Contributions of the Domains of the *Bacillus subtilis* Response Regulator Spo0A to Transcription Stimulation of the spoIIG Operon*

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Spo0A is a response regulator that controls entry into sporulation by specifically stimulating or repressing transcription of critical developmental genes. Response regulators have at least two domains: an output transcription regulation domain and a receiver domain that inhibits the output domain. Phosphorylation of the receiver domain relieves the inhibition. We examined the *in vitro* transcription activation mechanism for Spo0A, phosphorylated Spo0A (Spo0A−P), and a deletion mutant that consists solely of the C-terminal output domain (Spo0ABD).

Both Spo0A−P and Spo0ABD stimulated transcription from the *spoIIG* promoter 10-fold more efficiently than Spo0A. Spo0A−P and Spo0ABD induced DNA denaturation by RNA polymerase in the −10 recognition region, whereas Spo0A did not. DNase I footprint assays revealed that phosphorylation enhanced binding of intact Spo0A to the 0A boxes, while the binding of Spo0ABD was similar to that of Spo0A. Thus, activation of Spo0A by phosphorylation is not primarily due to enhanced DNA binding. The presence of a phosphorylated N terminus increased the stability of the ternary complex at the *spoIIG* promoter. We propose that the primary effect of phosphorylation is to expose an RNA polymerase interaction domain to promote transcription from *PspoIIG*.

Bacterial cells constantly monitor environmental conditions and adjust their metabolic state. Alterations in parameters such as cell density, temperature, nutrient status, and the presence of toxic compounds provide signals that trigger physiological responses to cope with these changes. One of the most striking examples of this adaptive ability occurs in *Bacillus subtilis* during continued starvation. A complex set of signals initiates a series of events leading to the remarkable morphological response of endospore formation (1–3).

At the core of the transition into the sporulation pathway is the phosphorelay, an elaborate form of a “two-component” regulatory system, the ultimate target of which is phosphorylation of the transcription regulator Spo0A (1, 4). Phosphate flux through the phosphorelay is controlled by the balance of activities of several sensor protein kinases, a kinase inhibitor, and a collection of specific protein phosphatases (5–7). Activation of Spo0A by phosphorylation leads to the expression of sporulation specific genes and repression of stationary phase regulators (Refs. 1 and 2 and references therein).

Spo0A is a member of the response regulator family of proteins (8–10). Examination of response regulators from many bacterial sources has revealed a common domain structure for these proteins. In the case of Spo0A, the C-terminal, output domain contains the minimal DNA binding activity and transcription activation properties (10, 11). Phosphorylation probably occurs on an aspartate residue located in the N terminus (Asp56) (4, 6) and relieves the N-terminal repression of the C-terminal domain (11). The result is the stimulation of the transcription regulatory activity of Spo0A (12–16).

We were interested in examining the mechanisms of transcription activation by phosphorylated Spo0A (Spo0A−P) and in identifying the domains within the C-terminal portion of Spo0A involved in this process. As a starting point, we used the *in vitro* assays established by Bird et al. (13) to compare the properties of unphosphorylated Spo0A, Spo0A−P, and the C-terminal, DNA binding portion of Spo0A (Spo0ABD). Phosphorylation of Spo0A increases its ability to stimulate both the initial rate of formation and maximum levels of complexes that are resistant to the inhibition by heparin at the σ^70-dependent *spoIIG* promoter (P*spoIIG*) (14). Some previous reports have suggested that Spo0A−P stimulates transcription from the *spoIIG* promoter by enhanced binding to the 0A boxes (15, 16).

Our results indicate that Spo0ABD was similar to Spo0A in DNA binding yet was able to stimulate both the initial rate of formation and maximum levels of heparin-resistant complexes at *PspoIIG*, similar to Spo0A−P. Furthermore, the pathway by which Spo0ABD and Spo0A−P stimulate transcription was similar. We demonstrate that Spo0A−P stimulated a step subsequent toRNA polymerase binding and activation of *PspoIIG* primarily involved the phosphorylation of RNA polymerase-promoter complexes, specifically in the −10 region of the promoter. Finally, we found an effect of stabilization of ternary complexes at *PspoIIG*, which we ascribe to the N-terminal domain of Spo0A−P.

EXPERIMENTAL PROCEDURES

Template DNA—The *spoIIG* template was isolated from the plasmid pUCIIGtpA (17) on a 600-bp DNA fragment generated by digestion with *Pvu*II. This fragment contained the *spoIIG* promoter, both upstream Spo0A binding sites, and the *trpA* transcription terminator approximately 160-bp downstream from the transcription start site. The *trpA* terminator functioned effectively *in vitro*. The fragment was isolated by electrophoresis, recovered by electroelution, and stored in 10

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‡ The abbreviations used are: Spo0A−P, phosphorylated Spo0A; Spo0ABD, the C-terminal fragment of Spo0A (amino acids 143–276); KAc, potassium acetate; Ac, CH₃COO; PspoIIG, promoter for *spoIIG* operon; bp, base pair(s); ApA, adenylyl adenosine.
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mm Hg, pH 8.0, 20 mM potassium acetate, 0.1 mM EDTA at 4 °C (13). The concentration of template fragment was determined using absorbance at 260 nm.

**RNA Polymerase Purification—**B. subtilis RNA polymerase was isolated as described by Dobinson and Spiegelman (18). Preparations were free of RNase and had a specific activity of >98% pure by Coomassie Blue staining of SDS-polyacrylamide gels. The polymerase concentration was determined using the Micro BCA assay (Sigma) using bovine serum albumin (Fraction V, Sigma) as a standard. The activity of the preparations was determined using single round transcription assays under DNA saturating conditions. Typically, preparations were found to be 30–50% active.

**Phosphorylation of Spo0A—**Spo0A and the proteins required to phosphorylate it were a gift of J. A. Hoch (Scripps Research Institute, La Jolla, CA). The phosphorylation of Spo0A was carried out as described previously (13). Samples of unphosphorylated Spo0A or Spo0ABD did not contain KlnA to ensure against low level phosphorylation during transcription initiation. A constant volume of appropriately diluted sample was added to transcription reactions.

**In Vitro Transcription Assays—**Standard transcription reactions (final volume, 20 µl) contained final concentrations of 40 nM RNA polymerase, 4.0 mM template, 80 mM potassium acetate, 0.4 mM each ATP, UTP, and CTP (fast protein liquid chromatography grade, Amersham Pharmacia Biotech); 0.005 mM [α-32P]GTP (800 Ci/mmol; NEN Life Sciences); and 1x transcription buffer (14). A 2-µl aliquot of an appropriate dilution of either Spo0A, Spo0A-P, or Spo0ABD was added to transcription samples containing (in 1× transcription buffer) template DNA, potassium acetate, and the initiating nucleotides ATP and GTP in a 14-µl volume. Following a 2-min incubation at 37 °C, reactions were initiated by adding 2 µl of RNA polymerase (diluted in 1× transcription buffer containing 30% glycerol (v/v)). The reactions were incubated for a further 2 min to allow the formation of initiated polymerase-promoter complexes, and RNA elongation was permitted by the addition of 2 µl of elongation mix (UTP, CTP, and heparin (10 µg/ml final concentration)). After 5 min, reactions were stopped by the addition of one-fifth the volume loading buffer (8.0 M urea, 180 mM Trizma base, 180 mM boric acid, 4.0 mM EDTA, 0.02% each bromophenol blue and xylene cyanole). Radiolabeled transcripts were separated from unincorporated nucleotides by electrophoresis through an 8% polyacrylamide gel containing 7 M urea, 45 mM boric acid, 45 mM Trizma base, and 0.5 mM EDTA. Labeled transcripts were visualized by autoradiography (Eastman Kodak Co.), and promoter activity was determined by measurement of the amount of full-length transcripts or on a Molecular Dynamics PhosphorImager SI using Imagequant software (version 1.0).

**RESULTS**

**A Shared Mechanism of Activation for Spo0A–P and Spo0ABD from Psps0IG—**Aliquots from serial dilutions of a complete phosphorelay reaction (Spo0A–P [open circles]) or a reaction that lacked both KlnA and ATP (Spo0A [open triangles]) or Spo0ABD (closed circles) were added to transcription reactions containing 80 mM potassium acetate, the initiating nucleotides ATP and GTP, and template DNA. Samples were incubated for 2 min at 37 °C, and transcription was initiated by the addition of RNA polymerase. After 2 min, a mixture of UTP, CTP, and heparin was added to block initiation but allow elongation of nascent transcripts, and transcriptional activity (expressed as the percentage of template transcribed) was determined as a function of the total activator concentration.

**FIG. 1. In vitro stimulation of transcription by Spo0A–P and Spo0ABD from Psps0IG.** Aliquots from serial dilutions of a complete phosphorelay reaction (Spo0A–P [open circles]) or a reaction that lacked both KlnA and ATP (Spo0A [open triangles]) or Spo0ABD (closed circles) were added to transcription reactions containing 80 mM potassium acetate, the initiating nucleotides ATP and GTP, and template DNA. Samples were incubated for 2 min at 37 °C, and transcription was initiated by the addition of RNA polymerase. After 2 min, a mixture of UTP, CTP, and heparin was added to block initiation but allow elongation of nascent transcripts, and transcriptional activity (expressed as the percentage of template transcribed) was determined as a function of the total activator concentration.

**Electrophoretic Mobility Shift Assays—**End-labeled DNA was prepared as described for the DNase I footprint reactions, except that the second digestion was performed with HinHI to give a 230-bp fragment. Reactions contained either Spo0A, Spo0A–P, or Spo0ABD at a final concentration of 400 nM and template DNA (105 cpm) in 1× transcription buffer in a final volume of 10 µl. The initiating nucleotides ATP and GTP (0.4 mM final concentration of each) were also present. Samples were incubated at 37 °C for 3 min to allow complex formation before the addition of one-third the volume of heparin loading buffer (100 µg/ml heparin, 20% glycerol (v/v)) in 1× transcription buffer. Samples were immediately loaded onto a running (20 mA) 4.5% acrylamide gel containing 40 mM acetic acid, 40 mM Trizma base, 1 mM EDTA, and 2% glycerol, and electrophoresis was continued for 2 h. The gels were dried and exposed to x-ray film (Kodak XAR) overnight.
sayes were used to assess the formation of heparin-resistant complexes (Fig. 2), addition of RNA polymerase to the spoIIG template resulted in a small amount of DNA being retarded. This paralleled the low base-line transcription. None of the forms of Spo0A alone formed an association with the promoter that was stable under the conditions used. Whereas no increase in retarded band intensity was observed when Spo0A was added to the polymerase-DNA mixture, the intensity of the shifted complex increased dramatically in samples containing Spo0A–P or Spo0ABD and RNA polymerase, in agreement with the transcription results. Thus, the C-terminal fragment of Spo0A contained the minimal machinery necessary for transcription from PspoIIG, and the N terminus of Spo0A was not required for formation of a transcriptionally active complex.

**Spo0A–P and Spo0ABD Modify RNA Polymerase-DNA Contacts in the −10 Region of the Promoter**—We expected the structural characteristics of complexes generated by Spo0A–P and Spo0ABD to be similar to each other and different from those containing Spo0A. We used potassium permanganate to probe transcription complexes (19) formed in the absence or presence of various initiating nucleotides: ATP, which allows formation of a potential dimer; ApA and GTP, which allow synthesis of a primer; and ATP and GTP, which allow synthesis of an 11-mer transcript (Fig. 3).

In the absence of initiating nucleotides, the addition of Spo0A to RNA polymerase-promoter complexes induced very little sensitivity of the −13 to +13 region of the promoter to modification by KMnO4. The inclusion of ATP, ApA, or ApA and GTP caused a slight increase in thymine reactivity in the −10 region of the promoter. This low level of modification may represent an association of the RNA polymerase with the promoter that was the source of the low level stimulation of transcription observed with Spo0A. In contrast, the inclusion of Spo0A–P to RNA polymerase-promoter complexes led to extensive denaturation of the promoter in the −3 to −13 region. Due to the presence of ATP in the phosphorelay reaction, all initiation reactions containing Spo0A–P also contained ATP. Although the first two bases in the transcript are adenines, the presence of ATP did not result in denaturation of the thymine at position +1. When ApA and GTP were added, the denatured region was extended to the +3 position. At this point, heparin resistance was attained (data not shown); thus, this state was dependent upon denaturation around the transcription start site and the initiation of RNA synthesis.

Spo0ABD stimulated denaturation in RNA polymerase-promoter complexes without nucleotide requirement. Strong KMnO4 reactivity was observed in the −3 to −13 region of the promoter. The addition of ATP or ApA did not alter the thymine reactivity pattern. This indicated that the −3 to −13 denatured region was not dependent on the presence of ATP. Under ApA plus GTP initiation conditions, the region of denaturation was propagated downstream to the +3 position. In the presence of ATP and GTP, complexes contained DNA that was denatured from −13 to +13, like those formed with Spo0A–P.

The observation that Spo0A/RNA polymerase-promoter complexes only weakly denatured the region upstream of +1 and could not efficiently initiate transcription indicated that melting of the promoter upstream of +1 is critical for stimulation of initiation at PspoIIG. With either Spo0ABD and Spo0A–P, RNA polymerase induced the same patterns of denaturation at PspoIIG and showed the same response to inclusion of ApA and GTP. On the basis of this comparison, we concluded that the general pathway of initiation was the same for Spo0A–P and Spo0ABD.

**A Lag in the Rate of Transcription Stimulation by Spo0ABD**—The data in Fig. 1 indicated that deletion of the N terminus of Spo0A did not reduce the maximum level of stimulation of transcription from the spoIIG promoter. However, the difference in stimulation between Spo0A–P and Spo0ABD at low protein concentrations suggested that N-terminal deletion might compromise the ability of Spo0ABD to increase the initial rate of initiation. To examine the rate of initiation, we measured the time course of heparin-resistant complex formation in reactions containing Spo0A, Spo0A–P, or Spo0ABD.

Initiation reactions were composed containing template DNA, one of the forms of Spo0A, and the initiation nucleotides ATP and GTP. RNA polymerase was added to start the initiation, and at various times, samples were removed and added to a mixture of UTP, CTP, and heparin to allow elongation by complexes that had initiated RNA synthesis (Fig. 4). In the presence of Spo0A, the initial rate of heparin-resistant complex formation was very low; however, the level of complexes continued to increase with extended incubation times. This indicated that Spo0A was ineffective at overcoming the rate-limiting step in heparin-resistant complex formation, although it could catalyze the transition to heparin resistance slowly. Al-
though both the final levels and the initial rate of formation were increased by Spo0A–P, the effect on the initial rate was most dramatic, in agreement with the results of Bird et al. (14). In the presence of Spo0ABD, the final level of open complexes formed was the same as with Spo0A–P, but a lag in the initial time course of complex formation was consistently observed. With Spo0A–P, accumulation of open complex formation was immediate, whereas reactions with Spo0ABD required 40–60 s to achieve the same level of transcription complexes. Maximum levels of initiation were reached after 3–5 min (data not shown).

A Phosphorylated N Terminus Does Not Alter the Temperature Dependence of spoIIG Transcription Initiation—If the observed difference between Spo0A–P and Spo0ABD in stimulation of the initial rates of formation of heparin-resistant complexes were due to an enthalpic barrier, the temperature dependence of spoIIG transcription stimulation by the two forms of Spo0A might differ. To test this possibility, transcription reactions containing 400 nM Spo0A–P or Spo0ABD were carried out at temperatures ranging from 0 to 42 °C (Fig. 5).

The temperature dependence profiles for transcription stimulated by Spo0A–P and Spo0ABD were virtually identical. Both forms of Spo0A stimulated transcription to the same extent at each of the temperatures tested. This suggested that the presence of a phosphorylated N terminus did not affect the rate-limiting temperature-dependent step necessary for PspoIIG transcription. Stimulation at temperatures below 30 °C was poor, and it was possible that the low level of transcription was due to reduced elongation rather than a failure to form a heparin-resistant complex. To eliminate the possibility that the temperature profile represented lower elongation, initiated complexes were formed at temperatures below 30 °C and then shifted to 37 °C for the elongation step. These assays yielded plots virtually identical to those above, indicating that elongation was not rate-limiting (data not shown).

A Phosphorylated N Terminus Contributes to Stable Ternary Complex Formation—The stability of protein-DNA and protein-protein interactions is strongly dependent on the reaction conditions, including the concentration and type of salt present. Cations interfere with protein-DNA interactions because protein binding requires the displacement of the cations from the DNA, an enthalpically unfavorable process. In addition, certain anions, such as Cl⁻, bind to proteins with low affinities of about 10⁻² M. These types of anions can compete with the DNA for positively charged regions on the protein. In contrast, at low concentration, anions such as Ac⁻ and Glu⁻ do not compete with DNA but can interfere with protein-protein interactions (21–24). Because anions can be used as probes to monitor protein-DNA and protein-protein interactions, we tested the effects of several salts on Spo0A-dependent stimulation (Fig. 6). Stimulation of transcription from PspspoIIG by Spo0A or Spo0ABD was inhibited above 40 mM KCl. Stimulation of transcription by Spo0A–P was less sensitive, but was still inhibited above 80 mM KCl. Similar inhibition profiles were seen with NaCl (data not shown). Transcription stimulation was considerably less sensitive to the presence of the organic salt potassium acetate (KAc). Stimulation by Spo0A or Spo0ABD was not inhibited until the organic salt concentration was above 80 mM, and stimulation by Spo0A–P was resistant to at least 120 mM KAc. We tested potassium glutamate as well and found that it also inhibited the transcription stimulation by Spo0ABD and Spo0A but not Spo0A–P (data not shown). Because a differential effect of Ac⁻ on transcription stimulation by the three forms of Spo0A was observed, we examined this further by testing the stability of these complexes to high salt challenge.

Initiation reactions were formed in 120 mM KAc on ice and then warmed to 37 °C. After a 1-min equilibration, aliquots were removed at various times and added to a mixture of the initiating nucleoside triphosphates. After 2 min more, a mixture of UTP, CTP, and heparin was added to allow a single round of elongation (Fig. 7). Transcription by RNA polymerase alone was used as a base line throughout the assay. Transcription from complexes formed with Spo0A or Spo0ABD dropped to the base line within 30 s, whereas complexes containing Spo0A–P decayed slowly. This indicated that the phosphorylated N terminus stabilized the uninitiated complex. We also examined the liability of the complexes by electrophoretic mobility shift assays. We observed that when initiating nucleoside triphosphates were omitted from reactions containing 120 mM KAc, only the Spo0A–P/RNA polymerase sample gave rise to a shifted complex (data not shown). This result indicated that whereas both Spo0A–P and Spo0ABD could activate transcription, the phosphorylated N terminus stabilized the transcription complex.

Spo0ABD Activates Transcription Despite Poor DNA Bind-
ing—The spoIIG promoter contains two 0A binding sites, each with two 0A consensus binding sequences (0A boxes (16)). Site 1 (−95 to −70) contains Spo0A boxes that are closer to the consensus recognition sequence than those in site 2 (−60 to −35). Consequently, site 1 has been deemed a strong binding site and site 2 a weak binding site (16). One model for the effect of phosphorylation on Spo0A is that it removes N-terminal inhibition of the C-terminal helix-turn-helix DNA binding motif, allowing DNA binding and thus stimulation (15, 16). If this were the case, then deletion of the N terminus, as in Spo0ABD, might lead to tight binding and thus transcription stimulation. To measure specific binding for Spo0A, Spo0A−P, and Spo0ABD, we examined the DNase I protection patterns of the three activator forms at the spoIG promoter. Occupancy of the site 1 and 2 0A boxes was assessed by measuring the intensity of 32P-labeled bands within the protected regions and normalizing them to bands outside the protected regions.

In accordance with previous reports, Spo0A did not exhibit a high affinity for the 0A boxes. Even at 800 nM, Spo0A only displayed 35% occupancy of site 1 and no occupancy of site 2 (Fig. 8A). The results with Spo0A−P demonstrated that phosphorylation enhanced binding to the spoIG promoter. Spo0A−P was able to completely protect both site 1 and site 2. Half maximum occupancy of sites 2 and 1 required 350 and 250 nM Spo0A−P, respectively (Fig. 8D). Spo0ABD, like Spo0A, displayed 32% occupancy of site 1 and no occupation of site 2 at 800 nM (Fig. 8C). Thus, concentrations of Spo0ABD that markedly increased transcription from the spoIG promoter (see Fig. 1) did not result in stable binding. Therefore, enhanced DNA binding was not primarily responsible for the stimulation properties of Spo0ABD, and by implication, activation of Spo0A by phosphorylation does not depend on increasing the interaction of the C terminus with the 0A boxes on the template.

DISCUSSION

Spo0A is the central regulator of sporulation initiation in B. subtilis and regulates endospore formation by specifically stimulating or repressing expression of developmental genes (1, 2, 10, 25). In vivo, the activity of Spo0A requires phosphorylation, and the level of activated Spo0A is tightly regulated by a complex signaling system that includes positive and negative regulators (1–3, 5, 6). N-terminal deletion mutants of Spo0A are constitutively active in vivo (26) and in vitro (Ref. 11 and this work). Thus, the C-terminal fragment of Spo0A is capable of effecting transcription stimulation, and the N-terminal fragment is a negative regulatory domain. Ultimately, analysis of the C terminus is the key to defining the domains within Spo0A that are responsible for transcription activation.

In this investigation, we have demonstrated that two activated forms of Spo0A, Spo0A−P and Spo0ABD, use a common mechanism to stimulate transcription from the spoIG promoter. Structural probing of activator–RNA polymerase–spoIG complexes using KMnO4 revealed that the activated forms of Spo0A, generated by either phosphorylation or N-terminal deletion, were capable of interacting with RNA polymerase to stimulate melting of the spoIG promoter upstream of the +1 site (Fig. 3). The patterns of the reactive thymines and changes in the patterns seen on addition of initiating nucleotides were the same for the two activated forms of Spo0A.

Previous reports have suggested that phosphorylation stimulated transcription from the spoIG promoter by enhancing binding to low consensus 0A boxes (15, 16). Experiments reported here also found that phosphorylation enhanced binding of Spo0A−P to the 0A boxes relative to unphosphorylated Spo0A. The increased binding could suggest that, like OmpR and NTRC (27–29), the primary point of regulation of Spo0A activity is the DNA affinity. However, no increase in binding was observed for Spo0ABD relative to Spo0A, although...
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Spo0ABD stimulated both the initial rate of formation and maximum levels of heparin-resistant complexes at PspoIIG. Because Spo0ABD showed the same transcription activation mechanism as Spo0A–P, we conclude that the enhanced stimulation of transcription caused by phosphorylation of Spo0A is not due to enhanced DNA binding per se, but that the primary effect of phosphorylation is to bring about a structural change that exposes RNA polymerase interaction domains within Spo0A that activate transcription from the spoIIG promoter.

The data in Fig. 6 indicate that a phosphorylated N terminus contributed to the stability of the Spo0A-RNA polymerase-promoter complex to challenge by organic ions but not inorganic ions (the hydrophobic effect). The hydrophobic effect (21–24) proposes that, relative to water, anions, such as Ac−, are preferentially excluded from hydrophobic regions of the surface of proteins. Increasing the overall salt concentration enhances an anion gradient, resulting in an entropically unfavorable formation of a water lattice at the protein surface. This unfavorable condition may be eliminated by protein association to shield these hydrophobic residues, leading to resistance to salt. If phosphorylation exposed a hydrophobic region on the N terminus of Spo0A–P it could provide the hydrophobic effect, whereas unphosphorylated Spo0A or Spo0ABD (which lacks the N terminus) would show less difference in salt sensitivity between inorganic and organic salts. The hydrophobic effect implies that it is possible to bury the hydrophobic residues. This could be accomplished by (i) a conformational change in each Spo0A–P, such as that demonstrated by Grimsley et al. (11); (ii) Spo0A–P self association leading to the formation of homo-oligomers, as suggested by Kobayashi and co-workers (30); or (iii) intermolecular association between Spo0A–P and RNA polymerase.

The higher DNA binding affinity of Spo0A–P compared with Spo0ABD (Fig. 8) was paralleled by an increased resistance to high anion concentrations by transcription complexes containing Spo0A–P compared with those containing Spo0ABD (Figs. 6 and 7). Thus, the increased DNA binding could account for the greater stability of transcription complexes containing Spo0A–P. In addition, the lower stability of complexes containing Spo0ABD may be the cause of the lag in heparin-resistant complex formation observed in the time course reactions (Fig. 4). However, our previous investigation of transcription initiation at the spoIIG promoter indicates that RNA polymerase binding is independent of Spo0A–P and that Spo0A–P binds to the binary complex of DNA and the polymerase (14). Thus, the effects on stabilization could be due to interactions between the N termini of Spo0A proteins or to interactions of the N terminus directly with the polymerase. This raises the possibility that the N terminus itself contributes to signal transduction by wild-type Spo0A.

In its stimulation of the spoIIG promoter, Spo0A acts as a class II activator, binding to the promoter in a region overlapping the −35 recognition sequence for RNA polymerase (31) and making contact with the σ subunit (32, 33). Spo0A also belongs to a class of activators (which include the regulators NTRC (34), NiaA (35), DctD (36), and N4SSB (37)) that function not through recruitment of RNA polymerase to the promoter but through modification of prebound RNA polymerase complexes. Some of this family of activators can cause an isomerization step (from a closed to an open complex) without themselves binding to DNA. We have not demonstrated this ability for Spo0A; however, strong DNA binding is not the primary requisite for its activity. Nevertheless, the Spo0A binding sites at PspoIIG have been shown to play a role in transcription regulation in vivo because mutation of the site 2 0A boxes toward consensus result in increased transcription (16). The 0A boxes may function to orient the activator for co-operative binding or simply increase the local concentration of Spo0A for interaction with the transcription apparatus.

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