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Effect of Chromosome Location of *Bacillus subtilis* Forespore Genes on Their *spo* Gene Dependence and Transcription by $E\sigma^F$: Identification of Features of Good $E\sigma^F$-Dependent Promoters

DONGXU SUN, PATRICIA FAJARDO-CAVAZOS,† MICHAEL D. SUSSMAN, FEDERICO TOVAR-ROJO, ROSA-MARTHA CABRERA-MARTINEZ, AND PETER SETLOW*

Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut 06030

Received 7 June 1991/Accepted 10 October 1991

Translational *lacZ* fusions to forespore genes of *Bacillus subtilis* were not expressed in *spoIIAC* (σ^F) or *spoIIIE* mutants when the *lacZ* fusions were integrated at the loci of the same genes or at the SPβ locus. However, some of these genes, including *gerA*, *gpr*, *spoIIIG* (σ^G), and *sspE*, were expressed in *spoIIIE* mutants and *spoIIIE spoIIIG* double mutants (but not in *spoIIAC* mutants) when the *lacZ* fusions were integrated at the *amyE* locus. When tested, the β-galactosidase made in these mutants was found only in the forespore, and the 5′ ends of the mRNAs produced in these mutants were identical to those in a Spo^+ background. Analysis of the in vitro transcription of forespore genes by RNA polymerase containing σ^F ($E\sigma^F$) revealed a direct correlation between good in vitro transcription by $E\sigma^F$ and expression at the *amyE* locus in *spoIIIE* mutants. This result suggests that forespore genes are transcribed by $E\sigma^F$ in *spoIIIE* and *spoIIIE spoIIIG* mutants. Comparison of the promoter regions of genes transcribed well and poorly by $E\sigma^F$ in vivo and in vitro showed that good transcription by $E\sigma^F$ was correlated with G residues at positions −15 and −16, a purine residue at position −13, and a T residue at position −7 relative to the start site of transcription. The importance of these residues in σ^F recognition was confirmed by analysis of the $E\sigma^F$-dependent transcription in vivo and in vitro of mutant *ssp* genes.

A number of *Bacillus subtilis* genes are expressed only in the forespore compartment of the sporulating cell (14, 16, 23, 27). Forespore genes include the following: the *gdh* operon, which codes for glucose dehydrogenase as well as a second protein of unknown function; the *gerA* operon, which codes for three proteins essential for alanine-triggered spore germination; the *gpr* gene, which codes for a protease which initiates the degradation of small, acid-soluble proteins during spore germination (31); the *spoIIIG* gene, which codes for the forespore sigma factor σ^G (11); the *spoVA* operon, which codes for five proteins needed to proceed beyond stage V of sporulation; and the *ssp* genes, which code for a family of small, acid-soluble spore proteins. The majority of these forespore genes are transcribed during sporulation by RNA polymerase containing σ^G ($E\sigma^G$) (4, 17, 23, 29). However, there is strong evidence that two of these genes, *gpr* and *spoIIIG*, are transcribed at least in part by RNA polymerase containing σ^F ($E\sigma^F$), the product of the *spoIIAC* gene (22, 28, 31). $E\sigma^F$ and $E\sigma^G$ have extremely similar promoter specificities, at least in vitro, and it is not yet clear what distinguishes a σ^F-dependent promoter from a σ^G-dependent promoter (18). Unlike σ^G, σ^F is synthesized before the forespore compartment is formed (32). However, $E\sigma^F$ activity is regulated in some fashion such that this enzyme only transcribes *gpr* and *spoIIIG* in the forespore (22).

Work in a number of laboratories has shown that the expression of the *gdh*, *gerA*, *gpr*, *spoVA*, and *ssp* genes (but not the *spoIIAC* gene) is blocked in *spoIIIE* mutants (4, 14, 16, 20, 31, 32). *spoIIIG* expression, as measured by the production of mRNA from the wild-type gene, or the expression of a translational *spoIIIG-lacZ* fusion integrated at the *spoIIIG* locus, is also blocked in *spoIIIE* mutants (5, 28). However, it was previously reported (11) and confirmed (28) that a *spoIIIG-lacZ* fusion integrated at the *amyE* locus is expressed in a *spoIIIE* mutant, suggesting that chromosomal context or location can alter the dependence of forespore gene expression on the *spoIIIE* gene product. Since the *spoIIIE* gene product may affect forespore gene expression by modulating $E\sigma^F$ activity (22), chromosomal context or location may alter gene transcription by $E\sigma^F$. Consequently, we have examined the expression of a number of forespore genes using translational *lacZ* fusions integrated at the loci of the same genes, at the *amyE* locus, and at the SPβ locus. We have found that the expression of a number of these genes becomes *spoIIIE* independent at the *amyE* locus, at which they are readily transcribed by $E\sigma^F$. Comparison of the promoters of these genes has allowed us to identify several features of a good σ^F-dependent promoter.

MATERIALS AND METHODS

Bacteria, plasmids, and DNA isolation. The *B. subtilis* strains and plasmids used in this work are listed in Table 1. The *Escherichia coli* strain used for the construction of all plasmids was RR101, and all cloning work was carried out as previously described (2). Plasmid DNA from *E. coli* strains was isolated and purified by CsCl density gradient centrifugation as described previously (2). *B. subtilis* and *E. coli* strains were grown at 37°C in 2× YT medium (2); *B. subtilis* sporulation was carried out at 37°C in 2× SG medium (7). *B. subtilis* and *E. coli* strains were made competent and transformed and transformants were selected as described previously (2, 3, 16). For the production of SPβ phage stocks, cells growing at 37°C in 2× YT medium were shifted to 50°C

* Corresponding author.
† Present address: Department of Microbiology and Immunology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107.

TABLE 1. *B. subtilis* strains and plasmids

Strain or plasmid	Relevant genotype or phenotype	Source and/or reference
Strains		
PS607	*spoIIIE36 trpC2*	Bacillus Genetic Stock Center
PS683	*spoIIIGΔ1 trpC2*	P. Stragier (11)
PS766	*trpC2* (pDG298 *spac-spoIIIG* Km^r)	18
PS832	Strain 168 trp^+ revertant	Laboratory stock
PS1063	*amyE::sspE-lacZ spoIIIE36 trpC2*	This work
PS1127	*amyE::sspE-lacZ spoIIIE36 spoIIIGΔ1110 trpC2*	This work
PS1128	*spoIIIE36 spoIIIGΔ1110 trpC2*	28
PS1175	*spoIIAC1 trpC2*	J. Errington
PS1287	*amyE::sspE-*2G*–lacZ*	This work
PS1301	*amyE::sspE-*2G*–lacZ spoIIIE36 trpC2*	This work
PS1484	*amyE::sspE-*2G*–lacZ spoIIIE36 spoIIIGΔ1110 trpC2*	This work
ZB307	SPβc2del2::Tn*917*::pSK1006	P. Zuber (33)
Plasmids		
pAAM81	*gerA-lacZ*	34
pKS27	2.9-kb *Eco*RI *cat* gene fragment from pMI1101 cloned in pGEM4	K. Sandman (13)
pPS300	*sspA-lacZ* in pJF751	16
pPS407	*sspB-lacZ* in pJF751	16
pPS435	*sspE-lacZ* in pJF751	16
pPS591	*sspE* promoter in pUC12	18
pPS689	*sspE-lacZ* in pKS27	This work
pPS706	*sspA* promoter in pUC18	18
pPS771	*sspB* promoter in pUC12	18
pPS896	*spoIIIG-lacZ* in pJF751	28
pPS918	*sspE-lacZ* in ptrpBG1	3
pPS939	*spoIIIG-lacZ* in ptrpBG1	28
pPS964	*gerA* promoter in pUC19	4
pPS966	*sspB-lacZ* in ptrpBG1	3
pPS995	ptrpBG1 with a 0.73-kb *Eco*RI-*Hin*dIII fragment replaced with an *Eco*RI-*Hin*dIII polylinker of pUC18	This work
pPS1027	*gpr-lacZ* in pJF751	31
pPS1216	*sspB-lacZ* in pKS27	This work
pPS1248	*sspA-lacZ* in ptrpBG1	This work
pPS1249	*gerA-lacZ* in ptrpBG1	This work
pPS1326	*gpr-lacZ* in ptrpBG1	30
ptrpBG1		25

for 10 min and then to 37°C for 1 h, the culture was centrifuged, and the supernatant fluid was passed through a 0.2-μm-pore-size filter and used for infection of other strains.

In vitro transcription. RNA polymerase containing σ^F but not σ^G or containing σ^G but not σ^F was prepared and purified through the heparin-agarose column step as described previously (18). In vitro transcription reactions were initiated by the addition of equivalent amounts of $E\sigma^F$ or $E\sigma^G$ [as measured by activity on poly(dAdT)] and carried out with heparin and a template excess as described previously (18, 28). Comparison of in vitro transcription on different genes was done with a mixture of both a test template and an *sspE* template (1 μg each). The templates used were as follows: *gerA*-pPS964 cut with *Hin*dIII, giving a 138-nucleotide (nt) transcript (4); *sspA*-pPS706 cut with *Pst*I, giving a 148-nt transcript (18); *sspB*-pPS771 cut with *Bam*HI, giving a 218-nt transcript (18); and *sspE*-pPS591 cut with *Eco*RI, giving a 195-nt transcript (18). The labeled nucleotide used for the mixture of *gerA* and *sspE* templates was [α-^{32}P]UTP; for *sspA* or *sspB* and *sspE* it was [α-^{32}P]CTP; however, for the *sspA-sspE* mixture, labeled CTP was present at 50 μM.

In vitro transcription from mutant *sspB* or *sspE* templates was carried out as described above. Each transcription reaction mixture contained a mutant template (1 μg) as well as the corresponding wild-type template (1 μg) as described previously (3). The mutant templates were those cloned in plasmid ptrpBG1 and cut with *Bam*HI, giving transcripts of 218 nt (*sspB*) and 97 nt (*sspE*). The wild-type templates were those cloned in pPS771 cut with *Pst*I (*sspB*) and pPS591 cut with *Eco*RI (*sspE*), giving transcripts of 230 and 195 nt, respectively (18). The labeled nucleotide used for *sspB* templates was [α-^{32}P]CTP, while [α-^{32}P]ATP was used for *sspE* templates. Transcripts were isolated, separated by electrophoresis on a 6% polyacrylamide–8 M urea sequencing gel, and detected by autoradiography, bands were cut out and counted, and counts were corrected for background as described previously (3, 18, 31).

Isolation and analysis of RNA. RNA was isolated from sporulating cells and purified as described by Mason et al. (15). The 5′ ends of various RNAs were determined by primer extension analysis as described previously (4, 18). The primer used for *sspE* mRNA was obtained by cleaving pPS918 with *Bam*HI and *Pst*I, labeling the 5′ ends with polynucleotide kinase and [γ-^{32}P]ATP, cutting with *Hph*I, and isolating the labeled 80-nt fragment containing the amino-terminal coding region of *sspE-lacZ* (9).

Construction of various translational *lacZ* fusions. (i) *gerA-lacZ*. Plasmid pAAM81 was cut with *Nhe*I and *Bss*HII, and the fragment containing the *gerA* control and coding region fused to *lacZ* was cloned in pPS995 cut with *Xba*I and *Bss*HII. The resulting plasmid, pPS1249, was isolated from

E. coli RR101, linearized with *Pst*I, and used to transform *B. subtilis* strains to a chloramphenicol resistance Amy$^-$ phenotype by integration at *amyE* (3, 25). Plasmid pPS1249 was cut with *Sac*I, and the 2.5-kb fragment carrying the *gerA* region fused to *lacZ* was ligated with *Sac*I-cut pKS27; after phenol extraction, the ligated mixture was cut with *Pst*I and used to transform strain ZB307 to chloramphenicol resistance by integration at the SPβ locus. SPβ phage were isolated from one transformant and used to infect other *B. subtilis* strains.

(ii) *gpr-lacZ*. A 93-nt fragment from nts 183 to 275 of the *B. subtilis gpr* sequence was synthesized by use of the polymerase chain reaction (30, 31). This fragment contains 42 nt upstream of the transcription start site and extends into the coding sequence (31). The 93-nt polymerase chain reaction fragment was cloned in plasmid pUC18, a clone with the orientation in frame with *lacZ* was isolated, and the correct sequence of the clone was confirmed by DNA sequence analysis (31). The fragment was excised from pUC18 as an *Eco*RI-*Hin*dIII fragment and cloned in plasmid ptrpBG1 cut with *Eco*RI and *Hin*dIII, giving plasmid pPS1326, in which the *gpr* control and coding region is fused in frame to *lacZ*. Plasmid pPS1326 was linearized with *Pst*I and used to transform *B. subtilis* strains to a chloramphenicol resistance Amy$^-$ phenotype by integration at *amyE*.

(iii) *spoIIIG-lacZ*. Plasmid pPS896 was used directly to transform strain ZB307 to a chloramphenicol resistance phenotype. Chromosomal DNA from several transformants was digested with *Eco*RI and *Eco*RV and subjected to Southern blot analysis with a *lacZ* probe (16) to confirm the correct integration at the SPβ locus. SPβ phage were prepared from one transformant and used to infect other *B. subtilis* strains.

(iv) *sspA-lacZ*. Plasmid pPS300 was cut with *Hin*dIII and *Cla*I, and the fragment carrying the *sspA* control and coding region fused in frame to *lacZ* was cloned in pPS995 cut with *Hin*dIII and *Cla*I. The resulting plasmid, pPS1248, was cut with *Pst*I and used to transform *B. subtilis* strains to a chloramphenicol resistance Amy$^-$ phenotype by integration at *amyE*.

(v) *sspB-lacZ*. Plasmid pPS966 was cut with *Hin*dIII and *Eco*RV, and the fragment carrying the *sspB* control and coding region fused in frame to *lacZ* was cloned in pKS27 cut with *Hin*dIII and *Pvu*II. The resulting plasmid, pPS1216, was linearized with *Cla*I and used to transform strain ZB307 to chloramphenicol resistance. Chromosomal DNA from several transformants was cut with *Eco*RV and *Xba*I and subjected to Southern blot analysis with a *lacZ* probe (16). One transformant with the correct chromosomal structure was used to produce SPβ phage, which were used to infect other *B. subtilis* strains.

(vi) *sspE-lacZ*. The *Hin*dIII-*Eco*RV fragment of pPS918 carrying the *sspE* control and coding region fused in frame to *lacZ* was cloned in *Hin*dIII-*Pvu*II-cut pKS27. The resulting plasmid, pPS689, was used to transform strain ZB307 to chloramphenicol resistance, and chromosomal DNA from several transformants was screened by *Bam*HI digestion and Southern blot analysis to confirm the correct integration at the SPβ locus. SPβ phage were isolated from one transformant and used to infect other *B. subtilis* strains.

For all translational *lacZ* fusions constructed, Southern blot analyses confirmed the predicted chromosomal structure of the integrated fusions (data not shown). Note also that the *lacZ* fusions at both SPβ and *amyE* had the same promoter sequences.

Construction of point mutations in the *sspE* promoter. The construction of *sspB* and *sspE* genes with mutations at position −13 or −7 or both of their promoters has been described previously (3). These mutations were present in plasmids pPS966 (*sspB*) and pPS918 (*sspE*), and mutant derivatives of these plasmids were used to transform *B. subtilis* strains to a chloramphenicol resistance Amy$^-$ phenotype by integration at *amyE*. To obtain mutant *sspE* genes integrated at SPβ, we cloned the *Hin*dIII-*Eco*RV fragments from various pPS918 derivatives in pKS27 and used the resultant mutant pPS689 derivatives to transform strain ZB307 to chloramphenicol resistance as described above. Chromosomal DNA of several transformants was screened by restriction enzyme digestion and Southern blot analysis as described above, and SPβ phage were isolated from a strain with a correct integration at the SPβ locus and used to infect other strains.

The point mutations at positions −15 and −16 of the *sspE* promoter were generated as described previously (3) with two different oligonucleotides. Both oligonucleotides were complementary to residues −28 to −5 relative to the transcription start site of the *sspE* gene (18), with one oligonucleotide replacing the G at position −15 with an A residue (*sspE*-OG) and the other oligonucleotide replacing the A at position −16 with a G residue (*sspE*-2G). The *sspE* gene fragment mutagenized was the 0.8-kb *Hin*dIII-*Bam*HI fragment from pPS918 which was cloned in pTZ19U (3). Mutants were identified by DNA sequencing as described previously (3), the mutant fragments were transferred back to pPS918 cut with *Hin*dIII and *Bam*HI, and the resulting mutant plasmids were used to transform *B. subtilis* strains to a chloramphenicol resistance Amy$^-$ phenotype by integration at *amyE*. The *Hin*dIII-*Eco*RV fragments from mutant derivatives of pPS918 were also cloned in pKS27, and the pPS689 mutant derivatives were used to integrate mutant *sspE-lacZ* fusions at SPβ as described above.

Analytical methods. Samples of sporulating cells were harvested, treated with lysozyme, and assayed for β-galactosidase and glucose dehydrogenase as described by Mason et al. (16). β-Galactosidase specific activities are given in Miller units unless otherwise stated. The *gerA-lacZ* fusion was assayed for β-galactosidase with methylumbelliferyl-β-D-galactoside as described previously (4). Cells for immunoelectron microscopy were isolated, fixed, embedded, sectioned, and postfixed as described previously (6). The sections were treated with rabbit antibody to β-galactosidase (1/500 to 1/1,000) and gold-labeled goat anti-rabbit gamma globulin, stained, and examined (6).

RESULTS

Expression of forespore genes in different chromosomal locations. As found previously, translational *lacZ* fusions to a number of forespore genes were not expressed in a *spoIIAC* or *spoIIIE* mutant when the *lacZ* fusions were at the loci of the same genes (4, 5, 11, 14, 16, 31) (Table 2). As expected, the expression of most of these *lacZ* fusions was also blocked in a *spoIIIG* mutant, since most of these genes are transcribed during sporulation only by Eσ^G (17, 18, 29) (Table 2). The two exceptions to this *spoIIIG* dependence were *gpr* and *spoIIIG*, both of which may be transcribed during sporulation in whole or in part by Eσ^F (22, 28, 31) (Table 2). This same dependence of forespore gene expression on *spoIIIE* and *spoIIIG* gene products was also seen when the *lacZ* fusions were integrated at the SPβ locus of the chromosome (Table 2).

When the *lacZ* fusions were integrated at the *amyE* locus,

TABLE 2. Expression of translational *lacZ* fusions integrated at different chromosomal loci in various genetic backgrounds[a]

Gene fused with *lacZ*	Gene expression (% maximum specific activity) at the indicated chromosomal locus and in the indicated background:								
	Same			*amyE*			SPβ		
	Wt	*spoIIIE*	*spoIIIG*	Wt	*spoIIIE*	*spoIIIG*	Wt	*spoIIIE*	*spoIIIG*
gerA	100	<10	<10	105	220	30	95	<10	<10
gpr	100	<4	25–75[b]	102	175	64	ND	ND	ND
spoIIIG	100	<4	104	98	96	54	96	<4	90
sspA	100	0.3	0.3	107	2.3	<0.3	ND	ND	ND
sspB	100	0.1	0.1	77	0.5	0.1	102	0.2	0.1
sspE	100	0.1	0.3	90	17	0.6	85	0.3	0.3

[a] Strains were isogenic, except for the noted differences, and were sporulated, harvested, and assayed as described in Materials and Methods. Plasmids carrying *lacZ* fusions for integration at the same locus were pAAM81, pPS300, pPS407, pPS435, PS896, and pPS1027; for integration at the *amyE* locus, the plasmids used were pPS918, pPS939, pPS966, pPS1248, pPS1249, and pPS1326; and for integration at the SPβ locus, phage from strain ZB307 carrying the appropriate *lacZ* fusion integrated at the SPβ locus were isolated and used to infect other *B. subtilis* strains. The *B. subtilis* strains used were PS832 (wild type), PS607 (*spoIIIE*), and PS683 (*spoIIIG*). Values are given as the percent maximum β-galactosidase specific activity of the fusion at the same locus in the wild-type (Wt) strain. These values were generally achieved at t_{5-6} of sporulation and were (in Miller units) as follows: *gpr*, 55; *spoIIIG*, 50; *sspA*, 618; *sspB*, 4,700; *sspE*, 967; values for *gerA* were determined by assays with methylumbelliferyl-β-D-galactoside. ND, not determined.

[b] This value varied significantly between experiments (31).

the level of expression in a wild-type background, the *spoIIAC* dependence, and the timing of gene expression during sporulation were essentially identical to those obtained when the *lacZ* fusions were integrated at the loci of the same genes (3, 28) (Tables 2 and 3 and data not shown). However, a number of genes (*gerA*, *gpr*, *spoIIIG*, and *sspE*) showed significant expression in a *spoIIIE* mutant (5, 11, 28) (Table 2). For two of these genes (*gerA* and *gpr*), the maximum level of expression in the *spoIIIE* mutant was higher than that in the wild-type background. The expression of the σ^G-dependent *ssp* genes integrated at the *amyE* locus was abolished in a *spoIIIG* mutant, while the expression of the *gpr* and *spoIIIG* genes was reduced by only ~40% (i.e., to an extent similar to that seen for their expression at the SPβ locus). The expression of *gerA* integrated at the *amyE* locus was reduced 70% in the *spoIIIG* mutant (Table 2). Surprisingly, the expression of *sspE* and *spoIIIG* at the *amyE* locus (*gerA* and *gpr* were not tested) was either unaffected (*spoIIIG*) or not dramatically reduced (*sspE*) in a *spoIIIE spoIIIG* double mutant (Table 3), in contrast to the results noted above with the *spoIIIG* mutant (Table 2) (11, 28).

Expression of forespore genes at the *amyE* locus in *spoIIIE* mutants. Analysis of the expression of a *spoIIIG-lacZ* fusion integrated at the *amyE* locus previously showed that the timing of β-galactosidase expression is similar in both a wild-type strain and a *spoIIIE* strain and begins at ~2.5 h of sporulation ($t_{2.5}$) (28). We obtained similar results for *gerA-lacZ*, *gpr-lacZ*, and *sspE-lacZ* fusions integrated at the *amyE* locus, although the time of initiation of β-galactosidase synthesis varied depending on the gene, from $t_{2.5}$ (*gpr-lacZ*) to t_3 (*sspE-lacZ*) (Fig. 1A and data not shown). It seems most likely that the transcription of *gerA-lacZ*, *gpr-lacZ*, *spoIIIG-lacZ*, and *sspE-lacZ* fusions in a *spoIIIE* background beginning at t_{2-3} of sporulation is carried out by RNA polymerase containing a σ factor other than σ^G, because σ^G is not made in *spoIIIE* mutants (28). The most likely candidate for this other σ factor is σ^F, since σ^F is made in *spoIIIE* mutants (32) and $E\sigma^F$ transcribes *gerA*, *gpr*, *spoIIIG*, and *sspE* templates well in vitro (4, 22, 28, 31; also see below) and, at least in some cases, in vivo (22). To examine this possibility in more detail, we determined the 5′ end of *sspE* mRNA produced from the *sspE-lacZ* fusion integrated at the *amyE* locus in both *spoIIIE* (Fig. 2) and *spoIIIE spoIIIG* (data not shown) mutants by primer extension analysis. While there was a significant background of short extension products in this experiment, it was clear that the 5′ end of the fully extended product formed with the RNA from the *spoIIIE* mutant was identical to that of the *sspE* mRNA generated from the locus of the same gene in a wild-type background (Fig. 2) (18). Similar results were obtained previously for *spoIIIG* mRNA (28). Both $E\sigma^F$ and $E\sigma^G$ have identical in vitro transcription start sites on *sspE* and *spoIIIG* templates, and these in vitro start sites are identical to the 5′ ends of the mRNAs made in vivo (18, 28). These data further implicate $E\sigma^F$ as transcribing *sspE* and *spoIIIG* in *spoIIIE* mutants.

Identification of features of a good $E\sigma^F$ promoter. Previous work showed that two genes (*gpr* and *spoIIIG*) whose expression at the *amyE* locus is high in a *spoIIIE* background are transcribed much more efficiently in vitro by $E\sigma^F$ than by $E\sigma^G$, in comparison with the σ^G-dependent *sspE* template (28, 31) (Table 4); this is also the case for a *gerA* template (Table 4). In contrast, both genes (*sspA* and *sspB*) which were expressed poorly if at all at the *amyE* locus in a *spoIIIE* mutant were poorer substrates in vitro for $E\sigma^F$ than for $E\sigma^G$ (Table 4). The *sspE* template was more active with $E\sigma^G$ in vitro (28, 29) but gave significantly higher in vitro transcription with $E\sigma^F$ than did the *sspA* or the *sspB* template (Table 4).

Comparison of the promoter regions for these genes indicated that those most active with $E\sigma^F$ in vivo and in vitro

TABLE 3. Expression of translational *lacZ* fusions at the *amyE* locus in various genetic backgrounds[a]

Gene fused with *lacZ*	Gene expression (% maximum specific activity) in the indicated background:		
	Wt	*spoIIIE spoIIIG*	*spoIIAC*
spoIIIG	100	106	<4
sspE	100	38	<0.6

[a] Strains were sporulated, harvested, and assayed as described in Materials and Methods. The plasmids used for integration of *lacZ* fusions at *amyE* were pPS896 and pPS918. The *B. subtilis* strains used were PS832 (wild type), PS1128 (*spoIIIE spoIIIG*), and PS1175 (*spoIIAC*). Values are given as the % maximum β-galactosidase specific activity (in Miller units) of the fusion in the wild-type (Wt) strain. These actual specific activities were as follows: *spoIIIG*, 50; *sspE*, 870.

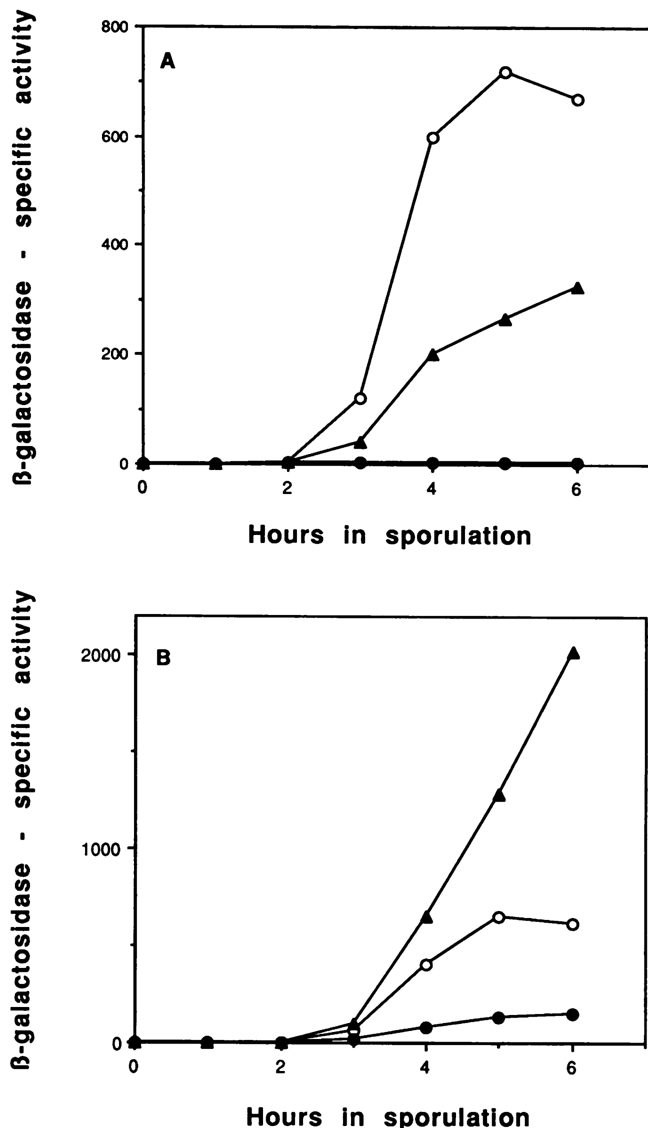

FIG. 1. Time course of β-galactosidase synthesis during sporulation of *sspE-lacZ* (A) or *sspE*-2G–*lacZ* (B) fusions in various genetic backgrounds. Plasmid pPS918 or the derivative of pPS918 carrying the *sspE*-2G mutation in which the C residue at position −15 was changed to a G residue was integrated at the *amyE* locus in various strains, the resultant strains were sporulated, and β-galactosidase specific activities (in Miller units) were determined as described in Materials and Methods. Symbols: ○, PS832 (wild type); ▲, PS607 (*spoIIIE*); ●, PS683 (*spoIIIG*).

(*gerA*, *gpr*, and *spoIIIG*) had G residues at positions −15 and −16 relative to the transcription start site (Fig. 3). In contrast, genes that were the least active with $E\sigma^F$ in vivo and in vitro (*sspA* and *sspB*) had no G residues at these positions, while the gene with intermediate activity with $E\sigma^F$ (*sspE*) had a G residue only at position −16 (Fig. 3). To test directly the importance of G residues at these positions in $E\sigma^F$ recognition and to determine whether the utilization of a promoter by $E\sigma^F$ would influence its expression from the *amyE* locus, we constructed two mutant *sspE* genes; one had G residues at both positions −15 and −16 (*sspE*-2G), and the other had a G residue at position −16 changed to a T

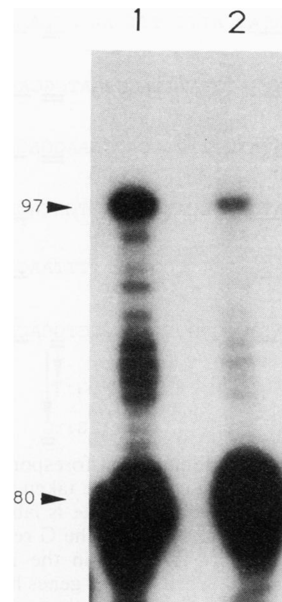

FIG. 2. Primer extension analysis of the 5′ end of *sspE* mRNA. RNAs from cells of strains PS832 (lane 1) and PS607 carrying plasmid pPS918 integrated at the *amyE* locus (lane 2) were isolated at t_4 of sporulation, purified, and subjected to primer extension analysis as described in Materials and Methods. The numbered arrows indicate the sizes of the primer (80 nt) and the extended product (97 nt), as determined by reference to a DNA sequencing ladder.

(*sspE*-0G) (Fig. 3). When integrated at the *amyE* or SPβ locus in a wild-type strain, *lacZ* fusions to all three *sspE* genes were expressed at the same time in sporulation and to similar levels (Fig. 1A and B, Table 5, and data not shown). The expression of the *sspE*-0G–*lacZ* fusion at the *amyE* locus and the *sspE*-2G–*lacZ* fusion at the SPβ locus was abolished in a *spoIIIE* mutant (Table 5). However, the expression of the *sspE*-2G–*lacZ* fusion at the *amyE* locus was elevated approximately twofold over that in the wild-type background, although β-galactosidase synthesis in this

TABLE 4. Relative in vitro transcription of forespore genes by $E\sigma^F$ and $E\sigma^G$[a]

Template	Relative transcription (% of maximum) by:	
	$E\sigma^F$	$E\sigma^G$
gerA	60	4
gpr	2,800[b]	270[b]
spoIIIG	120[c]	5[c]
sspA	<2	120
sspB	5	200
sspE	100[d]	100[d]

[a] In vitro transcription reactions with mixed templates were carried out and transcripts were analyzed and quantitated as described in Materials and Methods. Note that different labeled nucleotides were used for different template pairs and that, in some cases, suboptimal *sspE* transcription resulted. Relative transcription with a particular template was calculated as follows: (cpm in a gene transcript/cpm in the *sspE* transcript from the same reaction) × 100.

[b] Data were taken from reference 31.

[c] Data were taken from reference 28.

[d] While values for *sspE* transcription were set at 100%, *sspE* is actually transcribed more poorly by $E\sigma^F$ than by $E\sigma^G$ (3, 28).

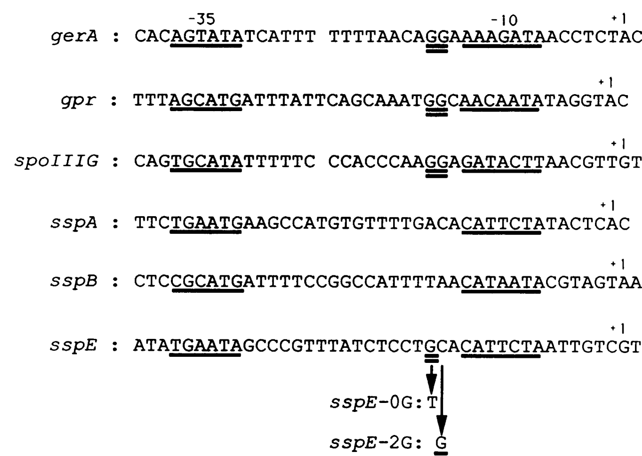

FIG. 3. Comparison of promoters of forespore genes. The promoter sequences of forespore genes were taken from references 4, 14, 26, and 29. The transcription start site is labeled +1, and −35 and −10 regions are singly underlined. The G residues at −15 and −16 are doubly underlined. The gaps in the *gerA* and *spoIIIG* sequences were introduced because these genes have a 17-nt spacer between the −10 and −35 regions; the other genes have an 18-nt spacer. The vertical arrows below positions −15 and −16 indicate residues in the *sspE* promoter altered to give the *sspE*-0G and *sspE*-2G mutant genes.

strain still began at t_3 (Fig. 1B and Table 5). There was also significant expression of the *sspE*-2G–*lacZ* fusion (again beginning at t_3) at either *amyE* or SPβ even in a *spoIIIG* mutant (Fig. 1B, Table 5, and data not shown). The expression of these *sspE* variants was abolished in a *spoIIAC* mutant, but the expression of the *sspE*-2G–*lacZ* fusion remained high in a *spoIIIE spoIIIG* mutant (Table 5). Control experiments showed that the *sspE*-2G gene in the wild-type background and in *spoIIIE* or *spoIIIE spoIIIG* mutants used in the same transcription start site as did the wild-type gene (data not shown). Analysis of $E\sigma^F$ and $E\sigma^G$ transcription in vitro on *sspE* templates with zero, one, or two G residues at positions −15 and −16 showed that both enzymes utilized the same in vitro start site on these

TABLE 6. Relative in vitro transcription of mutant *sspE* genes by $E\sigma^F$ and $E\sigma^G$ [a]

Base change	Position[b]	Relative transcription (% of maximum) by: $E\sigma^F$	$E\sigma^G$
None		100	100
C→G	−13	140	30
C→G and A→T	−13 and −7	77	2
C→G	−15	380	80
G→T	−16	20	20

[a] In vitro transcription reactions with mixed templates (wild type plus mutant) were carried out and quantitated as described in Materials and methods. Relative transcription with a particular template was calculated as follows: (cpm in the transcript from a particular template/cpm in the transcript from the wild-type template in the same reaction) × 100. For the wild-type template, the latter value was set at 100%.

[b] Relative to the transcription start site, +1 (Fig. 3).

templates and that $E\sigma^F$ had a significant preference for the *sspE*-2G template (Table 6 and data not shown).

While $E\sigma^F$ seems the likely holoenzyme which transcribes the *sspE*-2G–*lacZ* fusion in *spoIIIE* backgrounds, it is possible that σ^F-dependent gene expression under these circumstances is not confined to the forespore, since σ^F is made prior to the formation of the septum dividing the mother cell and the forespore (32). Consequently, we analyzed the intracellular distribution of β-galactosidase produced from the *sspE*-2G–*lacZ* fusion integrated at the *amyE* locus in wild-type, *spoIIIE*, or *spoIIIE spoIIIG* backgrounds using immunoelectron microscopy with anti-β-galactosidase antibody and gold-labeled second antibody (data not shown). Analysis of the number of gold particles decorating cells of strain PS683 (no *lacZ* fusion) harvested at ~t_5 of sporulation revealed approximately one or two particles over the forespore and two or three particle over the mother cell when 10 different cells were analyzed. In contrast, when 8 to 12 t_5 sporulating cells each of strains PS1287 (*sspE*-2G–*lacZ*::*amyE spo*$^+$), PS1301 (*sspE*-2G–*lacZ*::*amyE spoIIIE*), and PS1484 (*sspE*-2G–*lacZ*::*amyE spoIIIE spoIIIG*) were analyzed by immunoelectron microscopy, we found averages of 37, 25, and 32 gold particles, respectively, over the

TABLE 5. Expression of wild-type and mutant *ssp* genes in various genetic backgrounds[a]

Gene	Base change	Position[b]	Level of expression (%) of *ssp* genes in the indicated background: Wt	*spoIIIE*	*spoIIIG*	*spoIIIE spoIIIG*	*spoIIAC*
sspB	None		100	<2	<1	<2	<1
	A→T	−7	83	<2			
	C→G	−13	74	45			<1
	A→T and C→G	−7 and −13	14	70	<1		<1
sspE	None		100	18	<1	37	<1
	C→G	−13	46 (81)	21 (<1)	<1		
	A→T and C→G	−7 and −13	2 (2)	250 (<1)	<1		<1
	C→G	−15	89 (130)	270 (<1)	23 (18)	195	<1
	G→T	−16	78	<1	<1		

[a] Strains with *sspB* or *sspE-lacZ* fusions integrated at the *amyE* locus were grown and sporulated and samples were assayed for β-galactosidase as described in Materials and Methods. The plasmids used for integration of *lacZ* fusions at *amyE* were pPS918 and pPS966 and their mutant derivatives. For integration at SPβ, plasmid pPS689 and its mutant derivatives were used. SPβ phage were isolated from transformants and used to infect other *B. subtilis* strains: PS832 (wild type), PS1175 (*spoIIAC*), PS607 (*spoIIIE*), PS683 (*spoIIIG*), and PS1128 (*spoIIIE spoIIIG*). Values are the percent maximum β-galactosidase specific activity (in Miller units) found during sporulation relative to that in a wild-type (Wt) strain carrying the wild-type *ssp-lacZ* fusion. These actual specific activities were as follows: *sspB*, 3,700; *sspE*, 870. Values in parentheses are for *lacZ* fusions integrated at SPβ.

[b] Relative to the transcription start site, +1 (Fig. 3).

forespore and, again, only 2 or 3 gold particles over the mother cell. Since the forespore volume is approximately half that of the mother cell, this analysis indicates that the level of β-galactosidase accumulated from the *sspE*-2G–*lacZ* fusion integrated at the *amyE* locus is >10 to 15 times higher in the forespore than in the mother cell, even in *spoIIIE* or *spoIIIE spoIIIG* mutants. Previous work showed that β-galactosidase produced from wild-type *ssp-lacZ* fusions at *ssp* loci in wild-type strains is also located primarily, if not exclusively, in the forespore (6, 16). These data indicate that $E\sigma^F$ action on the *sspE* gene (and by analogy on the *gerA*, *gpr*, and *spoIIIG* genes) in a *spoIIIE* background is confined to the forespore. While this fact certainly does not prove that $E\sigma^F$ action in a wild-type (*spo*$^+$) strain is confined to the forespore, there is other evidence that this is the case (see reference 22).

We also noted that the promoters used most efficiently by $E\sigma^F$ in vitro invariably had a purine residue at position −13 and, in one case (*spoIIIG*), a T residue at position −7 (Fig. 3). Analysis of the expression of *sspB* and *sspE* mutant genes with these changes revealed that these mutant genes showed decreased expression in a wild-type background, whether integrated at SPβ or at *amyE* (Table 5). However, at *amyE* these mutant genes showed significant expression in a *spoIIIE* background and, in the case of the mutations at positions −7 and −13 in the *sspE* promoter, there was 125-fold higher expression in a *spoIIIE* background than in a wild-type background (Table 5). Analysis of the in vitro transcription of these mutant templates showed that *sspB* mutant genes exhibited no significant transcription by $E\sigma^F$ (data not shown). However, *sspE* mutant genes expressed well in a *spoIIIE* background exhibited increased $E\sigma^F$ transcription in vitro (Table 6).

DISCUSSION

It seems clear that the forespore-specific transcription of the *gerA*, *gpr*, *spoIIIG*, and *sspE* genes at the *amyE* locus in a *spoIIIE* mutant cannot be carried out by $E\sigma^G$, because the *spoIIIE* mutation blocks σ^G synthesis (28). Furthermore, for the genes tested, their expression at the *amyE* locus in a *spoIIIE* mutant was similar to that seen in a *spoIIIE spoIIIG* double mutant. The obvious candidate for the transcription of forespore genes at the *amyE* locus in a *spoIIIE* mutant is $E\sigma^F$. This holoenzyme transcribes the *gerA*, *gpr*, *spoIIIG*, and *sspE* genes in vitro with start sites identical to those of $E\sigma^G$, and the relative levels of expression of these and other genes at *amyE* in a *spoIIIE* mutant parallel the relative efficiencies of their transcription in vitro by $E\sigma^F$. In addition, σ^F is synthesized well before the septation event dividing the mother cell and the forespore and is synthesized in a *spoIIIE* mutant (32). While σF (or $E\sigma^F$) may well play a role in mother cell-specific events, such as the processing of pro-σ^E to its mature, active form (19), $E\sigma^F$ activity on *gpr* and *spoIIIG* genes appears to be confined to the forespore (11, 31). Similarly, the action of a mutant σ^F which can recognize the *sspB* promoter is also confined to the forespore (see reference 22). Clearly, σ^F activity is regulated such that it functions to direct the transcription of genes such as *gpr* and *spoIIIG* only in the forespore and 1 to 2 hr after σ^F synthesis. The mechanism of regulation of σ^F activity is not yet clear but appears to involve the action of the other two proteins derived from the *spoIIA* operon, SpoIIAA and SpoIIAB (22). SpoIIAB appears to be an antagonist of σ^F function, while SpoIIAA in turn antagonizes SpoIIAB action. The mechanism by which the SpoIIIE protein influences σ^F activity is even less clear but may reflect a requirement for SpoIIIE in the release of σ^F from the antagonistic effect of SpoIIAB. SpoIIIE may also be a general positive regulator in vivo for σ^F-dependent promoters. However, $E\sigma^F$ action in vitro does not require SpoIIIE (28, 29, 31).

From the discussion given above, it is clear why (although not necessarily how) the expression of σ^F- and σ^G-dependent forespore genes is abolished in a *spoIIIE* mutant. Indeed, such was the case with all genes tested at the same loci or at SPβ. However, the expression of a number of forespore genes integrated at the *amyE* locus in a *spoIIIE* mutant is much more difficult to explain. Clearly, the regulation of gene expression at *amyE* is different from that in other regions of the chromosome. In this regard, it is worth noting that *amyE* (map position, ~25°) is rather far removed from all the other loci tested (*sspB* and *sspE*, 65°; *spoIIIG*, 135°; SPβ, 180°; *gpr*, 210°; *sspA*, 266°; and *gerA*, 289°) (1, 21). Thus, it is possible that there is something unique about the structure of the chromosome in the *amyE* region which can influence its interaction with σ^F with or without SpoIIAB, and possibly this structure is dependent in some fashion on SpoIIIE. There is much evidence that the prokaryotic chromosome is composed of a number of separate domains, and it has been suggested that different domains may exhibit different accessibility to RNA polymerase (12, 26). Recent work in our laboratory has demonstrated a striking condensation of the forespore chromosome by an extremely early time in stage II of sporulation (24). One might speculate that chromosome accessibility to proteins, including RNA polymerase, could therefore be significantly different in the mother cell and forespore compartments, as forespore chromosome condensation could restrict the access of an enzyme to inner regions of the nucleoid. While forespore chromosome condensation does take place in a *spoIIIE* mutant, it is possible that the domain structure of the chromosome is altered in this mutant, such that the *amyE* region is now accessible to $E\sigma^F$.

While the basis for the utilization by $E\sigma^F$ of forespore-specific promoters at the *amyE* locus in *spoIIIE* mutants is unclear, this phenomenon has allowed more detailed analysis of features of promoters transcribed well by $E\sigma^F$. Clearly, one such feature is the presence of G residues at positions −15 and −16 of the promoter. Genes normally transcribed by $E\sigma^F$ during sporulation (*gpr* and *spoIIIG*) contain such residues, and the generation of an *sspE* mutant gene with G residues at positions −15 and −16 greatly increased its transcription by $E\sigma^F$ in vitro and in vivo. Indeed, this *sspE* mutant gene exhibited significant expression at *amyE* or SPβ even in a *spoIIIG* mutant background. However, it is not clear whether σ^F directly contacts residues at positions −15 and −16 or whether the identity of these residues has effects on the DNA structure in the −10 region. It has been noted previously that the identity of the residues at positions −15 and −16 of RNA polymerase containing σ^A promoters has a significant effect on their utilization and that G residues often appear at position −15 (8, 10). Two other features of efficiently utilized $E\sigma^F$ promotors are a purine residue at position −13 and a T residue at position −7. Thus, *sspB* and *sspE* mutant genes with these changes in their promoters showed good transcription in vitro by $E\sigma^F$ and high levels of expression at *amyE* in a *spoIIIE* mutant. In contrast, these mutant genes showed poor transcription by $E\sigma^G$. However, the presence of a purine residue at position −13 and a T residue at position −7 was not sufficient for $E\sigma^F$-dependent transcription during sporulation, as these mutant genes were expressed poorly if

at all in a *spoIIIG* mutant. Thus, the presence of G residues at positions −15 and −16 appears to be more fundamental for $E\sigma^F$ recognition.

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REFERENCES

1. **Amjad, M., J. M. Castro, H. Sandoval, J.-J. Wu, M. Yang, D. J. Henner, and P. J. Piggot.** 1991. An *Sfi*I restriction map of the *Bacillus subtilis* 168 genome. Gene **101:**15–21.
2. **Connors, M. J., J. M. Mason, and P. Setlow.** 1986. Cloning and nucleotide sequence of three genes for small, acid-soluble proteins from *Bacillus subtilis* spores. J. Bacteriol. **166:**417–425.
3. **Fajardo-Cavazos, P., F. Tovar-Rojo, and P. Setlow.** 1991. Effect of promoter mutations and upstream deletions on the expression of genes coding for small, acid-soluble spore proteins of *Bacillus subtilis*. J. Bacteriol. **173:**2011–2016.
4. **Feavers, I. M., J. Foulkes, B. Setlow, D. Sun, W. Nicholson, P. Setlow, and A. Moir.** 1990. The regulation of transcription of the *gerA* spore germination operon of *Bacillus subtilis*. Mol. Microbiol. **4:**275–282.
5. **Foulger, D., and J. Errington.** 1989. The role of the sporulation gene *spoIIIE* in the regulation of prespore-specific gene expression in *Bacillus subtilis*. Mol. Microbiol. **3:**1247–1255.
6. **Francesconi, S. C., T. J. MacAlister, B. Setlow, and P. Setlow.** 1988. Immunoelectron microscopic localization of small, acid-soluble spore proteins in sporulating cells of *Bacillus subtilis*. J. Bacteriol. **170:**5963–5967.
7. **Goldrick, S., and P. Setlow.** 1983. Expression of a *Bacillus megaterium* sporulation-specific gene during sporulation of *Bacillus subtilis*. J. Bacteriol. **155:**1459–1462.
8. **Graves, M. C., and J. C. Rabinovitz.** 1986. *In vivo* and *in vitro* transcription of the *Clostridium pasteurianum* ferredoxin gene: evidence for extended promoter elements in gram-positive organisms. J. Biol. Chem. **261:**11409–11415.
9. **Hackett, R. H., and P. Setlow.** 1987. Cloning, nucleotide sequencing, and genetic mapping of the gene for small, acid-soluble spore protein γ of *Bacillus subtilis*. J. Bacteriol. **169:**1985–1992.
10. **Henkin, T. M., and A. L. Sonenshein.** 1987. Mutations of the *Escherichia coli lacUV5* promoter resulting in increased expression in *Bacillus subtilis*. Mol. Gen. Genet. **209:**467–474.
11. **Karmazyn-Campelli, C., C. Bonamy, B. Savelli, and P. Stragier.** 1989. Tandem genes encoding sigma factors for consecutive steps of development in *Bacillus subtilis*. Genes Dev. **3:**150–157.
12. **Kellenberger, E.** 1990. Intracellular organization of the bacterial genome, p. 173–186. *In* K. Drlica and M. Riley (ed.), The bacterial chromosome. American Society for Microbiology, Washington, D.C.
13. **Kunkel, B., K. Sandman, S. Panzer, P. Youngman, and R. Losick.** 1988. The promoter for a sporulation gene in the *spoIVC* locus of *Bacillus subtilis* and its use in studies of temporal and spatial control of gene expression. J. Bacteriol. **170:**3513–3522.
14. **Mandelstam, J., and J. Errington.** 1987. Developmental sequences of gene expression controlling spore formation in *Bacillus subtilis*. Microbiol. Sci. **4:**238–244.
15. **Mason, J. M., P. Fajardo-Cavazos, and P. Setlow.** 1988. Levels of mRNAs which code for small, acid-soluble spore proteins and their *lacZ* fusions in sporulating cells of *Bacillus subtilis*. Nucleic Acids Res. **16:**6567–6583.
16. **Mason, J. M., R. H. Hackett, and P. Setlow.** 1988. Regulation of expression of genes coding for small, acid-soluble proteins of *Bacillus subtilis* spores: studies using *lacZ* gene fusions. J. Bacteriol. **170:**239–244.
17. **Nakatani, Y., W. L. Nicholson, K. D. Nietzke, P. Setlow, and E. Freese.** 1989. Sigma-G RNA polymerase controls forespore specific expression of the glucose dehydrogenase operon in *Bacillus subtilis*. Nucleic Acids Res. **17:**999–1017.
18. **Nicholson, W. L., D. Sun, B. Setlow, and P. Setlow.** 1989. Promoter specificity of σ^G-containing RNA polymerase from sporulating cells of *Bacillus subtilis*: identification of a group of forespore-specific promoters. J. Bacteriol. **171:**2708–2718.
19. **Partridge, S. R., D. Foulger, and J. Errington.** 1991. The role of σ^F in pre-spore-specific transcription in *Bacillus subtilis*. Mol. Microbiol. **3:**757–767.
20. **Piggot, P.** 1973. Mapping of asporogenous mutations of *Bacillus subtilis*: a minimum estimate of the number of sporulation operons. J. Bacteriol. **114:**1241–1253.
21. **Piggot, P. J.** 1989. Revised genetic map of *Bacillus subtilis* 168, p. 1–41. *In* I. Smith, R. A. Slepecky, and P. Setlow (ed.), Regulation of procaryotic development. American Society for Microbiology, Washington, D.C.
22. **Schmidt, R., P. Margolis, L. Duncan, R. Coppolecchia, C. P. Moran, Jr., and R. Losick.** 1990. Control of developmental transcription factor σ^F by sporulation regulatory proteins SpoIIAA and SpoIIAB in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **87:**9221–9225.
23. **Setlow, P.** 1989. Forespore-specific genes of *Bacillus subtilis*: function and regulation of expression, p. 211–221. *In* I. Smith, R. A. Slepecky, and P. Setlow (ed.), Regulation of procaryotic development. American Society for Microbiology, Washington, D.C.
24. **Setlow, P.** 1991. Changes in forespore chromosome structure during sporulation in *Bacillus* species. Semin. Dev. Biol. **2:**55–62.
25. **Shimotsu, H., and D. J. Henner.** 1986. Construction of a single-copy integration vector and its use in analysis of regulation of the *trp* operon of *Bacillus subtilis*. Gene **43:**85–94.
26. **Sinden, R. R., and D. E. Pettijohn.** 1981. Chromosomes in living *Escherichia coli* cells are segregated into domains of supercoiling. Proc. Natl. Acad. Sci. USA **78:**224–228.
27. **Stragier, P.** 1989. Temporal and spatial control of gene expression during sporulation: from facts to speculation, p. 243–254. *In* I. Smith, R. A. Slepecky, and P. Setlow (ed.), Regulation of procaryotic development. American Society for Microbiology, Washington, D.C.
28. **Sun, D., R. M. Cabrera-Martinez, and P. Setlow.** 1991. Control of transcription of the *Bacillus subtilis spoIIIG* gene, which codes for the forespore-specific transcription factor σ^G. J. Bacteriol. **173:**2977–2984.
29. **Sun, D., P. Stragier, and P. Setlow.** 1989. Identification of a new σ factor involved in compartmentalized gene expression during sporulation of *Bacillus subtilis*. Genes Dev. **3:**141–149.
30. **Sussman, M. D.** 1991. Ph.D. thesis, University of Connecticut Health Center, Farmington.
31. **Sussman, M. D., and P. Setlow.** 1991. Cloning, nucleotide sequence, and regulation of the *Bacillus subtilis gpr* gene, which codes for the protease that initiates degradation of small, acid-soluble proteins during spore germination. J. Bacteriol. **173:**291–300.
32. **Wu, J.-J., M. G. Howard, and P. J. Piggot.** 1989. Regulation of transcription of the *Bacillus subtilis spoIIA* locus. J. Bacteriol. **171:**692–698.
33. **Zuber, P., and R. Losick.** 1987. Role of AbrB in Spo0A- and Spo0B-dependent utilization of a sporulation promoter in *Bacillus subtilis*. J. Bacteriol. **169:**2223–2230.
34. **Zuberi, A. M., A. Moir, and I. M. Feavers.** 1987. The nucleotide sequence and gene organization of the *gerA* spore germination operon of *Bacillus subtilis*. Gene **51:**1–11.