J. Biochem. 124, 98-104 (1998)

Feedback Loops Involving Spo0A and AbrB in *In Vitro* Transcription of the Genes Involved in the Initiation of Sporulation in *Bacillus subtilis*

Masaya Fujita[1] and Yoshito Sadaie

Radioisotope Center, National Institute of Genetics, Mishima, Shizuoka 411-8540

Received for publication, January 28, 1998

Through mainly *in vivo* studies, the initiation of sporulation in *Bacillus subtilis* has been shown to depend on the phosphorylation of the Spo0A transcription factor mediated by the multicomponent phosphorelay *via* KinAB (C), Spo0F, Spo0B, and Spo0A in this order. RNA polymerase containing σ^A ($E\sigma^A$) or σ^H ($E\sigma^H$) transcribes the genes of the phosphorelay components. Phosphorylated Spo0A is also involved in their expression and is required for the induction of σ^H by repressing its repressor gene *abrB*. We have examined the effects of phosphorylated Spo0A (Spo0A-P) and AbrB on *in vitro* transcription of the genes involved in the Spo0A phosphorylation and initiation of sporulation. Spo0A-P repressed $E\sigma^A$-dependent transcription of the *kinC* and $E\sigma^H$-dependent transcription of *spo0A* and *kinA*. $E\sigma^H$-dependent transcription of *spo0F* was stimulated by Spo0A-P at low concentrations but was repressed by higher amounts of Spo0A-P. On the other hand, AbrB repressed $E\sigma^A$-dependent transcription of *spo0H* (σ^H gene), *kinC*, and *abrB*, although its effect was not strong. With the present results providing *in vitro* evidence for the roles of Spo0A-P and AbrB as transcriptional regulators, and other results described in the literature, the positive and negative feedback loops controlling the temporal expression of early sporulation genes are discussed.

Key words: *Bacillus subtilis*, RNA polymerase, sporulation, transcription factor.

The early gene expression required for the development of spores in *Bacillus subtilis* is regulated by the Spo0A transcription factor. Spo0A activity is dependent on phosphorylation. The immediate source for the phosphorylation of Spo0A is the phosphorelay signal transduction system (*1*), a more complicated variation of two-component signal transduction systems. The first step is the activation of protein kinases, predominantly the products of the *kinA* and *kinB* genes (*2, 3*), leading to ATP-dependent autophosphorylation. A third kinase, the product of the *kinC* gene, is required under some growth conditions (*4-6*). The phosphate group is then transferred to the Spo0F protein. Spo0F-P in turn becomes a phosphodonor for Spo0B, a phosphoprotein-phosphotransferase catalysing the transfer of phosphate to the Spo0A protein. Phosphorylated Spo0A is a transcription regulator that inhibits the transcription of certain genes including *abrB* (*7-10*), whose product (AbrB) represses the *spo0H* gene of the early-acting sporulation-specific sigma factor (σ^H) and activates the transcription of other genes including the *spoIIA* and *spoIIG* operons, which encode sporulation-specific RNA polymerase sigma factors (*9, 11-13*). The nature of the signals activating the kinases remains unclear.

Transcriptional regulation of the genes participating in the phosphorelay might well be one of main control mechanisms of Spo0A-P formation, leading to the initiation of sporulation. These genes are transcribed by σ^A- or σ^H-RNA polymerase. *kinA* has a σ^H-dependent promoter, while *kinB* and *kinC* have σ^A-dependent promoters. *spo0A* has two promoters, σ^H-dependent Ps and σ^A-dependent Pv. *spo0B*, *abrB*, and *spo0H* have σ^A-dependent promoters. *spo0F* has a σ^H-dependent P2 promoter (*14, 15*). As Spo0A-P, AbrB, and σ^H are involved directly or indirectly in the transcription of the phosphorelay genes, they, together with phosphorelay component genes, constitute the positive feedback loops (*16*). Most studies on the regulation of gene expression of the phosphorelay components have been based on genetic studies and on studies using *lacZ* fusions. The number of direct transcription analyses is insufficient.

In this communication, we describe *in vitro* transcription analyses of *spo0A*, and *spo0H*, and the genes involved in the phosphorelay using σ^A- and σ^H-RNA polymerase with purified Spo0A (or phosphorylated Spo0A) and AbrB proteins. Spo0A-P repressed transcription of the *spo0A*, *kinA*, and *kinC* genes, while transcription from the *spo0F* P2 promoter was first stimulated by Spo0A-P and then repressed by the higher amount of Spo0A-P. AbrB repressed transcription of *spo0H* and *kinC*. The results obtained support the notion of control circuits in which the Spo0A and AbrB proteins activate or repress the initial sporulation pathway.

[1] To whom correspondence should be addressed. Phone: +81-559-81-6880, Fax: +81-559-81-6870, E-mail: mafujita@lab.nig.ac.jp

Abbreviations: IPTG, isopropyl-β-D(−)-thiogalactopyranoside; LB, Luria broth; PMSF, phenylmethylsulfonyl fluoride.

MATERIALS AND METHODS

Overexpression and Purification of Spo0A and AbrB Proteins—The T7 promoter overexpression system of *Escherichia coli* was used for Spo0A and AbrB production. DNA fragments containing the Spo0A and AbrB protein-coding regions of *B. subtilis* chromosome were made by polymerase chain reaction (PCR) with LA-PCR kit (Takara Shuzo). The synthetic oligonucleotides 5′-TACATATGGA-GAAAATTAAAGTTTG-3′ (*Nde*I site is underlined, and boldface letters indicate the initiation codon) and 5′-TGCT-CGAGTTAAGAAGCCTTATGCTCTAAC-3′ (*Xho*I site is underlined, and boldface letters indicated the termination codon) were used to isolate *spo0A*; and 5′-TACATATGTT-TATGAAATCTACTGG-3′ (*Nde*I site is underlined, and boldface letters indicated the initiation codon) and 5′-TGC-TCGAGTTATTTAAGGTTTTGAA-3′ (*Xho*I site is underlined, and boldface letters indicated the termination codon) were used to isolate *abrB*. PCR was carried out for 30 cycles with 1 ng of *B. subtilis* JH642 DNA. Each cycle was run at 95°C for 1 min (denaturation), 55°C for 1.5 min (annealing), and 72°C for 2 min (extension). The PCR products were digested with *Nde*I and *Xho*I and inserted into the pET21b plasmid (Novagen) digested with *Nde*I and *Xho*I. Plasmids having the *spo0A* and *abrB* genes inserted downstream of the T7 promoter were selected and named pETSpo0A and pETAbrB, respectively. Each plasmid was transferred to *E. coli* BL21 (DE3). Cells carrying pETSpo0A or pETAbrB were grown in LB medium (1,000 ml) containing ampicillin (100 μg/ml) at 30°C. When the optical density at 600 nm (OD_{600}) reached 0.6, IPTG (1 mM) was added to the culture. After a 5-h incubation at 25°C, the cells were collected by centrifugation at 10,000× *g* for 10 min. All subsequent steps were based on the procedures of Strauch and Spiegelman for AbrB (*17*) and Asayama and Kobayashi for Spo0A (*18*) and were performed at 4°C. The pellet was resuspended in 20 ml of TGED buffer [50 mM Tris-HCl, (pH 8.0), 20% (v/v) glycerol, 1 mM EDTA, 0.3 mM DTT, 2 mM PMSF] containing lysozyme (100 μg/ml) and lysed by sonication. After centrifugation at 10,000×*g* for 40 min, the supernatant was removed and used as the crude extract. Solid ammonium sulfate was added slowly to the crude extract to a final concentration of 70%. After gentle stirring for 30 min, the suspension was centrifuged at 10,000×*g* for 30 min. The resulting protein pellet was resuspended in the TGED buffer (20 ml) and dialysed against the same buffer. The dialysate was applied to a DE52 column, then Heparin column chromatography was performed twice. Throughout these steps, the proteins were monitored by SDS-PAGE. Protein concentrations were determined using a Bio-Rad protein determination kit with BSA as the standard. Amino-terminal amino acid sequences of the purified proteins were determined with a gas-phase protein sequencer (Model 477A, Applied Biosystems).

In Vitro Phosphorylation of Spo0A—To phosphorylate Spo0A, acetyl phosphate, which has been used to specifically phosphorylate several response regulators by autophosphorylation (*13, 19-22*), was used as a low-molecular-weight phosphodonor. Spo0A (2 μM) was incubated with 50 mM acetyl phosphate in the transcription buffer [18 mM Tris-HCl, (pH 8.0), 10 mM NaCl, 10 mM $MgCl_2$, 20 μM EDTA, 10% (v/v) glycerol, 8 mM 2-mercaptoethanol] for 10 min at 37°C. The extent of autophosphorylation was not determined directly, but was monitored by the enhancement of the *spoIIG* transcription.

In Vitro Transcription—Purification of core RNA polymerase, σ^A and σ^H, reconstitution of holoenzyme, and the conditions for promoter-dependent *in vitro* transcription will be described elsewhere (accompanying paper). Either Spo0A (phosphorylated or non-phosphorylated form) or AbrB (Fig. 1) was incubated at 37°C for 3 min with 2 nM template DNA. For the transcription of *spoIIG* template, ATP (80 μM) and GTP (80 μM) were added to this reaction mixture. Following this incubation, which permitted Spo0A/Spo0A-P binding to the DNA, 20 nM RNA polymerase was added and the mixture was incubated for another 3 min. Without ATP and GTP, no transcription of *spoIIG* was observed. Transcription was initiated by the addition of a substrate-heparin mixture, and RNA synthesis was allowed to proceed for another 5 min. The reaction was terminated by adding stop solution (40 mM EDTA, 300 μg/ml yeast tRNA), and transcripts were precipitated with ethanol, dissolved in the sample buffer [80% (v/v) of formamide, 0.1% (w/v) SDS, 8% (v/v) glycerol, 8 mM EDTA, 0.05% (w/v) bromophenol blue, 0.05% (w/v) xylene cyanol], and analyzed by electrophoresis on polyacrylamide gels containing 8 M urea. The amounts of protein factors added to the first reaction mixtures are shown in the legends to Figs. 3 and 4. The linear DNA templates of *spoIIG* were prepared by PCR with primers 5′-GATCGTC-CGAGATGATTATG-3′ and 5′-CAGCCAAATGACATC-TA-3′. Other templates used will be described elsewhere (accompanying paper).

Gel Shift Assay—DNA fragments used in this assay are the same fragments used as templates for *in vitro* transcription. The 5′-ends of DNA were labeled using [γ-^{32}P]ATP and T4 polynucleotide kinase. Various amounts of AbrB, as indicated in the legend to Fig. 5, were incubated for 5 min at 37°C with 5′-end-labeled DNA fragment (0.1 pmol) and, if required, competitor DNA indicated in the legend to Fig. 5, in 25 μl binding buffer [10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 1 mM EDTA, 5% (v/v) glycerol, 50 μg/ml BSA, 50 μg/ml poly(dI-dC):poly(dI-dC), 1 mM DTT]. Reaction products were analyzed by electrophoresis through 4% polyacrylamide gel in 33 mM Tris-phosphate buffer (pH 7.8). Following electrophoresis, gels were analyzed with a Bioimage analyzer BAS2000.

RESULTS

Effect of Spo0A on the Transcription from the Promoters of the Genes Involved in the Initiation of Sporulation—We first analyzed the transcription of *spo0A* Ps, *spo0F* P2, and *kinA* by σ^H-RNA polymerase, and that of *kinC* promoter by σ^A-polymerase in the presence or absence of Spo0A/Spo0A-P *in vitro*. Acetylphosphate was used as a phosphate donor to make Spo0A-P. Template DNA (Fig. 2) and Spo0A/Spo0A-P were incubated for 3 min at 37°C, with initiating nucleotides ATP and GTP for *spoIIG*, and without nucleotides for the other promoters. RNA polymerase was then added and, after an additional 3 min, a mixture of ATP, CTP, GTP, [α-^{32}P]UTP, and heparin was added. The transcripts obtained in a single-round reaction for 5 min were quantified and used as an estimate of the number of

promoter-polymerase complexes capable of elongating RNA under the heparin challenge. The presence of initiating nucleotides is required for the formation of heparin-resistant complexes at the *spoIIG* promoter (*23*). However, for the other promoters tested in this report, the initiating nucleotides were not necessary for the formation of a stable complex between RNA polymerase and promoter DNA (data not shown). The results are illustrated in Fig. 3. As reported previously (*23*), the reaction that included Spo0A-P resulted in an obvious stimulation of transcription from the *spoIIG* promoter. Unphosphorylated Spo0A also stimulated transcription of *spoIIG*, but less effectively (compare lane 8 with lane 11 in the upper panel). On the other hand, transcription from *abrB* P2 was clearly inhibited by Spo0A-P, as described in the previous report (*10*). Previous *in vivo* and *in vitro* experiments indicated that there were two start sites, P1 and P2, for *abrB* transcription (*7*). Without initiating nucleotides, RNA from P1 was not detected under our reaction conditions (Fig. 3). This suggested that the *abrB* P1 promoter requires

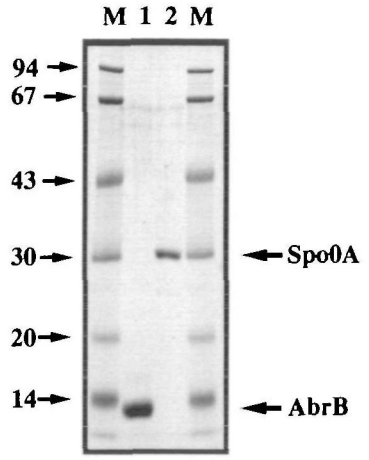

Fig. 1. **Purified AbrB and Spo0A.** AbrB (lane 1) and Spo0A (lane 2) were analyzed by SDS-PAGE. M, size markers of the indicated molecular masses (kDa$\times 10^{-3}$).

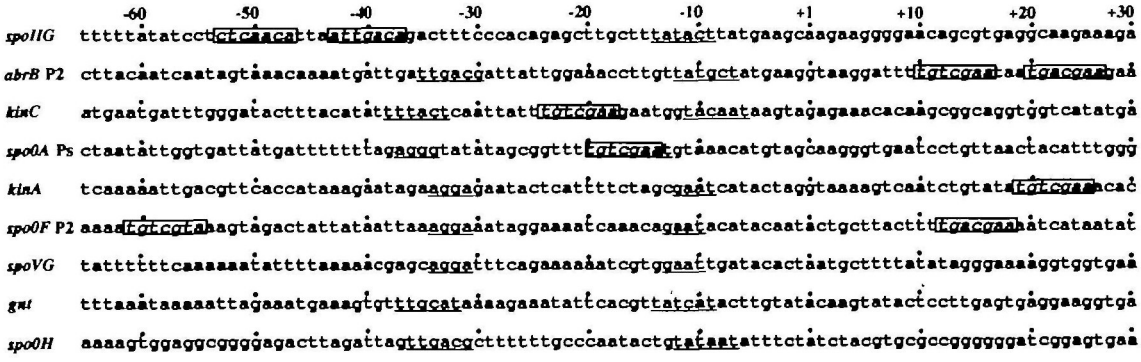

Fig. 2. **Sequence alignment of promoters analyzed in this study.** Transcription initiation site is indicated as +1. The promoter sequences (−35 and −10) proposed for each gene are underlined. σ^A promoters: *spoIIG*, *abrB* P2, *kinC*, *gnt*, and *spo0H*. σ^H promoters: *spo0A* Ps, *kinA*, and *spo0F* P2. The possible consensus Spo0A recognition sites (TGTCGAA, termed "0A box") are boxed.

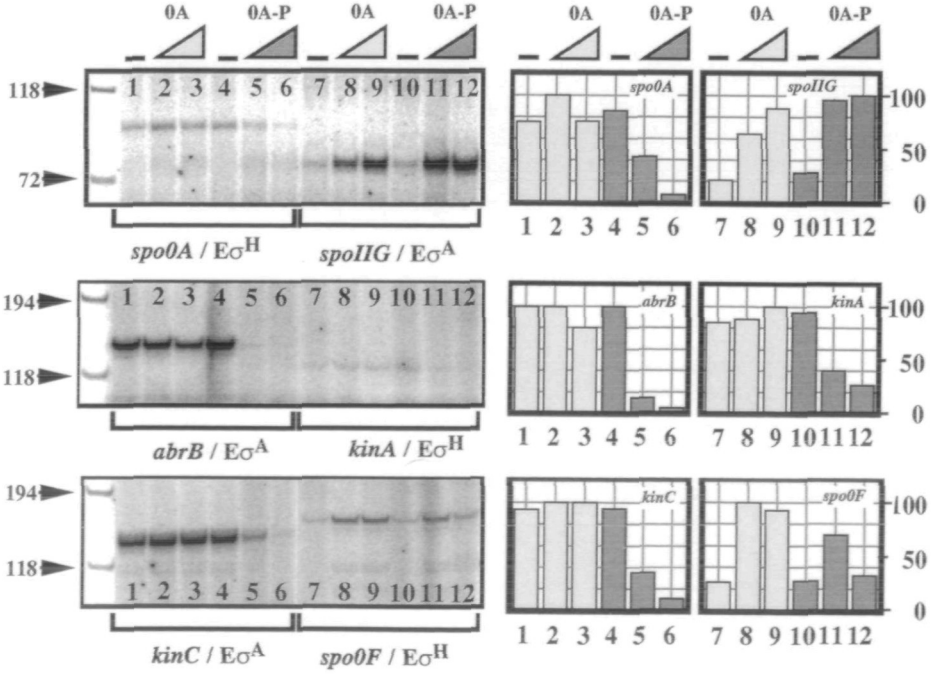

Fig. 3. **Effect of increasing inputs of Spo0A or Spo0A-P on *in vitro* transcription.** Each reaction mixture contained 2 nM linear DNA template and 20 nM RNA polymerase. Spo0A was phosphorylated by incubation with acetylphosphate as described under "MATERIALS AND METHODS." The amounts of Spo0A(-P) in the reactions were as follows: no Spo0A(-P) (lanes 1, 4, 7, and 10), 20 nM (lanes 2, 5, 8, and 11), 40 nM (lanes 3, 6, 9, and 12). Samples were subjected to electrophoresis in denaturing polyacrylamide gels followed by autoradiography (left panels). The peak areas measured from the autoradiograms are shown as histograms (right panels). The transcription levels were normalized to the maximum level and are expressed in arbitrary units.

initiating nucleotides to stabilize polymerase-promoter complexes in the presence of heparin.

Spo0A-P inhibited the transcription σ^H-dependent *spo0A* Ps and *kinA* promoters, and σ^A-dependent *kinC* promoter. Transcription of σ^H-dependent *spo0A* Ps promoter was not stimulated by Spo0A-P. On the other hand, Spo0A-P both stimulated and repressed transcription of σ^H-dependent *spo0F* P2 promoter: stimulation was observed at 20 nM Spo0A-P, and repression at 40 nM Spo0A-P (compare lane 11 with 12 in the lower panel). These results indicate that Spo0A-P is a negative regulator for the *spo0A* Ps, *kinA*, and *kinC* promoters and an ambivalent (positive/negative) regulator for the *spo0F* P2 promoter depending on its concentration, as suggested previously (*15, 16*).

Effect of AbrB on Transcription from the Promoters of the Genes Involved in the Initiation of Sporulation—AbrB is a DNA-binding regulatory protein that controls many developmentally regulated genes. DNase I footprinting of over 15 genes has revealed that AbrB can bind contiguously to stretches of DNA varying in length from 24 to over 100 bp, depending on the individual target (*17, 24-29*). However, examination of these regions has failed to reveal any obvious candidate for a consensus sequence, motif (*28*), or three-dimensional DNA structure (*30*). So far as we know, there are no reports that AbrB affects the transcription reaction directly *in vitro*. Thus, we examined the effect of AbrB on transcription of the *abrB*, *spo0H*, *kinC*, and *spoVG* promoters *in vitro*. Various amounts of AbrB were incubated with template DNA, then subjected to an *in vitro* single-round transcription assay (Fig. 4). The *gnt* promoter was used as a reference, since its expression is AbrB-independent (*31*). Upon preincubation of the template DNA with increasing amounts of AbrB, transcription from the respective promoters, *abrB*, *spo0H*, *kinC*, and *spoVG*, was progressively but not completely inhibited, while the *gnt*

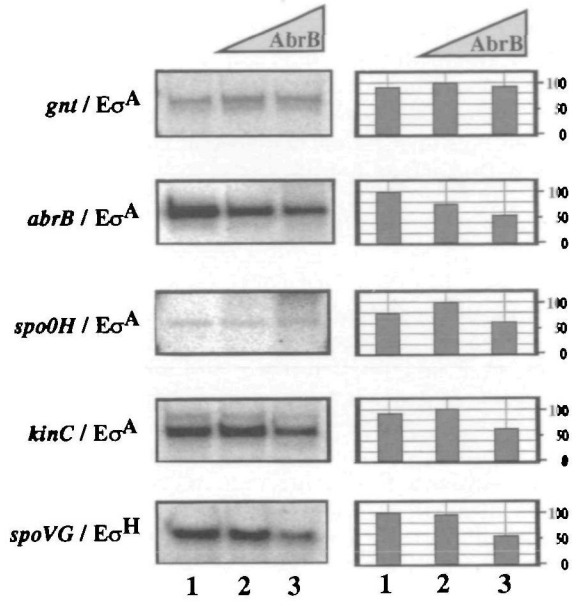

Fig. 4. **Effect of increasing inputs of AbrB on *in vitro* transcription.** Each reaction mixture contained 2 nM linear DNA template and 20 nM RNA polymerase. The amounts of AbrB in the reactions were as follows: no AbrB (lane 1), 0.3 μM (lanes 2), 3 μM (lanes 3). Samples were subjected to electrophoresis in denaturing polyacrylamide gels followed by autoradiography (left panels). The peak areas measured from the autoradiograms are shown as histograms (right panels). The transcription levels were normalized to the maximum level and expressed in arbitrary units.

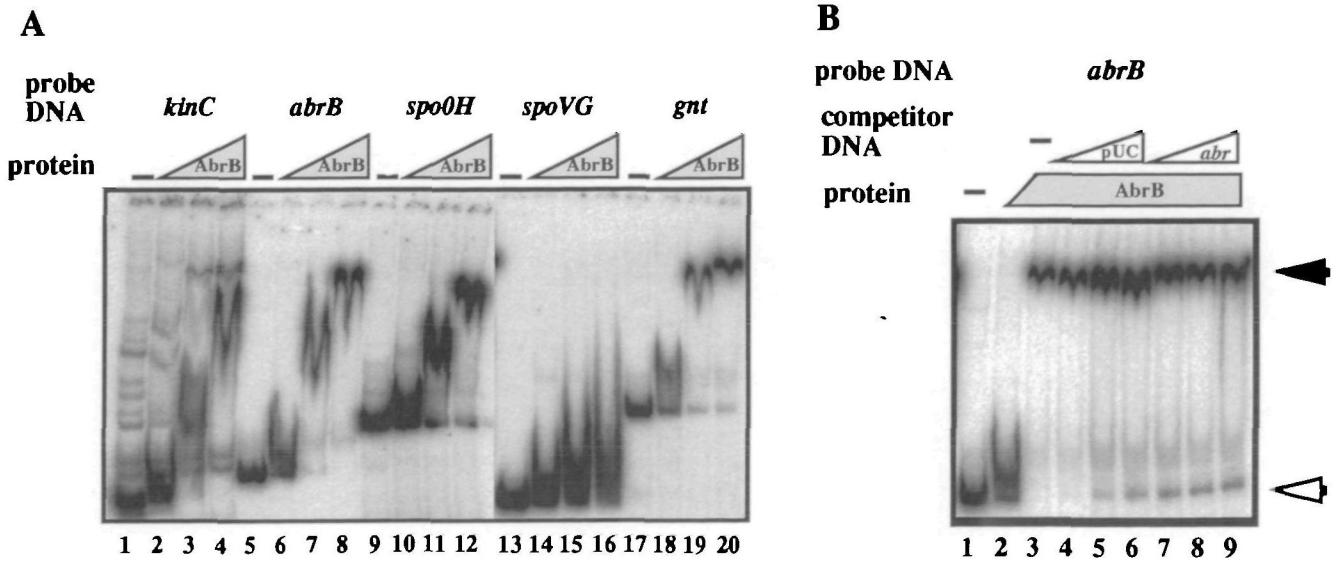

Fig. 5. **Binding of the AbrB protein to DNA fragments containing various promoters.** End-labeled DNA was incubated with various amounts of AbrB (molarity based upon the monomer form of AbrB) as described under "MATERIALS AND METHODS." (A) The lane assignments: no AbrB (lanes 1, 5, 9, 13, and 17), 0.3 μM (lanes 2, 6, 10, 14, and 18), 1.5 μM (lanes 3, 7, 11, 15, and 19), 3 μM (lanes 4, 8, 12, and 20). Probe DNAs used are indicated. (B) Binding specificity was examined by coincubation of labeled *abrB* promoter DNA with competitor DNAs. Reaction contained: no AbrB (lane 1), 0.3 μM (lane 2), 3 μM (lanes 3 to 9). Unlabeled pUC19 (lanes 4 to 6) and a *abrB* promoter (lanes 7 to 9) were added as a non-specific and a specific competitor, respectively: 10-fold molar excess (lanes 4 and 7), 20-fold molar excess (lanes 5 and 8), 50-fold molar excess (lanes 6 and 9). Open and closed arrowheads indicate free and shifted DNAs, respectively.

transcription was not inhibited at all.

Next, we examined the correlation between the inhibition of transcription by AbrB and the DNA-binding ability of AbrB. Using a gel retardation assay, we examined the binding ability of AbrB to DNA that was used as the template for *in vitro* transcription. As shown in Fig. 5A, all the DNA fragments including *gnt*, which was used as a reference, were shifted in the presence of AbrB, even at the protein concentration where transcription is not affected. Moreover, both specific and non-specific DNA competed for binding with AbrB (Fig. 5B).

DISCUSSION

The transcriptional regulation of the genes required for the initiation of sporulation has been the subject of much investigation. In this communication, we demonstrated that Spo0A-P and AbrB directly and selectively control transcription of genes involved in the initiation of sporulation.

In the *spo0A* Ps promoter, the upstream 0A box (−108 to −102 relative to the Ps start site, AGTCGAA) is thought to be required for transcription activation, whereas the downstream 0A box covering the −10 region of the promoter (−19 to −13, TGTCGAA) is postulated to repress the activation when Spo0A-P concentration reaches a certain threshold level (*32*). However, our results indicate that Spo0A-P synthesis was turned off when the Spo0A-P was added in a great amount, and the activation of transcription from Ps was not observed (Fig. 3). The location of the upstream 0A box might be too far from the Ps promoter to allow contact between Spo0A-P and σ^H-RNA polymerase; therefore, it is highly unlikely that the upstream 0A box contributes to the transcription from Ps promoter.

For the *spo0F* gene, Spo0A-P binding regions are located upstream and downstream of the σ^H-dependent promoter (P2) (Fig. 2) (*15, 16*). Both of these sites are thought to modulate the transcription of *spo0F* by feedback regulation (*15, 16*). The upstream region (−61 to −55 relative to the transcription start site of P2 promoter, TGTCGAA), rather than the downstream site (+13 to +19, TGACGAA), possesses an optimal sequence for Spo0A-P binding, and Spo0A-P actually binds more efficiently to the upstream site than to the downstream one (*15*). As shown in Fig. 3, Spo0A-P is both a positive and a negative effector of *spo0F* transcription, depending on its concentration. Stimulation of transcription by Spo0A observed on *spo0F* P2 promoter (Fig. 3, lanes 8 and 9 of *spo0F*) might be due to the higher affinity binding sequence of the upstream 0A box relative to the downstream 0A box. Some promoters possessing strong 0A binding sites, for example, *spo0F* P2 or *spoIIG*, may respond primarily to an increase in the amount of Spo0A protein, regardless of its phosphorylation state. It appears that some features of the sequence context that surrounds each 0A box may affect the relative affinities of phosphorylated and nonphosphorylated Spo0A.

Spo0A-P also acts as a repressor of the *kinA* and *kinC* genes (Figs. 2 and 3). This is probably because 0A boxes are located just downstream of the transcription start site of *kinA* and in the promoter region of *kinC*, as suggested previously (*1, 4*). Other reports also support our data: *kinA* expression increased continuously with the increase of σ^H during vegetative growth and declined after the onset of sporulation (*3*). *kinC* expression is induced after the onset of sporulation and shut off at T_3, whereas in the *spo0A* mutant it is expressed, although at a lower level, even after T_3 (*4*). Since *kinC* is also under the control of AbrB (Fig. 4), the transcription of *kinC* remains at a low level in the *spo0A* mutant, where the AbrB concentration is high.

Transcription of the *abrB*, *spo0H*, and *spoVG* promoters was weakly inhibited by AbrB, in contrast to the strong binding ability of AbrB to promoter DNA (Figs. 4 and 5). These unusual features of AbrB protein may be due to the lack of linearity in its binding with DNA, suggesting that the initial binding of the protein may be cooperative. The binding of any one AbrB molecule to its target is weak, and stronger binding will be observed only when the concentration of AbrB protein is sufficiently high so that the protein will also bind to the adjacent target sites. However, *in vivo*

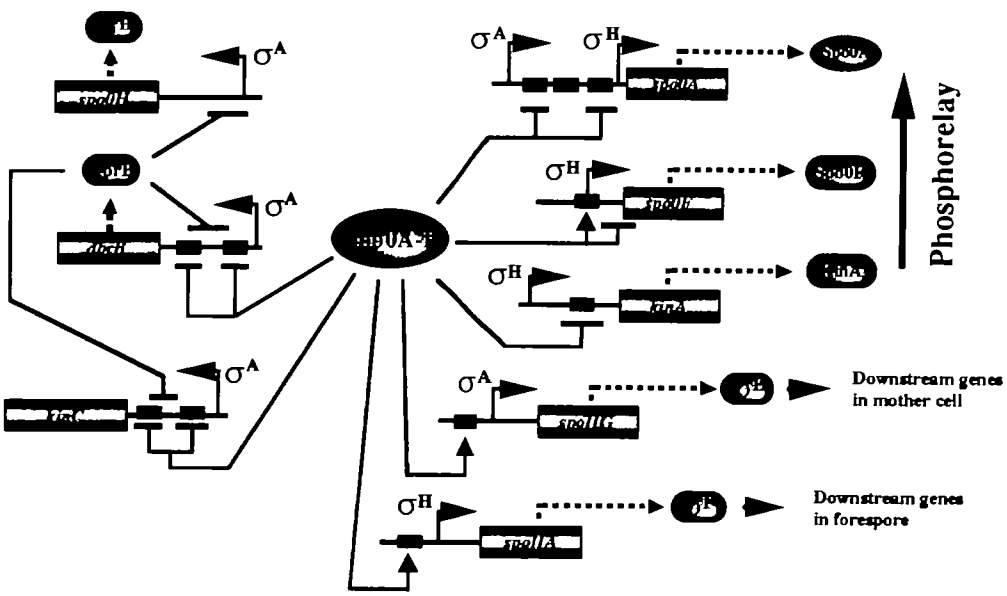

Fig. 6. **Proposed model of feedback loops controlling the initiation of sporulation.** The arrow indicates an activation event, while the T-shaped symbol indicates an inhibition event. Structural genes are denoted by boxes. Hatched arrows leading from the structural genes indicate production of proteins. Closed box indicates 0A box.

results with *lacZ* fusion genes strongly suggest that AbrB is involved in the repression of transcription from the above promoters (*31*). Therefore, the possibility that some other factors or conditions are required *in vitro* for the repressor activity of the AbrB protein cannot be excluded. Alternatively, most of the AbrB proteins in the reaction mixture might be in a denatured form and bind to DNA non-specifically, while a small fraction of AbrB proteins bind specifically to DNA and regulate the transcription.

Based on the results presented here and elsewhere (*1*), the following early sporulation events seems plausible (Fig. 6). The concentration of the components of the phosphorelay required for the production of Spo0A-P regulates the flow of phosphate. During exponential growth, RNA polymerase containing σ^A transcribes the *spo0A* Pv promoter. This transcription might occur at a low level, but it is sufficient to provide what can be termed an initiation-sensing concentration of intracellular Spo0A protein. This low amount of Spo0A protein enables AbrB to repress *spo0H* expression. As nutrients become depleted, unknown metabolic signals trigger the activation of protein kinases KinA, KinB, or KinC. The activated kinases undergo autophosphorylation and transfer the phosphate group to the Spo0A protein *via* the phosphorelay. The Spo0A-P, by virtue of its increased DNA binding affinity, binds at the 0A box located adjacent to the *abrB* promoter and represses its transcription. The resulting drop in AbrB levels leads to derepression of the transcription of the *spo0H* gene, which in turn results in the synthesis of more RNA polymerase containing σ^H. As σ^H-RNA polymerase transcribes *kinA*, *spo0A*, and *spo0F*, there is a rapid rise in the production of KinA, Spo0F, and Spo0A, resulting in an increase of phosphorylated Spo0A. This regulation, in which Spo0A-P acts as a positive regulator of the genes of the phosphorelay components, constitutes basically a positive feedback loop. This loop functions until elevated levels of Spo0A-P repress the expression of the genes for the phosphorelay components, such as *kinA*, *kinC*, *spo0F*, and *spo0A* itself. The change from positive to negative feedback loop reflects the progress of the sporulation program past the point where Spo0A-P is needed.

We thank R. H. Doi for critical reading of the manuscript.

REFERENCES

1. Hoch, J.A. (1993) Regulation of the phosphorelay and the initiation of sporulation in *Bacillus subtilis*. *Annu. Rev. Microbiol.* **47**, 441-465
2. Perego, M., Cole, S.P., Burbulys, D., Trach, K., and Hoch, J.A. (1989) Characterization of the gene for a protein kinase which phosphorylates the sporulation-regulatory proteins Spo0A and Spo0F of *Bacillus subtilis*. *J. Bacteriol.* **171**, 6187-6196
3. Antoniewski, C., Savelli, B., and Stragier, P. (1990) The *spoIIJ* gene, which regulates early development steps in *Bacillus subtilis*, belongs to a class of environmentally responsive genes. *J. Bacteriol.* **172**, 86-93
4. Kobayashi, K., Shoji, K., Shimizu, T., Nakano, K., Sato, T., and Kobayashi, Y. (1995) Analysis of a supressor mutation *ssb* (*kinC*) of *sur0B20* (*spo0A*) mutation in *Bacillus subtilis* reveals that *kinC* encodes a histidine protein kinase. *J. Bacteriol.* **177**, 176-182
5. LeDeaux, J. and Grossman, A.D. (1995) Isolation and characterization of *kinC*, a gene that encodes a sensor kinase homologous to the sporulation sensor kinases KinA and KinB in *Bacillus subtilis*. *J. Bacteriol.* **177**, 166-175
6. LeDeaux, J., Yu, N., and Grossman, A.D. (1995) Different roles for KinA, KinB, and KinC in the initiation of sporulation in *Bacillus subtilis*. *J. Bacteriol.* **177**, 861-863
7. Perego, M., Spiegelman, G.B., and Hoch, J.A. (1988) Structure of the gene for the transition state regulator, *abrB*: regulator synthesis is controlled by the *spo0A* sporulation gene in *Bacillus subtilis*. *Mol. Microbiol.* **2**, 689-699
8. Strauch, M.A., Webb, V., Spiegelman, G.B., and Hoch, J.A. (1990) The Spo0A protein of *Bacillus subtilis* is a repressor of the *abrB* gene. *Proc. Natl. Acad. Sci. USA* **87**, 1801-1805
9. Trach, K., Burbulys, D., Strauch, M., Wu, J.-J., Dhillon, N., Jonas, R., Hanstein, C., Kallio, P., Perego, M., Bird, T., Spiegelman, G., Fogher, C., and Hoch, J.A. (1991) Control of the initiation of sporulation in *Bacillus subtilis* by a phosphorelay. *Res. Microbiol.* **142**, 815-823
10. Greene, E.A. and Spiegelman, G.B. (1996) The Spo0A protein of *Bacillus subtilis* inhibits transcription of the *abrB* gene without preventing binding of the polymerase to the promoter. *J. Biol. Chem.* **271**, 11455-11461
11. Satola, S., Kirchman, P.A., and Moran, C.P., Jr. (1991) Spo0A binds to a promoter used by σ^A RNA polymerase during sporulation in *Bacillus subtilis*. *Proc. Natl. Acad. Sci. USA* **88**, 4533-4537
12. Bird, T.H., Grimsley, J.K., Hoch, J.A., and Spiegelman, G.B. (1993) Phosphorylation of Spo0A activates its stimulation of *in vitro* transcription from the *Bacillus subtilis spoIIG* operon. *Mol. Microbiol.* **9**, 741-749
13. Baldus, J.M., Green, B.D., Youngman, P., and Moran, C.P., Jr. (1994) Phosphorylation of *Bacillus subtilis* transcription factor Spo0A stimulates transcription from the *spoIIG* promoter by enhancing binding to weak 0A boxes. *J. Bacteriol.* **176**, 296-306
14. Predich, M., Nair, G., and Smith, I. (1992) *Bacillus subtilis* early sporulation genes *kinA*, *spo0F*, and *spo0A* are transcribed by the RNA polymerase containing σ^H. *J. Bacteriol.* **174**, 2771-2778
15. Asayama, M., Yamamoto, A., and Kobayashi, Y. (1995) Dimer form of phosphorylated Spo0A, a transcriptional regulator, stimulates the *spo0F* transcription at the initiation of sporulation in *Bacillus subtilis*. *J. Mol. Biol.* **250**, 11-23
16. Strauch, M.A., Wu, J-J., Jonas, R.H., and Hoch, J.A. (1993) A positive feedback loop controls transcription of the *spo0F* gene, a component of the sporulation phosphorelay in *Bacillus subtilis*. *Mol. Microbiol.* **7**, 967-974
17. Strauch, M.A., Spiegelman, G.B., Perego, M., Johnson, W.C., Burbulys, D., and Hoch, J.A. (1989) The transition state transcription regulator *abrB* of *Bacillus subtilis* is a DNA binding protein. *EMBO J.* **8**, 1615-1621
18. Asayama, M. and Kobayashi, Y. (1993) Signal transduction and sporulation in *Bacillus subtilis*: autophosphorylation of Spo0A, a sporulation initiation gene product. *Mol. Gen. Genet.* **238**, 138-144
19. Lukat, G.S., McCleary, W.R., Stock, A.M., and Stock, J.B. (1992) Phosphorylation of bacterial response regulator proteins by low molecular weight phospho-donors. *Proc. Natl. Acad. Sci. USA* **89**, 718-722
20. Wingrove, J.A. and Gober, J.W. (1994) A σ^{54} transcriptional activator also functions as a pole-specific repressor in *Caulobacter*. *Genes Dev.* **8**, 1839-1852
21. Hiratsu, K., Nakata, A., Shinagawa, H., and Makino, K. (1995) Autophosphorylation and activation of transcriptional activator PhoB of *Escherichia coli* by acetylphosphate *in vitro*. *Gene (Amst.)* **161**, 7-10
22. McCleary, W.R. (1996) The activation of PhoB by acetylphosphate. *Mol. Microbiol.* **20**, 1155-1163
23. Bird, T.H., Grimsley, J.K., Hoch, J.A., and Spiegelman, G.B. (1996) The *Bacillus subtilis* response regulator Spo0A stimulates transcription of the *spoIIG* operon through modification of RNA polymerase promoter complexes. *J. Mol. Biol.* **256**, 436-448
24. Furbass, R., Gocht, M., Zuber, P., and Marahiel, M.A. (1991) Interaction of AbrB, a transcriptional regulator from *Bacillus subtilis* with the promoters of the transition state-activated genes *tycA* and *spoVG*. *Mol. Gen. Genet.* **255**, 347-354
25. Slack, F.J., Mueller, J.P., Strauch, M.A., Mathiopoulos, C., and

Sonenshein, A.L. (1991) Transcriptional regulation of a *Bacillus subtilis* dipeptide transport operon. *Mol. Microbiol.* **5**, 1915-1925
26. Fisher, S.H., Strauch, M.A., Atkinson, M.R., and Wray, L.V., Jr. (1994) Modulation of *Bacillus subtilis* catabolite repression by transition state regulatory protein AbrB. *J. Bacteriol.* **176**, 1903-1912
27. Strauch, M.A. (1995) AbrB modulates expression and catabolite repression of a *Bacillus subtilis* ribose transport operon. *J. Bacteriol.* **177**, 6727-6731
28. Strauch, M.A. (1995) Delineation of AbrB-binding sites on the *Bacillus subtilis spo0H, kinB, ftsAZ,* and *pbpE* promoters and use of a derived homology to identify a previously unsuspected binding site in the *bsuB1* methylase promoter. *J. Bacteriol.* **177**, 6999-7002
29. Strauch, M.A. (1996) Dissection of the *Bacillus subtilis spo0E* binding site for the global regulator AbrB reveals smaller recognition elements. *Mol. Gen. Genet.* **250**, 742-749
30. Strauch, M.A. and Ayazifar, M. (1995) Bent DNA is found in some, but not all, regions recognized by the *Bacillus subtilis* AbrB protein. *Mol. Gen. Genet.* **246**, 756-760
31. Strauch, M.A. (1993) AbrB, a transition state regulator in *Bacillus subtilis and Other Gram-Positive Bacteria: Biochemistry, Physiology and Molecular Genetics* (Sonenshein, A.L., Hoch, J.A., and Losick, R., eds.) pp. 757-764, American Society for Microbiology, Washington, D.C.
32. Strauch, M.A., Trach, K., Day, J., and Hoch, J.A. (1992) Spo0A activates and represses its own synthesis by binding at its dual promoters. *Biochimie* **74**, 619-626