JOURNAL OF BACTERIOLOGY, Oct. 1990, p. 5631–5636
0021-9193/90/105631-06$02.00/0

Vol. 172, No. 10

Localization of a Second SigH Promoter in the *Bacillus subtilis sigA* Operon and Regulation of *dnaE* Expression by the Promoter

FENG-XIA QI AND ROY H. DOI*

Department of Biochemistry and Biophysics, University of California, Davis, California 95616

Received 10 April 1990/Accepted 16 July 1990

The presence of a second SigH promoter in the *sigA* operon of *Bacillus subtilis* was demonstrated by use of a promoter probe plasmid, a *sigH* deletion mutant, primer extension studies, and in vitro transcription with $E\sigma^H$ holoenzyme. Both SigH promoters were expressed at low levels even during the growth phase but were expressed at higher levels during the early stationary phase. Expression from the upstream SigH promoter allowed the expression of both *dnaE* and *sigA* genes; however, expression from the downstream SigH promoter, which was located in the ribosome-binding site of the *dnaE* gene, resulted only in the expression of the *sigA* gene, since the truncated *dnaE* ribosome-binding site could not be used for initiating translation. Thus, promoter switching during the early stationary phase resulted not only in expression from SigH promoters but also in differential expression of the genes in the *sigA* operon.

The expression of genes during sporulation of *Bacillus subtilis* is controlled in part by a number of σ factors that allow RNA polymerase holoenzymes to recognize promoters that control sets of developmental genes (6, 18, 21). Thus the sequential expression of σ genes allows a temporally controlled expression of sporulation genes. The question we have been addressing is, what type of regulation, if any, controls the expression of genes that are expressed during both growth and sporulation? The model system we have used for this study is the *sigA* operon of *B. subtilis*, in which there are three open reading frames coding for *P23* (unknown function, nonessential) (29), *dnaE* (DNA primase), and *sigA* (σ^A) (25).

Previous studies had shown that at least two SigA promoters and one SigH promoter were involved in the regulation of expression of the *sigA* operon, that the SigA promoters were used primarily during the exponential growth phase, and that the SigH promoter was used from the time of sporulation (T_0) to 3 h after sporulation (T_3) (26). This promoter switching allowed the operon to be expressed during the early stationary phase at a time when at least one stationary-phase gene, *aprE* (23), and the early sporulation genes *spoIIG* (14) and *spoIIE* (9) depended on the continued presence of SigA, the product of the last gene of the operon.

The current studies indicate the presence of a second SigH promoter downstream from the previously reported SigA and SigH promoters. This new promoter was observed during the mutation studies of the previously identified SigH promoter (P3), when it was found that deletion of the P3 from a fragment that contained only the known P3 promoter did not impair transcription initiation. By probing for promoters downstream of P3 and by using genetic studies and in vitro transcription analyses, we have found a new SigH promoter (P4) located immediately in front of the *dnaE* gene. Primer extension analyses located the transcription initiation site within the Shine-Dalgarno (SD) sequence of the mRNA for *dnaE*. When transcription was initiated from P4, the ribosomal binding site for *dnaE* was no longer functional and therefore only the mRNA for SigA was translated. Thus the synthesis of functional DNA primase mRNA was prevented when transcription was initiated at P4, but the synthesis of SigA remained unimpaired during early sporulation.

MATERIALS AND METHODS

Bacterial strains. DB2 (*B. subtilis* 168 *trpC2*) was used in RNA isolation for the primer extension analyses. DB403 (*rpC2 npr aprE epr*) (24) was used as a host for the promoter-probing and translational *lacZ* fusion assays.

Plasmid construction. The promoter probe plasmid pWP19 (26) was used for promoter-probing analyses. pWP19 is a pUB110 derivative that contains a promoterless subtilisin gene *aprE* preceded by a multiple cloning site derived from pUC19. pWP19HR was constructed by subcloning the *Hae*III-*Eco*RV fragment (Fig. 1) containing only the P4 promoter into pWP19. pBT143 is a pUB110 derivative that contains the *lacZ* gene from *E. coli* lacking the first 8 amino acids. The multiple cloning site was inserted in front of *lacZ* so that the insertion was in the third reading frame (1). pBTXR was constructed by cloning the *Xmn*I-*Eco*RV fragment (Fig. 1) into pBT143 in frame to the *lacZ* gene, so that *lacZ* was translated from the SD sequence of the *dnaE* gene that was under the control of both the P3 and P4 promoters. pBTHR was constructed in the same way as pBTXR but with the *Hae*III-*Eco*RV fragment inserted into pBT143, so that the *lacZ* gene was under the control of only the P4 promoter.

Plasmid pJ0H7 is a pBR322 derivative that contains the *spo0H* gene under the control of a inducible promoter, Pspc, and a downstream *lacI* gene (13). pAG58 is the vector without the *spo0H* insertion. Both plasmids were kindly provided by Healy and Losick.

RNA purification. *B. subtilis* DB2 cells were grown in liquid sporulation medium containing 0.1% glucose (2×SG) (16) at 37°C with shaking. Samples of 10 ml of cells were removed at the designed time points and centrifuged at 7,600 × *g* for 10 min at 4°C. The pellet was washed with 0.5 ml of SET buffer (20% sucrose, 50 mM Tris hydrochloride [pH 7.6], 50 mM EDTA) and suspended in 1.5 ml of SET with 2 mg of lysozyme per ml. The suspension was incubated at 37°C for 2 to 5 min depending on the cell age and centrifuged for 5 min at 4°C in a microfuge. The cell pellet was suspended in 0.5 ml of denaturation buffer (4 M guanidinium-thiocyanate, 0.05 M Tris hydrochloride [pH 7.6], 0.01 M

* Corresponding author.

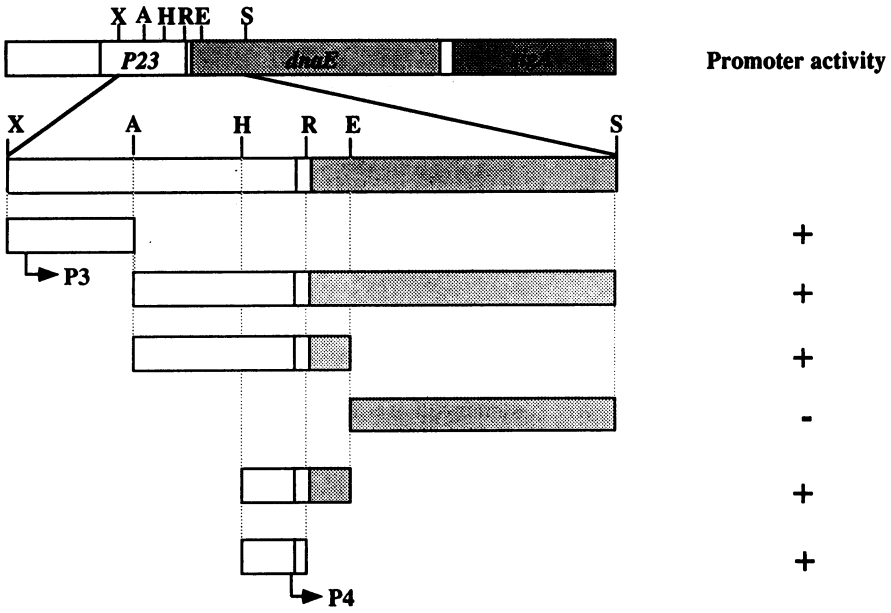

FIG. 1. Localization of the P4 promoter on the *Xmn*I-*Sst*I fragment of the *sigA* operon. The *Xmn*I-*Sst*I fragment contains *P23* and part of the *dnaE* gene. The fragment was first subcloned into pUC18 to form pUC18XS. Each bar represents the fragment that was used in promoter probing analyses with pWP19. The deletion analyses were performed by subcloning each fragment into pUC18 or pUC19 to acquire proper linkers. Then the fragment was subcloned into pWP19 and transformed into DB403. Promoter activities were measured by plating cells on 2×SG casein plates supplemented with 5 μg of kanamycin per ml. If the fragment contained a promoter that turned on the promoterless *aprE* gene, a clearing was observed. X, *Xmn*I; A, *Alu*I; H, *Hae*III; R, *Rsa*I; E, *Eco*RV; S, *Sst*I.

EDTA, 2% sodium dodecyl sulfate, and 0.14 M β-mercaptoethanol) (19) at 60°C, and 0.5 ml of hot phenol (60°C) was added. The mixture was vortexed for a few seconds and returned to a 60°C water bath; 0.2 ml of TEN buffer (10 mM Tris hydrochloride [pH 7.6], 1 mM EDTA, 10 mM NaCl) was added with 0.2 ml of chloroform-isoamyl alcohol (24:1). The mixture was vortexed for 30 s, put on ice for 30 s, and centrifuged for 3 min at 4°C in a microfuge. The water phase was extracted with phenol-chloroform for three or four times until the solution became clear. The RNA was precipitated with an equal volume of isopropanol at −20°C overnight. After the RNA pellet was washed with 70% ethanol and air dried, it was suspended in 200 μl of TEN buffer containing 0.5 μg of proteinase K per μl and incubated at 37°C for 30 min. The mixture was extracted with phenol-chloroform twice and precipitated with ethanol at −20°C for 2 h. The RNA pellet was suspended in 100 μl of DNase I solution (40 mM Tris hydrochloride [pH 8.0], 6 mM $MgCl_2$, 10 mM NaCl, 0.1 mg of RNase-free DNase I [Bethesda Research Laboratories, Inc.] per ml), incubated at 37°C for 1 h, and then extracted with phenol-chloroform twice. The RNA was precipitated with ethanol and quantitated by determining optical density at 260 nm. The quality of the RNA was checked by electrophoresis (1.5% agarose gel in TBE buffer [0.089 M Tris-borate, 0.089 M boric acid, 0.002 M EDTA]).

Primer extension. The primer used in the primer extension assay was FX130, which is complementary to the 5′ end of the *dnaE* gene with the sequence 5′-TCGGCCTTGCTTCTT TAATTGAACATAATC-3′. Primer extension was performed according to Inoue and Cech (12) with the following modifications: 100 μg of RNA was suspended in 30 μl of hybridization buffer with 10^5 cpm of labeled probe and denatured at 95°C for 3 min. The primer was annealed to the RNA template by slowly cooling down the mixture to 42°C in 1 to 2 h. Then 10 μl of reaction cocktail containing deoxynucleoside triphosphates, RNasin, Mg^{2+}, and avian myeloblastosis virus reverse transcriptase (Bethesda Research Laboratories) was added at final concentrations suggested by Inoue and Cech. The reaction mixture was incubated at 42°C for 1 h, extracted twice with phenol, and precipitated with ethanol. The pellet was washed with 70% ethanol twice, air dried, and suspended in 10 μl of 0.1 M NaOH solution. After denaturation at 95°C for 3 min, 5 μl of loading dye was added, and 5 μl was loaded on a 6% polyacrylamide sequencing gel containing 50% (wt/vol) urea.

DNA sequencing. The procedure for M13 sequencing supplied with the Sequenase kit (U.S. Biochemical Corp.) was followed exactly.

SigH isolation and in vitro transcription. Samples (20 ml) of *Escherichia coli* JM101 cells harboring pJ0H7 were grown in Luria broth medium containing 100 μg of ampicillin per ml to the midlog phase (70 Klett units) at 37°C with shaking. Isopropyl β-D-thiogalactoside was added to a final concentration of 1 mM, and the incubation was continued for 1.5 h. Cells were collected by centrifugation at 7,600 × *g* for 10 min at 4°C and suspended in 1 ml of SET buffer. Then 1 ml of sample application buffer (15) was added, mixed, and the suspension was boiled for 5 min. The cell lysate was vortexed for 20 min at room temperature and loaded onto a 12.5% sodium dodecyl sulfate–polyacrylamide gel (18 by 16 by 0.35 cm). The samples were run at 40 mA overnight at 4°C along with the prestained high-molecular-weight marker (Bethesda Research Laboratories). After electrophoresis, the gel was rinsed twice with double-distilled H_2O, stained with 0.25 M KCl–1 mM EDTA for 5 min at room temperature, and destained with double-distilled H_2O–1 mM EDTA for 10 min. The gel was sliced around 29 kilodaltons which is the expected mobility of the SigH protein (3). The protein elution from the gel and renaturation were performed exactly as described previously (10).

RNA polymerase reconstitution and in vitro transcription were performed as described previously (5). The in vitro

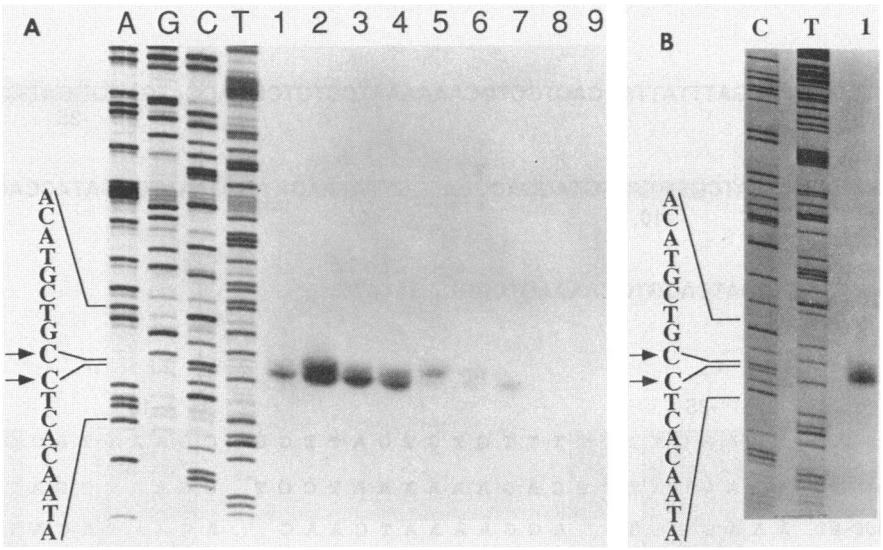

FIG. 2. Determination of transcription start site of P4 by primer extension analyses with in vivo and in vitro RNA. (A) Total RNA was isolated from DB2 (wild-type) cells at different growth stages as described in Materials and Methods. RNA (100 μg) from each stage was hybridized with the 5′-end-labeled primer FX130. After extension of the primer with reverse transcriptase, followed by ethanol precipitation, the mixture was subjected to electrophoresis in a 6% polyacrylamide sequencing gel. The template for generating size markers in a M13 sequencing reaction was mp18R1 in which a 3.5-kb fragment containing *P23* and *dnaE* genes and the upstream region was cloned into M13mp18 at the *Eco*RI site. The primer FX130 was also used in the M13 reaction. Lanes A, G, C, and T are the four reactions in the M13 sequencing reaction, and the sequence shown is the same as that of the bottom strand. The DNA sequence around the +1 site is shown. The arrowheads indicate the two transcription start sites (CC), which correspond to the two Gs in the ribosome-binding site of the mRNA for *dnaE*. Lanes: 1 through 7, RNA from cells in the log phase (T_1), T_0, T_1, T_2, T_3, T_4, and T_5, respectively; 8 and 9, RNA from IS233 (SigH$^-$) at T_0 and T_2, respectively. (B) RNA synthesized from a 500-μl reaction mixture with reconstituted Eσ^H was hybridized with the same primer as for the in vivo RNA. The template used in generating size markers by a M13 sequencing reaction was mp18R1P4, which contains the same *Eco*RI fragment as that in mp18R1 but has a mutation in the −10 region of P4 in which a *Bgl*II site is created by site-directed mutagenesis (Qi and Doi, unpublished results). Lanes: C and T, C and T reactions, respectively, of the M13 sequencing reaction; 1, in vitro RNA. The DNA sequence around the +1 site is shown; the arrowhead indicates the initiation site of the in vitro transcript.

RNA used in the primer extension assay was generated as described previously (4).

Template preparation for the in vitro runoff assay. The *Alu*I-*Eco*RV fragment (Fig. 1) was subcloned into pUC18 to form pUC18AR. pUC18AR was cut with *Pst*I and *Sst*I; a 580-base-pair fragment containing the promoter and part of the *dnaE* gene was isolated that gave a 79-base runoff transcript. pUC18AR was also cut with *Pvu*II; a 820-base-pair fragment was isolated that generated a 263-base runoff transcript. The template for generating in vitro RNA for primer extension was synthesized by the polymerase chain reaction with the standard reaction (11). The template pUC18XS was constructed by cloning the *Xmn*I-*Sst*I fragment (Fig. 1) into pUC18; the two primers were M13 reverse primer and FX130 (see above). The reaction was run at three segments: 95°C for 20 s, 50°C for 20 s, and 72°C for 30 s for 30 cycles in a Tempcycler 50 (Coy Laboratory Products Inc.).

Protease assay. Protease assays were performed as described previously (26).

β-Galactosidase assay. The β-galactosidase assay was performed as described previously (20). One unit was defined as (optical density at 420 nm × 10^3)/(Klett units × time × volume).

RESULTS

Localization of a new promoter on the *sigA* operon. As mentioned above, during mutagenesis of the P3 promoter a new promoter activity was found to be located on a fragment downstream of P3 to the *Sst*I site within the *dnaE* gene. By a series of deletion assays (Fig. 1), we narrowed the location of the promoter activity to a 170-base-pair *Hae*III-*Eco*RV fragment. S1 nuclease mapping showed that the +1 site was right in the SD sequence of the *dnaE* gene (data not shown). To confirm the S1 data, primer extension assays were performed with RNA isolated at different growth stages as described in Materials and Methods. The primer FX130 (see Materials and Methods) was used in the primer extension assays as well as in the M13 sequencing reactions to obtain size markers. The transcription from the new promoter started at the first two Gs in the SD sequence GGAG of *dnaE* (Fig. 2A and 3A). The primer extension data correlated very well with the S1 data. Taking the promoter-probe and primer extension analyses data together, we concluded that the *Hae*III-*Eco*RV fragment contained a new promoter that initiated within the SD sequence of the *dnaE* gene, and we named it P4.

Temporal expression of the new promoter P4. In our promoter extension analyses (Fig. 2A), we used the same amount of total RNA (100 μg) isolated from different growth stages (from the vegetative phase to 6 h after sporulation [T_6]) to hybridize with the primer FX130. The intensity of each band on the gel showed that the promoter was expressed at a low level during vegetative growth; the expression increased at T_0, reached a peak level at T_2 to T_3, and decreased from T_3 to T_5. During the localization of the new promoter, we constructed pWP19HR to measure the temporal expression of P4. The plasmid pWP19HR was introduced into a triple extracellular protease-negative strain, DB403 (24), and assayed for protease activity. Expression of the

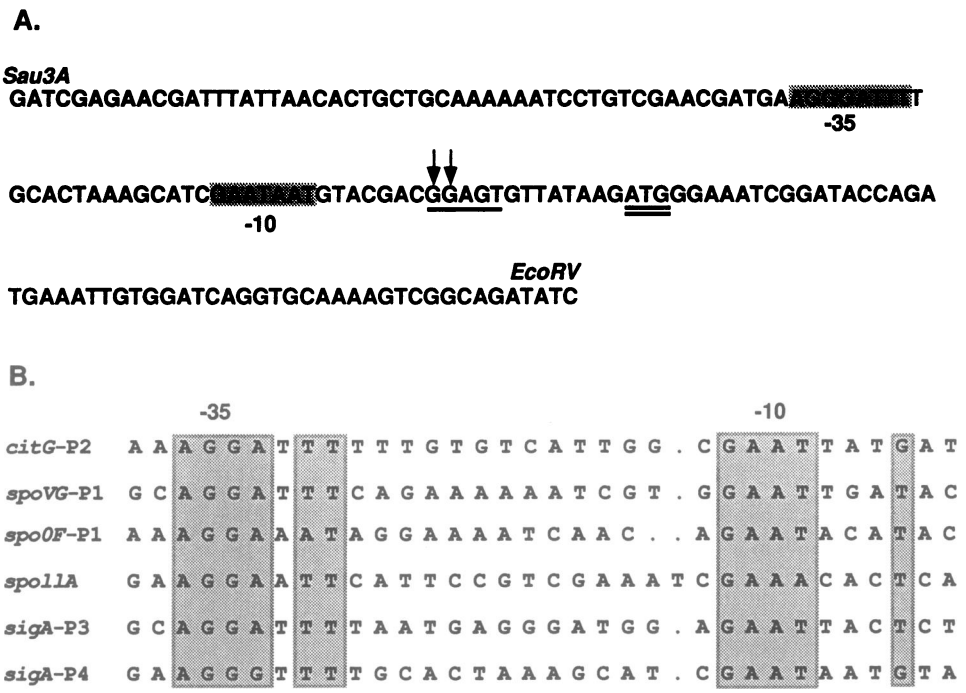

FIG. 3. (A) DNA sequence of the coding strand of the P4 promoter region on the *Sau*3A-*Eco*RV fragment. The −35 and −10 regions of P4 are shaded. The two arrowheads indicate the two Gs where transcription from P4 starts. The ribosome-binding site and the initiation codon for *dnaE* are underlined and double underlined, respectively. (B) Alignment of the P4 promoter region with that of the other known SigH promoters (2, 4, 8, 17, 28). The −35 and −10 regions are shaded.

promoter on the multicopy plasmid (Fig. 4) showed the same pattern as that of the in vivo RNA (Fig. 2A), indicating that the promoter expression patterns obtained in the primer extension analyses were not due to the variation in RNA quantitation but were a true reflection of promoter expression in vivo.

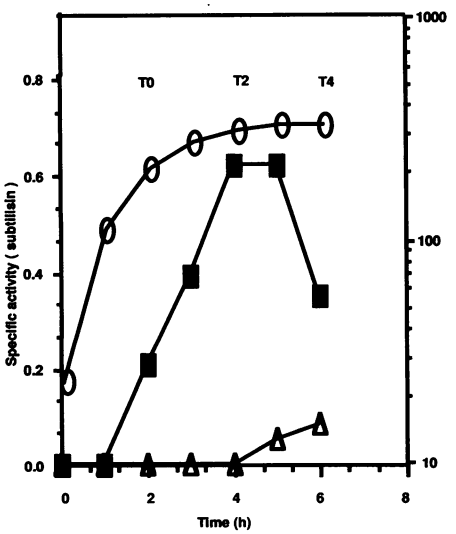

FIG. 4. Activity of P4 promoter assayed in a transcriptional gene fusion to *aprE*. DB403 cells containing the plasmids pWP19HR and pWP19 were grown in 2×SG medium at 37°C with shaking. Samples were taken from each strain at each designed time point and assayed for protease activity. The average value of three parallel samples was plotted on the graph. Symbols: ○, growth of the cells as measured by Klett units; ■, DB403(pWP19HR) (P4); △, DB403+ (pWP19) (vector). T_0, T_2, and T_4 are hours at or after the onset of sporulation.

SigH is required for the in vivo expression of promoter P4. Computer analysis of the *Hae*III-*Eco*RV fragment upstream of the +1 site of P4 showed −35 and −10 regions homologous to the consensus SigH promoters (Fig. 3B). To determine whether P4 was another SigH promoter, we wanted to test it for promoter activity in a *sigH* null mutant strain, IS233 (27). Since IS233 secretes extracellular proteases, which would obscure the protease activity generated from pWP19HR containing the subtilisin reporter gene, we introduced the *sigH* null mutation into a triple extracellular protease-negative background strain, DB403. When pWP 19HR was introduced into the new *sigH* null mutant derivative of DB403 and assayed for protease activity on a 2×SG casein plate with 5 μg of kanamycin, no protease activity was observed. In contrast, pWP19HR in a $SigH^+$ background showed a very high protease activity (data not shown).

To confirm the protease assay data, we isolated total RNA from IS233 at the T_0 and T_2 stages and performed a primer extension assay. The same amount of RNA (100 μg) was used in the assay with RNA from DB2 cells. Transcription from the P4 promoter in IS233 cells was totally absent as compared with that in DB2 cells (Fig. 2A, lanes 8 and 9). We concluded that expression of the P4 promoter in vivo was absolutely dependent on *sigH* gene activity.

Use of the P4 promoter by $E\sigma^H$ in vitro. Since computer sequence analysis and in vivo genetic studies suggested that P4 was a SigH promoter, we reasoned that it would be transcribed by the σ^H-containing RNA polymerase in vitro. The SigH protein was isolated from *E. coli* JM101 cells harboring pJ0H7 and reconstituted with RNA polymerase core enzyme as described in Materials and Methods. To

ensure that the transcription was carried out specifically by $E\sigma^H$ and not by some nonspecific proteins from *E. coli*, JM101 cells harboring just the vector pAG58 were used as a control in protein isolation and RNA polymerase reconstitution in the same way as for cells harboring pJ0H7. Two DNA templates, *Pst*I-*Sst*I and *Pvu*II fragments containing the same promoter but with different downstream regions (see Materials and Methods), were used in the in vitro runoff transcription experiments. Reconstituted $E\sigma^H$ with σ^H isolated from *E. coli* cells harboring pJ0H7 gave a transcript of 79 bases from the *Pst*I-*Sst*I fragment and a transcript of 263 bases from the *Pvu*II fragment, both of which were as we expected. In contrast, RNA polymerase reconstituted with proteins isolated from cells containing only the vector pAG58 did not produce any transcripts from either template (data not shown).

To confirm that the in vitro transcript was initiated at the same site as the in vivo RNA, we performed a primer extension analysis with in vitro RNA synthesized in a 500-μl reaction with unlabeled nucleoside triphosphates as described previously (4). The primer used was FX130, which was also used in the primer extension analysis with in vivo RNA. The same primer was also used to generate size markers in an M13 sequencing reaction with the template mp18R1P4, which contained the same *Eco*RI site as that in mp18R1 but with a mutation in the −10 region of P4 (Fig. 2). As shown in Fig. 2B, the transcription started at the same site in vitro as in vivo (Fig. 2A). Taking the in vivo and in vitro data together, we concluded that P4 was a second SigH promoter in the *sigA* operon.

Function of P4 in the expression of *dnaE*. One of the features of the P4 promoter caught our attention. The transcription started at the first two Gs of the SD sequence GGAG of the *dnaE* gene. We reasoned that the transcript from the P4 promoter may not be translated into DnaE, since the 5′ end of the transcript was too short for binding a ribosome (22).

To test this hypothesis, we constructed a *dnaE* translational fusion to the β-galactosidase gene on the plasmid pBT143. As described in Materials and Methods, pBTXR contained the *Xmn*I-*Eco*RV fragment, which included the P3 and P4 promoters. pBTHR contained the *Hea*III-*Eco*RV fragment with only the P4 promoter. Both plasmids were transformed into DB403 cells and assayed for β-galactosidase activity. If both P3 and P4 transcripts can be translated into DnaE, we should see higher β-galactosidase activity from pBTXR and lower activity from pBTHR. Alternatively, if P4 transcripts cannot be translated into DnaE, we should see high β-galactosidase activity from pBTXR but no activity from pBTHR. Indeed, cells harboring pBTHR showed no β-galactosidase activity compared with the background, whereas cells containing pBTXR showed a dramatic increase in β-galactosidase activity (Fig. 5). When Fig. 5 is compared with Fig. 4, which shows results of a transcriptional fusion assay of P4 promoter to *aprE*, it is obvious that the zero activity of P4 in its translational fusion assay to *lacZ* is not due to the inactivity of the promoter but to the impairment of the ribosome-binding site for the mRNA of the *dnaE* gene in the transcript initiated from P4. Therefore, we concluded that the transcript from the P4 promoter was only used for the translation of *sigA*, the third gene of the operon.

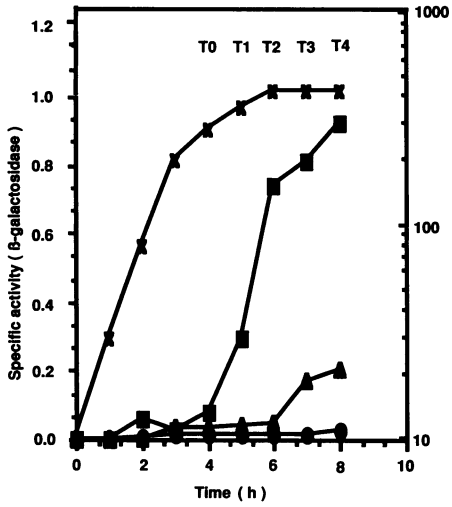

FIG. 5. Determination of the translational ability of P4 transcripts by a *lacZ* translational gene fusion. DB403 cells containing pBTXR, pBTHR, and pBT143 were grown in 2×SG medium at 37°C with shaking. A sample (1 ml) of cells was removed from each strain at each designed time point and assayed for β-galactosidase activity. The average value taken from three parallel samples was plotted on the graph. Symbols: ×, average growth curve of the three strains as measured by Klett units; ■, DB403(pBTXR) (P3 and P4); ▲, DB403 (pBT143) (vector); ●, DB403(pBTHR) (P4 alone). T_1, T_2, T_3, and T_4 are hours after the onset (T_0) of sporulation.

DISCUSSION

The identification of another SigH promoter in the *sigA* operon demonstrates an increasing complexity in the regulation of this operon. It is now apparent that two SigH promoters are utilized to express the *sigA* operon at low levels even during growth and at higher levels during early sporulation (the low level expression of the P3 promoter during growth will be reported elsewhere). Transcription from P3 allows the expression of both *dnaE* and *sigA*, whereas transcription from P4 results in only *sigA* expression.

Thus, two types of regulation are occurring during promoter switching at the end of the exponential phase of growth. In the first case, the cell reduces transcription from SigA promoters and takes advantage of the higher levels of SigH holoenzyme to switch to SigH promoters that control expression of the *sigA* operon and other early-stationary-phase genes. At the same time, the transcription from P4, the downstream SigH promoter, favors the expression of one gene (i.e., *sigA*) over that of another gene (i.e., *dnaE*) in the operon. This situation occurs because the location of the P4 promoter results in a truncated ribosome-binding site for the *dnaE* mRNA and prevents its translation into DnaE as demonstrated by the fusion gene experiments (Fig. 5). Since a final round of DNA replication occurs in the cell at around T_2 (7), the need for further synthesis of DNA primase is reduced at this time, whereas the requirement for SigA continues to late stages of sporulation. SigA-directed transcription has been shown to occur as late as T_5 (23). The time of expression from P4 was found to occur as late as T_5 (Fig. 2A), and thus SigA synthesis must also occur at this late stage. In fact, Chang and Doi (unpublished results) have shown by use of anti-SigA antiserum that SigA is present in late-stationary-phase cells, and we have shown by use of a *sigA* gene probe that mRNA for SigA is also present in late-sporulation-phase cells (Qi and Doi, unpublished results). These results also suggest that SigA is synthesized and functions late in sporulation.

Our current analysis with the primer extension method for

identifying the start point of transcription and quantitating the transcripts has been much more sensitive and accurate than our previous studies, in which we used the S1 nuclease mapping technique (26). Furthermore, we were able to detect low levels of transcription from both SigH promoters even during growth (Fig. 2A and unpublished results). However, as shown previously, most transcription of the *sigA* operon during vegetative growth occurs from the P1 and P2 SigA promoters. The level of transcription from P1 and P2 drops sharply at the early stationary phase T_0 and becomes almost undetectable at T_1 (26; Qi and Doi, unpublished results), a time at which transcription from both SigH promoters starts to increase. Based on the expression patterns of both SigH promoters P3 and P4, we suggest that they play an important role during early sporulation for the synthesis of σ^A. The finding that at least two sporulation-specific genes, *spoIIE* and *spoIIG*, are transcribed by $E\sigma^A$ during early sporulation indicates that σ^A plays a significant role in sporulation. The question that remains is, how critical is the promoter switching observed from P1 P2 to P3 P4 for the synthesis of σ^A and for $E\sigma^A$ function during sporulation? We are currently addressing this question.

ACKNOWLEDGMENTS

We thank Judy Healy and Richard Losick for providing us with the SigH-producing plasmids pAG58 and pJ0H7 and Ban-Yang Chang for providing the RNA polymerase core enzyme.

This research was supported in part by Public Health Service grant GM19673 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Alonso, J. C.** 1988. New plasmid vectors for the construction of translational gene fusion in *Bacillus subtilis*. Gene **65**:325–328.
2. **Banner, C. D. B., C. P. Moran, Jr., and R. Losick.** 1983. Deletion analysis at a complex promoter for a developmentally regulated gene from *Bacillus subtilis*. J. Mol. Biol. **168**:351–365.
3. **Carter, L., III, and C. P. Moran, Jr.** 1986. New RNA polymerase σ under *spo0* control in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **83**:9438–9442.
4. **Carter, L., III, L.-F. Wang, R. H. Doi, and C. P. Moran, Jr.** 1988. *rpoD* operon promoter used by σ^H-RNA polymerase in *Bacillus subtilis*. J. Bacteriol. **170**:1617–1621.
5. **Chang, B.-Y., and R. H. Doi.** 1990. Overproduction, purification, and characterization of *Bacillus subtilis* RNA polymerase σ^A factor. J. Bacteriol. **172**:3257–3263.
6. **Doi, R. H., and L.-F. Wang.** 1986. Multiple procaryotic RNA polymerase sigma factors. Microbiol. Rev. **50**:227–243.
7. **Dunn, G., P. Jeffs, N. H. Mann, D. M. Torgersen, and M. Young.** 1978. The relationship between DNA replication and the induction of sporulation in *Bacillus subtilis*. J. Gen. Microbiol. **108**:189–195.
8. **Feavers, I. M., V. Price, and A. Moir.** 1988. The regulation of the fumarase (*citG*) gene of *Bacillus subtilis* 168. Mol. Gen. Genet. **211**:465–471.
9. **Guzman, P., J. Westpheling, and P. Youngman.** 1988. Characterization of the promoter region of the *Bacillus subtilis spoIIE* operon. J. Bacteriol. **170**:1598–1609.
10. **Hager, D. A., and R. R. Burgess.** 1980. Elution of proteins from sodium dodecyl sulfate-polyacrylamide gels, removal of sodium dodecyl sulfate, and renaturation of enzymatic activity, results with sigma subunit of *Escherichia coli* RNA polymerase, wheat germ DNA topoisomerase, and other enzymes. Anal. Biochem. **109**:76–86.
11. **Innis, M. A., and D. H. Gelfand.** 1990. Optimization of PCRs, p. 3–12. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (ed.), PCR protocols, a guide to methods and applications. Academic Press, Inc., New York.
12. **Inoue, T., and T. R. Cech.** 1985. Secondary structure of the circular form of the *Tetrahymena* rRNA intervening sequence: a technique for RNA structure analysis using chemical probes and reverse transcriptase. Proc. Natl. Acad. Sci. USA **82**:648–652.
13. **Jaacks, K. J., J. Healy, R. Losick, and A. D. Grossman.** 1989. Identification and characterization of genes controlling the sporulation-regulatory gene *spo0H* in *Bacillus subtilis*. J. Bacteriol. **171**:4121–4129.
14. **Kenney, T. J., K. York, P. Youngman, and C. P. Moran, Jr.** 1989. Genetic evidence that RNA polymerase associated with σ^A factor uses a sporulation-specific promoter in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **86**:9109–9113.
15. **Laemmli, U. K.** 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
16. **Leighton, T. J., and R. H. Doi.** 1971. The stability of messenger ribonucleic acid during sporulation in *Bacillus subtilis*. J. Biol. Chem. **246**:3189–3195.
17. **Lewandoski, M., E. Dubnau, and I. Smith.** 1986. Transcriptional regulation of the *spo0F* gene of *Bacillus subtilis*. J. Bacteriol. **168**:870–877.
18. **Losick, R., and J. Pero.** 1981. Cascades of sigma factors. Cell **25**:582–584.
19. **Maniatis, R., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual, p. 192. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 352–355. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Moran, C. P., Jr.** 1989. Sigma factors and regulation of transcription, p. 167–184. *In* I. Smith, R. A. Slepecky, and P. Setlow (ed.), Regulation of procaryotic development. American Society for Microbiology, Washington, D.C.
22. **Moran, C. P., Jr., N. Lang, S. F. J. LeGrice, G. Lee, M. Stephens, A. L. Sonenshein, J. Pero, and R. Losick.** 1982. Nucleotide sequences that signal the initiation of transcription and translation in *Bacillus subtilis*. Mol. Gen. Genet. **186**:339–346.
23. **Park, S.-S., S.-L. Wong, L.-F. Wang, and R. H. Doi.** 1989. *Bacillus subtilis* subtilisin gene (*aprE*) is expressed from a σ^A (σ^{43}) promoter in vitro and in vivo. J. Bacteriol. **171**:2657–2665.
24. **Wang, L.-F., R. Brückner, and R. H. Doi.** 1989. Construction of a *Bacillus subtilis* mutant deficient in three extracellular proteases. J. Gen. Microbiol. **35**:487–492.
25. **Wang, L.-F., and R. H. Doi.** 1986. Nucleotide sequence and organization of *Bacillus subtilis* RNA polymerase major sigma (σ^{43}) operon. Nucleic Acids Res. **14**:4293–4307.
26. **Wang, L.-F., and R. H. Doi.** 1987. Promoter switching during development and the termination site of the σ^{43} operon of *Bacillus subtilis*. Mol. Gen. Genet. **207**:114–119.
27. **Weir, J., E. Dubnau, N. Ramakrishna, and I. Smith.** 1984. *Bacillus subtilis spo0H* gene. J. Bacteriol. **157**:405–412.
28. **Wu, J., M. G. Howard, and P. J. Piggot.** 1989. Regulation of transcription of the *Bacillus subtilis spoIIA* locus. J. Bacteriol. **171**:692–698.
29. **Zuberi, A. R., and R. H. Doi.** 1990. A mutation in *P23*, the first gene in the RNA polymerase σ^A (σ^{43}) operon, affects sporulation in *Bacillus subtilis*. J. Bacteriol. **172**:2175–2177.