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NOTES

Activation of *spo0A* Transcription by σ^H Is Necessary for Sporulation but Not for Competence in *Bacillus subtilis*

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***spo0A* and *spo0H* are needed for the initiation of sporulation and for the development of genetic competence in *Bacillus subtilis*. Transcription of *spo0A* initiates from two promoters, Pv and Ps. Pv is active during vegetative growth and is recognized by RNA polymerase containing σ^A. Expression from Ps increases during sporulation and depends on σ^H, the *spo0H* gene product. A deletion mutation, *spo0AΔPs*, that removes the promoter controlled by σ^H blocked sporulation but had no detectable effect on competence. These results indicate that expression of *spo0A* from Ps is necessary for sporulation and that the requirement for *spo0H* in competence development is not due to its role in expression of *spo0A*.**

Under conditions of nutrient deprivation, cells of *Bacillus subtilis* enter into a developmental pathway that leads to the production of dormant, heat-resistant endospores. Sporulation involves the production of two distinct cell types with different developmental fates (reviewed in reference 11). Sporulation is not the only form of development in *B. subtilis*. When cells are grown in minimal glucose medium, a fraction of the cells in a culture differentiate to become competent to take up exogenous DNA (reviewed in reference 9).

Both sporulation and competence development are controlled in part by the *spo0A* gene product, a transcription factor that functions as an activator and a repressor, depending on the location of the target binding site. Spo0A is a response regulator of the large family of two-component regulatory systems (23). The activity of Spo0A is controlled by phosphorylation (6), and the phosphorylated (activated) form has a higher affinity for DNA than does the nonphosphorylated form (4, 5, 31, 32). Several proteins are required for the phosphorylation of Spo0A, including the histidine protein kinases KinA (3, 24), KinB (33), and KinC (19) and the phosphotransfer proteins Spo0F and Spo0B. One or more kinases autophosphorylate on a histidine residue and serve as substrate to donate phosphate to Spo0F. Spo0B then acquires the phosphate from Spo0F~P, and finally phosphate is transferred from Spo0B~P to Spo0A (6). A threshold concentration of Spo0A~P appears to be necessary for the initiation of sporulation (8).

Transcription of *spo0A* is controlled by two promoters, Pv and Ps (7). Pv is expressed during vegetative growth and is transcribed by RNA polymerase containing the major sigma factor σ^A. Ps is induced during sporulation and requires σ^H as well as Spo0A~P for expression (26, 30). σ^H, the *spo0H* gene product, is also needed for the development of competence and the expression of competence genes (2, 17).

Previous work had characterized the role of Pv in sporulation (7). Pv is not needed for sporulation under normal conditions in the absence of glucose. However, mutations that prevent transcription of *spo0A* from Pv cause sporulation to be much more sensitive to glucose inhibition than is normal, probably because Ps is partly repressed when cells are grown in glucose. The roles of Ps, the promoter upstream of *spo0A* that is controlled by σ^H, in sporulation and competence are not known.

Construction of *spo0AΔPs*. To test the role of *spo0APs* in competence and sporulation, we constructed a deletion mutation that removed the sporulation promoter, Ps. This mutation is contained in plasmid pSK5 and was first made by deleting DNA located between the *Hpa*I (26 bp upstream of the *spo0A* initiation codon) and *Ssp*I (102 bp upstream of the initiation codon) sites in pJF1361 (Fig. 1). pSK5 (Fig. 1), containing the *spo0AΔPs* mutation, was recombined into the *B. subtilis* chromosome by a single crossover selecting for resistance to chloramphenicol. Both Spo^+ (~3%) and Spo^- (~97%) transformants were obtained, indicating that the *spo0AΔPs* mutation caused a defect in sporulation. The few Spo^+ transformants were expected to result when integration of the plasmid occurred in the 207 bp upstream of *spo0AΔPs*.

In order to remove the plasmid and leave behind a single copy of *spo0A* containing the promoter deletion, we grew a Spo^+ transformant in the absence of chloramphenicol to allow for loss of the integrated plasmid and screened for colonies that became Spo^-. These were most likely to result from loss of the plasmid by single crossover, leaving behind the mutant allele, or from a gene conversion of $spo0A^+$ to *spo0AΔPs*. Spo^- isolates that had lost the plasmid became sensitive to chloramphenicol, and one isolate (strain AG1242) was chosen for further analysis.

PCR analysis was done to confirm that the *spo0AΔPs* mutation had been recombined into the chromosome of strain AG1242 and that the $spo0AP^+$ allele was no longer present. Preparations of chromosomal DNA from AG1242 (*spo0AΔPs*) and the otherwise isogenic strain JH642 ($spo0A^+$) and plasmid DNA from pSK5 (*spo0AΔPs*) and pJF1361 ($spo0AP^+$) were used as templates for PCR with primers KS-3 and KS-6. KS-3 begins ~130 bp downstream of the *Cla*I site, and KS-6 extends to the *Bgl*II site in *spo0A* (Fig. 1). The PCR products generated with AG1242 and pSK5 (*spo0AΔPs*) DNA as templates were indistinguishable in size on an agarose gel and were smaller

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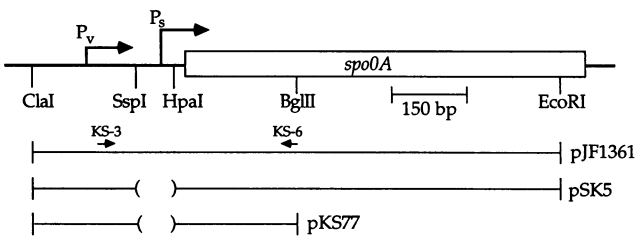

FIG. 1. Restriction map of *spo0A* region and plasmids used. pJF1361 was obtained from J. Hoch (13) and contains *spo0A* DNA from *Cla*I to *Eco*RI cloned into the *Cla*I and *Eco*RI sites of the integrative vector pJH101 (12). Integration of pJF1361 and its derivatives into the chromosome by single crossover results in one intact copy of the *spo0A* structural gene and one copy truncated at the *Eco*RI site. pSK5 was made from pJF1361 by deleting from *Ssp*I to *Hpa*I. Because of the presence of two *Ssp*I sites in the vector sequences, in addition to the site in the *spo0A* promoter region, the *Ssp*I digest was partial. pKS77 was made from pSK5 by deleting from *Bgl*II to *Eco*RI. The approximate locations of the primers KS-3 and KS-6 are indicated. These primers were used for PCR to verify that the *spo0AΔPs* mutation replaced *spo0AP*$^+$ in strain AG1242.

than those from the wild-type and pJF1361 (*spo0AP*$^+$) DNA (data not shown). We did not detect a PCR product that had the mobility of the *spo0AP*$^+$ fragment from AG1242. These results indicate that the *spo0AΔPs* mutation had replaced *spo0AP*$^+$ in the chromosome of strain AG1242.

Sporulation phenotypes caused by *spo0AΔPs*. The *spo0AΔPs* mutation caused a severe defect in sporulation. When sporulation was induced in nutrient sporulation medium (28) or in minimal medium by the addition of decoyinine (22), the sporulation frequency of the mutant was reduced to $<2 \times 10^{-8}$ to $\sim 5 \times 10^{-7}$ of the frequency of the isogenic wild type. The *spo0AΔPs* mutant was phenotypically different from a *spo0A* null mutant. For example, in qualitative plate assays, in contrast to a *spo0A* null mutant, the *spo0AΔPs* mutant produced extracellular proteases and antibiotics.

To determine the nature of the developmental defect caused by the *spo0AΔPs* mutation, we measured the expression of several genes (*spoVG*, *spoIIA*, *spoIIE*, and *spoIIG*) that require *spo0A* for expression. In all cases, expression was monitored by using fusions to *lacZ*. Transcription of *spoVG* is indirectly activated by Spo0A~P via repression of *abrB* (38). That is, AbrB directly represses transcription of *spoVG*, and repression

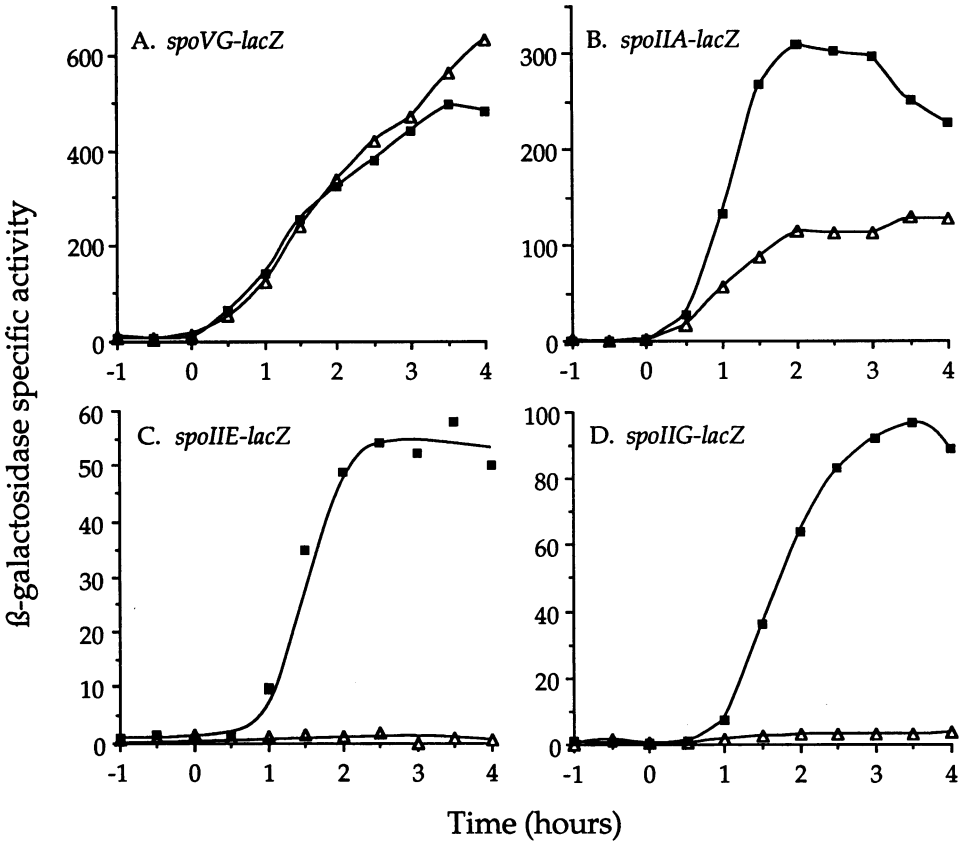

FIG. 2. Effect of *spo0AΔPs* mutation on sporulation gene expression. Strains containing indicated fusions were grown in minimal S7 medium, with 50 instead of 100 mM MOPS (morpholinepropanesulfonic acid) (17, 34), with 1% glucose, 0.1% glutamate, and other amino acids at 40 μg/ml. Sporulation was induced by the addition of decoyinine (22) to a final concentration of 500 μg/ml when the culture reached an optical density at 600 nm of between 0.4 and 0.6. Samples were taken at the indicated times for determination of β-galactosidase specific activity ([ΔA_{420} per minute per milliliter of culture per OD_{600}] × 1,000 [16, 17]). The indicated *spo-lacZ* fusions were in wild-type cells (usually strain JH642 [25]) (closed squares) or the *spo0AΔPs* mutant (strain AG1242) (open triangles). (A) *spoVG-lacZ* in wild-type cells (strain AG130 [14]) and *spo0AΔPs* (strain AG1252). The *spoVG-lacZ* fusion is a translational fusion contained in the specialized transducing phage SPβ (37). (B) *spoIIA-lacZ* in wild-type cells (strain SIK86 [16]) and *spo0AΔPs* (strain AG1257). The *spoIIA-lacZ* fusion is a transcriptional fusion contained in SPβ (35). (C) *spoIIE-lacZ* in wild-type cells (strain AG659) and *spo0AΔPs* (strain AG1250). The *spoIIE-lacZ* fusion is a transcriptional fusion that is integrated into the *spoIIE* locus by single crossover (15). (D) *spoIIG-lacZ* in wild-type cells (strain SIK122 [16]) and *spo0AΔPs* (strain AG1253). The *spoIIG-lacZ* fusion is a transcriptional fusion contained in SPβ (18).

of *abrB* by Spo0A~P is necessary for induction of *spoVG* expression. In contrast, transcription of *spoIIA*, *spoIIE*, and *spoIIG* is directly activated by Spo0A~P (4, 5, 27, 32, 36).

Expression of *spoVG* in the *spo0AΔPs* mutant was indistinguishable from that in wild-type cells (Fig. 2A). In contrast, expression of *spoIIA* was reduced in the mutant (Fig. 2B), and expression of *spoIIE* (Fig. 2C) and *spoIIG* (Fig. 2D) was undetectable. In addition, expression of *abrB-lacZ* appeared normal in the *spo0AΔPs* mutant (data not shown), as expected on the basis of results with *spoVG*. These results indicate that expression of Spo0A from the vegetative promoter, Pv, is sufficient for normal regulation of *spoVG* (and *abrB*) and partial expression of *spoIIA*. In contrast, increased expression of *spo0A* from Ps is required for expression of *spoIIE* and *spoIIG*. These results are consistent with those of previous work indicating that a higher threshold concentration of Spo0A~P is needed for expression of *spoII* genes than for expression of *spoVG* (8).

Because Spo0A~P is needed for expression of *kinA* and *spo0F*, the products of which are required for full activation of Spo0A, it was possible that the effect of *spo0AΔPs* on sporulation was due to decreased expression of these two genes. To test this, we crossed the *spo0AΔPs* mutation into a strain that contained the *rvtA11* allele of *spo0A*. *rvtA11* bypasses the requirement for *kinA* and *spo0F* for sporulation (20, 29) and seems to make Spo0A a direct substrate for KinC (19). To ensure that the recombinant that contained the *spo0AΔPs* mutation still contained the *rvtA11* mutation, we deleted DNA in pSK5 from the *Bgl*II site in *spo0A* to the *Eco*RI site at the junction of *spo0A* and the cloning vector (Fig. 1). This created pKS77, which had the *spo0AΔPs* mutation and only a fragment of *spo0A* ending at the *Bgl*II site. Since the *rvtA11* mutation is downstream of the *Bgl*II restriction site in *spo0A*, integration of pKS77 into the *rvtA11* mutant could not generate a *spo0A*$^+$ allele.

pKS77 was integrated into the *rvtA11* strain by single crossover, and both Spo$^+$ (~25%) and Spo$^-$ (~75%) transformants were obtained. The distribution of Spo$^+$ and Spo$^-$ transformants was similar to that obtained when pKS77 was integrated into wild-type cells (~30% Spo$^+$ and ~70% Spo$^-$). Since the parent strain (*spo0AP*$^+$ *rvtA11*) was Spo$^+$, the Spo$^-$ transformants resulted from the *spo0AΔPs rvtA11* double mutation. If the sporulation defect caused by the *spo0AΔPs* mutation was due solely to decreased expression of *kinA* and *spo0F*, then the *spo0AΔPs rvtA11* double mutant should be Spo$^+$ and all transformants should have been Spo$^+$. The frequency of sporulation of the *spo0AΔPs rvtA11* double mutant (strain AG1285) was similar to that of the *spo0AΔPs* single mutant, indicating that the sporulation defect caused by the *spo0AΔPs* mutation did not result simply from decreased expression of *kinA* and *spo0F*.

Competence development is normal in the *spo0AΔPs* mutant. The *spo0AΔPs* mutation did not cause a defect in competence development. Both *spo0A* and *spo0H* are required for the development of competence (2), and it was possible that the role of *spo0H* in competence development was due to its role in expression of *spo0A*. Expression of *comG-lacZ* (an operon induced during and required for competence development [1, 2]) in the *spo0AΔPs* mutant was indistinguishable from that in wild-type cells (Fig. 3). In addition, the *spo0AΔPs* mutation had little or no effect on the actual transformation frequency. In a direct, side-by-side comparison of the *spo0AΔPs* mutant and the wild-type cells grown in minimal glucose medium, the transformation efficiency was ~0.02% for the mutant and ~0.01% for the wild type. We conclude from these results that the *spo0H* gene product, σ^H, plays a role in competence development independent of its role in expression of *spo0A* and that increased expression of *spo0A* from Ps is not required for competence development.

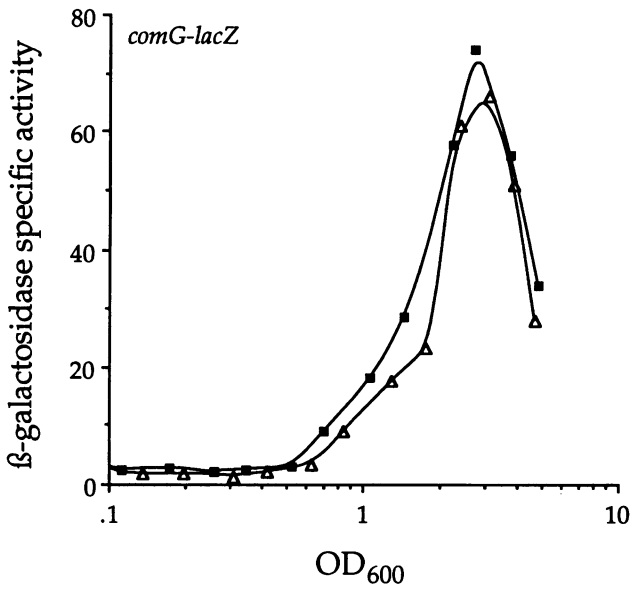

FIG. 3. Expression of *comG-lacZ* normal in *spo0AΔPs* mutant. Strains JMS107 (wild type; *comG-lacZ*) (closed squares) and AG1256 (*spo0AΔPs comG-lacZ*) (open triangles) were grown in minimal medium as described in the legend to Fig. 2, except that no decoyinine was added. At various times during growth, samples were taken for determination of β-galactosidase specific activity. Under these conditions, competence is maximal during late exponential growth and declines as cells enter stationary phase (10, 21). The end of exponential growth occurred at an optical density at 600 nm (OD_{600}) of ~3 in this experiment. The *comG-lacZ* fusion is a transcriptional fusion located in the chromosome at the *amyE* locus (21).

A lower intracellular concentration of Spo0A~P may be needed for competence development than for sporulation. This is consistent with the notion that the primary role of Spo0A in competence is to inhibit transcription of *abrB* (2). The amount of Spo0A~P needed for inhibition of *abrB* is less than that needed to activate expression of *spoII* genes, as evidenced by the effects of various kinase mutations on gene expression in bulk culture (33) and in single cells (8). Interestingly, the amount of Spo0A~P needed for expression of *spoIIA* appears to be less than that needed for expression of *spoIIE* and *spoIIG*. This could be due to possible differences in binding affinities at the different sites or perhaps to differences in interactions with RNA polymerase. *spoIIA* is transcribed by RNA polymerase containing σ^H (35), while *spoIIE* and *spoIIG* are transcribed by RNA polymerase containing σ^A (18, 36).

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