Activation of the *Bacillus subtilis* spoIIG Promoter Requires Interaction of Spo0A and the Sigma Subunit of RNA Polymerase

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*Bacillus subtilis* Spo0A activates transcription from both σ^70^- and σ^H^-dependent promoters. Baldus et al. (2) identified two amino acid substitutions in the carboxyl terminus of σ^H^, K356E and H359R, that specifically impaired Spo0A-activated transcription in vivo. To test the model in which the K356E and H359R substitutions in σ^H^ interfere with the interaction of Spo0A and σ^H^, we examined the effects of alanine substitutions at these positions in σ^H^ on σ^H^-'s ability to direct transcription in vivo and in vitro. We found that alanine substitutions at these positions specifically reduced expression from the σ^H^-dependent, Spo0A-dependent promoters, spoIIG and spoIIE, in vivo. Furthermore, we found that stimulation of spoIIG promoter activity by Spo0A in vitro was reduced by the single substitutions H359A and H359R in σ^H^.

A growing body of evidence supports the idea that site-specific DNA binding proteins activate promoters in bacteria by contacting RNA polymerase (reviewed in reference 4). The site on RNA polymerase that is contacted by the activator protein appears to depend in part on the site on DNA at which the activator protein is bound (16). The *Escherichia coli* cyclic AMP receptor protein (CAP) interacts with the C-terminal domain (CTD) of the RNA polymerase alpha subunit when CAP is bound at a site centered about 61 bp upstream from the transcription start point (−61) (reviewed in reference 11), whereas CAP bound at a site centered at about position −41 activates transcription by contacting the N-terminal domain of the σ subunit (25; reviewed in reference 5). Other activator proteins contact the σ subunit of RNA polymerase, FNR (a protein that is structurally similar to CAP), PhoB, MalT, and cl from phage lambda bind to sites that overlap the −35 region of promoters, and probably contact the σ subunit of RNA polymerase (reference 5 and references therein: 9, 22–24). Some promoters contain multiple binding sites for activators. In some of these cases the two coactivators appear to interact directly with the RNA polymerase (6, 18, 29). At other promoters one coactivator affects DNA binding by the second coactivator, which is the only one to make direct contact with RNA polymerase (26).

Spo0A from *Bacillus subtilis*, which is required for the initiation of sporulation, binds the spoIIG promoter at two sites known as 0A boxes, centered at positions −37 and −87 (reviewed in reference 15; 1, 27). Binding of Spo0A at these sites stimulates utilization of the spoIIG promoter by RNA polymerase containing σ^H^, a homolog of *E. coli* σ^70^ (1, 3, 20, 27, 28). It is not known whether spoIIG promoter activation involves interactions between Spo0A and RNA polymerase. Baldus et al. (2) found that spoIIG promoter activity was reduced in mutants of *B. subtilis* in which σ^H^ contained one of two single amino acid substitutions (glutamate substituted for lysine at position 356, K356E, or arginine substituted for histidine at position 359, H359R). These observations led to the suggestion that spoIIG promoter activation involves an interaction between Spo0A and σ^H^.

The two amino acid substitutions in σ^H^ that reduced spoIIG promoter activity lie near the region of σ^H^ that makes sequence-specific contacts with the base pairs at the −35 region of its cognate promoters (Fig. 1). Other amino acid substitutions in this region of σ^H^ and other sigma factors have been shown to change the specificity of promoter utilization by RNA polymerase containing these mutant sigma factors (12, 19, 32). An alternative explanation for the results of Baldus et al. (2) could be that K356E or H359R slightly changes the specificity of promoter recognition by RNA polymerase, resulting in changes in gene expression that block the initiation of sporulation, and indirectly reduces spoIIG promoter activity. To determine whether the amino acyl residues at positions 356 and 359 of σ^H^ are required specifically for activation of the spoIIG promoter by Spo0A, we examined the effects of single mutations to alanine at these positions on promoter utilization in vivo. We also examined the effects of amino acid substitutions at these positions on stimulation of spoIIG promoter activity in vitro by purified Spo0A.

Mutant sigA alleles were made in vitro by using a multistep PCR procedure described by Cormack (8) and used to replace the wild-type (wt) allele in the *B. subtilis* chromosome by transformation of strain JH642 to kanamycin resistance as described previously (2). Strains EUC9603 and EUC9604 express the H359A and K356A forms of σ^H^, respectively. The isogenic wt parent of these strains produced 4 × 10^8 heat-resistant spores/ml, as did EUC9604. Sporulation of EUC9603 was severely reduced, resulting in only 1.3 × 10^3 heat-resistant spores/ml.

To monitor the effects of each amino acid substitution on the activation of specific promoters, strain EUC9603, strain EUC9604, and the wt parent were lysogenized with SPβYI, specialized transducing phages that carried *lacZ* transcription fusions to one of four different promoters. We examined the accumulation of β-galactosidase in cultures of these strains during growth and sporulation as described previously (2) (Fig. 2). The H359A mutation completely abolished activity from the two Spo0A-dependent, σ^H^-dependent promoters, spoIIG (Fig. 2) and spoIIE (data not shown). The K356A mutation

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Spo0A-dependent promoter
cultivating the Spo0A-independent
transcription of Spo0A, since the unphosphorylated form of Spo0A (data not shown).

whose activity requires Spo0A (1, 33), is active in these strains
polymerase through these mutations by in vitro transcription. We isolated the RNA
ability to interact with Spo0A, we tested the effects of these mutations by in vitro transcription. We isolated the RNA
holoenzyme or H359A
spoIIG promoter. These levels of spoIIG and spoIIIE promoters the activity of the Spo0A-independent, σ^A-dependent promoter tms was unaffected by the H359A and K356A substitutions in σ^A (Fig. 2). There was also no effect of the amino acid substitutions in σ^A on the Spo0A-dependent, σ^H-dependent spoIIA promoter (Fig. 2). The results show that the side chains of H359 and K356 are important for wt levels of Spo0A-dependent spoIIG and spoIIIE promoter activity. On the other hand, these side chains are not required for activity of the σ^H-specific, Spo0A-independent tms promoter. Moreover, it seems unlikely that these changes in σ^A affected expression of Spo0A, since the σ^H-specific spoIIA promoter, whose activity requires Spo0A (1, 33), is active in these strains (Fig. 2).

To eliminate the possibility that the effects of these changes in σ^A on utilization of the spoIIG promoter were caused by some unanticipated indirect effect on σ^A activity, other than its ability to interact with Spo0A, we tested the effects of these mutations by in vitro transcription. We isolated the RNA polymerase σ^H holoenzyme (31) from EUC9605 (wt σ^H), EUB9403 (H359R σ^H) (2), and EUC9603 (H359A σ^H) cell, harvested at late exponential phase in Luria-Bertani medium. The Spo0A activator protein required in our minimal in vitro system was purified from the overproducing E. coli strain JM109(pGO16) (1). However, Spo0A produced in E. coli is the unphosphorylated form, and its efficiency in activation of spoIIG transcription is very low (1, 3). Studies of the domain structure of Spo0A have revealed an amino-terminal domain which undergoes specific aspartate phosphorylation and a CTD able to specifically bind to DNA (13). We used the CTD Spo0A as the activator in the in vitro experiments. Preparation of this activation domain was performed by trypsin cleavage of Spo0A and subsequent separation of proteolytic products by affinity chromatography on heparin, as described in reference 13. For the same concentration, the CTD Spo0A was observed to stimulate three times more specific spoIIG transcripts than the unphosphorylated form of Spo0A (data not shown).

In vitro transcription experiments were performed by co-incubating the Spo0A-independent tms promoter (10) and the Spo0A-dependent promoter spoIIG −35C (27) with wt σ^H holoenzyme or H359A σ^H holoenzyme in the presence of variable concentrations (0 to 400 nM) of the CTD Spo0A (Fig. 3a). Regardless of CTD Spo0A concentrations, wt and H359A holoenzymes were able to generate tms transcripts with equal efficiencies (Fig. 3a). However, this was not the case for spoIIG transcription. To be able to more easily compare results from different enzyme preparations and from different incubations, we compared the amount of spoIIG transcript to the tms transcripts and plotted this ratio as a function of CTD Spo0A.
in vitro strongly supports the model in which close contact at positions 356 and 359 result from subtle changes in the amino acids with large side chains (K356E and H359R) and is essential for efficient utilization of the spoIIG promoter. Results obtained with the enzyme harboring the H359R substitution in the reaction mixtures contained 1,122 kcpm, 161 kcpm, and 83 kcpm at high CTD Spo0A concentrations were 1,122 kcpm, 161 kcpm, and 83 kcpm and those at low CTD Spo0A concentrations were 195 kcpm, 67 kcpm, and 72 kcpm for transcription with wt σ^A RNA polymerase, H359A σ^A RNA polymerase, and H359R σ^A RNA polymerase, respectively. For the tms transcripts, the counts for high and low CTD Spo0A concentrations, respectively, were 155 and 224 kcpm when H359A σ^A RNA polymerase was used, and 91 and 224 kcpm when H359R σ^A RNA polymerase was used.

The K356A substitution also reduced spoIIG promoter activity in vivo (albeit to a lesser degree than the effect of the H359A substitution). The strain containing the K356A mutation retained 50% of wt spoIIE activity and 17% of normal spoIIG activity. Though low, these amounts of promoter activation still produced a sporulation-proficient strain. When the lysine at 356 was mutated to a glutamic acid, a more dramatic Spo0A-specific effect was observed (2). These results are consistent with a model in which Spo0A contacts σ^A near position 359 because the alanine substitution is unlikely to have long-range effects on σ^A structure.

The 356 to 359 region of spoIIG (Fig. 1) which presents a net positive charge. This region might electrostatically interact with a negatively charged subregion of Spo0A. A homologous region in σ^D is suspected to be involved in electrostatic interactions with transcriptional activators. For example, transcriptional activation by CAP’s AR3 (a CAP derivative with a substitution of K52) at class II CAP-dependent promoters and by cI at the F_KM promoter may involve ionic interactions (reference 5 and references therein; 7, 22).

Our results indicate that an interaction between Spo0A and σ^A is required for spoIIG promoter activation. Spo0A binds to two sites on the spoIIG promoter, and it is not known at which site Spo0A is bound when it contacts the 356-to-359 region of σ^A. Other activators that bind at sites upstream from position

![FIG. 3. (a) Comparison of in vitro transcription experiments at the spoIIG promoter with B. subtilis RNA polymerase (40 nM) containing σ^A wt (lanes 1 to 5) and σ^A H359A (lanes 6 to 10). Increasing concentrations of the CTD Spo0A were used for activation: lanes 1 and 6, no CTD Spo0A; lanes 2 and 7, 50 nM; lanes 3 and 8, 100 nM; lanes 4 and 9, 200 nM; lanes 5 and 10, 400 nM. Shown is an autoradiograph of radiolabeled transcripts that were subjected to electrophoresis in 5% (wt/vol) polyacrylamide gel. Arrows indicate the positions of transcripts from the spoIIG and tms promoters. (b) Quantification of the data in panel a. The spoIIG signal is standardized by division with the tms signal from the same lane and plotted against increasing concentrations of the CTD Spo0A. Circles, wt σ^A RNA polymerase; squares, H359A σ^A RNA polymerase; triangles, H359R σ^A RNA polymerase. Results obtained with the enzyme harboring the H359R substitution in σ^A4 are indicated by triangles. The absolute counts at high CTD Spo0A concentrations were 1,122 kcpm, 161 kcpm, and 83 kcpm and those at low CTD Spo0A concentrations were 195 kcpm, 67 kcpm, and 72 kcpm for transcription with wt σ^A RNA polymerase, H359A σ^A RNA polymerase, and H359R σ^A RNA polymerase, respectively. For the tms transcripts, the counts for high and low CTD Spo0A concentrations, respectively, were 155 and 224 kcpm when H359A σ^A RNA polymerase was used, and 91 and 224 kcpm when H359R σ^A RNA polymerase was used.](http://jb.asm.org/Content/0179/11/5607.FIG.3.1.jpg)
Donnelly, C. E., and A. L. Sonenshein.

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REFERENCES


