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Transcription of *Bacillus subtilis* Subtilisin and Expression of Subtilisin in Sporulation Mutants†

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The start point for transcription of the subtilisin (*aprE*) gene was determined by primer extension analysis and was found to be at a point significantly different from that identified in a previously published report (S. L. Wong, C. W. Price, D. S. Goldfarb, and R. H. Doi, Proc. Natl. Acad. Sci. USA 81:1184–1188, 1984). An *aprE-lacZ* fusion was used to analyze expression of the promoter. Deletion analyses of the promoter were performed to determine the extent of the upstream region necessary for activity. This was found to be between −52 and −41 with respect to the transcription start site. Expression of the *aprE-lacZ* fusion was unimpaired in a mutant deleted for the σ^B subunit of RNA polymerase. Mutations in the gene for the σ^H subunit of RNA polymerase decreased expression of the *aprE-lacZ* fusion to approximately 25% of that of the wild type. These results leave the identity of the sigma factor responsible for transcription of this gene in question. Mutations in the *spo0A* gene drastically decreased the activity of the *aprE* promoter and its upstream deletion derivatives, while the *abrB* gene, a phenotypic suppressor of *spo0* mutations, restored activity of the *aprE* promoter in all of the deletion derivatives. Thus, inhibition of transcription by the *spo0A* mutation and its restoration by an *abrB* mutation could not be separated from the promoter of the *aprE* gene.

The synthesis of subtilisin at the onset of stationary phase is a hallmark for the initiation of sporulation-specific transcription. Subtilisin production starts very early in the stationary phase and is detectable in the first hour after a culture leaves exponential growth. Little is known of the effectors that bring about its synthesis at this stage of the developmental cycle. Curtailed production of proteases, along with inability to produce other stationary-phase-associated products such as phosphatases and extracellular antibiotic, has long been a phenotype of early *spo0* mutations (6). *spo0A* mutations, in particular, result in deficient expression of protease (4). The protease deficiency and most of the other phenotypes of *spo0A* mutations can be overcome by mutations at an unlinked locus called *abrB*; however, the sporulation deficiency phenotype is not suppressed (15). Mutations at the *abrB* locus suppress mutations at either the *spo0A* or the *spo0B* locus and are not allele specific, suggesting that suppression occurs by bypassing of the requirement for *spo0* genes, not by restoration of their function (15).

Even though subtilisin is not itself essential for sporulation (14), its regulation by the sporulation process makes it an attractive target for more detailed studies of sporulation-related regulation. In a previous study, we used a subtilisin gene (*aprE*) fusion to the β-galactosidase gene (*lacZ*) to quantitate the effects of various *spo0* mutations on subtilisin expression (4). In this study, we examined the effects of defined deletions of the *sigB* (encoding σ^B [σ^{37}]) and *sigH* (encoding σ^H [σ^{30}]) genes on expression of the *aprE-lacZ* fusion. In addition, the *aprE* promoter was defined by both deletion analysis and determination of the transcription start point. Finally, the relationship between the *spo0A* locus and its suppressor locus, *abrB*, in subtilisin transcription was analyzed.

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MATERIALS AND METHODS

Strains. The *Bacillus subtilis* strains used in this study are listed in Table 1. Strain IS233 was a gift of I. Smith. The *spo0H Hin*d deletion was described by Weir et al. (16). Strains BH-1 and ML-1 contain chloramphenicol insertions in *sigH* (*spo0H*) and *sigB*, respectively, and were provided by Richard Losick. The rest of the strains were constructed for this study. All of the pSG35.1 derivatives were originally transformed into BG125, with selection for chloramphenicol resistance. The Amy$^-$ nature of the transformants was checked by growth of colonies overnight on a nutrient broth plate containing 1% (wt/vol) starch and staining of the plate with a solution of 0.5% (wt/vol) iodine and 1% (wt/vol) potassium iodine. DNA was extracted from each of the BG125 derivatives and transformed into strain JH642, JH646, or JH646MS, with selection for chloramphenicol resistance. *Escherichia coli* MM294 (F$^-$ *supR44 endA1 thi-1 hsdR4*) was used as a host for plasmid constructions. *E. coli* JM101 [Δ(*lac-pro*) *supE thi* F′ *traD36 proAB lacI*^{q}ZΔM15] was used as a host for phage M13.

Plasmids and plasmid constructions. Plasmids pUC18 (9), ptrpBG1 (13), pSG35 (4), and pJF751 (5) were used for construction of the plasmids used in this study. Plasmid pJM783 was constructed to allow construction of transcriptional fusions to the *lacZ* gene. The polylinker region of pJH751 was replaced with a polylinker that precedes the ribosome-binding site and initiation codon of the *spoVG* gene fused in frame to the *lacZ* gene. The *spoVG-lacZ* fusion was derived from Tn*917-lac* (10). Plasmid pJM818 was constructed by placing the approximately 750-base-pair (bp) *Eco*RI-*Bam*HI fragment containing the *aprE* promoter region from pSG35 into the *Eco*RI and *Bam*HI sites of pJF751. Plasmid pSG35.1 was constructed by replacing the *trpE-lacZ* fusion of ptrpBG1 with the *aprE-lacZ* fusion of pSG35. This was accomplished by isolation of the approximately 1,500-bp *Eco*RI-*Cla*I fragment of pSG35 containing the *aprE* promoter and the amino-terminal portion of *lacZ* and its ligation into the unique *Eco*RI and *Cla*I sites of ptrpBG1. Plasmids

TABLE 1. Strains

Strain	Genotype[a]
JH642	*trpC2 phe-1*
JH646	*trpC2 phe-1 spo0A12*
JH646MS	*trpC2 phe-1 spo0A12 abrB15*
JH703	*trpC2 phe-1 spo0AΔ204*
JH12421	*trpC2 sigB* (Cm^r) *amyE*::[*aprE'-'lacZ* pC194 *cat*]
JH12424	*trpC2 phe-1 spo0HΔHind amyE*::[*aprE'-'lacZ* pC194 *cat*]
JH12464	*trpC2 phe-1 amyE*::[*aprE'-'lacZ* pC194 *cat*]
JH12465	*trpC2 phe-1 aprE*::pJM818
JH12472	*trpC2 phe-1spo0A12 amyE*::[*aprE'-'lacZ* pC194 *cat*]
JH12473	*trpC2 phe-1 spo0A12 aprE*::pJM818
JH12496	*trpC2 thr-5 his-1 spo0H* (Cm^r) *amyE*::[*aprE'-'lacZ* pC194 *cat*]
IS233	*trpC2 phe-1 spo0HΔHind*
BH-1	*trpC2 sigH*::*cat*
ML-1	*trpC2 sigB*::*cat*
BG125	*trpC2 thr-5 his-1*

[a] The *aprE'-'lacZ* fusions are the SG35.5 derivatives described in Fig. 4.

SG35.4, pSG35.5, and pSG35.6 are derivatives of pSG35.1 that have deletions of the upstream region of the *aprE* promoter. Their general structure is shown in Fig. 1.

β-Galactosidase assays. Strains were streaked on a tryptose blood agar base (Difco Laboratories, Detroit, Mich.) plate for overnight growth at 32°C. Cells were scraped from the plate and suspended in prewarmed (37°C) Schaeffer medium (12) at an optical density at 525 nm of approximately 0.1. The cultures were grown at 37°C with vigorous aeration. Samples were removed at 0.5-h intervals for determination of optical density and β-galactosidase specific activity. β-Galactosidase assays were done as previously described (4), and all points were determined in triplicate. Milligrams of cellular extract were determined from a previously calculated standard curve of optical density versus milligrams of protein.

DNA manipulations. The procedures used for isolation, analysis, and construction of plasmid DNAs were carried out as previously described (13). DNA sequencing was conducted by the dideoxy chain termination method of Sanger et al. (11), with single-stranded M13 phage as templates (20).

Deletions of the *aprE* promoter were created by various methods using synthetic linkers, *Bal* 31 exonuclease digestion, or simply subcloning of appropriate restriction fragments. Each deletion derivative was subcloned into pUC18 in such a manner as to recreate an *Eco*RI site upstream of the promoter and a *Bam*HI site downstream of the promoter. The endpoints of all of the deletions were sequenced. Oligonucleotides were provided by the Genentech Organic Synthesis Group. Restriction enzymes, T4 DNA ligase, *Bal* 31 exonuclease, reverse transcriptase, and the Klenow fragment of DNA polymerase I were purchased from commercial sources and used as recommended by the suppliers.

mRNA extraction, mapping, and Northern (RNA) blot analysis. Cultures of 100 ml were grown in Schaeffer medium (12) until t_4, harvested by centrifugation, suspended in 0.5 ml of 10 mM Tris hydrochloride–1 mM EDTA (pH 8), and frozen in liquid nitrogen. The frozen cells were homogenized in a coffee grinder. After homogenization, the mixture was added to 2 ml of a solution of 200 mM Tris hydrochloride (pH 8.5), 50 mM EDTA, 250 mM NaCl, 6% (wt/vol) *para*-aminosalicylic acid (Sigma Chemical Co., St. Louis, Mo.), and 1% tri-isopropylnaphthalenesulfonic acid (Eastman Kodak Co., Rochester, N.Y.) and deproteinized by extractions with an equal volume of phenol-chloroform (1:1) until the interface was clear. Ethanol (2 volumes) was added to the aqueous phase, and the precipitate was collected by centrifugation.

A 30-base oligonucleotide complementary to codons 21 to 31 of the subtilisin-coding sequence was used for the reverse transcriptase reactions. RNA (50 μg) and primer (240 ng) were dissolved in 10 μl of 50 mM Tris hydrochloride (pH 8)–1 mM EDTA–0.4 M NaCl. They were heated to 95°C for 2 min and allowed to cool at room temperature for 10 min. RNasin (1 μl; Promega Biotech) was added. A 10-μl volume of 2× reverse transcriptase buffer (200 mM Tris [pH 8.3], 20 mM NaCl, 100 mM KCl, 20 mM dithiothreitol, 200 μg of each deoxynucleotide triphosphate per ml, 200 μg of actinomycin D per ml, 2,000 U of RNasin per ml) was added. One microliter of deoxycytidine 5'-[γ-^{32}P]triphosphate (3,000 Ci/mmol, 10 mCi/ml; Amersham Corp., Arlington Heights, Ill.), and 12.5 U of reverse transcriptase (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) were added, and the reaction mixture was incubated at 42°C for 2 h. Subsequently, the reaction mixture was extracted with an equal volume of phenol-$CHCl_3$. A 1/10 volume of 3 M sodium acetate and 2 volumes of ethanol were added, and the precipate was collected by centrifugation. The reverse tran-

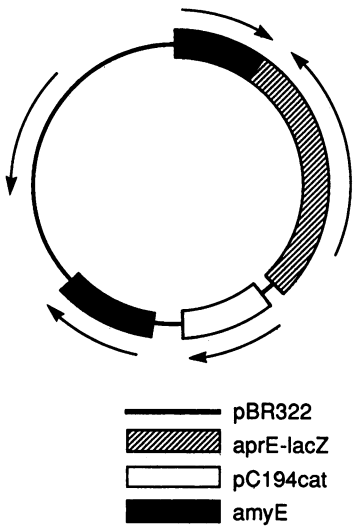

FIG. 1. General structure of the *aprE-lacZ* plasmids used in this study. The construction of these plasmids is described in Materials and Methods. Arrows indicate direction of transcription.

scriptase reactions were analyzed by electrophoresis on a standard sequencing gel. The same primer was used in a sequencing reaction on an M13 template of the subtilisin gene, and those reactions were loaded on the same gel for size standards.

Northern analyses were performed as described by Maniatis et al., with formaldehyde gels (7). Molecular weight standards were purchased (catalog no. 5620SA; Bethesda Research Laboratories, Inc., Gaithersburg, Md.). RNA was blotted onto Nytran filters (Schleicher & Schuell, Inc., Keene, N.H.). The oligonucleotide used for the reverse transcriptase experiments was labeled by phosphorylation with polynucleotide kinase and 5'-[γ-^{32}P]dATP (5,000 Ci/mmol; Amersham). Hybridization and washing conditions were as previously described (19).

Bacterial transformation and transduction. *E. coli* transformation was performed by the calcium shock procedure (3). Transformants were selected on Luria-Bertini plates supplemented with carbenicillin at 50 μg/ml. *B. subtilis* was transformed by a previously published procedure (1), with selection on tryptose blood agar base plates supplemented with chloramphenicol at 5 μg/ml. Transformation into the *amyE* gene by double-crossover integration was checked by showing that all of 50 transformants were Amy$^-$ (13). Many of the transformants were also checked by Southern analysis as previously described (13).

RESULTS

Subtilisin expression in a single-copy vector. In previous studies to determine the effects of *spo0* mutations on the expression of subtilisin, we used an integrative vector that was recombined into the chromosome by a Campbell-type insertion (4). Such vectors have the complication that they are subject to amplification, resulting in tandem duplications, depending to some degree on the concentration of antibiotic in the selective medium. To ensure that differences in copy number were not a factor in the interpretation of the present studies, we carried out most of the studies with derivatives of the single-copy integration vector ptrpBG1 (13). A general scheme of these vectors is shown in Fig. 1. These vectors have a chloramphenicol resistance gene and a promoterless β-galactosidase gene located between two halves of the α-amylase gene of *B. subtilis*. Constructions to generate *lacZ* fusions place the promoter to be assayed in a direction opposite from α-amylase transcription. Transformation of such vectors with *B. subtilis* with chloramphenicol selection results in a double-crossover event that generates a single-copy insertion within the α-amylase gene. Besides ensuring that each strain analyzed has a single copy of the *aprE-lacZ* fusion, this vector allows analysis of promoter deletions. Deletion analyses are less feasible with a Campbell-type vector, since such integrants generally have the entire recipient sequence upstream of the integration point.

A comparison of the β-galactosidase profiles of identical *aprE-lacZ* fusions in the single-copy integration vector and in the Campbell integration vector (4) showed almost identical profiles, suggesting that the previous studies were not unduly influenced by copy number problems (data not shown). A single-copy translational fusion was compared with a Campbell integration transcriptional fusion (Fig. 2), and almost identical profiles were seen in both the wild-type and *spo0A* strains. This is consistent with the notion that the inhibition of subtilisin expression by *spo0A* mutations occurs at the level of transcription. Since identical results were obtained with both systems, we felt confident in comparing directly the results of transcription and translation fusions in this system. The data presented in this work are based on translational fusions unless otherwise indicated. To facilitate comparisons between strains, the average rate of β-galactosidase accumulation during the first 2 h after the end of exponential growth was calculated from the β-galactosidase activity profiles. This method was also thought to reflect the activity of the *aprE* promoter in the various strains more accurately than would simple selection of a time point at some hour after the end of exponential growth, since some of the *spo0* strains begin to lyse within 2 to 3 h after the end of exponential growth.

Location of the start site of transcription. To determine the exact start site of transcription of the *aprE* gene, primer extension experiments were carried out on RNAs extracted from cells of cultures grown to 2 h after the end of exponential growth. The reverse transcripts were run on a sequencing gel along with sequencing ladders from DNA of the same region generated with the same oligonucleotide primer. The result of this experiment is shown in Fig. 3. The major site of transcription initiation was found to be the adenine nucleotide indicated at +1 in Fig. 4. An additional potential start site was detected further downstream at approximately +31. Scans of these gels indicated that 10% or less of the reverse transcripts terminated at this downstream location. We suspect that this putative additional transcriptional start site is artifactual. Although it was not reproduced well in the figure, there is a ladder of bands at this region rather than a single discrete band at the upstream location. The amount of

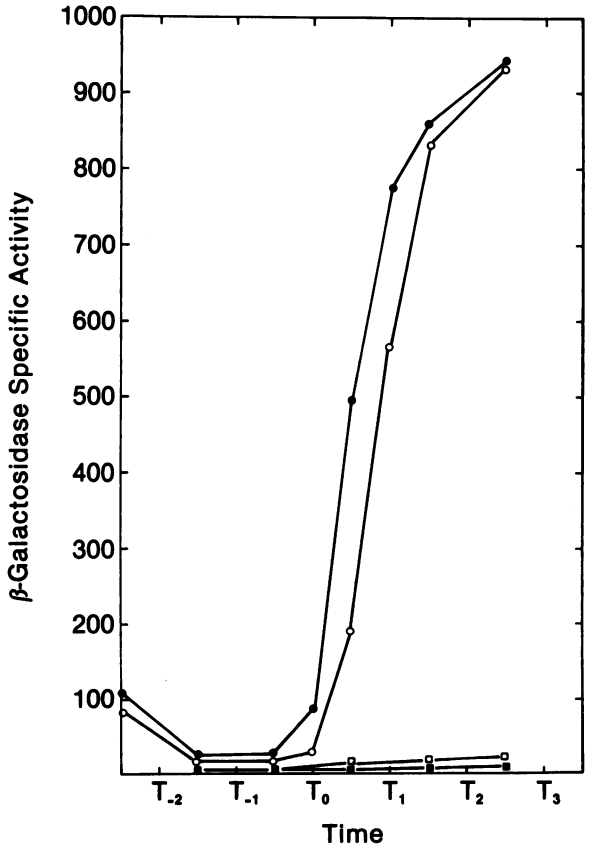

FIG. 2. Comparison of transcriptional and translational fusions of *aprE-lacZ*. Cultures were grown and assayed as described in Materials and Methods. Symbols: ○, JH12464; □, JH12472; ●, JH12465; ■, JH12473.

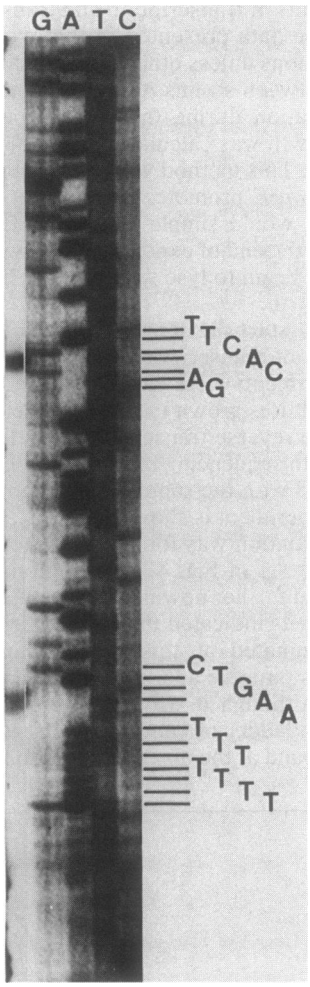

FIG. 3. Determination of the start point of transcription of the *aprE* promoter. Primer extension analysis was carried out on mRNA preparations as described in Materials and Methods. The source of mRNA was BG125. The sequencing ladder, derived from the same primer, is shown on the right, with the G, A, T, and C reactions from left to right. The sequence indicated is that of the strand other than that of the sequencing gel.

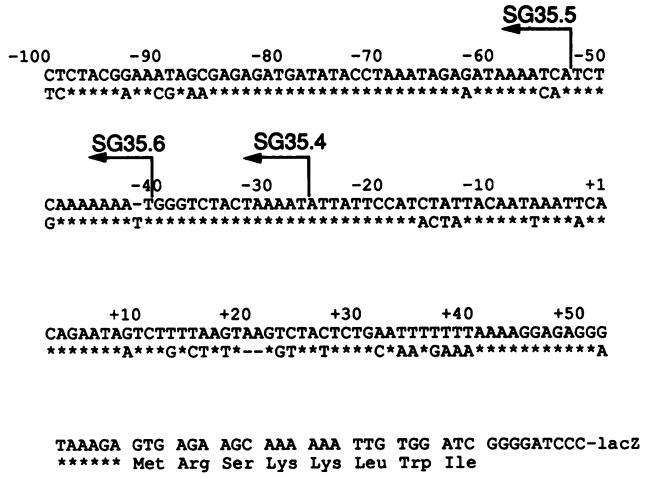

FIG. 4. Nucleotide sequence of the *aprE* promoter. The sequence of the "*B. amyloliquefaciens*" promoter (17; J. Wells, personal communication) is shown below the *B. subtilis* sequence (14). Asterisks indicate identity, and dashes indicate gaps. The endpoints of the deletions described in the text are indicated by arrows pointing toward the deleted region. The start point of transcription is indicated as +1. The point of fusion to *lacZ* for all of the transcriptional vectors is indicated.

these downstream bands also varied in different mRNA preparations. The *aprE* promoter deletion analysis described below was also more consistent with the upstream start site.

Deletion analysis of the *aprE* promoter. To define the DNA sequences necessary for *aprE* promoter function, we generated a series of deletions of the *aprE* promoter (Fig. 4). These deletions were placed in the *aprE-lacZ* amylase vector, and the β-galactosidase activity profiles were determined in strain JH642. Intermediate deletions between the SG35.1 derivative with an endpoint at approximately −600 and the SG35.5 derivative with an endpoint at −52 were essentially equivalent to the strains used in this study. The SG35.5 deletion to −52 retained full activity of the promoter (Table 2). The SG35.6 deletion to −41 showed a large decrease in β-galactosidase accumulation, and a further deletion to −26 (SG35.4) completely abolished promoter activity. These deletions defined the DNA region needed for promoter activity to between −41 and −52, and these limits seem to be in reasonable agreement with the transcription start point defined above.

Effect of sigma factor mutations on *aprE-lacZ* fusion expression. In a previous analysis of the *aprE* promoter, Wong et al. (18) showed data that suggested that the *aprE* promoter was transcribed in vitro by σ^B (σ^{37}) containing RNA polymerase. Recently, mutations that interrupt the genes that encode both σ^B (*sigB* [2]) and σ^H (σ^{30}) (*spo0H* [16]) have become available. Thus, it was of interest to examine the expression of the *aprE-lacZ* fusion in strains that carry these deletions. The SG35.5 derivative of the *aprE-lacZ* fusion was transferred into strains that carry mutations in either the *sigB* or *spo0H* gene. The β-galactosidase activity profile was determined, and the results are shown in Fig. 5. An interruption of the *sigB* gene had no effect on the expression of the *aprE-lacZ* fusion. The same *sigB* mutation completely abolished expression of the *ctc* gene, which is known to be transcribed by σ^B containing RNA polymerase (R. Losick, personal communication). Two mutants that carry deletion mutations in the *sigH* gene were examined. In both cases, the rate of *aprE-lacZ* expression was 25 to 30% of that seen in a wild-type strain (Fig. 5).

Site of action of the *spo0A* and *abrB* mutations on *aprE* expression. The *spo0* genes influence the levels of expression of *aprE* and a wide variety of other genes. Loss of the function of the *spo0A* gene product results in inability to express subtilisin at the end of exponential growth. The results presented in Fig. 2 showed that the *spo0A* mutation

TABLE 2. Initial rates of β-galactosidase accumulation from subtilisin promoter deletions

Deletion	Endpoint	Initial rate of β-galactosidase accumulation (U/mg per h)
SG35.1	~−600	490
SG35.5	−52	707
SG35.6	−41	83
SG35.4	−26	<15

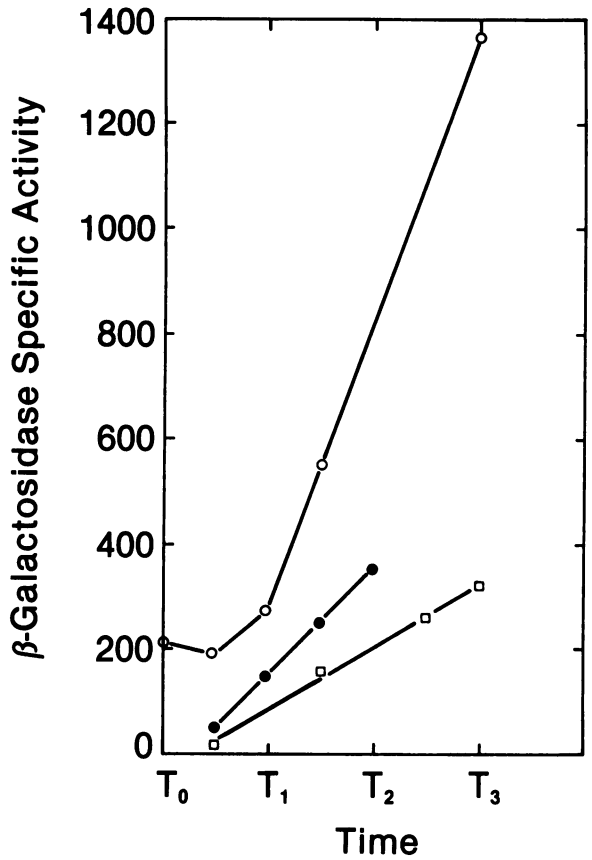

FIG. 5. Effect of sigma factor mutations on *aprE-lacZ* expression. Cultures were grown and assayed as described in Materials and Methods. Symbols: ○, JH12421 (*sigB* deletion); ●, JH12496 (*spo0H* null mutation); □, JH12424 (*spo0H* deletion).

affects transcriptional and translational fusions to *aprE* equally. Northern analysis of mRNAs extracted from strains that carry *spo0A* or *spo0H* (*sigH*) showed that the level of *aprE* mRNA in these strains was reduced compared with that of the wild-type strain (Fig. 6). These results indicate that these mutations most likely act by decreasing transcription of the *aprE* promoter.

The deletion derivatives previously described allowed us to determine whether the site of *spo0A* action could be distinguished from the promoter activity per se. Each of the deletion plasmids was integrated into strain JH646 (*spo0A12*), and the effect of the mutation on *aprE-lacZ* expression was determined. The results of these analyses (Table 3) showed that the *spo0A* mutation severely reduced expression from all of the promoters.

The *spo0A* effect can be suppressed by *abrB* mutations which act in an allele-nonspecific manner. Since protease production has long been a prototype gene for this suppression, we were curious about whether the deletion analysis could pinpoint a site of action for this *abrB* suppression. The various *aprE-lacZ* fusions were transformed into strain JH646MS (*spo0A12 abrB15*), and the β-galactosidase activity profiles were determined. Inhibition of *aprE-lacZ* expression by *spo0A* was reversed in all cases by the *abrB* suppressor (Table 3). Deletion SG35.6, which has weak promoter activity, could be restored to nearly wild-type levels by the *abrB15* mutation. On the other hand, deletion

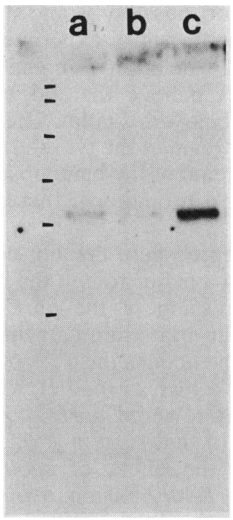

FIG. 6. Quantitation of the *aprE* mRNAs in *spo0A* and *spo0H* mutants. The procedures for mRNA extraction and Northern analysis are described in Materials and Methods. Lanes: a, BH-1; b, JH703; c, BG125. The molecular sizes of the standards indicated on the left are, from top to bottom, 9,500, 7,500, 4,400, 2,400, 1,400, and 300 bp.

SG35.4, which results in an inactive promoter, could not be reactivated by the *abrB* suppressor.

DISCUSSION

A previous characterization of the *aprE* promoter by Wong et al. (18) differs substantially from the results presented here. They reported dual transcription start points at the locations corresponding to +26 and +41 (Fig. 4). These start sites were described both in vivo and for an in vitro transcription reaction by σ^B RNA polymerase. We have confidence in the start point reported in this study, as we feel that it is supported by several additional pieces of evidence. Analysis of the very similar "*Bacillus amyloliquefaciens*" subtilisin promoter localized the start point to an identical location (C. Moran, personal communication). The nucleotide sequence of this promoter is highly conserved between these two organisms (Fig. 4). The deletion analysis was consistent with the mRNA mapping. A deletion to −41 begins to abrogate promoter function, and one at −26 completely eliminates promoter function. As discussed in Results, we think that the minor potential start site we detected at +31 in the primer extension experiments is artifactual. The *aprE* mRNA can potentially be folded into a weakly associated stem-loop structure just upstream of this region, and there might be a degradation intermediate de-

TABLE 3. Initial rates of β-galactosidase accumulation from subtilisin promoter deletions in *spo0A* and *spo0A abrB* strains

Deletion	Initial rate of β-galactosidase accumulation (U/mg per h) of *B. subtilis* with the following genotype:		
	spo$^+$	*spo0A12*	*spo0A12 abrB3*
SG35.1	490	<15	
SG35.5	707	<15	740
SG35.6	83	<15	480
SG35.4	<15	<15	<15

tected at this point or, possibly, some stalling of the reverse transcriptase in the in vitro experiments. However, the finding that the −26 deletion is completely inactive as a promoter makes us believe that no biologically relevant transcription starts below this point. The −26 deletion would be 51 and 67 bp upstream of the two start sites of Wong et al. (18) and 56 bp upstream of the band observed at +31, which seems adequate for a promoter if these sites were actually used in vivo.

The results presented here are not consistent with a σ^B holoenzyme playing a major role in the in vivo transcription of *aprE*. The expression of the *aprE-lacZ* fusion is not significantly different in a strain carrying an interruption of the *sigB* gene, which encodes the σ^B protein (2). Deletions of the *sigH* gene (also known as *spo0H*), which encodes the σ^H protein, significantly lowered *aprE-lacZ* expression to just less than one-third of that seen in a wild-type strain. In the previous study of the effect of *spo0* mutations on the expression of an *aprE-lacZ* fusion, a *sigH* mutation showed the same reduction in expression as *spo0B*, *spo0E*, and *spo0F* mutations (4). These results suggest to us that this reduction of expression in a *sigH* mutant is a secondary consequence of the sporulation defect rather than a specific lack of the sigma factor necessary for transcription of *aprE*. However, it remains possible that the σ^B holoenzyme transcribes this promoter along with another unidentified sigma factor-containing holoenzyme. We feel that the sigma factor responsible for the in vivo transcription of this gene remains unidentified.

The *abrB* mutations were originally described as partial suppressors of *spo0A* mutations; that is, they were able to bypass the requirement for the *spo0A* gene product in the transcription of certain gene products, including subtilisin. Two recent papers have explored the effect of *abrB* mutations on the *spoVG* and *tycA* promoters in *B. subtilis* (8, 21). The authors of these papers speculated that the *abrB* gene acts as a negative regulatory factor that is inactivated by the *spo0* gene system. The *spo0A* gene then acts in some way to inactivate the *abrB* gene product at the end of exponential growth. In the case of the *spoVG* gene promoter, a mutation in an upstream T-A box can relieve the inhibition of the *spoVG* promoter by a *spo0A* mutation, and it was speculated that this might be a binding site for negative regulation by the *abrB* gene product. There is a similar T-A box upstream of the *tycA* promoter. There is no T-A box sequence upstream of the *aprE* promoter, and deletion of the upstream region does not eliminate repression of the *aprE* promoter by a *spo0A* mutation. This seems effectively to eliminate any upstream region as the site of action of *abrB* repression activity. There are several T-A sequences within the *aprE* promoter which might serve as a site for *abrB* inhibition if this protein acts on the subtilisin promoter in the manner speculated for the *spoVG* promoter.

The *tycA* promoter, which is normally expressed as the cells reach stationary phase, becomes constitutive in an *abrB* mutant (8). The *spoVG* promoter, on the other hand, is still temporally regulated (21). The *aprE* promoter acts like the *spoVG* promoter in this regard; in no case did we see expression of the *aprE* promoter during exponential growth.

A logical explanation for these phenomena, proposed by Zuber and Losick (21), is that the *abrB* inactivation is the sole control for *tycA*, whereas the expression of *spoVG* and *aprE* has more than one level of control by *spo0A* function. How direct any of these interactions are, whether *abrB* in fact directly interacts with the *aprE* promoter, and how the *spo0A* gene product acts to antagonize *abrB* are still very much open questions. The recent isolation of the *abrB* gene (Perego and Hoch, unpublished data) should facilitate this analysis. We feel that the other major pieces of the puzzle that are still missing are the identity of the sigma factor responsible for *aprE* transcription and the effects of *spo0A* and *abrB* mutations on the activity of the factor.

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LITERATURE CITED

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