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Temporal Regulation of the *Bacillus subtilis* Early Sporulation Gene *spo0F*

UMA BAI,[1] MARK LEWANDOSKI,[2]† EUGENIE DUBNAU,[1] AND ISSAR SMITH[1,2]*

Department of Microbiology, The Public Health Research Institute,[1] and Department of Microbiology, New York University Medical Center,[2] New York, New York 10016

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The initiation of sporulation in *Bacillus subtilis* depends on seven genes of the *spo0* class. One of these, *spo0F*, codes for a protein of 14,000 daltons. We studied the regulation of *spo0F* by using *spo0F-lacZ* translational fusions and also measured Spo0F protein levels by immunoassays. *spo0F-lacZ* and Spo0F levels increased as the cells entered the stationary phase, and this effect was repressed by glucose and glutamine. Decoyinine, which lowers GTP levels and allows sporulation in the presence of normally repressing levels of glucose, induced *spo0F-lacZ* expression and raised Spo0F levels. The expression of *spo0F-lacZ* was dependent on *spo0A*, *-0B*, *-0E*, *-0F*, and *-0H* genes, a *spo0H* deletion causing the strongest effect. In most respects, the *spo0F* gene was regulated in a manner similar to that of *spoVG*. However, the presence of an *abrB* mutation did not relieve the dependence of *spo0F* gene expression on *spo0A*, as it does with *spoVG* (P. Zuber and R. Losick, J. Bacteriol. 169:2223–2230, 1987).

Nutrient depletion in *Bacillus subtilis* leads to a process of differentiation which results in spores. The initiation of this temporally ordered developmental process is under the control of at least seven genetic loci known as the *spo0* genes, mutations which block the sporulation at the earliest discernible stage, stage 0 (29). The products of *spo0A*, *-0B*, -0E, *-0F*, *-0H*, and other genes play an important role in the triggering of sporulation affecting the transcription of a wide variety of genes. The *spo0F* gene has been cloned and sequenced (35, 38) and codes for a protein with an approximate M_r of 14,000 (24). The amino acid sequence of the Spo0F protein shows extensive similarity with Spo0A and several other regulatory proteins, which are part of highly conserved two-component environmentally responsive signaling systems found in procaryotes (35).

Several *lacZ* fusions have been constructed with *B. subtilis* spore genes in order to study the interactions among these genes and others turned on early in the sporulation process. A *spoVG-lacZ* fusion has been used by Zuber and Losick (41) to study the epistatic interaction between *spoVG* and other sporulation genes. *spo0B-lacZ* fusions appear to be expressed at a maximum during vegetative growth and at reduced levels during sporulation (4, 9). Yamashita et al. (37) have shown that the expression of *spo0A-lacZ* and *spo0F-lacZ* was elevated at T_0 (the beginning of the sporulation process) and required functional *spo0B*, *-0E*, *-0F*, and *-0H* genes. The *sof-1* mutation, a missense mutation in the *spo0A* structural gene (12, 16), restored *spo0A-lacZ* and *spo0F-lacZ* expression that was impaired by *spo0B* or *-0F* mutations. It has also been shown that the glucose repression of *spo0A-lacZ* fusion activity could be reversed by the addition of decoyinine (36). *spo0E* is induced at the end of logarithmic growth, and its expression is dependent on *spo0A* (26). Studies on *spo0H-lacZ* in *Bacillus licheniformis* and *B. subtilis* have shown β-galactosidase activity to be at low levels during vegetative growth and to increase 5- to 10-fold by T_0 (7) (Weir et al., unpublished experiments). Expression of the *B. subtilis spo0H* gene is dependent on functional *spo0A*, *-0B*, *-0E*, *-0F*, and *-0H* genes (Weir et al., unpublished results).

In an attempt to further understand the regulation of *spo0F* gene expression, we made various translational *spo0F-lacZ* fusions (19) and analyzed these fusions under different growth conditions and in different genetic backgrounds. We have also measured the levels of the *spo0F* gene product, Spo0F, during growth.

MATERIALS AND METHODS

Bacterial strains and media. The bacterial strains and plasmids used in this study are listed in Table 1. IS385, containing a large deletion of the *spo0F* regulatory sequences and the NH_2-terminal region of the structural gene, was previously described (18). The *spo0F-lacZ* fusion strains, consisting of translational fusions between *spo0F* and *lacZ*, integrated into the chromosome at the *spo0F* gene locus by Campbell-like insertion, have been previously described (19). IS688 was constructed by isolating chromosomal DNA from IS414, which contains a *spoVG-lacZ* plasmid, pZL207, integrated, via a homologous recombination event, in JH642. This latter strain has been previously described (41). The DNA was used to transform IS75, selecting for chloramphenicol resistance (Cm^r). One of the transformants was saved as IS688. Plasmid pIS134 was constructed by cloning the *Eco*RI (filled in)-*Hin*dIII fragment from pIS22 containing the intact *spo0F* gene (18) into *Mbo*I (filled in)-*Hin*dIII-cut pIM13 (23). *B. subtilis* strains were grown on solid media containing tryptose blood agar base (Difco Laboratories). Strains were grown in Schaeffer liquid nutrient sporulation medium (NSM) (32). In some experiments, NSM was supplemented with 2% glucose and 0.2% glutamine. For experiments in which sporulation was induced by the addition of decoyinine, cells were grown in S6C liquid medium (11), which contains 1% glucose. β-Galactosidase activity was assayed as described by Miller (22). The specific activity was expressed in units per milligram of protein or in Miller units (22) as indicated.

Preparation of antiserum, protein extracts, and Western

* Corresponding author.
† Present address: Department of Anatomy, University of California at San Francisco, San Francisco, CA 94143.

TABLE 1. Bacterial plasmids and strains

Plasmid or strain	Relevant phenotype	Source or reference
pIS112	Cm^r	19
pIS134	Cm^r *spo0F*	This work
pIS143	Cm^r *spo0F-lacZ*	This work and reference 19
pZL207	Cm^r *spoVG-lacZ*	41
IS75	*metB5 hisA1 leuA8*	
IS385	*metB5 hisA1 leuA8 spo0FΔSacI*	18
IS414	*trpC2 pheA1*(*spoVG*::pZL207)	41
IS438	*metB5 hisA1 leuA8*(*spo0F*::pIS143)	This work
IS440	*trpC2 pheA1*(*spo0F*::pIS143)	This work
IS465	*trpC2 spo0BΔPst*(*spo0F*::pIS143)	This work
IS513	*trpC2 pheA1 sigB*::*ermC*(::pIS143)	This work
IS528	*trpC2 pheA1 abrB703 spo0AΔ204* (*spo0F*::pIS143)	This work
IS529	*trpC2 pheA1 abrB703*(*spo0F*::pIS143)	This work
IS530	*trpC2 pheA1 spo0AΔ204*(*spo0F*:: pIS143)	This work
IS553	*trpC2 pheA1 spo0HΔHind*(*spo0F*:: pIS143)	This work
IS557	*trpC2 pheA1 spo0F221*(*spo0F*::pIS143)	This work
IS688	*metB5 hisA1 leuA8*(*spoVG*::pZL207)	This work
IS1324	*trpC2 pheA1*(pIS134)	This work

immunoblot analysis. The peptide, NH_2-Cys-Ile-Arg-Asp-Ala-Val-Lys-Lys-Tyr-Leu-Pro-Leu-Lys-Ser-Asn-COOH, corresponding to the carboxyl terminus of Spo0F, was synthesized by Biosearch and attached to keyhole limpet hemocyanin (as a carrier) via the coupling of cysteine's SH to the carrier's amino groups. (The NH_2 terminal cysteine residue is not a normal constituent of this region of Spo0F.) The coupled peptide was then injected into rabbits for raising the antibody to Spo0F. French pressure cell extracts were prepared from *B. subtilis* strains grown under different conditions. Cells were collected and washed in harvest buffer (50 mM Tris hydrochloride [pH 8.0], 10 mM EDTA, 10% [vol/vol] glycerol, 1.0 M KCl, 1.7 mM phenylmethylsulfonylfluoride) and were suspended in buffer I (10 mM Tris hydrochloride [pH 8.4], 1 mM EDTA, 1.7 mM phenylmethylsulfonylfluoride, 10 mM $MgCl_2$, 0.3 mM dithiothreitol). The lysate, prepared by passage through a French pressure cell twice at 1,250 lb/in^2, was centrifuged at 12,000 × *g* for 40 min, and the supernatant was electrophoresed on a 15% sodium dodecyl sulfate-polyacrylamide gel. The proteins were then transferred to nitrocellulose paper. Blotting and immunotechniques were performed as described by Blake et al. (3) with the Spo0F antibody and alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G antibodies (Calbiochem-Behring). The Spo0F signal was measured by scanning the nitrocellulose paper on a Bio-Rad 2D model 620 video densitometer. Standard curves relating Spo0F concentration to densitometer signals were prepared by separating different amounts of pure Spo0F protein by gel electrophoresis and immunoblotting-alkaline phosphatase assay, as described above. These data indicated that the amounts of Spo0F visualized in the in vivo growth experiments were in the linear range of the analyses.

RESULTS

Regulation of *spo0F* expression. An obvious strategy to determine the role of the *spo0F* gene in sporulation is to first study the regulation of its expression and to measure the levels of its gene product during growth and differentiation. The effects of growth conditions on these levels and the epistatic interaction with other early sporulation genes can also be analyzed. For these studies we constructed translational fusions between the *spo0F* gene and the eighth codon of *lacZ*, so that β-galactosidase activity could be used as a reporter for *spo0F* expression. These constructs were integrated into the *B. subtilis* chromosome, by Campbell integration, at the *spo0F* locus (19). We also made antibodies against Spo0F by using as antigen a synthetic oligopeptide corresponding to the C-terminal end of the *spo0F* open reading frame. These antibodies enabled us to directly measure in vivo Spo0F levels.

IS440, a *B. subtilis* strain containing a *spo0F-lacZ* translational fusion, together with a wild-type copy of *spo0F*, was grown in NSM. At intervals, samples were removed for determination of β-galactosidase activity and for immunodetection of Spo0F. As shown in Fig. 1A, induction of reporter gene activity occurred at the end of exponential growth, T_0. (T_0 is defined experimentally as the point at which the optical density of a culture stops increasing. T_{-1} and T_1 refer to 1 h before T_0 and 1 h after, respectively.) Enzyme activity increased at T_1 and decreased thereafter. Levels of Spo0F also increased but reached a peak somewhat earlier than the reporter gene activity (at T_0) and then decreased (Fig. 1A and B). This difference in times of peak activity may reflect differential stability of the intact Spo0F protein and the *spo0F-lacZ* fusion protein.

The time course of *spo0F-lacZ* expression and levels of Spo0F indicated that these levels increased greatly at T_0, when the sporulation process was initiated (Fig. 1). We next studied the levels of Spo0F in comparison with expression of the *spoVG* gene. Increased expression of *spoVG* is one of the earliest indicators of the initiation of sporulation (41).

For these studies we used IS414, a *B. subtilis* strain containing an integrated *spoVG-lacZ* translational fusion. As Fig. 2A illustrates, the specific activity of *spoVG*-directed β-galactosidase in NSM remained at a relatively low level during vegetative growth and was induced approximately 15-fold at T_1, after which it began to level off. Spo0F measured at the same time points (Fig. 2A and B) shows a similar pattern of increase.

Effects of different environmental conditions on *spo0F* expression. Since glucose and easily utilizable nitrogen sources inhibit sporulation and expression of early sporulation genes like *spoVG* (14, 40), we tested the effects of these metabolites on *spo0F* expression. Two isogenic strains, IS438, containing an integrated *spo0F-lacZ* fusion, and IS688, with a similar *spoVG* construct, were grown in NSM with or without glucose and glutamine (Fig. 3). *spoVG* expression was severely repressed by glucose, and a combination of glucose and glutamine completely inhibited gene expression (Fig. 3B), confirming earlier observations (14, 40). Identical results were observed with *spo0F* expression (Fig. 3A). Glutamine alone had little or no effect on gene expression. Immunoassays for Spo0F showed that the presence of glucose severely decreased the levels of this protein (data not shown).

Effect of decoyinine on *spo0F* expression. Decoyinine induces sporulation in a glucose-rich medium by inhibiting guanine nucleotide biosynthesis (11). *spoVG* expression is also induced by decoyinine (42). To determine the effect of decoyinine on *spo0F-lacZ* expression, β-galactosidase activity of IS440, containing a *spo0F-lacZ* fusion, was measured in S6C, a glucose-rich minimal medium, in the presence or absence of decoyinine (Fig. 4). Expression of *spo0F-lacZ*

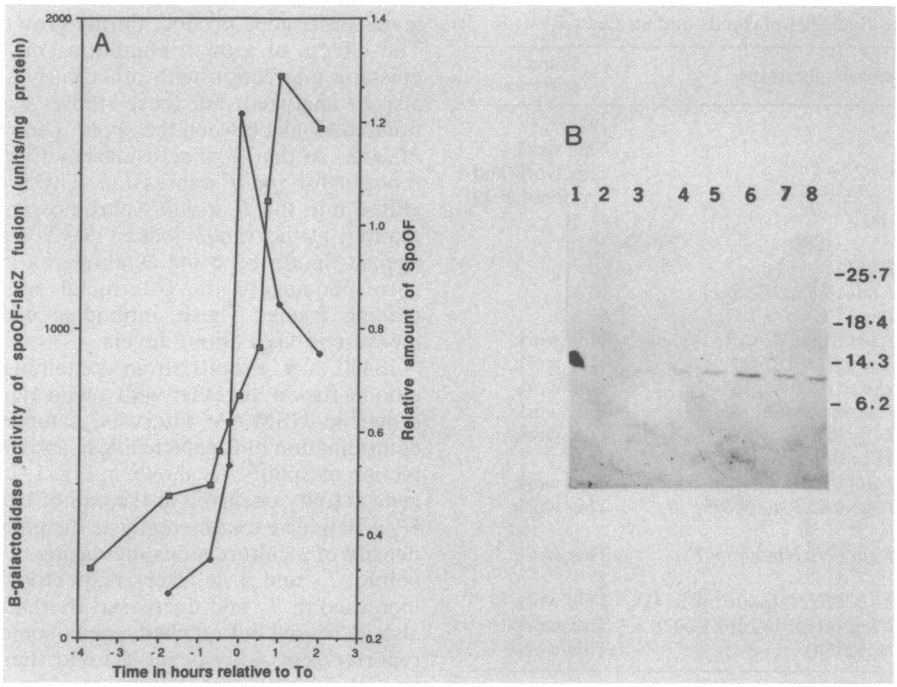

FIG. 1. *spo0F-lacZ* expression and Spo0F levels during growth. (A) β-Galactosidase activity of IS440 (containing an integrated *spo0F-lacZ* gene fusion) was assayed during growth in NSM. ⊡, β-Galactosidase activity; ◆, Spo0F protein levels derived from densitometer tracings of the immunoblots illustrated in panel B. (B) Total cellular proteins were isolated from aliquots of IS440 grown in NSM, as in panel A. Samples (50 μg of protein) from different time points were electrophoresed on a sodium dodecyl sulfate–15% polyacrylamide gel. The proteins from the gel were transferred to nitrocellulose and probed with anti-Spo0F antibodies, as described in Materials and Methods. Lane 1, IS1324 (multicopy *spo0F* plasmid); lane 2, IS385; lanes 3 through 8, IS440 at $T_{-1.75}$, $T_{-0.7}$, $T_{-0.25}$, T_0, T_{+1}, and T_{+2}, respectively. In these experiments, IS385 containing a large deletion in the *spo0F* gene was a negative control, while IS1324, which carries a multicopy plasmid, pIS134, with the intact *spo0F* gene, was a positive control (compare lanes 1 and 2). The numbers on the right-hand ordinate indicate the molecular mass (in kilodaltons) of protein standards electrophoresed on an adjacent lane.

remained low unless decoyinine was added, and in this case, the expression of *spo0F* was induced sixfold (Fig. 3A) compared with the levels of *spo0F-lacZ* activity in the control culture. An increase was noted at the earliest time point, 15 min after addition of the drug. Spo0F levels also increased after the addition of decoyinine (Fig. 4B and C).

Effects of *sigB* and *sigH* mutations. P_1, the major temporally regulated promoter of *spo0F*, resembles the consensus sequences recognized by $E\sigma^H$ (33). However, there is some similarity to $E\sigma^B$ promoters. To resolve this point, *spo0F* expression was measured in strains, grown in NSM, which were missing these σ factors.

The *sigH* deletion, *spo0HΔHind* (in IS553), abolished the stationary-phase induction of *spo0F-lacZ*, while the *sigB* gene disruption, *sigB*::*ermC*, in IS*513* had no effect (Fig. 5). In contrast to *spo0F-lacZ* expression, the *sigB*::*ermC* disruption abolished β-galactosidase activity of the *ctc-lacZ* gene (data not shown), confirming results of the Losick laboratory which showed that *ctc* is transcribed by σ^B containing RNA polymerase (13). These data along with the sequence of the *spo0F* P_1 promoter (18, 33) suggest that RNA polymerase containing σ^H transcribes this promoter in vivo.

Effects of *spo0* mutations. Maximal induction of *spo0F-lacZ* expression occurred before or at the very beginning of sporulation. Thus, other *spo0* genes, which affect the cell at this stage, may have an effect on *spo0F-lacZ* regulation. Cultures of *spo0* mutant strains containing the *spo0F-lacZ* fusion along with isogenic *spo0*$^+$ strains were sampled for β-galactosidase specific activity throughout growth in NSM and in S6C medium, with or without decoyinine. Figure 6 illustrates that intact *spo0A*, *-0B*, *-0H*, and -0F genes are required for the full induction by decoyinine of *spo0F-lacZ* in S6C medium, with the *spo0H* mutant having the strongest effect. Identical results were obtained in the NSM experiments, but in these experiments it was shown that *spo0E* mutations also caused an inhibition of *spo0F-lacZ* expression (data not shown). These latter results confirm earlier studies (37). Thus, decoyinine induction of sporulation results in the elevated expression of *spo0F*, as also occurs in NSM medium. However, addition of the drug cannot bypass the requirement for other *spo0* genes.

Effect of *abrB*. *abrB* mutations, which suppress some of the pleiotropic phenotypes of *spo0A* lesions, also suppress the *spo0A* dependence of the expression of *spo0H*, *spoVG*, and *spo0E* genes (7, 27, 42). To test whether the *abrB* mutation has a similar effect on the *spo0A* dependence of *spo0F*, whose major promoter resembles the *spoVG* P_1 promoter, the *spo0F-lacZ* activity was measured in three isogenic strains: one with both the *spo0AΔ204* and *abrB703* mutations (IS528), one with the *abrB703* mutation (IS529), and the other with the *spo0AΔ204* mutation (IS530). It was observed that the *spo0A* strain (IS530) and the *spo0A* and *abrB703* strain (IS528) had the same low levels of *spo0F-lacZ* expression (Fig. 7). This indicates that *abrB703* could not bypass the *spo0A* requirement for *spo0F* expression. The *abrB703* mutation alone did not have a dramatic effect on *spo0F-lacZ* expression when compared with a wild-type strain (compare IS440 in Fig. 1 with IS528 in Fig. 7).

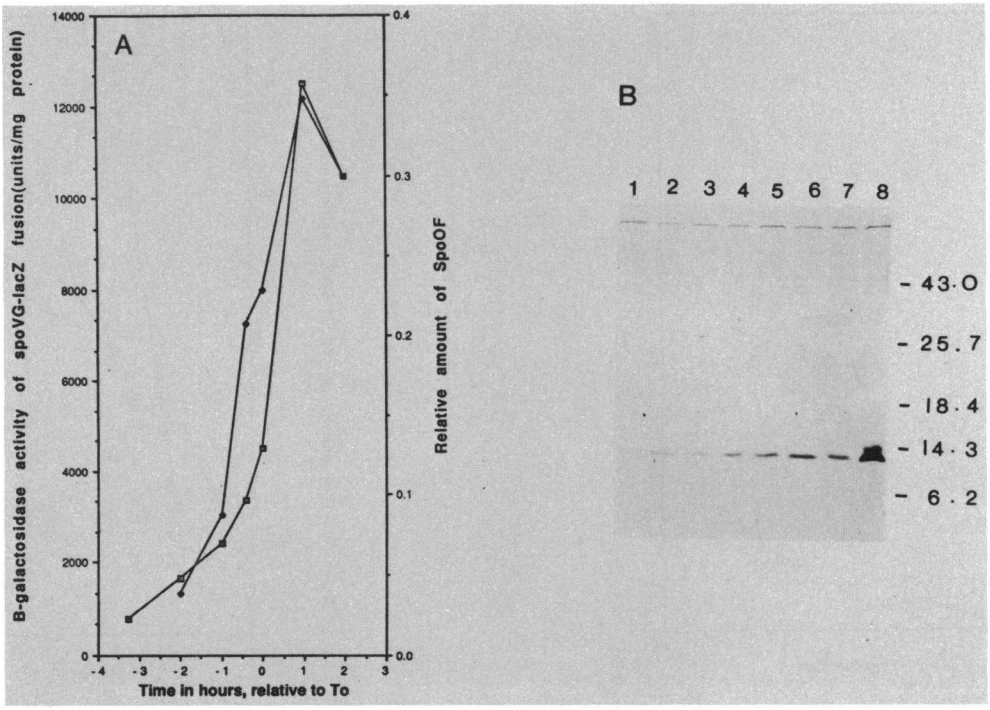

FIG. 2. *spoVG-lacZ* expression and Spo0F levels during growth. (A) β-Galactosidase activity of IS414 (containing an integrated *spoVG-lacZ* fusion) was assayed during growth in NSM, as in Fig. 1A. Quantitative analysis of Spo0F was done with 50 μg of crude extract proteins by using the data obtained from the Western immunoassays, as described in Fig. 1B. ⊡, β-Galactosidase activity; ◆, Spo0F. (B) Immunoblotting analysis of Spo0F was carried out as described previously for Fig. 1. Lane 1, IS385; lanes 2 through 7, IS414 at −2.0, −1.0, −0.5, 0.0, 1.0, and 2.0 h, relative to T_0; lane 8, IS1324. The numbers to the right represent molecular mass, in kilodaltons, of standard proteins, run in an adjacent lane.

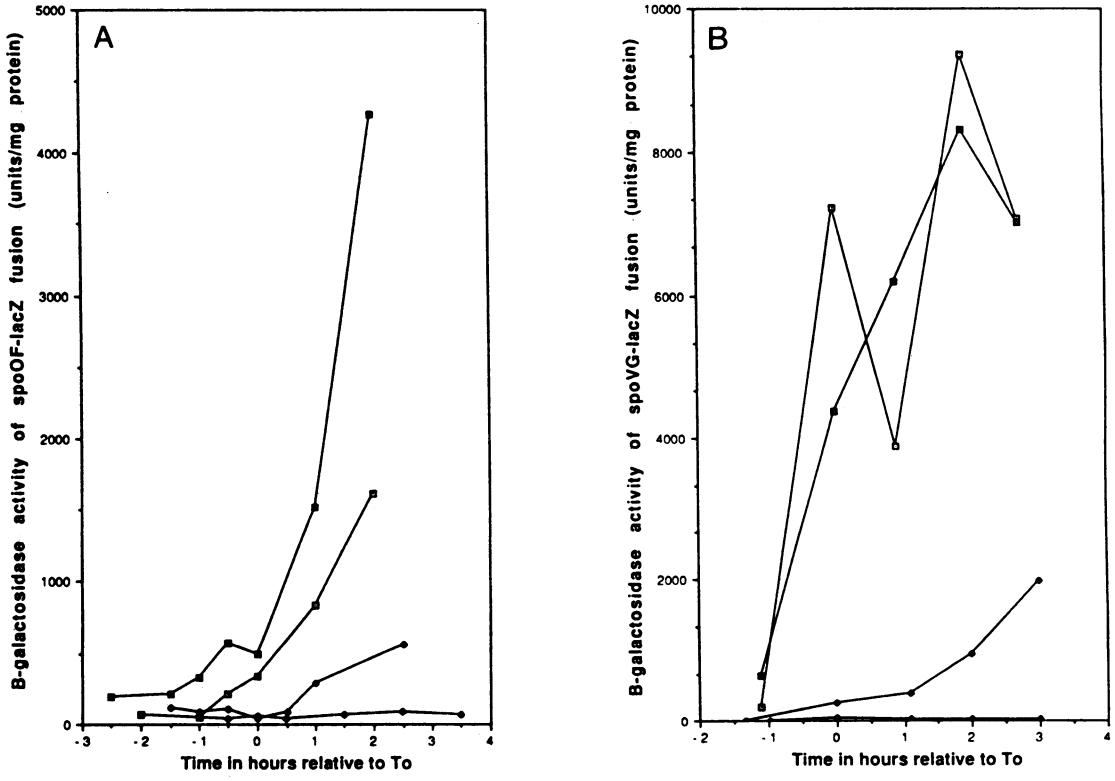

FIG. 3. Effects of glucose and glutamine on *spo0F* and *spoVG* gene expression. Strains IS438, containing a *spo0F-lacZ* fusion, and IS688, with a *spoVG-lacZ* fusion, were grown in NSM medium (⊡), NSM containing 2.0% glucose (◆), NSM containing 0.2% glutamine (■), or NSM containing both glucose and glutamine (◆). At intervals, samples were removed and assayed for β-galactosidase activity, as described above. (A) *spo0F*-driven β-galactosidase activity in IS438. (B) *spoVG*-driven enzyme activity in IS688.

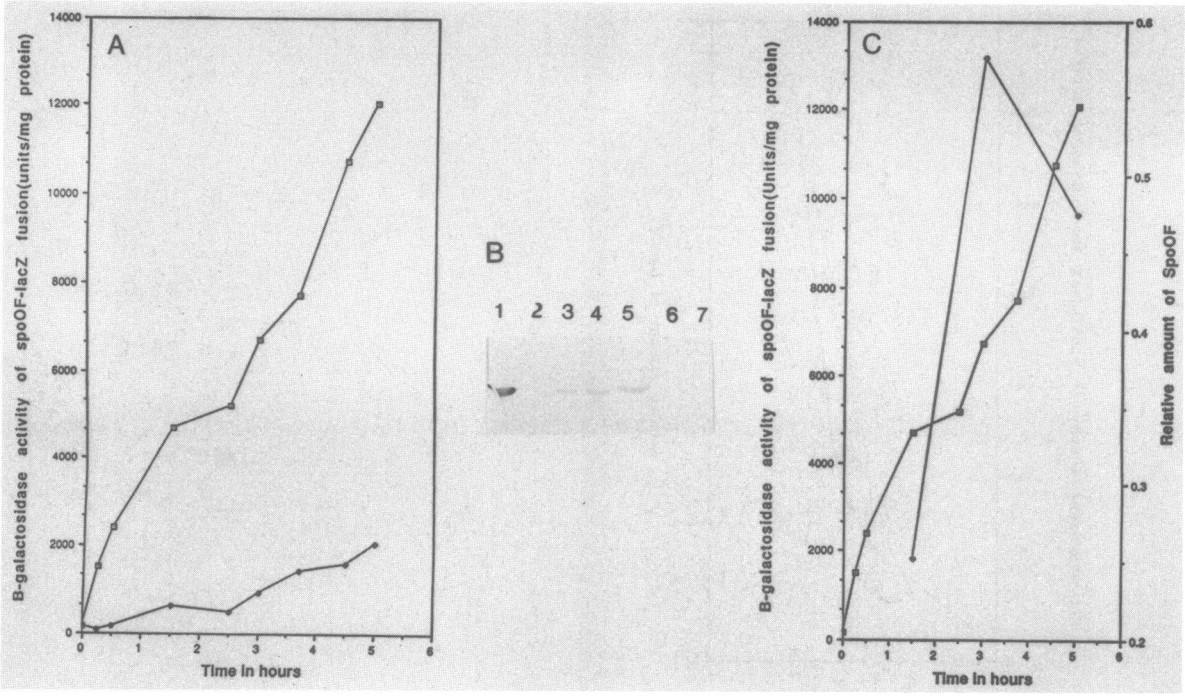

FIG. 4. Effect of decoyinine on *spo0F-lacZ* regulation and on Spo0F levels. β-Galactosidase activity of IS440 was assayed during growth in S6C. When the turbidity of the culture reached 50 Klett units, decoyinine was added to a final concentration of 0.5 mg/ml. Samples were removed at different time intervals for β-galactosidase assays and for determination of Spo0F content, by Western immunoassay, as described in Fig. 1. (A) ⊡, β-Galactosidase activity of IS440 in the presence of decoyinine; ◆, β-galactosidase activity in the absence of decoyinine. (B) Western blotting of Spo0F. Lane 1, IS1324; lane 2, IS440 at 50 Klett units, prior to the addition of decoyinine; lanes 3 through 5, IS440 at 1.5, 3.0, and 5.0 h after adding decoyinine; lane 6, IS440, at 5 h, in the absence of decoyinine; lane 7, IS385. (C) Spo0F was quantitated from the Western immunoblot in panel B, as described before, and the values were plotted along with β-galactosidase activity determinations. ◆, Spo0F with decoyinine; ⊡, β-galactosidase activity with decoyinine.

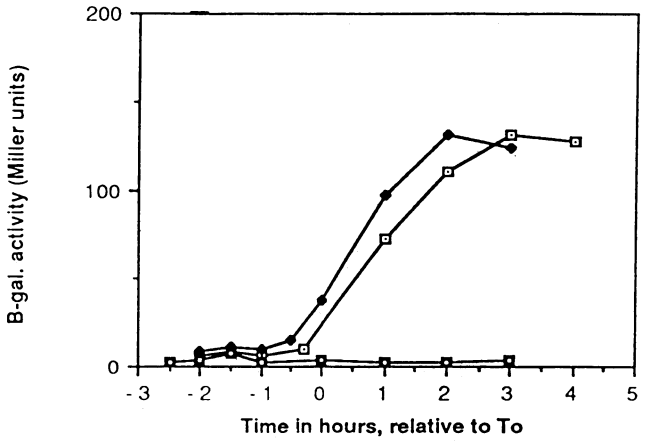

FIG. 5. *spo0F-lacZ* regulation in mutant strains deleted for σ^B or σ^H. (A) *spo0F*-driven β-galactosidase activity of various strains containing an integrated *spo0F-lacZ* plasmid, pIS143, in the wild-type locus was assayed during growth in NSM. The results of β-galactosidase assays are plotted against time. In this experiment the specific activity of β-galactosidase was calculated by using the optical density at 660 nm of the cells, giving Miller units, rather than activity per milligram of protein as in all other assays. The former method gives normalized specific activity values approximately 1/10 of those for which the latter calculation was used. ⊡, IS440 (wild type); ◆, IS513 (*sigB*); ■, IS553 (*sigH*).

DISCUSSION

A *spo0F-lacZ* translational fusion integrated into the wild-type *spo0F* locus was used to study *spo0F* regulation in a variety of environmental and genetic contexts. In all cases, the induction of *spo0F*-driven β-galactosidase and the elevation of Spo0F levels from a basal vegetative level correlated with sporulation. Thus, β-galactosidase and Spo0F were induced in the sporulation medium NSM but were repressed in S6C or in NSM supplemented with glucose and glutamine (Fig. 1 and 3). The addition of decoyinine to S6C medium, which induces sporulation (20), induced *spo0F-lacZ* expression and raised Spo0F levels (Fig. 4). Yamashita et al. obtained similar results with *spo0A-lacZ* fusions (36). Induction of *spo0F-lacZ* activity was dependent on *spo0* genes in NSM and in S6C medium plus decoyinine (i.e., *spo0A*, *-0B*, *-0E*, *-0F*, and *-0H*), with the greatest loss of induction caused by a *spo0H* deletion (Fig. 5). Our results on *spo0* dependence are in agreement with those of Yamashita et al. (37). The correlations of enhanced *spo0F-lacZ* expression and higher levels of Spo0F with the onset of sporulation suggest that an increase in this protein is necessary for the initiation of sporulation. This conclusion was also suggested by results of experiments with deletions in the upstream regulatory region of *spo0F* (19).

Lewandoski et al. (18) found that two *spo0F* mRNA species, RNA I and RNA II, were present during exponential growth. At the end of the exponential phase, the level of RNA I increased 3- to 5-fold, whereas RNA II was no longer detectable. This pattern continued for at least 3 h into the stationary phase. In NSM medium, the pattern of *spo0F-*

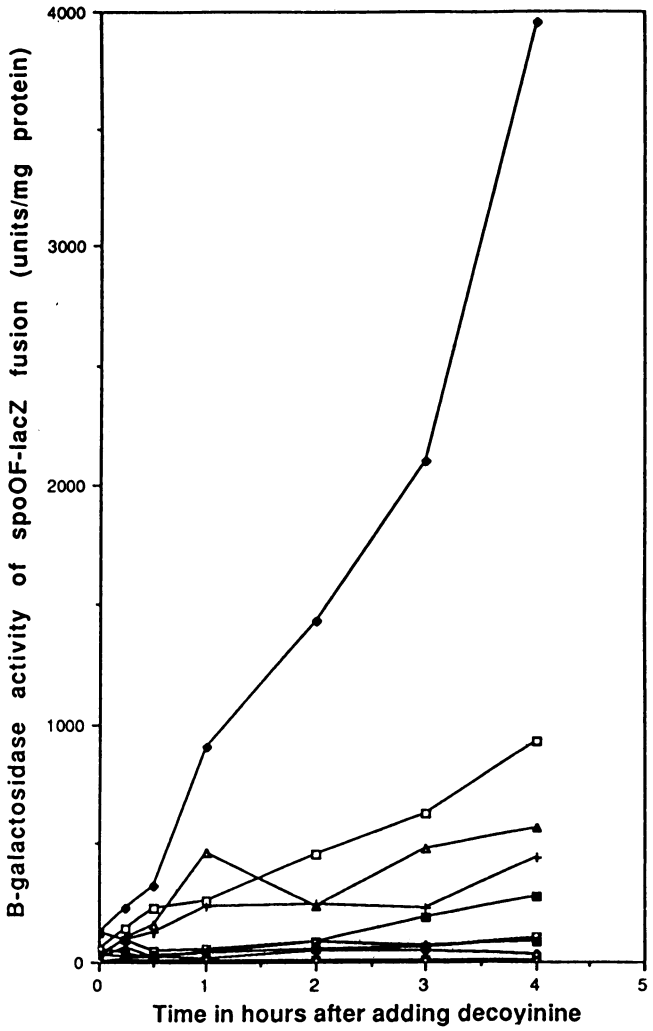

FIG. 6. *spo0F-lacZ* regulation in *spo0* strains. β-Galactosidase activity of various strains was assayed during growth in S6C medium with or without decoyinine. The strains all contained the *spo0F-lacZ* plasmid, pIS143, integrated into the wild-type locus and are isogenic except for the following mutations: IS438 (wild type) with decoyinine (◆) and without decoyinine (⊡); IS465 (*spo0BΔPst*) with decoyinine (═) and without decoyinine (■); IS530 (*spo0AΔ204*) with decoyinine (□) and without decoyinine (■); IS553 (*spo0HΔHind*) with decoyinine (◆) and without decoyinine (■); and IS557 (*spo0F221*) with decoyinine (▲) and without decoyinine (▲).

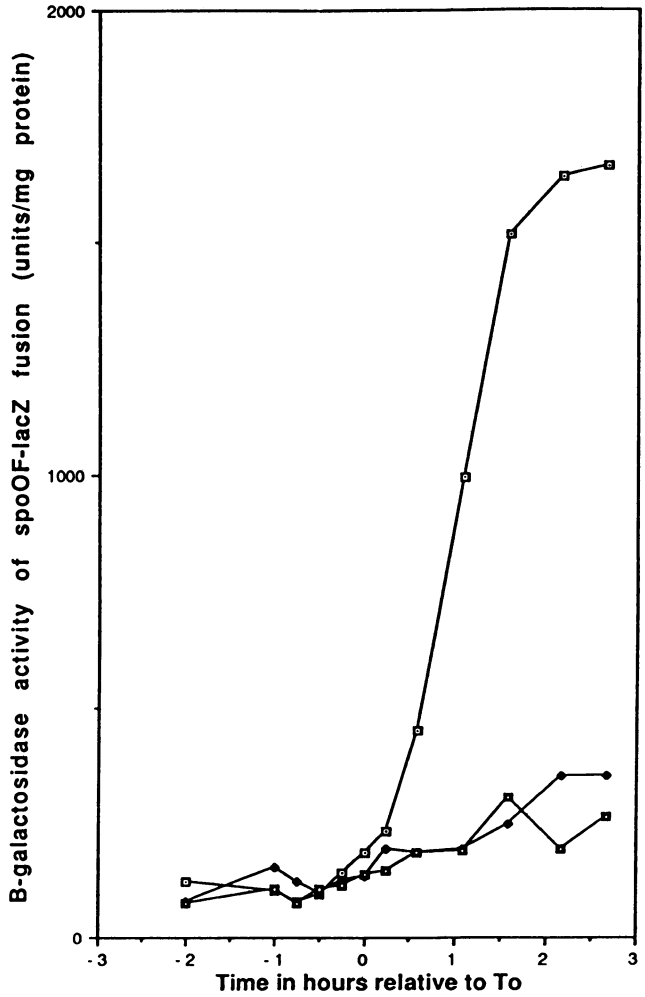

FIG. 7. Effect of *abrB703* on Spo0F expression. *spo0F-lacZ* β-galactosidase activity of various strains was assayed during growth in NSM. All strains contained a *spo0F-lacZ* fusion integrated into the wild-type locus and are isogenic except for the following mutations: *spo0AΔ204*, *abrB703*, or both *abrB703* and *spo0AΔ204*. IS528 (*spo0A*, *abrB*) (◆); IS529 (*abrB*) (⊡); IS530 (*spo0A*) (■).

driven *lacZ* expression and the level of Spo0F roughly correlates with that of the steady-state levels of *spo0F* RNA I transcripts (18). This stationary-phase induction is shared by all mRNAs initiated from σ^H promoters during growth of cells in NSM medium (2, 5, 30, 34). That Spo0F also follows the same pattern of increase during sporulation as RNA I suggests that this RNA, which is transcribed from a $E\sigma^H$-like promoter, codes for Spo0F (33). This view is further strengthened by the findings that (i) *spo0F* expression is dependent on a functional *spo0H* gene (37) (Fig. 5 and 6) and (ii) mutational alteration of the RNA I promoter (from G to A in the −11 region of the promoter) results in a drastic reduction of *spo0F-lacZ* activity (M. Predich and I. Smith, unpublished results). The equivalent nucleotide residue is essential for $E\sigma^H$ transcription of *spoVG* (39).

It is of interest to compare the regulation of the early sporulation genes, *spo0F*, *spoVG* and *spo0A*. All are induced at the end of exponential growth, in rich media, and all are repressed by glucose. Decoyinine can bypass the repressive effects of glucose in minimal media (36, 40) (Fig. 4). In addition, both *spo0F* and *spoVG* are regulated by dual promoters (15, 18), and there is indirect evidence that *spo0A* is also controlled by two promoters (36).

The temporal regulation of the above genes in rich media, the glucose and glutamine repressibility of their expression, and the effect of decoyinine in reversing the glucose effect are similar to the regulation of *citB* (10, 31). It is possible that the product of the *citB* group, aconitase, is necessary for the regulation of *spo0F*, *spoVG*, and *spo0A*. However, *citB* mutations have no effect on the expression of *spoVG* (10) or *spo0F* (E. Dubnau and I. Smith, unpublished data). In addition, *citB* expression does not require intact *spo0H* and *spo0A* genes (6), which are essential for the expression of the early sporulation genes (6). This indicates that while *citB* and the early sporulation genes respond similarly to certain nutrients, they are regulated by different pathways.

lacZ fusion data demonstrate that the *spo0A*, *-0B*, *-0E*, and *-0F* genes are expressed during vegetative growth, and with the onset of sporulation, expression of *spo0A* and *spo0E*, like that of *spo0F*, sharply increases, while that of *spo0B* gradually decreases (4, 9, 26, 37). Yamashita et al. (37) have found that *spo0F-lacZ* induction was unaffected by *spo0B* and *-0F* mutations, if the strain also carried the *sof-1* mutation. Hoch et al. (12) have suggested that either the Spo0A protein, modified by the *sof-1* mutation, carries out functions accomplished by products of these three *spo0* genes or the three Spo0 proteins somehow modify an inactive form of Spo0A to an active form. Several genes, *spo0H* (7), *spoVG* (42), *aprE* (8), *tycA* (21), and *spo0E* (26), which require *spo0A*, are actually repressed by AbrB, and the *spo0A* product is required to reverse this repression (27). Thus, a *spo0A-abrB* double mutation can relieve the *spo0A* dependence of these genes. However, this is not the case with *spo0F* (Fig. 7). Possibly the activated Spo0A protein is acting as a positive regulator for *spo0F*, either indirectly or directly by binding to the *spo0F* promoter.

The Spo0F protein shares amino acid sequence homology with other signal transduction receivers (33, 35). When overproduced, Spo0F inhibits sporulation (17, 18, 28). This might prevent modification of Spo0A, which is apparently needed for sporulation. It has recently been shown that Spo0F is phosphorylated in vitro (25). We have also shown that a high-copy plasmid containing the *spoIIJ* gene, when introduced into a strain unable to sporulate due to overproduction of Spo0F, restored sporulation to normal levels (I. Smith and P. Stragier, unpublished observation). *spoIIJ* has a derived amino acid sequence resembling that of bacterial transmitters (autokinases) of the two-component systems (1), and it has been demonstrated that purified SpoIIJ can phosphorylate Spo0F in vitro (25). These data also suggest that unphosphorylated Spo0F can inhibit sporulation and that phosphorylation of this protein can eliminate this effect.

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