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A Region in *Bacillus subtilis* σ^H Required for Spo0A-Dependent Promoter Activity

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Spo0A activates transcription in *Bacillus subtilis* from promoters that are used by two types of RNA polymerase, RNA polymerase containing the primary sigma factor, σ^A, and RNA polymerase containing a secondary sigma factor, known as σ^H. The region of σ^A near positions 356 to 359 is required for Spo0A-dependent promoter activation, possibly because Spo0A interacts with this region of σ^A at these promoters. To determine if the amino acids in the corresponding region of σ^H are also important in Spo0A-dependent promoter activation, we examined the effects of single alanine substitutions at 10 positions in σ^H (201 to 210). Two alanine substitutions in σ^H, at glutamine 201 (Q201A) and at arginine 205 (R205A), significantly decreased activity from the Spo0A-dependent, σ^H-dependent promoter *spoIIA* but did not affect expression from the σ^H-dependent, Spo0A-independent promoters *citGp2* and *spoVG*. Therefore, promoter activation by Spo0A requires homologous regions in σ^A and σ^H. A mutant form of Spo0A, S231F, that suppresses the sporulation defect caused by several amino acid substitutions in σ^A did not suppress the sporulation defects caused by the Q201A and R205A substitutions in σ^H. This result and others indicate that different surfaces of Spo0A probably interact with σ^A and σ^H RNA polymerases.

Spo0A is a DNA binding protein in *Bacillus subtilis* that is essential for the initiation of endospore formation (reviewed in reference 6). Spo0A activates transcription from promoters that are used by two types of RNA polymerase, RNA polymerase containing the primary sigma factor, σ^A, and RNA polymerase containing a secondary sigma factor, known as σ^H (12, 15, 17). Three sporulation-specific promoters that are activated by Spo0A have been characterized extensively: *spoIIG* and *spoIIE*, which are σ^A dependent, and *spoIIA*, which is σ^H dependent (7, 18, 20). Spo0A binds to these promoters at sites overlapping the −35 region and may interact with the sigma subunit of RNA polymerase. Baldus et al. (1) found that transcription from the σ^A-dependent, Spo0A-dependent promoters *spoIIG* and *spoIIE* was reduced in mutants of *B. subtilis* in which σ^A contained either of two single-amino-acid substitutions, lysine at position 356 replaced by glutamate (K356E) or histidine at position 359 replaced by arginine (H359R). However, these substitutions had no effect on the use of a Spo0A-independent promoter, *tms*. Moreover, alanine substitutions at positions 356 and 359 had similar Spo0A-specific effects (14). A single-amino-acid substitution in Spo0A, serine at position 231 replaced by a phenylalanine (S231F), was recently shown to partially suppress the effect of the H359R mutation in σ^A (2). These results support the hypothesis that *spoIIG* and *spoIIE* promoter activation by Spo0A requires the region of σ^A near positions 356 to 359, possibly because Spo0A interacts with this region of σ^A at these promoters.

To determine if the amino acids in the corresponding region of σ^H are also important in Spo0A-dependent promoter activation, we examined the effects of single alanine substitutions at 10 positions in σ^H, from 201 to 210 (Fig. 1). We expected that strains in which an alanine substitution in σ^H resulted in loss of interaction with Spo0A would exhibit reduced sporulation efficiency because utilization of σ^H-dependent, Spo0A-dependent promoters such as *spoIIA* is required for endospore formation. Moreover, if the alanine substitution in σ^H specifically prevented its interaction with Spo0A, then the σ^H mutant would retain the ability to direct transcription from promoters such as *citG* and *spoVG*, since use of these promoters does not require direct interaction with Spo0A (4). The *sigH* allele on plasmid pJB3 was mutagenized in vitro by a multiple-step PCR procedure (3) and used to replace the wild-type allele in the *B. subtilis* chromosome by transformation of strain JH642 (Table 1) to spectinomycin resistance as described previously (1). Spectinomycin-resistant transformants resulted from a single crossover event between the plasmid-encoded *sigH* allele and the chromosomal *sigH* allele. Four *sigH* mutant alleles, which resulted in the substitution of alanine for amino acids at positions 203, 207, 208, and 209 in σ^H, produced transformants that appeared to sporulate as efficiently as the wild-type strain on DSM agar (13). Six alleles of *sigH* produced strains that appeared sporulation deficient (*spo*) on DSM agar (Fig. 1). Of these, four alleles (producing alanine substitutions at positions 202, 204, 206, and 210 in σ^H [Fig. 1]) appeared to inactivate all σ^H-dependent promoter activity (measured as described in reference 1; data not shown) and were not further studied. The remaining two alleles, which resulted in substitutions at 201 and 205 in σ^H, each caused a specific decrease in the Spo0A-dependent, σ^H-dependent *spoIIA* promoter activity, while the Spo0A-independent, σ^H-dependent promoters *spoVG* and *citGp2* appeared to be active (data not shown).

In order to examine the quantitative effects of these amino acid substitutions in σ^H, we isolated derivatives of our *sigH* mutants that had lost the integrated plasmid but retained the mutant *sigH* allele. To isolate these strains, we screened for sporulation-deficient (*spo*; determined as described in reference 1), spectinomycin-sensitive mutants after transformation of *B. subtilis* JH642 (*trpC2 phe-1*) to tryptophan prototrophy with a mixture of 40 ng of *B. subtilis* ZB307A chromosomal DNA (Table 1) and 2 μg of the pJB3*sigHQ201A* and pJB3*sigHR205A* plasmids. Transformants were selected on minimal plates for

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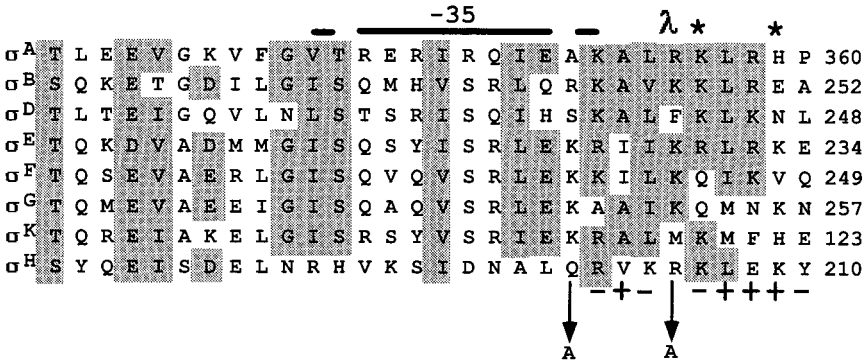

FIG. 1. Amino acid alignment of the −35 recognition regions in the carboxyl terminus of sigma factors in *B. subtilis*. Conserved regions are shaded according to Sun et al. (16). The position number for the last amino acid shown in each sigma factor is indicated. σ^{70} of *E. coli* is not shown, but σ^{70} and σ^{A} are almost identical in this region. Amino acid substitutions are indicated by the arrow from the wild-type amino acid to the altered amino acid. The + and − signs below the σ^{H} sequence indicate the phenotypes of alanine substitutions at that amino acid in σ^{H}. A + sign indicates an allele which produced a functionally wild-type σ^{H} protein, and a − sign designates a completely inactive σ^{H} protein. The λ indicates an amino acid substitution at 596 in σ^{70}, R596H, which specifically suppresses the D38N mutation in λ cI (8, 9). The asterisk indicates amino acid substitutions in σ^{A} (K356E and H359R) which specifically prevent transcription from Spo0A-dependent promoters (1).

sporulation containing 50 μg of phenylalanine and screened for spectinomycin sensitivity and a *spo* phenotype. DNA sequence analysis demonstrated that the *spo* mutants, but none of the Spo^{+} isolates, contained the Q201A or R205A mutations in *sigH*. Two of these *spo* strains, EUC97Q1 and EUC97R1, which express σ^{H} Q201A and R205A, respectively, produced only about 1% of the spores made by the strain containing wild-type *sigH* (Table 2).

To monitor the effects of each amino acid substitution on the activation of specific promoters, we isolated an isogenic set of strains containing operon fusions of *lacZ* to *spoIIG*, *spoIIA*, or *spoVG* or to *citG* as described previously (1) (Table 1). The σ^{H} Q201A and R205A mutations decreased the expression from the σ^{H}-dependent, Spo0A-dependent promoter, *spoIIA*, more than 10-fold (Fig. 2A). The amount of activity in these strains is similar to that measured in strains expressing a *Spo0A* nonsense allele, which retains only 5% of wild-type *spoIIA* activity (2). However, the strains expressing the *sigHQ201A* and *sigHR205A* alleles displayed wild-type levels of the σ^{H}-dependent, Spo0A-independent promoters *citGp2* and *spoVG* (Fig. 2B and C). To determine if *spoIIA* promoter activity was indirectly reduced in the *sigH* mutant strains because of a decreased level of Spo0A, we measured the activity of the σ^{A}-dependent, Spo0A-dependent promoter *spoIIG* and found it to be about 25% of the activity measured in the isogenic wild-type strain (data not shown). These levels are significantly higher than those in an otherwise isogenic strain expressing a *spo0A* nonsense allele (7.4% of wild-type *spoIIG* activity [2]). These results suggest that the side chains of Q201 and R205 of σ^{H} are important for wild-type levels of Spo0A-dependent, σ^{H}-dependent *spoIIA* promoter activity. On the other hand, these amino acid residues are not required for activity of the Spo0A-independent, σ^{H}-dependent promoters *citGp2* or *spoVG*.

The sporulation-defective phenotype caused by an amino acid substitution in σ^{A} (H359R) is suppressed by a single-amino-acid substitution (S231F) in Spo0A (2). We wished to determine whether the S231F form of Spo0A suppressed the *spo* phenotype of the σ^{H} mutant strains. We transformed strains EUC97Q1, EUC97R1, and the wild-type parent, JH642, with plasmids pCB2*spo0A*wt as a control and pCB2*spo0AS231F* as

TABLE 1. Bacterial strains and bacteriophages

Strain or phage	Derivation or genotype	Reference or source
Strain		
JH642	*trpC2 phe-1*	J. A. Hoch
AM878	*amyE*::ϕ(*citGp2-lacZ cat*)	4
ZB307A	Spβc2del2::Tn*917*::pSK10δ6	21
EUC97Q1	JH642 transformed with pJB3*sigHQ201A*	This work
EUC97R1	JH642 transformed with pJB3*sigHR205A*	This work
EUC9729	JH642 transformed with pCB2*spo0A*wt[a]	This work
EUC9733	JH642 transformed with pCB2*spo0AS231*F	This work
EUC9791	EUC97Q1 transformed with pCB2*spo0A*wt	This work
EUC9792	EUC97Q1 transformed with pCB2*spo0AS231F*	This work
EUC9793	EUC97R1 transformed with pCB2*spo0A*wt	This work
EUC9794	EUC97R1 transformed with pCB2*spo0AS231F*	This work
EUC97Q2	EUC97Q1 transduced with SPβ *spoIIA-lacZ*	This work
EUC97Q3	EUC97Q1 transformed with AM878 chromosomal DNA	This work
EUC97Q4	EUC97Q1 transduced with SPβ *spoVG-lacZ*	This work
EUC97R2	EUC97Q1 transduced with SPβ *spoIIA-lacZ*	This work
EUC97R3	EUC97R1 transformed with AM878 chromosomal DNA	This work
EUC97R4	EUC97R1 transduced with SPβ *spoVG-lacZ*	This work
Bacteriophage		
SPβ *spoIIA-lacZ*		19
SPβ *spoVG-lacZ*		21
SPβ *spoIIG-lacZ*		11

[a] wt, wild-type allele.

TABLE 2. Effects of mutations on sporulation

Strain	Allele		No. of heat-resistant spores/ml
	sigH	*spo0A*	
JH642	wt[a]	wt	6.0×10^{8}
EUC97Q1	*Q201A*	wt	1.1×10^{6}
EUC97R1	*R205A*	wt	7.7×10^{5}
EUC9729	wt	wt[b]	4.0×10^{8}
EUC9733	wt	*S231F*[b]	1.1×10^{9}
EUC9791	*Q201A*	wt[b]	1.4×10^{7}
EUC9792	*Q201A*	*S231F*[b]	4.9×10^{7}
EUC9793	*R205A*	wt[b]	5.2×10^{6}
EUC9794	*R205A*	*S231F*[b]	6.2×10^{7}

[a] wt, wild-type allele.
[b] Strain was transformed with pCB2*spo0A*.

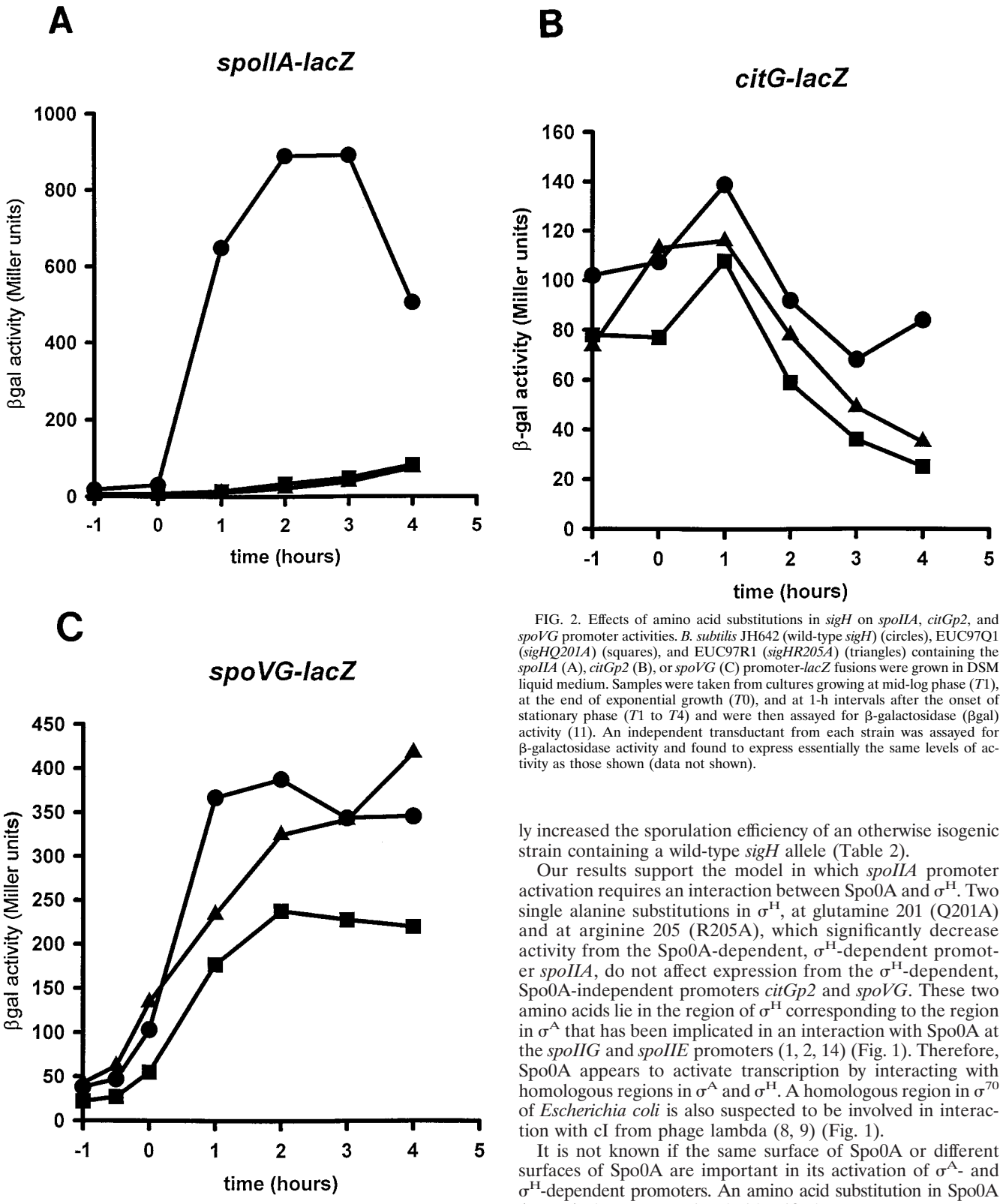

FIG. 2. Effects of amino acid substitutions in *sigH* on *spoIIA*, *citGp2*, and *spoVG* promoter activities. *B. subtilis* JH642 (wild-type *sigH*) (circles), EUC97Q1 (*sigHQ201A*) (squares), and EUC97R1 (*sigHR205A*) (triangles) containing the *spoIIA* (A), *citGp2* (B), or *spoVG* (C) promoter-*lacZ* fusions were grown in DSM liquid medium. Samples were taken from cultures growing at mid-log phase (*T*1), at the end of exponential growth (*T*0), and at 1-h intervals after the onset of stationary phase (*T*1 to *T*4) and were then assayed for β-galactosidase (βgal) activity (11). An independent transductant from each strain was assayed for β-galactosidase activity and found to express essentially the same levels of activity as those shown (data not shown).

described previously (2). Analysis of the sporulation efficiency of the resultant strains (Table 2) indicated that the *spo0AS231F* allele failed to suppress the *spo* phenotype caused by the amino acid substitutions in σ^H. However, the *spo0AS231F* allele slightly increased the sporulation efficiency of an otherwise isogenic strain containing a wild-type *sigH* allele (Table 2).

Our results support the model in which *spoIIA* promoter activation requires an interaction between Spo0A and σ^H. Two single alanine substitutions in σ^H, at glutamine 201 (Q201A) and at arginine 205 (R205A), which significantly decrease activity from the Spo0A-dependent, σ^H-dependent promoter *spoIIA*, do not affect expression from the σ^H-dependent, Spo0A-independent promoters *citGp2* and *spoVG*. These two amino acids lie in the region of σ^H corresponding to the region in σ^A that has been implicated in an interaction with Spo0A at the *spoIIG* and *spoIIE* promoters (1, 2, 14) (Fig. 1). Therefore, Spo0A appears to activate transcription by interacting with homologous regions in σ^A and σ^H. A homologous region in σ^{70} of *Escherichia coli* is also suspected to be involved in interaction with cI from phage lambda (8, 9) (Fig. 1).

It is not known if the same surface of Spo0A or different surfaces of Spo0A are important in its activation of σ^A- and σ^H-dependent promoters. An amino acid substitution in Spo0A (S231F) that was previously identified as a suppressor mutation of the sporulation defect caused by an amino acid substitution in σ^A, H359R (2), displayed little or no suppression of the sporulation defects of the σ^H mutants Q201A and R205A. Alanine substitutions in the 229 to 233 region of Spo0A did not reduce *spoIIA* transcription (2), and Hatt and Youngman (5)

have isolated five additional mutant forms of Spo0A in the region of 227 to 240 that prevented *spoIIG* and *spoIIE* transcription, without affecting transcription from *spoIIA*. Therefore, another region of Spo0A may interact with σ^H RNA polymerase to stimulate its activity. An amino acid substitution in Spo0A, A257T, has been found to prevent *spoIIA* transcription but not *abrB* repression (10). These results are consistent with the model that different amino acids in Spo0A are involved in the activation of σ^H and σ^A RNA polymerases. The ability of response regulators to interact with multiple sigma factors may increase the variety of responses made by bacteria with a limited number of transcription factors.

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