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α-Helix E of Spo0A Is Required for σ^A- but Not for σ^H-Dependent Promoter Activation in *Bacillus subtilis*

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At the onset of endospore formation in *Bacillus subtilis*, the DNA binding protein Spo0A activates transcription from two types of promoters. The first type includes the *spoIIG* and *spoIIE* promoters, which are used by σ^A-RNA polymerase, whereas the second type includes the *spoIIA* promoter, which is used by RNA polymerase containing the secondary sigma factor σ^H. Previous genetic analyses have identified specific amino acids in α-helix E of Spo0A that are important for activation of Spo0A-dependent, σ^A-dependent promoters. However, these amino acids are not required for activation of the σ^H-dependent *spoIIA* promoter. We now report the effects of additional single-amino-acid substitutions and the effects of deletions in α-helix E. The effects of alanine substitutions revealed one new position (239) in Spo0A that appears to be specifically required for activation of the σ^A-dependent promoters. Based on the effects of a deletion mutation, we suggest that α-helix E in Spo0A is not directly involved in interaction with σ^H-RNA polymerase.

Initiation of endospore formation in *Bacillus subtilis* is controlled by the DNA binding protein Spo0A, which activates transcription from several promoters, including *spoIIG* (11), *spoIIE* (17), and *spoIIA* (2, 15), by binding to sites near the −35 region of these promoters. The *spoIIG* and *spoIIE* promoters are used by σ^A-RNA polymerase, whereas the *spoIIA* promoter is used by RNA polymerase containing the secondary sigma factor σ^H. Previous studies conducted by Buckner et al. (1) and Hatt and Youngman (4) identified a 14-amino-acid region in the C terminus of Spo0A (from residues 227 to 240) important for activation of Spo0A-dependent, σ^A-dependent promoters. Specifically single-amino-acid substitutions at positions G227, I229, S233, F236, and V240 result in reduced ability to stimulate transcription of σ^A-specific promoters while having no effect on stimulation of σ^H-dependent promoters (1, 4). In addition to these mutations that specifically impair σ^A-dependent promoter activation, Buckner et al. (1) also report that a mutant form of Spo0A (S231F) suppresses the sporulation defect of H359R and several other substitutions in σ^A, again suggesting that the region around residue 231 in Spo0A is important for σ^A-dependent promoter activation. Interestingly, all mutations in Spo0A affecting the ability of σ^A-dependent RNA polymerase to activate transcription cluster in α-helix E, a flexible helix in the C terminus of the protein that is positioned away from the core structure of the protein (7, 18) (Fig. 1). Taken together these results suggest that α-helix E perhaps contacts σ^A-RNA polymerase when bound to promoters to stimulate transcription.

It is not known whether all the amino acid residues in α-helix E that are involved in stimulation of σ^A-directed transcription have been identified or whether any of the amino acids in α-helix E play a direct role in activation of σ^H-RNA polymerase. Therefore, we examined the effects of additional single-amino-acid substitutions and the effects of deletions in α-helix E on activation of σ^A- and σ^H-dependent promoters.

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MATERIALS AND METHODS

Bacterial strains and culture media. Routine microbiological manipulations and procedures were carried out by standard techniques as described by Harwood and Cutting (3). The concentrations of antibiotics used for selection on Luria broth or Difco sporulation media (DSM) were 5 μg/ml for choramphenicol, 100 μg/ml for spectinomycin, and 100 μg/ml for ampicillin. Cultures were in grown in Luria broth, and sporulation was induced by nutrient exhaustion in DSM. Competent cells were prepared and transformed by the two-step method as described by Harwood and Cutting (3).

The *B. subtilis* strains used (Table 1) are all derivatives of JH642 and contain the *trpC2* and *phe-1* alleles. Plasmids derived from pCB2 (1) were used for inserting various mutations at the wild-type *spo0A* locus.

In order to create the *spo0A* deletion strain (EUAKB78), the 5′-flanking DNA of *spo0A* was PCR amplified with primers 0AUS5FOR (*Hin*dIII end) and 0ADS5REV (*Bcl*I end) and was cloned into *Hin*dIII-*Bcl*I-digested pCB3 (1) to generate plasmid pAK53. pAK53 was linearized with *Sca*I and was transformed into JH642 as previously described by Buckner et al. (1). Chromosomal DNA was isolated from a spectinomycin (100 μg/ml)-resistant transformant by using the Qiamp DNA Mini Kit (Qiagen Inc., Valencia, Calif.) and was subjected to PCR to determine if the gene replacement of spectinomycin for *spo0A* occurred. The following PCR primers were used in combination, 0AUUS5FOR and SpecREV or 0ADS2REVand SpecFOR, to confirm the allelic replacement of the *spo0A* with the spectinomycin gene.

The QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, Calif.) was used to create mutations in Spo0A that resulted in single alanine substitutions. Briefly, pCB2 was used to create single alanine substitutions from amino acid positions 234 to 239 of Spo0A. The combination of FOR and REV primers listed in Table 2 was used to make the single-amino-acid substitutions, and the resulting plasmids for each mutation were subjected to sequencing by using the 0AUS4FOR and 0A3CREV primers to ensure the presence of the desired mutation.

Overlapping PCR was used to create three different deletions within the coding sequence of Spo0A. In deletion 1, amino acids from positions 225 to 244 were deleted, and in deletion 2, amino acids from positions 233 to 241 were deleted, while in the third class of deletion amino acids from positions 229 to 241 were deleted. In the first round of PCR, the 5′ end of the coding sequence (from the starting methionine to either amino acid position S225, S233, or I229) was amplified with forward primer 0AUSFOR in combinations with reverse primer 0ADS3REV, 0ADS4REV, or 0ADS5REV. This generated three PCR products that contained overlapping complementary regions at their 3′ end to primers

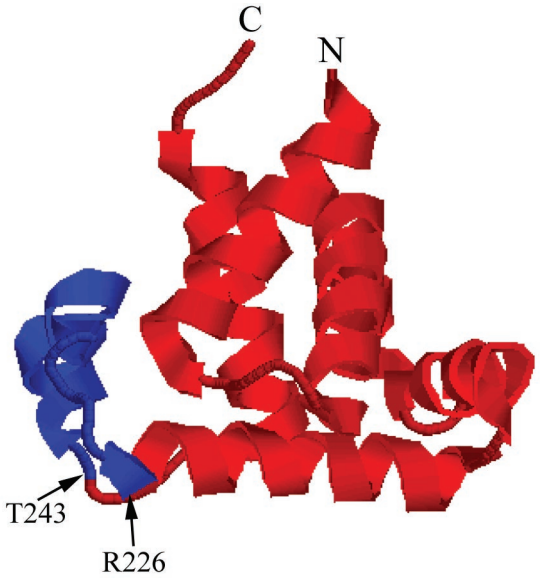

FIG. 1. Ribbon diagram of the C-terminal, DNA binding domain of Spo0A. The structure of the *Bacillus stearothermophilus* Spo0A is based on coordinates from Lewis et al. (7) and is generated by using RasMol version 2.7.1.1. The α-helix E is colored blue, and the rest of the structure is red. Regions of the helix deleted in *B. subtilis* Spo0A are delineated by the numbered amino acid positions. Amino acid positions R226 and T243 correspond to amino acid positions R218 and S235 in *B. stearothermophilus* Spo0A, respectively.

0AUS2FOR, 0AUS3FOR, and 0AUS4FOR primers, respectively. In the second round, the 3′ end of the Spo0A coding sequence (from amino acid positions A244 or S241 to S267) was amplified with primer 0AUS2FOR, 0AUS3FOR, or 0AUS4FOR in combination with 0ADS6REV. In the third step, the products of the first- and second-round primers were combined and reamplified with outer primers 0AUSFOR and 0ADS6REV. This gave PCR products that now contained the entire *spo0A* sequence with deletions incorporated within them. The PCR products obtained from the third round were digested with *Bcl*I and *Hin*dIII and were cloned into *Bam*HI-*Hin*dIII-digested pCB3, thus giving rise to three different integrational vectors (pAK21, pAK22, and pAK23). To confirm that the deletions were in frame with the coding sequence, plasmids pAK21, pAK22, and pAK23 were sequenced with primer 0A3CREV.

TABLE 1. Bacterial strains used in this study

B. subtilis strain or bacteriophage	Genotype	Source or reference
Strains		
JH642	*trpC2pheA1*	J. Hoch
EUAKB18	Wt*spo0A-spec*	This work
EUAKB78	Δ*spo0A*::*spec*	This work
EUAKB56	S234A-Spo0A	This work
EUAKB55	L235A-Spo0A	This work
EUAKB57	F236A-Spo0A	This work
EUAKB54	G237A-Spo0A	This work
EUAKB20	Y238A-Spo0A	This work
EUAKB19	Y238K-Spo0A	This work
EUAKB21	Y238D-Spo0A	This work
EUAKB58	T239A-Spo0A	This work
EUAKB38	Spo0AΔ1	This work
EUAKB39	Spo0AΔ2	This work
EUAKB40	Spo0AΔ3	This work
EUAKB82	Suppressor to EUAKB38, V8A+Spo0AΔ1	This work
Bacteriophages		
SPβ*spoIIG-lacZ*		10
SPβ*spoIIA-lacZ*		16
SPβ*abrB-lacZ*		19

TABLE 2. Oligonucleotides used for PCR, sequencing, and mutagenesis

Name	Sequence (5′–3′)
0AUS5FOR	CCCAAGCTTAACAGAAAATCAAAACGAAGCTG
0ADS5REV	GCGTGATCACACGTTTCTTCCTCCCCAAATG
0AUUS5FOR	GGATGCTGTCAGAAGCAGGAATC
SpecREV	CCACTCTCAACTCCTGATCC
0ADS2REV	CTTAGTCGGCTACCGCCTGTC
SpecFOR	GGATCAGGAGTTGAGAGTGG
0AUS4FOR	CATTTGGGGAGGAAGAAAC
0A3CREV	CGCGGATCCTTAAGAAGCCTTATGCTC
0AUSFOR	AAGCAAGCTTACTGCCGGAGTTTCCGGA
0ADS3REV	AGCTTTGCCTCCGCCGCTCCATGCCACTTCAATTGC
0ADS4REV	TGTCATGCTGCCTCCGGAAATGGAATCAATGTTTCC
0ADS5REV	TGTCATGCTTACCTCCAATGTTTCCTCTGCTCCATGC
0AUS2FOR	TGGAGCGGCGGAGGCAAAGCTAAACCTACCAACAGTG
0AUS3FOR	TCCATTTCCGGAGGCAGCATGACAAAAGCTAAACC
0AUS4FOR	GGAAACATTGGAGGTAGCATGACAAAAGCTAAACC
0ADS6REV	TCTAGGGTTGATCATGCTTCGTGATCC
S234AFOR	TGATTCCATTTCCGCGTTGTTTGGTTATAC
S234AREV	GTATAACCAAACAACGCGGAAATGGAATCA
L235AFOR	GATTCCATTTCCTCGGCGTTTGGTTATACTG
L235AREV	CAGTATAACCAAACGCCGAGGAAATGGAATC
F236FOR	CATTTCCTCGTTGGCTGGTTATACTGTCAGC
F236AREV	GCTGACAGTATAACCAGCCAACGAGGAAATG
G237AFOR	CATTTCCTCGTTGTTTGCTTATACTGTCAGCATG
G237AREV	CATGCTGACAGTATAAGCAAACAACGAGGAAAT
Y238AFOR	CCATTTCCTCGTTGTTTGGTGCTACTGTCAGCATGAC AAAAGC
Y238AREV	GCTTTTGTCATGCTGACAGTAGCACCAAACAACGAGG AAATGG
Y238DFOR	CCATTTCCTCGTTGTTTGGTGATACTGTCAGCATGAC AAAAGC
Y238ADREV	GCTTTTGTCATGCTGACAGTATCACCAAACAACGAGG AAATGG
Y238KFOR	CCATTTCCTCGTTGTTTGGTAAAACTGTCAGCATGAC AAAAGC
Y238KREV	GCTTTTGTCATGCTGACAGTTTTACCAAACAACGAGG AAATGG
T239AFOR	CGTTGTTTGGTTATGCTGTCAGCATGACAAAAG
T239AREV	CTTTTGTCATGCTGACAGCATAACCAAACAACG

Each mutant derivative of pCB2 was linearized with *Sca*I and was transformed into competent JH642. Chromosomal DNA was prepared from spectinomycin-resistant colonies and was subjected to PCR with primer sets 0AUUSFOR and SpecFOR and 0ADSREV2 and SpecREV to indicate that recombination occurred at the correct location on the chromosome. The resulting PCR fragment was then sequenced with 0AUS4FOR and 0A3CREV to confirm the presence of the desired mutation.

In order to measure the effects that Spo0A mutations had on σ^A- and σ^H-RNAP holoenzyme-transcribed promoters, each of the mutant strains was transduced with an SPβ lysate containing either an *spoIIA-lacZ*, *spoIIG-lacZ*, or *abrB-lacZ* reporter, as previously described by Henriques et al. (5). All strains used are listed in Table 1.

Sporulation assay. Sporulation was induced by medium exhaustion in DSM as described previously (12). Sporulation efficiency was determined in 30-h cultures as the total number of heat-resistant (80°C for 20 min) CFU compared with the total number of CFU before heat treatment. Data presented were from representative experiments. Similar results were obtained in at least three independent experiments.

β-Galactosidase activity. Cultures were grown in duplicate in DSM with the appropriate antibiotics to initiate sporulation. Two 300-μl aliquots of each culture were collected, i.e., one to measure the optical density and the other to assay for β-galactosidase activity. Enzymatic activity is reported in Miller units (5).

In vivo mutagenesis with EMS. The strain to be mutagenized was plated on DSM agar containing the appropriate antibiotics and was incubated for 36 h at 37°C. A sterile piece of filter paper with 3 drops of ethyl methanesulfonate (EMS) (1.7 mg/ml; Sigma) was placed at the center of the plate. The cultures

TABLE 3. Effects of alanine substitutions for amino acid residues 234 to 239 within wild-type *B. subtilis* Spo0A

Strain	Genotype	No. of CFU (ml^{-1})[a]	No. of heat-resistant spores (ml^{-1})[b]	β-Galactosidase activity (Miller units)[c] for:		
				IIA:lacZ	*IIG:lacZ*	*arbB:lacZ*
EUAKB18	Isogenic wild type	9×10^8	6×10^8	87 ± 6	26 ± 3	20 ± 3
EUAKB78	Null	15×10^8	No spores	14 ± 0	9 ± 0.11	265 ± 31
EUAKB56	S234A	8×10^8	11×10^8	219 ± 11	16 ± 2	31 ± 9
EUAKB55	L235A	5×10^8	4×10^8	165 ± 22	23 ± 0.1	39 ± 8
EUAKB57	F236A	9×10^8	10×10^3	37 ± 3	7 ± 0.5	37 ± 10
EUAKB54	G237A	15×10^8	7×10^8	213 ± 5	23 ± 0.6	34 ± 1
EUAKB20	Y238A	6×10^8	3×10^8	513 ± 72	30 ± 2	20 ± 1
EUAKB58	T239A	5×10^8	15×10^4	127 ± 19	14 ± 0.4	19 ± 5

[a] Cultures were grown for 30 h at 37°C, serially diluted, and plated for counting.
[b] Same cultures were heated for 20 min at 80°C, serially diluted, and plated for counting.
[c] β-Galactosidase activity of the promoter fusions at 3 h after the end of the stationary phase. Shown is the average from two experiments.

were further incubated for 24 h at 37°C, and the plates were exposed to chloroform vapor for 15 min to kill all nonsporulating cells. The plate contents were incubated for 48 h at 37°C to allow any spores to germinate. Colonies were seen only with the EMS-mutagenized strain EUAKB38 carrying the deletion 1 derivate of Spo0A. Single colonies (15) were picked from DSM plates and were streaked out to Luria broth plates. Chromosomal DNA was prepared from each of these colonies and was used to transform EUAKB11 (wild type-Spo0A; *spoIIA-lacZ*). The transformants were selected for spectinomycin resistance (the marker for the *spo0A*) and were scored for blueness. These transformations revealed that all the suppressor mutations were linked to the *spo0A* gene.

Chromosomal DNA was prepared from each of 15 strains, and the *spo0A* gene was amplified with primers 0AUS4FOR and 0A3CREV. The resulting PCR product was sequenced with primers to identify the position of the new mutation. All sequencing was done at the Emory Core DNA facility (Emory University, Atlanta, Ga.).

RESULTS AND DISCUSSION

Identification of a new position in α-helix E of Spo0A that is required for σ^A-dependent promoter activation. To determine whether additional amino acids in α-helix E are involved in stimulation of σ^A-dependent promoters and if any of these amino acids play a role in stimulation of σ^H-directed transcription, we isolated mutants that produced single alanine substitutions at each position from 234 to 239 in Spo0A. To assay the effects of the single alanine substitutions in Spo0A on expression of Spo0A-regulated promoters, we transduced these mutants with specialized SPβ phage lysates that carried either fusions of *spoIIG-lacZ* (an Spo0A-activated, σ^A-dependent promoter), *spoIIA-lacZ* (an Spo0A-activated, σ^H-dependent promoter), or *abrB-lacZ* (an Spo0A-repressed promoter). We also isolated isogenic transductants of a strain containing a spectinomycin marker linked to the wild-type *spo0A* allele and of a strain carrying a deletion of the *spo0A* locus. All of the strains were cultured in DSM, and the accumulation of β-galactosidase was monitored during endospore formation. Three of the single alanine substitutions (L235A, G237A, and Y238A) had little effect on the expression of *spoIIG-lacZ* (Table 3). These mutants also formed heat-resistant spores at frequencies similar to that of the wild-type strain (Table 3). However, substitution of mutations F236A and T239A resulted in reduced expression of *spoIIG-lacZ* and spore formation (Table 3; Fig. 2). The T239A substitution caused increased expression of *spoIIA-lacZ* and had little to no effect on expression of *abrB-lacZ* (Table 3; Fig. 2). These latter results indicate that T239A replaced Spo0A functions as least as well as wild-type Spo0A in activating *spoIIA* transcription and in repressing *abrB* tran-

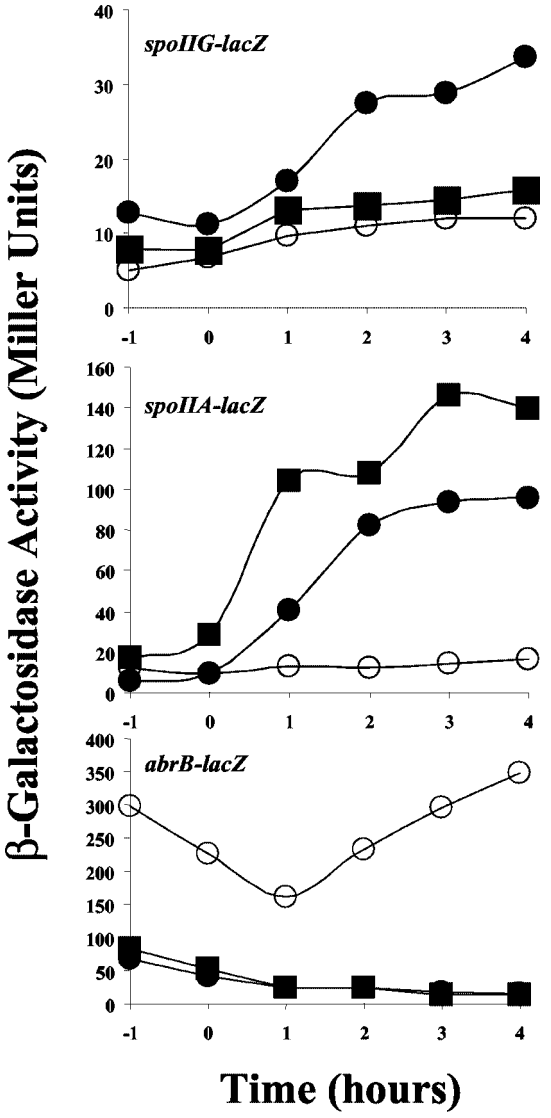

FIG. 2. Effect of substitution T239A on the expression of *spoIIA-lacZ*, *spoIIG-lacZ*, and *abrB-lacZ* transcriptional fusions. DSM cultures of each transduced strain, i.e., EUAKB18 (wild-type–0A ●), EUAKB58 (T239A-0A ■), and EUAKB78 (Null-0A ○), were harvested at hourly intervals beginning at about 1 h before the end of the exponential growth, which is indicated as 0 on the time scale. The collected samples were assayed for β-galactosidase activity indicated in Miller units.

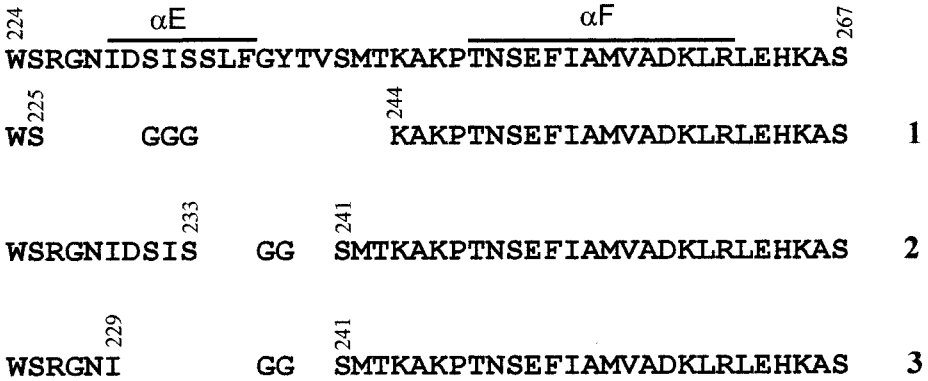

FIG. 3. Amino acid sequence alignment of the C-terminal domains from Spo0A and helix E deletion derivatives. Shown is an alignment of the amino acid sequences from positions 224 to 267 in wild-type *B. subtilis* Spo0A and the homologous positions in three deletion-substitution derivatives of Spo0A. Amino acids regions deleted in the three mutants are represented by gaps, and the corresponding positions in wild-type Spo0A are numbered. The two or three glycine residues that were substituted for the deleted amino acids have been aligned arbitrarily. The α-helix E and α-helix F of C-Spo0A are represented by bars above the amino acid sequence.

scription. Therefore, the T239A substitution probably does not grossly alter structure, stability, or phosphorylation properties of the protein. Evidently, the side chain of T239 is required specifically for activation of the σ^A-dependent *spoIIG* promoter.

Substitution of alanine for the phenylalanine at position 236 (F236A) of Spo0A also reduced the expression of *spoIIG-lacZ* (Table 3). However, this substitution also reduced expression of *spoIIA-lacZ* (Table 3). This substitution did not prevent repression of *abrB-lacZ* expression; therefore, this form of Spo0A probably accumulates and binds DNA like wild-type Spo0A. Substitution of serine for phenylalanine at position 236 is also reported to have similar σ^A- and σ^H-dependent effects in an independent screen conducted by Hatt and Youngman (4). However, since replacement of phenylalanine at position 236 with either serine or an alanine reduced transcription from both the σ^A-dependent and σ^H-dependent promoters, it is not clear whether this substitution defines a position in Spo0A that is directly involved in σ^A or σ^H promoter activation or whether the substitution has a subtle, indirect effect on accumulation or phosphorylation of the protein. In summary, the effects of the alanine substitutions revealed one new position (T239) that appears to be specifically required for activation of a σ^A-dependent promoter and another amino acid (F236) that affects both σ^A- and σ^H-dependent promoter activation. However, in the latter case we cannot eliminate the possibility of an indirect involvement of F236 in promoter activation.

α-Helix E of Spo0A is not required for σ^H-dependent promoter activation. Experimental results (1, 4) previously identified amino acid substitutions in α-helix E of Spo0A that specifically reduced activation of σ^A-dependent promoters; however, no substitutions have been described in this region that specifically affect σ^H-dependent promoter activation. Therefore, it is not known whether α-helix E is involved directly in σ^H-dependent promoter activation. To test the role of α-helix E, we used oligonucleotide-directed mutagenesis to create alleles encoding three different deletions within this helix (Fig. 3). The deletion limits and the placements of linker glycine residues were designed to minimize the effect of deletions on the remaining structure (Fig. 3). We then tested the effects of the deletions on expression of the Spo0A-regulated promoter-*lacZ* fusions. All three deletions abolished transcription from the *spoIIA* and *spoIIG* promoters (data not shown). However, the three deletion derivatives of Spo0A repressed expression of *abrB-lacZ* (data not shown), suggesting that these deletion derivatives of Spo0A retained their ability to bind DNA.

We expected that deletion of α-helix E would prevent activation of σ^A-dependent promoters, since several amino acids in this region have been shown to be essential for σ^A-dependent promoter activation. However, deletion of α-helix E also impaired the ability of Spo0A to activate σ^H-directed transcription. In order to explore further the possibility that α-helix E may be essential for stimulating σ^H-directed transcription, we sought to identify intragenic suppressors of the deletions. A selection for sporulation-proficient derivatives of the α-helix E deletion strains failed, probably because sporulation would require restoration of both σ^A-directed and σ^H-directed transcription. However, during this procedure we discovered that a single valine (GTT)-to-alanine (GCT) substitution at position 8 (V8A) in the deletion 1 derivative of Spo0A suppressed the effect of the deletion on σ^H-dependent *spoIIA-lacZ* expression (Fig. 4). The V8A substitution did not suppress the effect of the deletion on *spoIIG-lacZ* (Fig. 4) expression, nor did it restore formation of heat-resistant spores (data not shown). Therefore, α-helix E in the V8A-substituted Spo0A is not required for activation of the σ^H-dependent *spoIIA* promoter, while α-helix E is required for activation of the σ^A-dependent *spoIIG* promoter.

Since α-helix E is not required for stimulation of σ^H-directed transcription, at least not by the V8A-substituted Spo0A, how does the deletion of α-helix E affect σ^H-directed transcription and how does the V8A substitution suppress the effect of the deletion on σ^H-directed transcription? Immunoblot analyses of Spo0A accumulation during sporulation revealed that the deletion derivatives of Spo0A accumulated to levels that were at least two- to fourfold lower than that of wild-type Spo0A (data not shown). These lower levels of accumulation may have been caused by small decreases in stability of the deletion derivatives of Spo0A and may have been compounded by the requirement of Spo0A for stimulating transcription of its own structural

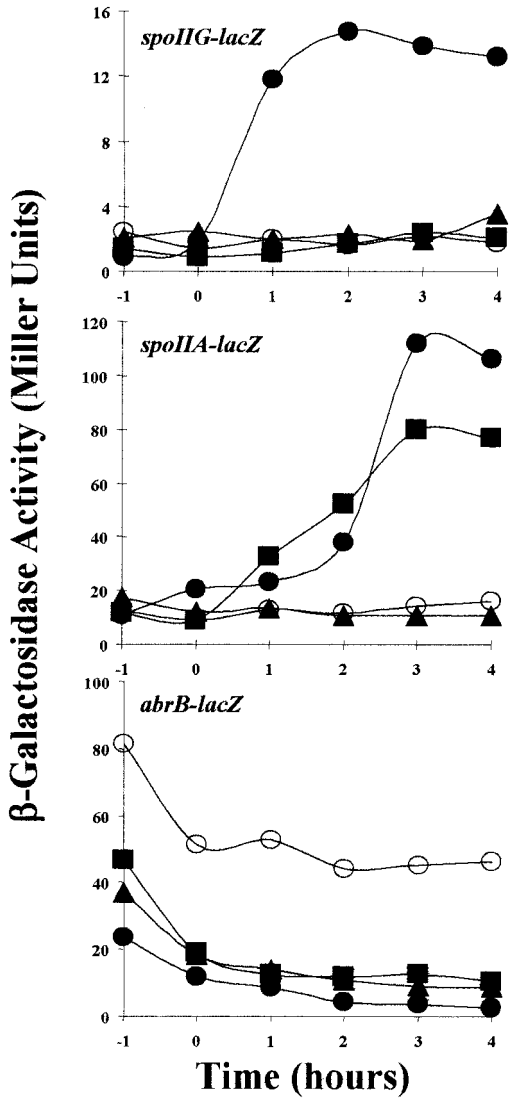

FIG. 4. Effect of deletion mutant Spo0A alleles on expression of *spoIIA-lacZ*, *spoIIG-lacZ*, and *abrB-lacZ* transcriptional fusions. DSM cultures of each transduced strain, i.e., EUAKB18 (wild-type–0A ●), EUAKB38 (Spo0AΔ1 ▲), EUAKB82 (Spo0AΔ1+V8A ■), and EUAKB78 (null-0A ○), were harvested at hourly intervals beginning at about 1 h before the end of the exponential growth, which is indicated as 0 on the time scale. The collected samples were assayed for β-galactosidase activity indicated in Miller units.

gene (14). However, the small decrease in accumulation of the Spo0A deletion derivatives probably played no role in the reduction of Spo0A-dependent promoter activation. Immunoblot analyses showed that the V8A substitution did not substantially increase the accumulation of Spo0A (data not shown). Nevertheless, the V8A substitution restored activation of the *spoIIA* promoter by the deletion 1 derivative Spo0A. One possible explanation for these results is that the V8A substitution increases the fraction of the Spo0A that is phosphorylated, which allows the protein to stimulate transcription more efficiently. Consistent with this hypothesis is the observation by Stephenson et al. (13) that N12 of Spo0A is critical for interaction with the Spo0E phosphatase. Two highly conserved aspartate residues, D10 and D11, that form part of the acid pocket at the phosphorylation site are located between N12 and V8 (6). Therefore, it is possible that the V8A substitution may reduce interaction with the Spo0E phosphatase, resulting in higher levels of Spo0A phosphorylation. The equivalent residue of Spo0A V8 is normally a hydrophobic residue in response regulator receiver domains and lies in the first element of the secondary structure, a β-strand that contributes to the positioning of the β1-α1 loop containing the residue D10-N12, and so this is our preferred explanation.

Other possible mechanisms by which the V8A substitution restores activation of σ^H-directed transcription by the α-helix E deletion derivative of Spo0A would include creation of an interaction between the N-terminal domain of Spo0A and RNA polymerase that compensates for an interaction with RNA polymerase that was lost upon deletion of α-helix E or a model in which the V8A affects interaction between the N- and C-terminal domains of Spo0A. We cannot eliminate the former model, but it seems unlikely that substitution of valine for alanine, which effectively removes side chain volume, would establish a new interaction between proteins and seems likelier that the alanine substitution would eliminate an interaction, such as between Spo0A and Spo0E. We also cannot completely eliminate the latter model. However, if the V8A substitution affects the interaction between the C- and N-terminal domains of Spo0A, the effect on the conformation of the C-terminal domain would likely be very small. This effect would not likely be great enough to compensate for the absence of α-helix E if this helix plays a direct role in stimulating σ^H-RNA polymerase. Therefore, we conclude that α-helix E in Spo0A probably is not directly involved in interaction with σ^H-RNA polymerase. If α-helix E does not interact with σ^H-RNA polymerase, then another region of Spo0A probably interacts with σ^H-RNA polymerase. Presently the best candidate for a region of Spo0A that interacts with σ^H-RNA polymerase is at the extreme C terminus, where amino acid substitutions at positions 257, 258, and 260 have been shown by Rowe-Magnus et al. (9) and Perego et al. (8) to reduce activation of the σ^H-dependent promoter *spoIIA*. However, as Rowe-Magnus et al. (9) discuss in their paper, they could not eliminate an indirect role for this region in activation of σ^H-dependent promoters.

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