Repression of transcription of the abrB gene is essential to expression of many of the postexponential genes in Bacillus. The repression is due to the activity of the response regulator protein Spo0A. We have used in vitro transcription and DNase I and hydroxyl radical footprinting to explore the mechanism of transcription inhibition. Spo0A binds to specific DNA sequences (0A boxes), and two such boxes are found downstream of the tandem promoters for the abrB gene. The data indicate that both RNA polymerase and Spo0A bind simultaneously to a DNA fragment containing the promoters and the 0A boxes. The Spo0A prevents the polymerase from inducing DNA strand denaturation at the promoter for the abrB gene.

When Bacillus subtilis reaches the end of logarithmic growth, it enters a transition state in which cells express genes designed to search for alternative nutrient sources. These genes, such as proteases, amylases are under the direct control of a set of proteins termed transition state regulators (1–3). One such regulator is the AbrB protein. AbrB has a positive effect on transcription of some genes, such as hpr (4) and represses transcription of genes such as tycA (5), spoVG (6), and aprE (2, 7, 8).

The abrB gene is transcribed during vegetative growth, and during transition and subsequently sporulation, its transcription is repressed by the product of the spo0A gene (9). The repression is due to the binding of Spo0A protein to two consensus binding sites (TGTCCAG, termed 0A boxes), which are located between positions +11 and +17 and positions +21 and +27, relative to the downstream (P2) of two reported transcription initiation start sites (P1 and P2; Ref. 10). Spo0A is a member of the response regulator class of proteins (11, 12), and its activity is modulated by phosphorylation (13, 14). The regulation of phosphorylation of Spo0A ties the level of Spo0A-P1 to multiple inputs reflecting a variety of aspects of cell physiology (13, 15, 16). Spo0A is both a transcription repressor and a transcription activator, and in this paper we address how Spo0A represses transcription.

The structure of the abrB promoter region suggested three possible models for Spo0A repression of abrB transcription. One model is the formation of a DNA loop between a Spo0A binding site that is upstream of the promoter (at position −214, relative to P2) and the Spo0A binding sites downstream of P2. The DNA loop would block RNA polymerase access to the two abrB promoters. In the second model, binding of Spo0A to the 0A boxes would exclude polymerase binding to the abrB promoter by steric hindrance. In the third model, both RNA polymerase and Spo0A bind simultaneously to the promoter region; however, the polymerase is prevented from initiating, or possibly elongating, RNA synthesis.

MATERIALS AND METHODS

Preparation of DNA—Escherichia coli strain DH5α was used as host to produce plj M5134 DNA, which contains an 804-bp DNA fragment extending from +703 to +37, relative to the P2 promoter start site (Fig. 1 and Ref. 9). Plasmid was prepared by cleared lysis and cesium chloride centrifugation as described by Sambrook et al. (17). An 804-bp EcoRI/HindIII fragment and a 107-bp EcoRI/Asp700 fragment were isolated as described by Bird et al. (18) for use as templates.

Labeled DNA fragments for protection assays were made by first digesting plj M5134 with EcoRI. To label the coding strand, the DNA was treated with the Klenow fragment of DNA polymerase I in 1 × Klenow buffer containing 35 mM dTTP, 5 μCi of [α-32P]dATP (DuPont NEN, 3000 Ci/mmole), and Klenow enzyme. The Klenow reaction was terminated, and the fragment was released with HindIII. To label the template strand, the EcoRI-cut DNA was treated with calf intestinal alkaline phosphatase and then labeled with 0.6–0.8 mCi of [γ-32P]ATP (ICN, 7000 Ci/mmole) using T4 polynucleotide kinase. After terminating the kinase reaction the fragment was released with HindIII. The labeled fragments were isolated by electrophoresis (19). The position of the bases in the footprints was determined using the end-labeled DNA fragments treated with various restriction enzymes to generate specific size markers.

Phosphorelay Reactions—Phosphorelay reactions used to phosphorylate Spo0A were carried out in 1 × transcription buffer (40 mM Hepes, pH 8.0, 5 mM magnesium acetate, 0.1 mM EDTA, 0.1 mM dithiothreitol, 0.1 mg/ml bovine serum albumin (19), using stocks of recombinant protein provided by J. A. Hoch (Research Institute of the Scripps Clinic, La Jolla, CA). Phosphorylation of Spo0A was followed with [γ-32P]ATP (18). Typically, Spo0A was 60% phosphorylated.

Transcription Assays—Transcription assays were done as described by Bird et al. (18) with the following modifications. Initiating reactions contained 1 × transcription buffer (18), 4 nM DNA fragment, 0.4 mM ATP, 10 μM GTP, 3 μCi of [α-32P]GTP (DuPont NEN, 800 Ci/mmole), 80 mM potassium acetate, various concentrations of Spo0A-P(-0.4 mM) and 0.4 mM UTP when indicated. The mixture was incubated for 3 min at 37 °C. RNA polymerase (100 nM) was added, and after 2 min a mixture containing heparin (10 μg/ml final concentration) and nucleotides that would permit elongation was added. Total reaction volume after the addition of all components was 20 μl. Elongation was allowed to continue for 5 min and terminated by the addition of 10 μl of 1 M urea in 0.25 × TBE (17), 0.1% xylene cyanol, 0.1% bromphenol blue. Samples were heated for 2 min to 100 °C and electrophoresed through a 12% acrylamide (40% acrylamide, 1.38% bisacrylamide), 7 nM urea, 0.25 × TBE gel at 60 V/cm. Gels were autoradiographed overnight on Kodak XRP or Kodak XAR film. Gel slices corresponding to bands on the autoradiograms.
Spo0A Inhibition of abrB Transcription

RESULTS

Characteristics of in Vitro Transcription from the abrB P1 and P2 Promoters

In vitro transcription reactions were used to define conditions for structural studies. Spo0A or Spo0A-P was incubated with a linear DNA fragment containing the abrB promoter in transcription buffer containing either of two sets of nuclease triphosphates: 1) ATP and GTP (AG initiation), or 2) ATP, UTP, and GTP (AUG initiation). The polymerase was added, allowed to bind, and then challenged with a mixture containing the polymerase inhibitor heparin and nucleotides that would allow RNA chain elongation (either UTP plus CTP or only CTP). After 5 min, to allow completion of RNA chains, the products were separated by electrophoresis and the number of transcripts was determined. Previous in vivo experiments indicated that there were two start sites for abrB transcription (9). The same sites were found in vitro (10). AUG initiation conditions permit synthesis of a 13-nucleotide RNA from P2 and a 6-nucleotide RNA from P1, while AG initiation conditions provide only the initiating nucleotide for each promoter. Transcription from P2 was much more efficient than was transcription from P1, with P2 accounting for 80% of the transcripts. Both promoters were inhibited by Spo0A(-P) in vitro.

An example of the transcription inhibition by Spo0A and Spo0A-P is shown in Fig. 2. Transcription from both P1 and P2 was inhibited over a narrow range of Spo0A(-P) concentrations, and Spo0A-P was more efficient at inhibiting transcription (usually 2–5-fold). Transcription from both P1 and P2 was 90% inhibited by 400 nM Spo0A and 200 nM Spo0A-P. Order of addition experiments showed that under single round AG or AUG initiation conditions, the polymerase could be added before Spo0A or Spo0A-P and the response regulator could still inhibit transcription. This result was the first indication that the effect of Spo0A(-P) was not due to exclusion of polymerase binding. Five different preparations of recombinant Spo0A and two different preparations of RNA polymerase were tested, and all gave essentially identical results. Low inputs of Spo0A appeared to stimulate transcription slightly. The cause for this apparent stimulation is unknown.

There is a Spo0A binding site 219 bp upstream of the abrB P2 promoter initiation site fragment, which might participate in DNA loop formation to inhibit abrB transcription (Fig. 1). We tested transcription of a template that had been shortened by cleavage with Asp700, removing the 219 Spo0A binding site. Transcription inhibition by Spo0A(-P) was unaffected by this change in template, indicating that the loop model did not explain Spo0A inhibition of abrB transcription (Fig. 3). We thus tested whether Spo0A prevented RNA polymerase binding to a DNA fragment containing the promoter.

DNase I Probing of Spo0A(-P)-RNA Polymerase-abrB Promoter Complexes

To determine whether polymerase exclusion explained Spo0A(-P) inhibition of the abrB promoter, we used DNase I and hydroxyl radical protection assays under AG and...
AUG initiation conditions, in the presence of RNA polymerase and increasing concentrations of Spo0A(-P). If Spo0A(-P) excluded the polymerase, then its addition should replace the protection pattern seen with the polymerase alone with a pattern seen with Spo0A(-P) alone. Both strands were examined with both reagents, and the conclusions from all assays were identical. For brevity, only selected data are shown.

Protections of the abrB promoter from DNase I digestion by Spo0A(-P) is shown in Fig. 4. The level of protection of the DNA was not linearly dependent on Spo0A(-P) input, and the two OA boxes downstream of the promoter region appeared to fill simultaneously. Spo0A protected the abrB promoter at 400 nM but not 350 nm, while Spo0A-P protected the abrB template at 350 nm but not 300 nm. This behavior has been observed previously (10). When the DNA was labeled at the 3' end of the coding strand, Spo0A protected DNA between positions +4 and +28, while Spo0A-P protection extended to −4 and +32. On the template strand, Spo0A provided weak protection between +6 and +28 even at high concentrations, while Spo0A-P protected positions between −3 and +32 (data not shown).

To examine the patterns of protection of Spo0A(-P) in the presence of RNA polymerase, mixtures of Spo0A(-P), RNA polymerase, and labeled DNA were treated with DNase I. Fig. 5A shows the DNase I digestion pattern observed with DNA labeled at the 3' end of the coding strand. Spo0A-P protected positions −4 to +34 as seen in Fig. 4, and Spo0A was less effective at protecting DNA. Between positions −5 and −50, there was no effect of adding Spo0A(-P) on the DNase I digestion pattern.

Under AG initiating conditions (−UTP, Fig. 5A, left lanes), RNA polymerase alone protected several groups of bases. Positions −10 to −25 were well protected, while positions −14 to −23 were partially protected. Upstream of position −25, the promoter DNA contained regions that were inherently resistant to DNase I digestion; however, it was clear that the polymerase provided protection at positions −27, −28, −30, and −43 by the significant reductions in cleavage at these sites. DNase I cleavage at position −24 was enhanced by the presence of RNA polymerase. Under AG initiating conditions (+UTP, right lanes), the DNase I digestion pattern observed when polymerase alone was added was similar to the pattern under AG initiating conditions, despite the fact that when UTP was included the polymerase could transcribe an RNA 13 bases long. A summary of the protection by Spo0A(-P) and by RNA polymerase alone is presented in Fig. 7.

To examine the question of exclusion, DNase I digestion reactions included RNA polymerase and either Spo0A or Spo0A-P. In all experiments, Spo0A(-P) was added and allowed to bind to the DNA first, and then polymerase was added and the mixture was subjected to a short DNase I treatment. Under AG initiating conditions, the effects of Spo0A differed from those of Spo0A-P. Spo0A addition increased protection at the −4 site and in the regions −27 to −33 and −37 to −44. At the same time, protection between +20 and +25 was slightly reduced. When Spo0A-P was added, the protection pattern between −35 and −43 and between −27 and −33 did not change relative to the pattern seen with polymerase alone. The addition of Spo0A-P increased protection between −14 and −20, while the protection between −1 and −12 decreased.

Fig. 5B shows scans of selected lanes from AUG initiating conditions in Fig. 5A. Comparison of the control (scan 5) and the Spo0A-P alone (scan 4) shows that between −4 and −50 the scans were identical, so that Spo0A-P had no effect on the DNase I digestion upstream of the −4 position. The presence of RNA polymerase alone (scan 1) dramatically altered the scan between positions −25 and −50. The peaks in the region −10 to −25 were significantly reduced. Other changes induced by the polymerase by binding included the exposure of the base at −24 (increase in the peak height), the protection of the bases at −27 and −28 (disappearance of the peaks on the shoulder of the −24 peak), and the protection in the region of −43. When Spo0A and RNA polymerase were added together (scan 2), the pattern between −1 and −50 was essentially unchanged from that of the polymerase alone. The addition of Spo0A-P altered the pattern of protection seen with the polymerase, since the protection between −1 and −10 decreased slightly, although the polymerase was still clearly present, since these bases were not protected by Spo0A-P (scan 4). Furthermore, between −15 and −50, the protection by Spo0A-P plus polymerase was identical to that of the polymerase alone.

Several conclusions can be drawn from the data in Fig. 5. First, the areas of DNA protected by RNA polymerase alone, especially upstream of position −14, were not deprotected by the addition of Spo0A(-P). Thus, under conditions of complete transcription inhibition, it was evident that the polymerase was still bound to the promoter. If Spo0A-P blocked transcription by exclusion, the polymerase footprint should have been replaced by the Spo0A(-P) footprint. For example, the height of the −24 peak should have been reduced, and the peaks at −27 and −28 should have reappeared. Since the protection pattern seen with the polymerase was retained, we conclude that Spo0A(-P) did not deplete the polymerase from the template. Second, the addition of Spo0A-P caused some changes in areas of protection afforded by the polymerase alone. For example, adding Spo0A-P increased protection in the −14 to −20 region.
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FIG. 5. DNase I treatment of DNA labeled on the coding strand. A, pJ M5134 was cut with EcoRI and labeled with the Klenow fragment of DNA polymerase. The labeled fragment was released with HindIII, isolated, and used in DNase I protection assays. AG and AUG initiating conditions were tested. When present, the polymerase was 100 nM; Spo0A inputs were 100, 200, and 400 nM, or 400 nM when present alone, and the DNA concentration was 2 nM. Base pairs relative to the start site for transcription from P2 are indicated. The positions that are multiples of 10 are marked by short dashes, and intervening positions that are multiples of 5 are marked by dots. At the right can be seen one of the fragments used as a molecular weight marker for this gel, which runs at position +16.

FIG. 6. Hydroxyl radical treatment of DNA labeled on the template strand. pJ M5134 was cut with EcoRI and labeled with the polynucleotide kinase. The labeled fragment was released with HindIII, isolated, and used in hydroxyl radical protection assays. AG and AUG initiating conditions were tested. When present, the polymerase was 100 nM; Spo0A inputs were 100, 200, and 400 nM, or 400 nM when present alone, and the DNA concentration was 2 nM. Base pairs relative to the start site for transcription from P2 are indicated. The positions that are multiples of 10 are marked by short dashes, and intervening positions that are multiples of 5 are marked by dots. At the right can be seen one of the fragments used as a molecular weight marker for this gel, which runs at position +16.

under AG initiating conditions and the −10 to −20 region under AUG initiating conditions. Since these nucleotides were not affected by binding of Spo0A-P alone, the changes can only be accounted for by having Spo0A-P and the polymerase bound to the same DNA. Finally, the interaction of Spo0A and Spo0A-P with the bound polymerase was not the same, although both inhibited transcription. The patterns of the protected bands in the −1 to −10 region (Fig. 5B, compare scans 2 and 3) are most illustrative of these differences. The significance of these changes is not clear as yet, but we presume they reflect differences in the structure of phosphorylated and unphosphorylated Spo0A.

An indication of the mechanism for Spo0A-P inhibition of abrB transcription was obtained by examining the hydroxyl radical cleavage pattern using DNA labeled at the 5′ end of the template strand (Fig. 6). Spo0A-P protected two 3-bp areas (+8, +9, +10 and +18, +19, +20). Minor areas of protection were noted on either side, and Spo0A protected the same areas, although less effectively. Under AG initiating conditions (−UTP, right lanes) the polymerase alone induced strong hypersensitive sites between −5 and −2. Hypersensitive sites have been seen in other examples of hydroxyl radical treatment and have been interpreted as reflecting exposure of the DNA backbone due to DNA strand denaturation (see “Discussion”). In addition, the polymerase protected several groups of bases spaced at nearly 10-bp intervals (see Fig. 7). Particularly noteworthy is the protection at −40 to −43. Under AUG initiation conditions, the hypersensitive sites shifted to +3 to +7, while the protection pattern between −10 and −45 was effectively identical to that seen under AG initiation conditions. Addition of Spo0A or Spo0A-P reduced or eliminated the hypersensitive sites under both AG and AUG initiation conditions, with Spo0A-P being more effective. In all tests, the addition of Spo0AP(-P) had no obvious effect on the protection pattern between −10 and −45.

The change in hydroxyl radical hypersensitivity in Fig. 6 is further evidence that the addition of Spo0A(-P) affected the interaction of the polymerase and the abrB promoter, since Spo0A(-P) itself had no effect on these positions. Furthermore, the change in the pattern in the presence of both Spo0A(-P) and RNA polymerase indicates that the patterns observed are not the summation of interactions of the polymerase and Spo0A(-P) with separate populations of DNA, since only by binding to the same DNA strands as the polymerase could Spo0A(-P) reduce the hypersensitivity to hydroxyl radical. Finally, the change in the hypersensitivity occurred without affecting the protection of the upstream regions of promoter by the polymerase, con-
Spo0A Inhibition of abrB Transcription

The experiments we reported were designed to distinguish between three models for repression of abrB transcription by Spo0A. In the first model, Spo0A binding to a site at position −219, relative to the abrB P2 promoter and to the 0A boxes downstream of the P2 start site, would induce a formation of a DNA loop, which would inhibit transcription. This model is consistent with the interpretation that Spo0A(-P) did not displace the polymerase when it inhibited transcription at the abrB promoter.

DISCUSSION

The experiments we reported were designed to distinguish between three models for repression of abrB transcription by Spo0A. In the first model, Spo0A binding to a site at position −219, relative to the abrB P2 promoter and to the 0A boxes downstream of the P2 start site, would induce a formation of a DNA loop, which would inhibit transcription. Transcription regulation by DNA loop formation is well documented in Gram-negative bacteria (23, 24). However, Spo0A inhibition of in vitro transcription of the abrB promoters was unchanged when the upstream site had been removed, arguing strongly against the loop model.

The second model predicted that Spo0A binding to the downstream 0A boxes would exclude RNA polymerase binding to the promoter region of abrB. The paradigmatic examples of this model are the regulation of the Pγ promoter of bacteriophage λ by d(25, 26) and the regulation of the trp promoter by the Trp repressor (27). In the third model, Spo0A would prevent transcription but would not exclude the RNA polymerase from binding to the abrB promoter. Since the DNase I and hydroxyl radical protection assays showed RNA polymerase protection of the abrB promoter region in the presence of Spo0A and Spo0A-P at concentrations that substantially inhibited transcription, these experiments support the third model for Spo0A repression of abrB transcription.

The hydroxyl radical assays showed the template strand was hypersensitive to cleavage in the presence of RNA polymerase. To explain the increased sensitivity of the abrB promoter, we propose that strand separation exposed the sugar phosphate backbone of the template strand, increasing its accessibility to the hydroxyl radical. The hypersensitive region shifted as the predicted transcript length at abrB P2 changed from 1 to 13 nucleotides, reflecting the expected movement of the active site of RNA polymerase. We examined the hydroxyl radical footprint at another promoter (from phage φ29) and found both the hypersensitivity and a change in the location of hypersensitive sites when the polymerase could elongate RNA further (data not shown). Similar sensitivity to hydroxyl radical attack was noted for E. coli RNA polymerase at the T7 A1 promoter (28), and the hypersensitivity was also interpreted as reflecting exposure of the template strand due to denaturation of the DNA. However, these hypersensitive sites are not found at all promoters (for example, see Ref. 29). The hypersensitivity of the abrB promoter was inhibited by the addition of Spo0A(-P), indicating that transcription inhibition at the P2 promoter results from preventing the formation of a complex with the DNA strands denatured. Since the assays we used contained the initiating nucleotides, we cannot pinpoint more precisely which step in initiation was blocked. In spite of the block to strand separation, the hydroxyl radical assays showed that regions of the promoter protected solely by the polymerase were still protected even when Spo0A(-P) was present. This is consistent with the data from the DNase I assays that Spo0A(-P) does not displace the polymerase from the abrB promoter. We presume that when a polymerase is bound at P2 but cannot elongate RNA, it prevents transcription from P1, although we have not directly tested this hypothesis.

One of the most extensively investigated transcription repressors is the lac repressor (LacI). While LacI has been shown to block initiation, conflicting findings about the precise step affected have been reported (see Ref. 30 and the references therein). A recent study by Schlax et al., carried out under conditions where formation of the initiated complexes was reversible, supported a model in which LacI and RNA polymerase compete for free promoter (30). LacI appeared to block the formation of a complex with denatured DNA strands as we found for Spo0A(-P) inhibition at abrB P2, but for the lac case, there was no evidence for ternary complexes of repressor, DNA, and polymerase. The reactions conditions used by Schlax et al., are similar to the AG initiation conditions used in the work reported here in terms of concentration of polymerase, DNA,
and repressor and the reversible state of the initiation complexes. In contrast, other studies, which have looked at the inhibition mechanism of Lac by examining stable initiation complexes, suggested that Lac and the polymerase were bound to the promoter simultaneously (31–33). We would expect that the AUG initiation conditions at the abrB promoter would reflect a stable initiated state. While there were differences in the interaction of Spo0A(-P) and RNA polymerase, depending on the initiation conditions, the general finding that Spo0A(-P) inhibited transcription while binding along with the polymerase to the abrB promoter appeared to be independent of the reaction conditions.

Binding of Spo0A to the OA Boxes at the abrB Promoter—Protection of DNA from hydroxyl radical by proteins binding across a minor groove in B-DNA shows a pattern of protection in which regions on the two strands are offset by two to three bases. In contrast, binding across the major groove produces the areas of protection offset by at least seven bases. Proteins that bind to one side of DNA will show protected areas that are spaced by 10-base pair periodicity (34). Binding of Spo0A(-P) to the OA-boxes protected several 3-nucleotide regions with 1–2-base offsets on the two DNA strands and separated by 10 base pairs (Fig. 7). These data suggest that the protection was due to binding across the minor groove of the DNA. Spo0A binding to one side of the DNA has been suggested previously (10).

Spo0A has been proposed to be a member of the helix-turn-helix family of DNA binding protein (35, 36). The DNA binding by these proteins involves sequence-specific interactions by one helix (usually the C-terminal helix) and interactions with the phosphates in the minor groove by the other helix (reviewed in Ref. 37). The hydroxyl radical footprint thus indicates that the N-terminal part of the Spo0A helix-turn-helix region would make strong contact with the +7 to +10 region and +17 to +20 region of the abrB P2 promoter. This is compatible with proposed sequence-specific binding of Spo0A to the OA boxes, which are between positions +11 and +17 and positions +21 and +27. Methylation protection experiments have shown that residues at positions +12, +20, +22, and +23 (on the coding strand) and positions +14 and +24 on the template strand are protected by Spo0A (10). At higher inputs of Spo0A(-P), additional binding sites appear in the hydroxyl radical footprint, separated by 10-bp intervals. These indicate that Spo0A may tend to “line up” on the DNA. While the physiological significance of this binding is not known, we believe it represents the tendency of Spo0A to self-associate on the DNA. Transcription and protection assays were sensitive to small changes in concentration of Spo0A(-P) (Figs. 2–4), and this sensitivity is probably necessary for Spo0A phosphorylation to have an effect on abrB transcription in vivo.

The appearance of hypersensitive sites in the DNase I assays (Fig. 4) indicated the Spo0A binding altered the conformation of the DNA particularly on the 5′ side of the OA box. Furthermore, the 5′ boundary of the footprint with phosphorylated Spo0A was quite different from that seen with unphosphorylated Spo0A, a difference that may be due to changes in the structure of the protein that occurred upon phosphorylation.

Binding of the RNA Polymerase to the abrB Promoter—Protection of abrB DNA from hydroxyl radical attack by RNA polymerase in the region upstream from the promoter between −46 and −6 appeared to be mainly on one side of the DNA. The protected areas on the two DNA strands were offset by 2–4 nucleotides, suggesting that binding occurred in the minor groove at each site. The regions between −6 and +20 were protected on both strands of the abrB promoter. Protection of the T7 A1 promoter from hydroxyl radical cleavage by E. coli RNA polymerase showed that the enzyme interacts with one side of the promoter DNA between −52 and −11 and between 13 and +18 on both strands (29, 38). The DNA binding of E. coli at the T7 A1 and B. subtilis RNA polymerase at the abrB2 promoter resulted in similar patterns of protection, including the hypersensitive sites on the template strand. Protection of the bacteriophage λ P2 promoter by E. coli RNA polymerase was recently studied for two forms of open complexes (29). The major difference from the complex we observed at the abrB promoter was the lack of hydroxyl radical hypersensitivity at P_R. At both P_R and abrB, bases near positions −38 and −48 were hypersensitive to DNase I. This was interpreted as evidence for wrapping of the DNA around the polymerase in the case of P_R (29). With other Bacillus RNA polymerase-promoter complexes, DNase I-hypersensitive sites near position −24 and over all protection patterns similar to those seen in Fig. 5 have been detected (39, 40).

Straney and Crothers (41) proposed a model for transcription initiation that included a stressed intermediate structure created by transcription that occurs before RNA polymerase releases upstream contacts at the promoter. E. coli RNA polymerase protected the lacI8 UV5 promoter from DNase I digestion between −54 and +23. Upon formation of abortive 6- or 8-mer transcripts, protection extended to +26 without RNA polymerase shifting from its interactions with upstream DNA. The upstream boundary for RNA polymerase protection of the abrB promoter region from DNase I remained unchanged during transcript elongation of 1 or 13 nucleotides, while the downstream protected region moved (Figs. 5–7). These results are similar to those obtained by Straney and Crothers (41) for the stressed intermediate complex formed at lacI8 UV5, suggesting that a similar stressed intermediate complex may form at abrB.

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The Spo0A Protein of *Bacillus subtilis* Inhibits Transcription of the *abrB* Gene without Preventing Binding of the Polymerase to the Promoter
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