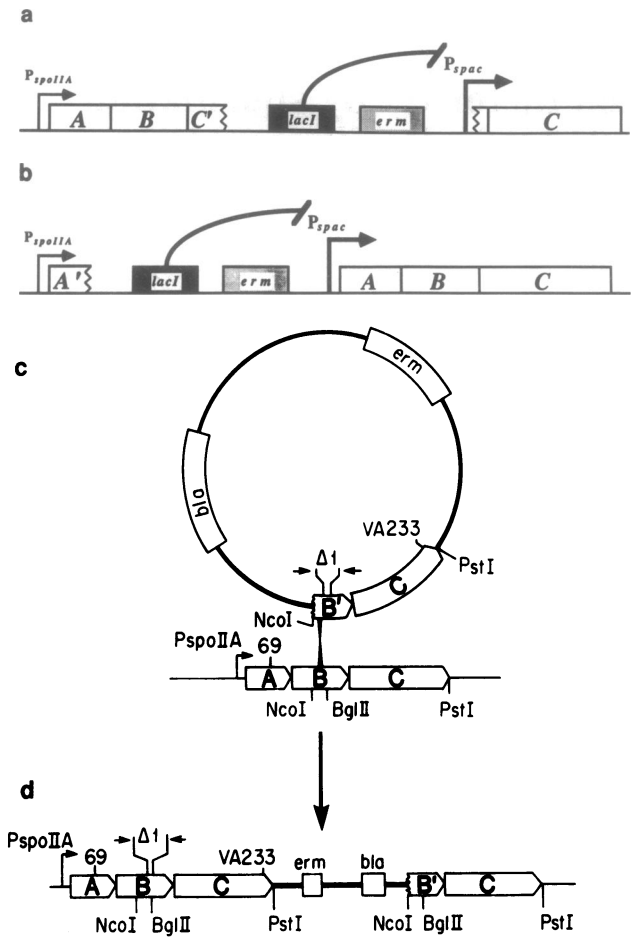

FIG. 1. Molecular genetic manipulations of the *spoIIA* operon. (*a* and *b*) Structures of fusions of the IPTG-inducible promoter P_{spac} to *spoIIAC* and to the entire *spoIIA* operon, respectively. The P_{spac}–*spoIIAC* fusion was created by the insertion of plasmid pRS11 into the chromosome, whereas the P_{spac}–*spoIIA* operon fusion was created by the insertion of pRS7 into the chromosome. The diagrams are not drawn to scale except for the relative sizes of *spoIIAA*, *-AB*, and *-AC*. Restriction maps of P_{spac} and the vector region have been reported (13, 14). (*c*) Recombination between hybrid plasmid pPM23 or pPM24, bearing a segment of *spoIIA* DNA extending from the *Nco* I site within *spoIIAB* to a *Pst* I site just downstream of *spoIIAC*, and the corresponding region of *spoIIA* DNA in the chromosome. The recombination results in the replacement of sequences downstream of the recombination site by corresponding plasmid-borne sequences. (*d*) Structure of chromosomal DNA in the vicinity of integrated plasmid. The vector backbone (thick line) is not drawn to scale. A, B, and C are abbreviations for *spoIIAA*, *spoIIAB*, and *spoIIAC*, respectively. A′, B′, and C′ are abbreviations for truncated copies of *spoIIAA*, *spoIIAB*, and *spoIIAC*, respectively.

mum accumulation within 2 hr after the addition of the inducer and was then rapidly depleted from the cells. We conclude that σ^F has the capacity to direct efficient transcription from the *spoIIIG* promoter when its synthesis is artificially induced in vegetative cells.

Interestingly, cells containing the P_{spac}–*spoIIAC* fusion form small colonies that lyse after 1–2 days when grown on solid medium in the presence of IPTG. We attribute this toxicity to overexpression of *spoIIAC* because (*i*) colony formation in the absence of IPTG was normal, (*ii*) a control strain in which P_{spac} was inserted in the opposite orientation to that of *spoIIAC* was fully viable (though Spo^-) in the presence of IPTG, and (*iii*) the introduction of pRS11 into cells containing a *spoIIAC* mutation (*spoIIAC1*) gave rise to transformants that were insensitive to IPTG.

SpoIIAB Is an Inhibitor of σ^F-Directed Gene Expression. We also attempted to induce σ^F-directed transcription of

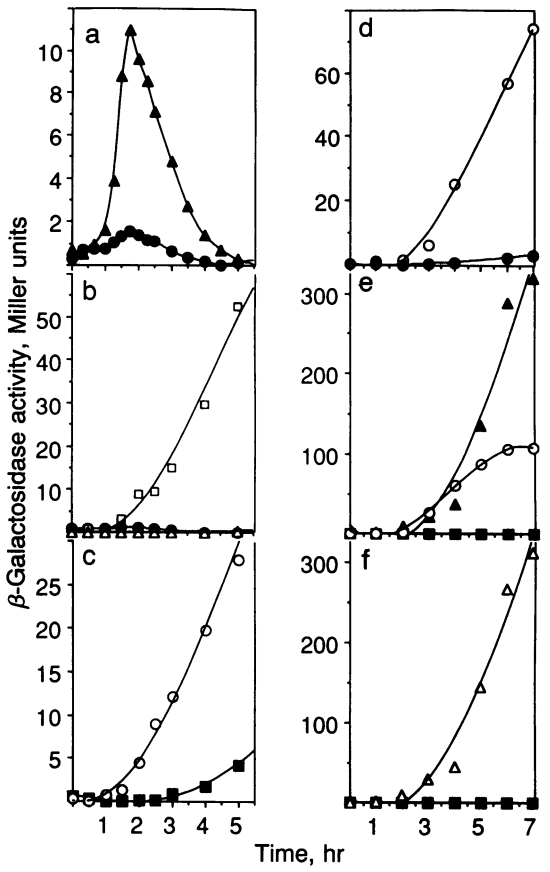

FIG. 2. Gene fusion-directed β-galactosidase synthesis. (*a–c*) IPTG-dependent induction of *spoIIIG*-directed β-galactosidase synthesis. Cells were grown to an OD_{595} of 0.3 in DS medium (16, 17). Parallel cultures of induced and uninduced cells were then established by splitting each culture into two portions and adding IPTG (1 mM) to one portion. Samples were removed and assayed (18) for β-galactosidase at the indicated times after IPTG addition. The level of β-galactosidase activity in the uninduced cells was subtracted as background from the β-galactosidase activity of the cells that were exposed to IPTG. Strains used in the experiments of *a–c* were isogenic except as indicated and contained the mutation *spoIIIGΔ1*, a *spoIIIG–lacZ* transcriptional fusion at the *amyE* locus, and an integrated copy of pRS11 to create a P_{spac} fusion to *spoIIAC* or an integrated copy of pRS7 to create a P_{spac} fusion to the *spoIIA* operon. The strains of *a* contained pRS11 and pRS7 integrated into the chromosomes of $spoIIA^+$ bacteria to create a P_{spac} fusion to *spoIIAC* (▲, strain RS79) and a P_{spac} fusion to the *spoIIA* operon (●, strain RS78), respectively. The strains of *b* contained pRS7 integrated into $spoIIA^+$ and *spoIIA* mutant bacteria to create P_{spac} fusions to the wild-type *spoIIA* operon (●, strain RS78) and to operons bearing the mutations *spoIIAA69* (△, strain RS110) or *spoIIABΔ1* (□, strain RS150). The strains of *c* contained pRS7 integrated into *spoIIABΔ1* mutant cells that harbored pHDAB (■, strain RS165) or pJ89 (○, strain RS162). (*d–f*) *sspB*-directed β-galactosidase synthesis during sporulation of a σ^F altered-specificity mutant. Cells were grown in DS medium and β-galactosidase specific activities were determined at the indicated times after the end of the exponential phase of growth. The strains contained *spoIIIGΔ1*, *sspB–lacZ*, and *spoIIA* operons of the following genotypes: $spoIIAA^+$ $spoIIAB^+$ $spoIIAC^+$ (●, strain PM10) and $spoIIAA^+$ $spoIIAB^+$ *spoIIAC-VA233* (○, strain PM73) in *d*; $spoIIAA^+$ $spoIIAB^+$ *spoIIAC-VA233* (○, strain PM276), *spoIIAA69* $spoIIAB^+$ *spoIIAC-VA233* (■, strain PM282), and $spoIIAA^+$ *spoIIABΔ1 spoIIAC-VA233* (▲, strain PM274) in *e*; and *spoIIAA69* $spoIIAB^+$ *spoIIAC-VA233* (■, strain PM282) and *spoIIAA69 spoIIABΔ1 spoIIAC-VA233* (△, strain PM279) in *f*.

spoIIIG–lacZ by use of a fusion of P_{spac} to the entire *spoIIA* operon. The P_{spac}–*spoIIA* operon fusion was created by use of the plasmid pRS7, which contains a fusion of P_{spac} to a fragment of *spoIIAA* that extends from just upstream of the *spoIIA* transcription start site (position −31) to codon 59 of

the *spoIIAA* open reading frame. Integration of pRS7 into the chromosome by single-reciprocal recombination created a fusion of P_{spac} to the entire *spoIIA* operon, thereby leaving only a truncated copy of *spoIIAA* under the control of the normal *spoIIA* promoter (Fig. 1*b*). Addition of IPTG to cells bearing the P_{spac}–*spoIIA* operon fusion was therefore expected to induce transcription of the entire operon, including the promoter-distal genes. To our surprise, however, little *spoIIIG*-directed β-galactosidase synthesis was observed following the addition of IPTG to cells bearing the P_{spac}–*spoIIA* operon fusion (Fig. 2*a*). Among the possible explanations for the observed inability of the P_{spac}–*spoIIA* operon fusion to direct *spoIIIG–lacZ* expression is that the induction of SpoIIAA or SpoIIAB synthesis by IPTG addition was interfering with σ^F-directed gene expression.

Therefore, to examine the possible effects of SpoIIAA and SpoIIAB on σ^F-directed *spoIIIG–lacZ* expression, we constructed fusions of P_{spac} to operons bearing the missense mutation *spoIIAA69* (19) or the in-frame deletion *spoIIABΔ1* (11). To create fusions of P_{spac} to the mutant *spoIIA* operons, plasmid pRS7 (see Fig. 1*b*) was inserted into the chromosomes of a *spoIIAA69* mutant and a *spoIIABΔ1* mutant. To monitor σ^F-directed gene expression, the fusions of P_{spac} to the mutant *spoIIA* operons were introduced by DNA-mediated transformation into *spoIIIGΔ1* mutant cells bearing a *spoIIIG–lacZ* transcriptional fusion at the *amyE* locus.

spoIIAA69 had little effect on *spoIIIG–lacZ* expression (Fig. 2*b*); thus, addition of IPTG to cells containing the P_{spac} fusion to the mutant operon bearing *spoIIAA69* caused the same low level of β-galactosidase synthesis as was observed following induction of the P_{spac}–*spoIIA* wild-type operon fusion. In contrast, however, the addition of IPTG to cells bearing the P_{spac}–*spoIIABΔ1* mutant operon fusion caused a dramatic induction of β-galactosidase synthesis (Fig. 2*b*). We conclude that IPTG-induced expression of *spoIIAB* in the P_{spac}–*spoIIA* operon fusion is responsible for the impaired expression of *spoIIIG–lacZ*.

Next, we asked whether the *spoIIAB* gene product acts in trans to block σ^F-directed gene expression. This question was investigated by introducing plasmid pHDAB into cells containing the P_{spac} fusion to the *spoIIABΔ1* mutant operon. pHDAB is a multicopy plasmid bearing wild-type copies of the *A* and *B* cistrons, which are transcribed from a vegetatively expressed promoter (11). The level of β-galactosidase in cells bearing pHDAB was much lower than that observed in otherwise identical cells containing in place of pHDAB the plasmid vector pJ89 (Fig. 2*c*). We conclude that *spoIIAB* encodes a diffusible product (SpoIIAB) that acts in trans to inhibit σ^F-directed gene expression.

The results of Fig. 2*a* showed that the level of β-galactosidase in cells containing P_{spac}–*spoIIAC* began to decrease rapidly 2 hr after induction by IPTG. We can now explain this observation by hypothesizing a *spoIIAB*-dependent shutoff in *spoIIIG–lacZ* expression shortly after the cells begin to sporulate (the drop in β-galactosidase levels presumably being due to proteolysis of preexisting enzyme). In cells containing a direct fusion of P_{spac} to *spoIIAC*, *spoIIAB* (and *spoIIAA*) remains under the control of the *spoIIA* operon promoter (Fig. 1*a*) and hence expression of *spoIIAB* is expected to switch on in early stationary phase. In confirmation of our hypothesis, β-galactosidase in P_{spac}–*spoIIAC*-containing cells of a *spoIIABΔ1* mutant was found to continue to accumulate for several hours after the addition of IPTG (data not shown).

Toxicity Due to the *spoIIABΔ1* Mutation. While manipulating *spoIIABΔ1*, we discovered that cells containing the deletion mutation readily accumulated suppressor mutations in *spoIIAC* that blocked σ^F function. When separated from these second-site mutations by backcross experiments, the *spoIIABΔ1* mutation was found to cause the formation of colonies that lysed after 1–2 days. We attribute the toxic effect of *spoIIABΔ1* to the stimulation of σ^F activity caused by the absence of the *spoIIAB* product.

SpoIIAB Inhibits σ^F-Directed Gene Expression During Sporulation. Having demonstrated that SpoIIAB inhibits σ^F-directed gene expression in cells that had been engineered to transcribe the *spoIIA* operon during growth, we next asked whether SpoIIAB is also an inhibitor of σ^F-directed gene expression in cells undergoing sporulation—that is, in cells in which *spoIIA* is induced during sporulation under the control of its normal promoter, P_{spoIIA}. Since *spoIIIG–lacZ* is expressed at only a low level during sporulation, we took advantage of the finding (P.M., unpublished results) that the substitution of alanine for valine at residue 233 of σ^F (as a consequence of mutation *spoIIAC-VA233*) converts the *spoIIAC* product to the promoter specificity of σ^G. (σ^F and σ^G are very similar to each other in their predicted amino acid sequences, and the valine to alanine substitution increases the similarity of the putative "−35" recognition helix of σ^F to that of σ^G; refs. 15 and 20.) As a result of the valine to alanine substitution, mutant σ^F is able to direct transcription efficiently from the promoter for *sspB*, a strongly expressed gene that is normally under the control of σ^G (7).

Fig. 2*d* shows an experiment in which the *spoIIAC-VA233* mutant gene was used to replace the wild-type *spoIIAC* gene in the chromosome of a *spoIIIG* mutant containing an *sspB–lacZ* fusion. (The *spoIIIG* mutation was included to prevent σ^G-directed transcription of the gene fusion.) The replacement was carried out by recombinational insertion of plasmid pPM7 to create strain PM73. pPM7 contains a 967-base-pair segment of *spoIIA* DNA extending from a *Bgl* II site in the middle of *spoIIAB* to a *Pst* I site immediately downstream of the mutant *spoIIAC* gene. A control strain (PM10) was constructed by inserting into the chromosome a plasmid (pPM3) that was identical to pPM7 except that it contained a wild-type *spoIIAC* gene. Active expression of the *sspB–lacZ* fusion commenced at about hour 2 of sporulation in *spoIIIG* mutant cells bearing the *spoIIAC-VA233* mutation (Fig. 2*d*). This activity was dependent on the mutant σ^F protein, as little *sspB–lacZ* expression was detected in *spoIIIG* mutant cells bearing a wild-type copy of *spoIIAC*. An advantage of the *spoIIAC-VA233/sspB–lacZ* system is that it ensured in the sporulation experiments to follow that we were monitoring the expression of a gene under the direct control of σ^F.

To investigate the effect of SpoIIAB on σ^F-directed gene expression during sporulation, we constructed a *spoIIIG* mutant strain (PM274) containing the *spoIIABΔ1* mutation and the σ^F altered-specificity mutation *spoIIAC-VA233*. PM274 was constructed by inserting into the chromosome (Fig. 1 *c* and *d*) a plasmid (pPM23) that contains a segment of *spoIIA* DNA (extending from the *Nco* I site near the beginning of *spoIIAB* to the *Pst* I site just downstream of *spoIIAC*) bearing *spoIIABΔ1* and *spoIIAC-VA233*. As a control, strain PM276 was constructed by inserting into the chromosome a plasmid (pPM24) that was identical to pPM23 except for the absence of the *spoIIABΔ1* mutation. We then monitored σ^F-directed gene expression in the mutant cells by use of the *sspB–lacZ* fusion. The spoIIABΔ1 mutation markedly increased the rate of *sspB*-directed β-galactosidase synthesis above that observed in otherwise isogenic cells that were *spoIIAB*$^+$ (Fig. 2*e*). This finding agrees with the view that SpoIIAB interferes with the synthesis or activity of σ^F in cells undergoing sporulation.

SpoIIAA Is an Inhibitor of the Synthesis or Activity of SpoIIAB. We next wished to investigate the role of SpoIIAA, the product of the promoter-proximal member of the *spoIIA* operon, on σ^F-directed gene expression during sporulation. We therefore constructed a *spoIIIG* mutant strain (PM282) containing *spoIIAA69* and the σ^F altered-specificity mutation *spoIIAC-VA233*. PM282 was constructed by inserting pPM24

(above) into the chromosome of a *spoIIAA69* mutant. The presence of the *spoIIAA69* mutation caused a complete block in *sspB*-directed β-galactosidase synthesis (Fig. 2*e*). Thus, SpoIIAA is required for σ^F-directed gene expression, the effect of the *spoIIAA69* mutation being opposite to that of *spoIIABΔ1*.

A simple hypothesis to explain the observation that *spoIIAA* and *spoIIAB* mutations had opposite effects on σ^F-directed gene expression is that SpoIIAA is an antagonist of SpoIIAB or otherwise reverses the inhibitory effect of SpoIIAB on σ^F-directed gene expression. Thus, in the absence of functional SpoIIAA, σ^F-directed gene expression is blocked by the inhibitory action of SpoIIAB. As a test of this hypothesis we created a *spoIIAA69 spoIIABΔ1* double mutant (PM279) with the expectation that the *spoIIABΔ1* mutation should relieve the block in σ^F-directed gene expression caused by the *spoIIAA69* mutation. In other words, if SpoIIAA is an antagonist of SpoIIAB, then *spoIIABΔ1* should be epistatic to *spoIIAA69*. PM279 was constructed by inserting the *spoIIABΔ1*-bearing plasmid pPM23 (above) into the chromosome of a *spoIIAA69* mutant. A high level of β-galactosidase synthesis was observed in cells of the *spoIIAA69 spoIIABΔ1* double mutant (Fig. 2*f*) and, indeed, the pattern of *sspB–lacZ* expression in the double mutant was indistinguishable from that observed in *spoIIAA*$^+$ cells containing the *spoIIABΔ1* mutation (Fig. 2*e*).

Finally, to address the question of whether the effects of *spoIIAA* and *spoIIAB* mutations on σ^F-directed transcription of *sspB–lacZ* are a general feature of σ^F-directed gene expression, we constructed strains similar to those described above, except that they contained *lacZ* fusions to *spoIIIG* (7) and to *gpr* (12). Our results demonstrated that *spoIIIG–lacZ* and *gpr–lacZ* expression was (*i*) enhanced in *spoIIABΔ1* mutant cells, (*ii*) prevented in *spoIIAA69* mutant cells, and (*iii*) restored by the introduction of *spoIIABΔ1* into *spoIIAA69* mutant cells. Thus, the observation that *spoIIAA* and *spoIIAB* mutations have opposite effects and that *spoIIABΔ1* is epistatic to *spoIIAA69* applies to the capacity of σ^F to direct the transcription of three sporulation genes.

SpoIIAA and SpoIIAB Do Not Exert their Effects on σ^F-Directed Gene Expression at the Level of Transcription or Translation of *spoIIAC*. We interpret our results to indicate that SpoIIAB is an inhibitor of σ^F-directed gene expression and that SpoIIAA blocks or otherwise reverses the effect of SpoIIAB. Are the effects of SpoIIAA and SpoIIAB exerted at the level of the expression of *spoIIAC*? Northern hybridization experiments (ref. 21; R.S., unpublished results) demonstrate that the accumulation of the 1.6-kilobase *spoIIA* operon mRNA is unimpaired in *spoIIAA69* mutant cells. If SpoIIAB were an inhibitor of *spoIIAC* transcription (for example, if it blocked read-through transcription from the preceding gene in the operon), a marked decrease in the amount or size of the polycistronic message should have been detected. As an independent way to monitor *spoIIAC* expression, we constructed a gene fusion in which *spoIIAC* was joined in frame at its 180th codon to *lacZ*. Fig. 3 shows that *spoIIAA69* caused no detectable impairment in the expression of the gene fusion.

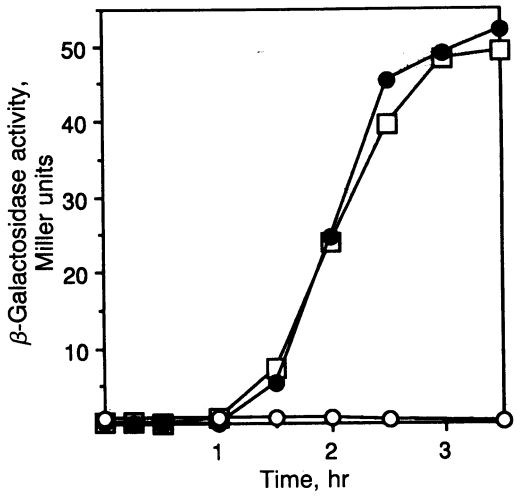

FIG. 3. *spoIIAC–lacZ*-directed β-galactosidase synthesis. β-Galactosidase activity was measured during sporulation in DS medium of *spoIIAC–lacZ* fusion-bearing cells of strain LD1 (□), of the *spoIIAA69* mutant strain LD2 (●), and of the *spo0H* mutant strain LD4 (○). The strains were constructed by integrating into the chromosomes of otherwise isogenic *spo*$^+$ and *spo*$^-$ cells plasmid pLD1, which contained a 730-base-pair fragment of *spoIIA* DNA, extending from a *Bgl* II site in *spoIIAB* to a *Bcl* I site at the 180th codon of *spoIIAC*, that was joined in-frame (at the *Bcl* I site) to *E. coli lacZ*.

DISCUSSION

We have monitored σ^F-directed gene expression during growth in cells that had been engineered to transcribe *spoIIA* in response to IPTG and during sporulation in cells in which transcription of *spoIIA* was under its normal sporulation control. Our principal observations are that (*i*) overexpression of *spoIIAB* during growth causes strong inhibition of σ^F-directed gene expression, (*ii*) a mutation in *spoIIAB* stimulates σ^F-directed gene expression during sporulation, (*iii*) a mutation in *spoIIAA* prevents σ^F-directed gene expression during sporulation, and (*iv*) a mutation in *spoIIAB* relieves the block in σ^F-directed gene expression caused by a mutation in *spoIIAA*. We infer from these observations that the products of the *spoIIA* operon constitute a regulatory system in which SpoIIAB inhibits σ^F-directed gene expression and SpoIIAA prevents or reverses the inhibition of σ^F-directed gene expression caused by SpoIIAB.

How does the regulatory system work? Because a mutation in *spoIIAA* does not block the accumulation of *spoIIA* operon transcripts or the expression of a *spoIIAC–lacZ* gene fusion, we infer that the regulatory system does not operate at the level of the transcription of the σ^F structural gene or the translation of its mRNA, but rather at the level of σ^F action. Thus, SpoIIAA and SpoIIAB could modulate the activity of σ^F protein or affect the capacity of σ^F–RNA polymerase to interact with cognate promoters. For example, SpoIIAB could inhibit σ^F by direct interaction to form an inactive complex or by chemical modification. Alternatively, SpoIIAB could be a repressor that blocks the capacity of σ^F–RNA polymerase to initiate transcription from σ^F-recognized promoters. Similarly, SpoIIAA could inhibit SpoIIAB by direct interaction to form an inactive complex or by chemical modification. Alternatively, SpoIIAA could interact with σ^F to protect it from or reverse the effect of SpoIIAB.

These considerations suggest two possible relationships among the products of the *spoIIA* operon. One is a hierarchical cascade in which SpoIIAA antagonizes the action of SpoIIAB, and SpoIIAB, in turn, blocks the capacity of σ^F to switch on transcription from its target promoters:

$$\text{SpoIIAA} \curvearrowright \text{SpoIIAB} \curvearrowright \sigma^F$$

An alternative possibility is that SpoIIAB inhibits σ^F by converting it to an inactive form, σ^{F*}, and that SpoIIAA reverses (or counteracts) the effect of SpoIIAB by converting σ^{F*} back to the active form of the σ factor:

$$\sigma^F \underset{\text{SpoIIAB}}{\overset{\text{SpoIIAA}}{\rightleftharpoons}} \sigma^{F*}$$

Kalman *et al.* (22) have discovered that the structural gene for the non-sporulation σ factor σ^B is preceded in the same operon by two genes, *V* and *W*, whose products bear a striking similarity in their predicted amino acid sequences to those of SpoIIAA and SpoIIAB, respectively. *V* and *W* are theorized to control the activity of σ^B. By analogy with the role of SpoIIAA and SpoIIAB in σ^F-directed gene expression, it seems likely that *V* and *W* constitute a system of regulation of σ^B-directed gene expression in which *V* inhibits (or reverses) the action of *W*, which, in turn, inhibits the action of σ^B.

An important contribution of our current work is the discovery that the *spoIIABΔ1* mutation causes impaired cell viability. Cell death is presumably due to the enhanced level (or prolonged duration) of σ^F activity, because *spoIIAB* mutant cells readily accumulate second-site mutations in *spoIIAC* that block σ^F activity and that suppress the lethal effect of the *spoIIABΔ1* mutation. This is consistent with earlier work (23) indicating that expression of a cloned copy of *spoIIAC* is toxic in *E. coli* and with our present finding that overexpression of *spoIIAC* causes death in *B. subtilis*. These observations explain the absence of *spoIIAB* mutations in the large collection of *spoIIA* operon alleles that were identified in traditional screens for sporulation mutants; *spoIIAB* mutants would have been lost because of their inviability or because they had accumulated *spo* mutations in *spoIIAC*. Moreover, the compensatory effect of *spoIIAC* mutations in *spoIIABΔ1* mutant cells suggests that σ^F activity is the ultimate target of SpoIIAB.

Recently, Rather *et al.* (11) isolated a *spoIIAB* missense mutation (*spoIIAB1*) of a special kind while screening for mutants with enhanced expression of a gene (*gdh*) that is under the direct control of the forespore factor σ^G. Unlike the deletion mutation *spoIIABΔ1*, the *spoIIAB1* missense mutation does not impair viability or sporulation or significantly stimulate σ^F-directed gene expression (unpublished results). These observations suggest that, in addition to being an inhibitor of σ^F, SpoIIAB may be an antagonist of the synthesis or activity of σ^G. If so, the nature of the inhibitory effect on σ^F may be qualitatively different than the inhibitory effect on σ^G, since *spoIIAB1*-altered SpoIIAB protein is fully capable of antagonizing σ^F-directed gene expression but is impaired in its capacity to prevent σ^G-directed gene expression.

There are several possible roles for the SpoIIAA/SpoIIAB/SpoIIAC regulatory system in the program of sporulation gene expression. *B. subtilis* cells enter sporulation as a response to conditions of nutritional deprivation. SpoIIAA may be a sensor that blocks or reverses the action of SpoIIAB in response to certain nutritional signals, thereby ensuring that σ^F (and σ^G; ref. 11) becomes active only when conditions appropriate for sporulation are met.

A second, more intriguing possibility is that the regulatory system plays a role in the establishment of compartment-specific gene expression. Setlow and coworkers (refs. 7 and 12; D. Sun, R. M. Cabrera-Martinez, and P. Setlow, personal communication) have shown that σ^F directs the transcription *in vitro* of *spoIIIG* and certain other genes (e.g., *gpr*) that are known to be selectively expressed in the forespore. The use of the IPTG-inducible promoter P_{spac} has enabled us to show that σ^F can drive the transcription of *spoIIIG* (present results) and *gpr* (R.S., unpublished results) *in vivo*. The *spoIIA* operon is known to be induced prior to the formation of the septum that partitions the sporangium into mother-cell and forespore compartments (24). We speculate that SpoIIAB partially or completely inhibits σ^F-directed transcription in the predivisional cell and then in the mother-cell after the sporulation septum is formed. In these cell types SpoIIAA might be in an inactive state. Some feature of the forespore might activate SpoIIAA, causing it to interfere with the action of SpoIIAB and thereby relieve the inhibition of σ^F function. In support of our model, recent experiments (P.M., unpublished results) indicate that σ^F-directed gene expression is prevented by a mutation in the forespore regulatory gene *spoIIIE* (25) and that the block in σ^F-directed gene expression caused by the *spoIIIE* mutation is relieved by *spoIIABΔ1*. Finally, we note that since SpoIIAB may be an inhibitor of σ^G, as well as σ^F, forespore-specific inactivation of SpoIIAB could also be a mechanism for restricting σ^G-directed gene expression to the forespore.

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