Overproducing the *Bacillus subtilis* Mother Cell Sigma Factor Precursor, Pro- σ^K , Uncouples σ^K -Dependent Gene Expression from Dependence on Intercompartmental Communication

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During sporulation of *Bacillus subtilis*, proteolytic activation of pro- σ^K and ensuing σ^K -dependent gene expression normally require the activity of many sporulation gene products. We report here that overproducing pro- σ^K at the onset of sporulation substantially uncouples σ^K -dependent gene expression from its normal dependency. Overproducing pro- σ^K in strains with a mutation in *spoIIIG*, *spoIIIA*, *spoIIIE*, or *spoIVB* partially restored σ^K -dependent gene expression in the mother cell and resulted in accumulation of a small amount of polypeptide that comigrated with σ^K , but these mutants still failed to form spores. In contrast, sporulation of *spoIVF* mutants was greatly enhanced by pro- σ^K overproduction. The products of the *spoIVF* operon are made in the mother cell and normally govern pro- σ^K processing, but overproduction of pro- σ^K appears to allow accumulation of a small amount of σ^K , which is sufficient to partially restore mother cell gene expression and spore formation. This *spoIVF*-independent mechanism for processing pro- σ^K depends on σ^E , an earlier-acting mother cell-specific sigma factor. The *spoIIID* gene, which encodes a mother cell-specific DNA-binding protein that is normally required for pro- σ^K production, was shown to be required for efficient pro- σ^K processing as well. *bof* (bypass of forespore) mutations bypassed this requirement for *spoIIID*, suggesting that SpoIIID is less directly involved in pro- σ^K processing than are *spoIVF* gene products. However, *bof spoIIID* double mutants overproducing pro- σ^K still failed to sporulate, indicating that SpoIIID serves another essential role(s) in sporulation in addition to its multiple roles in the production of σ^K .

Bacillus subtilis is a gram-positive bacterium that undergoes sporulation in response to nutrient deprivation. The first morphological change observed during the sporulation process is the synthesis of an asymmetrically positioned septum that partitions the cell into two unequal compartments, the mother cell and the forespore. After further morphogenesis, the process culminates with the lysis of the mother cell and the release of a dormant spore that is highly resistant to harsh environmental conditions (26). Accompanying the morphological changes during sporulation is a highly regulated program of gene expression. This regulation is achieved, in part, by the ordered synthesis and activation of a cascade of sigma factors, each of which confers on core RNA polymerase the ability to transcribe a set of genes during a certain period and in a particular location (i.e., the mother cell or the forespore) (25).

One of the sigma factors involved in mother cell-specific gene expression is σ^K . σ^K -containing RNA polymerase transcribes genes encoding proteins required for the synthesis of the spore cortex and coat during morphological stages IV and V, respectively (4, 21, 45, 46). Production of active σ^K involves multiple levels of regulation. First, the gene that encodes σ^K , *sigK*, is generated via a mother cell-specific chromosomal DNA rearrangement (41). This site-specific recombination event requires *spoIVCA*, which encodes a putative recombinase (23, 34, 38), and *spoIIID*, which encodes a small, DNA-binding protein (11, 22, 41). The SpoIIID protein may participate in the rearrangement directly and/or indirectly, since SpoIIID stimulates *spoIVCA* transcription in vitro by σ^E RNA polymerase (11), a form of RNA polymerase that is active in the mother cell during the early stages of sporulation (8). A second level of regulation in the production of σ^K involves transcrip-

tion of the *sigK* gene. SpoIIID is required for the initial transcription of *sigK* by σ^E -containing RNA polymerase (11, 24). SpoIIID also greatly stimulates later transcription of *sigK* by σ^K -containing RNA polymerase (21). A third level of regulation in the synthesis of active σ^K involves proteolytic processing. The primary translation product of *sigK* is an inactive precursor, pro- σ^K , that is processed to active σ^K by removing 20 amino acids from the N terminus (21, 27).

Several lines of evidence suggest that processing of pro- σ^K to σ^K is a developmentally regulated event that couples mother cell gene expression to forespore development. First, mother cell-specific gene expression is blocked in several mutants that are defective in forespore-specific gene expression (e.g., *spoIIIA*, *spoIIIE*, *spoIIIG*, and *spoIVB* mutants) (6, 24, 36, 46) because processing of pro- σ^K is blocked in these mutants (27). Second, deletion of the pro-amino acid coding sequence from the *sigK* gene bypasses the requirement for *spoIIIG* (encoding the forespore-specific sigma factor, σ^G) in mother cell-specific gene expression (5). Third, mutants that had been isolated on the basis of their ability to relieve the dependence of mother cell gene expression on forespore regulatory proteins show restoration of pro- σ^K processing (5). These bypass of forespore (*bof*) mutations map to two loci, *spoIVF* and *bofA* (5, 7, 35). The *bof* mutations that map to *spoIVF* are special alleles of this locus because other mutations in *spoIVF* prevent pro- σ^K processing (27). Hence, the products of the mother cell-expressed *spoIVF* operon are thought to be intimately involved in processing of pro- σ^K (5, 7, 27).

We showed previously that pro- σ^K overproduced from a plasmid at the onset of sporulation fails to activate σ^K -dependent genes until it is processed to active σ^K at about the fourth hour of development (27). We reasoned that this system should allow us to determine whether *spoIIID* is required for pro- σ^K processing, since the normal requirements for SpoIIID

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TABLE 1. *B. subtilis* strains used

Strain ^a	Genotype	Reference or source
PY79	<i>spo</i> ⁺	44
AG514 ^b	$\Delta spoOA::cat$ <i>abrB703 trpC1 pheA1</i>	A. Grossman
AG665 ^b	<i>spoOH::cat trpC2 pheA1</i>	17
AG185 ^c	<i>spoIIAC1 trpC2</i>	A. Grossman
PEW ^b	<i>spoIIIGAΔ5 trpC2 pheA1</i>	19
EU8701 ^b	$\Delta sigE::erm$ <i>trpC2 pheA1</i>	20
SC615	<i>spoIIIA53</i>	5
BK395	<i>spoIIID83</i>	24
BK541	<i>spoIIIDΔerm</i>	22
SC622	<i>spoIIIE36</i>	6
BK338	<i>spoIIIGΔ1</i>	5
BK754	<i>spoIVB165</i>	4
KS179	<i>spoIVFA::Tn917ΩHU179</i>	37
SC834	<i>spoIVFB152</i>	5
KI834 ^b	<i>bofA::Tn917Δlac::pTV21Δ2 trpC2 pheA1</i>	16
BSL011	$\Delta spoOA::cat$ (pSL1)	This study
BSL021	<i>spoOH::cat</i> (pSL1)	This study
BSL50	<i>spoIIID83 bofA::Tn917Δlac::pTV21Δ2 (cat)</i>	This study
SC1044	<i>spoIIID83 bofB8 chr::Tn917Ω HU144</i>	5
BSL51	<i>spoIVFΔAB::cat</i>	This study

^a All strains were derived from the wild-type strain PY79 unless noted otherwise.

^b Derived from JH642 (*trpC2 pheA1*).

^c Derived from 168 (*trpC2*).

in producing pro- σ^K would be circumvented by producing pro- σ^K from a plasmid. As a control, we also tested a *spoIIIG* mutant, which is blocked for pro- σ^K processing when pro- σ^K is produced at the normal level (27). To our surprise, we found that overproducing pro- σ^K in a *spoIIIG* mutant appears to allow a small amount of σ^K to be produced, resulting in substantial expression of σ^K -dependent genes. This result prompted us to carefully examine the effects of overproducing pro- σ^K during sporulation. We found that pro- σ^K overproduction bypasses the normal communication between forespore and mother cell, including the normal requirement for *spoIVF* gene products in governing pro- σ^K processing. We also show that *spoIIID* is required for normal pro- σ^K processing and that *bof* mutations can alleviate this requirement for *spoIIID*.

MATERIALS AND METHODS

Bacterial strains. *Escherichia coli* AG115 [*araD139 Δ(ara,leu)7697 ΔlacX74 galU galK hsr hsm⁺ strA* (F' *proAB lacI^qZ::Tn5*)] was obtained from A. Grossman (Massachusetts Institute of Technology) and served as the host for construction and maintenance of plasmids. The *B. subtilis* strains used in this study are listed in Table 1. Competent *B. subtilis* cells were prepared and transformed as described previously (9). Transformants containing pSL1 (27) were selected on LB agar (29) containing kanamycin sulfate (5 μg/ml). The use of specialized transducing phages SPB::*cotA-lacZ*, SPB::*cotD-lacZ*, and SPB::*gerE-lacZ* has been described elsewhere (5, 6, 46).

Strains BSL011 and BSL021 were constructed by transforming competent pSL1-containing PY79 cells with chromosomal DNA isolated from strains AG514 and AG665, respectively. Transformants were selected on LB agar containing kanamycin sulfate (5 μg/ml) and chloramphenicol (5 μg/ml).

Strain BSL50 was constructed by transforming competent

BK395 cells with chromosomal DNA isolated from strain KI834. Transformants were selected on LB agar containing chloramphenicol (5 μg/ml). Chloramphenicol-resistant transformants were transferred to Difco sporulation (DS) agar (43) containing chloramphenicol (5 μg/ml). After 24 h of incubation at 37°C, the plate was incubated for 3 days at room temperature to allow sporulation. Two types of colonies were observed. A small number of brown-pigmented (Pig⁺) colonies arose, presumably as a result of complementation of the *spoIIID83* mutation in BK395 by congression. The brown pigment is characteristic of colonies in which cells are undergoing sporulation and expressing *cotA* (36). Most of the colonies were Pig⁻, presumably because they retained the *spoIIID83* mutation. One such transformant was isolated and named BSL50.

Strain BSL51 has the *spoIVF* operon replaced by a DNA fragment containing a *cat* gene. To construct this strain, a derivative of pDG148 (39), pSL4, which contains the isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible P_{spac} promoter but lacks the *EcoRI* fragment containing the origin of replication and kanamycin resistance gene that function in *B. subtilis*, was linearized with *SalI* and ligated to a 1.9-kbp *BglII-SalI* fragment containing the entire *spoIVF* operon from pSC227, which is identical to pSC224 (7) except it contains the *bofB8* mutation (3). The unligated ends were filled in with the Klenow fragment of DNA polymerase I, and the blunt ends were ligated with T4 DNA ligase. The resulting plasmid, pSL12, has the P_{spac} promoter fused to the *spoIVF* operon. A 2.1-kbp *EcoRV-SalI* fragment containing the *spoIVF* operon was released from pSL12 and ligated to pBR322 digested with *EcoRI* and *SalI*. The unligated ends were rendered blunt with the Klenow fragment of DNA polymerase I, and ligation was continued with T4 DNA ligase. The resulting plasmid, pSL18, has a unique *EcoRI* site near the 3' end of *spoIVFB*. pSL18 was linearized with *EcoRI* and then partially digested with *SspI* to release a 3.9-kbp vector fragment that retained about 100 bp upstream of the -35 region of the *spoIVF* promoter and 110 bp downstream of the *EcoRI* site near the 3' end of *spoIVFB*. This vector fragment was gel purified and ligated to a 1.5-kbp *SmaI-EcoRI* fragment containing the *cat* gene from pMI1101 (44). The resulting plasmid, pSL19, has the *cat* gene flanked by regions upstream and downstream of the *spoIVF* operon. pSL19 was linearized with *PvuII*, gel purified, and used to transform competent PY79 cells. Transformants were selected on LB agar containing chloramphenicol (5 μg/ml). Southern blot analysis confirmed that one of the transformants, BSL51, has the *spoIVF* operon replaced by the 1.5-kbp DNA fragment containing the *cat* gene (data not shown).

Sporulation. Sporulation of *B. subtilis* strains was induced by nutrient exhaustion in DS medium at 37°C (36). Overproduction of pro- σ^K during sporulation of pSL1-containing strains was induced at the onset of sporulation (*T*₀) with 1 mM IPTG. Formation of heat-resistant spores was assayed by collecting 1 ml of *B. subtilis* culture taken 24 h after the initiation of sporulation (*T*₂₄), incubating the culture at 80°C for 15 min, plating serial dilutions on LB agar, and counting colonies after 24 h of incubation at 37°C.

Western blot (immunoblot) analysis. Samples (1 ml) were collected at hourly intervals during sporulation. Whole-cell extracts were prepared as described previously (27), and protein concentrations were determined by the method of Bradford (1). Whole-cell extracts (10 μg of protein) were separated by sodium dodecyl sulfate (SDS)-12.5% polyacrylamide gel electrophoresis and electroblotted to a poly(vinylidene difluoride) membrane (31). Immunoblot analysis using polyclonal anti-pro- σ^K antibodies, which detect both pro- σ^K and

TABLE 2. Ability of *B. subtilis* strains to form heat-resistant spores in the absence and presence of pro- σ^K overproduction

Relevant genotype	Sporulation ^a	
	No plasmid	pSL1
<i>spo</i> ⁺	100	120
<i>spoIVFA::Tn917ΩHU179</i>	2×10^{-1}	25
<i>spoIVFB152</i>	4×10^{-2}	41
<i>spoIVFΔAB::cat</i>	2×10^{-4}	23

^a The number of heat-resistant spores was determined as described in Materials and Methods and is expressed as a percentage of that observed for the wild-type *spo*⁺ strain PY79, which produced about 3×10^8 spores per ml. Strains containing pSL1 were initially selected on LB agar containing kanamycin sulfate (5 μ g/ml), and the selection was removed during sporulation in DS medium. IPTG (1 mM) was added to all cultures at *T*₀ of sporulation. The results are averages of at least two independent experiments for each strain. The numbers of viable cells prior to heat treatment were found to be similar for all cultures (data not shown).

σ^K , was performed as described elsewhere (27). Two different methods were used to detect the primary anti-pro- σ^K antibodies on blots. Method 1 employed alkaline phosphatase-conjugated secondary antibody (Bio-Rad) and a color reaction involving 5-bromo-4-chloro-3-indolyl phosphate as the substrate in the presence of nitroblue tetrazolium, as instructed by the manufacturer (Bio-Rad). Method 2 employed horseradish peroxidase-conjugated secondary antibody (Bio-Rad) and an enhanced chemiluminescence detection system, which was used as instructed by the manufacturer (Amersham). Method 2 was about twofold more sensitive for detecting σ^K than method 1.

Measurement of β -galactosidase activity. Samples (1 ml) were harvested at hourly intervals during sporulation, pelleted, and stored at -70°C prior to the assay. Cells were permeabilized by treatment with toluene (32); except where noted, cells were pretreated with SDS and then treated with lysozyme or were just treated with lysozyme (30). The specific activity of β -galactosidase was determined by the method of Miller (32), using *o*-nitrophenol- β -D-galactopyranoside as the substrate. One unit of enzyme hydrolyzes 1 μ mol of substrate per min per unit of initial cell optical density at 595 nm.

RESULTS

Overproducing pro- σ^K does not interfere with sporulation of wild-type *B. subtilis*. To assess the effects of overproducing pro- σ^K in wild-type *B. subtilis* cells, a multicopy plasmid, pSL1 (27), which has the intact *sigK* gene fused to an IPTG-inducible promoter (*P*_{spac}), was introduced into the wild-type strain PY79. As a control, the plasmid from which pSL1 was derived, pDG148, was also introduced into PY79. These strains, and PY79 without plasmid, were then lysogenized with an SP β phage bearing a σ^K -dependent gene fusion, *cotD-lacZ*. Sporulation of these strains was carried out in DS medium, and IPTG (1 mM) was added to induce pro- σ^K synthesis at the onset of sporulation (*T*₀). The presence of either pSL1 (Table 2) or pDG148 (data not shown) had no deleterious effect on formation of heat-resistant spores.

To measure the levels of pro- σ^K and σ^K , whole-cell extracts were prepared at hourly intervals during sporulation and subjected to Western blot analysis using anti-pro- σ^K antibodies. The levels of pro- σ^K and σ^K in the strain containing the control plasmid, pDG148 (Fig. 1A), were comparable to the levels in the strain without plasmid, reported previously (27). Pro- σ^K was first observed at 3 h into the sporulation process (*T*₃) and reached a plateau at *T*₄ to *T*₆. σ^K was first observed at

FIG. 1. Overproduction of pro- σ^K does not interfere with σ^K accumulation or *cotD-lacZ* expression in wild-type cells. (A) Western blot analyses of whole-cell extracts (10 μ g of protein) prepared from sporulating wild-type (WT) strain PY79 containing the control plasmid (pDG148) or the plasmid that allows pro- σ^K overproduction (pSL1), performed as described in Materials and Methods by using anti-pro- σ^K antibodies and detection method 1. Lane numbers correspond to the number of hours after the initiation of sporulation. The positions of pro- σ^K and σ^K are indicated. (B) *cotD*-directed β -galactosidase activity during sporulation of PY79 (●), PY79 containing pDG148 (□), and PY79 containing pSL1 (Δ). Each strain was lysogenized with phage SP β ::*cotD-lacZ*. Points are averages of three determinations, and error bars indicate 1 standard deviation of the data.

*T*₄ and reached a maximum at *T*₆. In the strain containing pSL1, pro- σ^K was present at *T*₀, i.e., at the time of addition of IPTG, and increased thereafter until *T*₄. Western blot analysis of serially diluted *T*₄ cell extract indicated that this strain accumulated at least 10-fold more pro- σ^K than did strains with the control plasmid or without plasmid (data not shown). Even without the addition of IPTG, pro- σ^K was present at *T*₀ in the strain containing pSL1 and about fivefold more pro- σ^K was produced during sporulation than in strains with the control plasmid or without plasmid, suggesting that the *P*_{spac} promoter is not fully repressed during growth or sporulation (data not shown). Even in the strain containing pSL1, however, no σ^K was detected until *T*₄ (Fig. 1A), the time when σ^K appeared in strains with the control plasmid (Fig. 1A) or without plasmid (27). This result is consistent with the idea that processing of pro- σ^K is developmentally regulated (5, 27). Furthermore, since overproducing pro- σ^K did not substantially increase the amount of σ^K accumulated during sporulation, the availability of pro- σ^K does not appear to be the limiting factor for σ^K accumulation.

Expression of a *cotD-lacZ* fusion requires active σ^K RNA polymerase (27, 46). Figure 1B shows that *cotD*-directed β -galactosidase activity increased at *T*₅ and reached a similar maximum level in the strain producing pro- σ^K from pSL1 and in strains with the control plasmid (pDG148) or without plasmid. The increase in *cotD*-directed β -galactosidase activity

FIG. 2. Overproduction of pro- σ^K uncouples pro- σ^K processing and *cotD-lacZ* expression from forespore control. (A) Levels of pro- σ^K and σ^K in a pSL1-containing *spoIIIGΔ1* mutant during sporulation. Western blot analysis using anti-pro- σ^K antibodies and detection method 2 was performed as described in Materials and Methods except that 15 μ g of total protein was loaded in each lane. Lane numbers correspond to the number of hours after the initiation of sporulation. The unmarked lane contained 5 μ g of total protein from wild-type PY79 cells harvested at T_6 during sporulation, a time when both pro- σ^K and σ^K are present and could serve as markers. (B) *cotD*-directed β -galactosidase activity during sporulation of pSL1-containing PY79 (Δ ; same data as shown in Fig. 1), *spoIIIA53* ($\bullet$), *spoIIIGΔ1* ($\blacktriangle$), *spoIVB165* ($\circ$), and *spoIIIE36* ($\blacksquare$) strains. Points are averages of three determinations, and error bars indicate 1 standard deviation of the data. The *spoIIIGΔ1* mutant containing pDG148 ($\square$) served as a negative control.

coincided with the appearance of σ^K , not pro- σ^K , consistent with the idea that pro- σ^K is inactive and must be processed to produce active σ^K (27).

Taken together, these results demonstrate that overproduction of pro- σ^K at or before the onset of sporulation does not interfere with the production of active σ^K at the normal developmental time or with the formation of heat-resistant spores in wild-type cells.

Overproducing pro- σ^K uncouples pro- σ^K processing and *cotD-lacZ* expression from forespore control. The product of the *spoIIIG* gene, σ^G , must be active in the forespore compartment in order for pro- σ^K to be converted to σ^K in the mother cell (5, 27). We therefore anticipated that overproduction of pro- σ^K in a *spoIIIG* mutant would have no effect on sporulation or σ^K -dependent gene expression. To our surprise, we found that a small amount of a polypeptide of the expected size for σ^K was detected by Western blot analysis beginning at T_4 during sporulation of a pro- σ^K -overproducing *spoIIIGΔ1* mutant (Fig. 2A, the faint band immediately below the dark pro- σ^K band). The level of this polypeptide remained about the same at T_4 through T_8 of sporulation, and we estimated the level to be between 10 and 20% of the level observed in wild-type cells at T_6 of sporulation (data not shown). About 1 h after the appearance of this polypeptide, *cotD-lacZ* expres-

FIG. 3. Levels of pro- σ^K and σ^K in mutants overproducing pro- σ^K during sporulation. Western blot analysis using anti-pro- σ^K antibodies and detection method 2 was performed as described in Materials and Methods except that 15 μ g of total protein from cells harvested at T_6 during sporulation was loaded in each numbered lane. The pSL1-containing mutants were *spoIVFA::Tn917ΩHU179* (lane 1), *spoIVFB152* (lane 2), *spoIIIA53* (lane 3), *spoIIIE36* (lane 4), *spoIVB165* (lane 5), *spoOA::cat* (lane 6), Δ *sigE::erm* (lane 7), and *spoIIIDΔerm* (lane 8). Alternate lanes (without numbers) contained 5 μ g of total protein from wild-type PY79 cells harvested at T_6 during sporulation, a time when both pro- σ^K and σ^K are present and could serve as markers.

sion began to increase and reached about half of the wild-type level by T_8 (Fig. 2B). Overproduction of pro- σ^K did not restore spore-forming ability to the *spoIIIG* mutant, however, presumably because σ^G -dependent, forespore-specific gene expression is required for heat-resistant spore formation (data not shown).

We infer that the polypeptide comigrating with σ^K and detected by anti-pro- σ^K antibodies has σ^K activity, based on the correlation between its appearance and expression of the σ^K -dependent *cotD* gene. The fact that the putative σ^K appears with similar timing as σ^K in wild-type cells suggests that it is produced by a similar mechanism. However, we cannot exclude the possibility that it is a closely related, but improperly processed, polypeptide(s) with σ^K activity. The sensitive detection method used for the Western blot shown in Fig. 2A revealed several polypeptides smaller than σ^K that could be breakdown products of pro- σ^K . However, neither these polypeptides nor pro- σ^K itself is likely to account for the *cotD-lacZ* expression seen in the *spoIIIG* mutant, since the small polypeptides and pro- σ^K were present in other mutants (i.e., *sigE*, *spoOH*, *spoIIAC*, and *spoIIGA* mutants) overproducing pro- σ^K , yet these mutants failed to increase *cotD-lacZ* expression above a low level (see below).

Other mutants that are defective in forespore-specific gene expression showed similar responses to overproduction of pro- σ^K . Overproduction of pro- σ^K in *spoIIIA53*, *spoIIIE36*, and *spoIVB165* mutants during sporulation resulted in accumulation of a low level of a polypeptide that comigrates with σ^K (Fig. 3, lanes 3 to 5). The timing and level of *cotD-lacZ* expression in the *spoIIIA* and *spoIVB* mutants was similar to that in the *spoIIIG* mutant, and the level was somewhat lower in the *spoIIIE* mutant (Fig. 2B). As for the *spoIIIG* mutant, overproduction of pro- σ^K did not enhance the ability of these mutants to produce heat-resistant spores (data not shown).

Overproducing pro- σ^K from pSL1 appeared to uncouple *cotD-lacZ* expression in the mother cell from dependence on forespore events. However, it was possible that *cotD-lacZ* was being expressed in the forespore, since pro- σ^K was synthesized prior to the formation of the asymmetric septum and pSL1 was expected to be present in both compartments. As measured by differential sensitivity to SDS (4) and release by lysozyme treatment (30), however, *cotD-lacZ* expression occurred predominantly, if not exclusively, in the mother cell (data not shown).

We also considered the possibility that the apparent uncoupling resulted from a titration effect of DNA present in the multicopy plasmid, pSL1, rather than from pro- σ^K overpro-

duction. For example, pSL1 contains several binding sites for SpoIIID, a DNA-binding protein that has been shown to repress transcription of *cotD* (11, 12, 21). We constructed a deletion derivative of pSL1, called pSL1Kp, that retained the known SpoIIID binding sites, including the promoter region and the first 40 codons of the *sigK* open reading frame, but removed the rest of *sigK* and 556 bp of downstream *B. subtilis* DNA. A pSL1Kp-containing *spoIIIGΔ1* mutant did not increase *cotD-lacZ* expression above the background level during sporulation (data not shown). The wild-type strain PY79 bearing pSL1Kp showed the same level and timing of *cotD-lacZ* expression (data not shown) as the wild type without plasmid (Fig. 1B). These results are inconsistent with the idea that titration of SpoIIID is responsible for uncoupling *cotD-lacZ* expression from its normal dependence on forespore events.

Overproducing pro- σ^K rescues sporulation of *spoIVF* mutants. The partial rescue by overproduced pro- σ^K of defects in forespore gene expression mutants provoked us to examine the effects of overproducing pro- σ^K in *spoIVF* mutants. *spoIVF* is a two-cistron operon whose products have been proposed to reside in the outer forespore membrane and govern the processing of pro- σ^K in response to a signal from the forespore (4, 5, 7, 27). A transposon insertion mutation in the A cistron (*spoIVFA::Tn917ΩHU179*) or a missense mutation in the B cistron (*spoIVFB152*) (2, 7, 37) blocks processing of pro- σ^K (27) and expression of σ^K -dependent genes (6, 24, 36, 46), yet both mutants sporulate at low frequency (Table 2). A null mutant (*spoIVFΔAB::cat*), in which both cistrons were deleted and replaced by a *cat* gene (see Materials and Methods), was also tested. In comparison with the other two *spoIVF* mutants, the *spoIVFΔAB::cat* mutant exhibited a more severe sporulation defect (Table 2). Like the other *spoIVF* mutations, the *spoIVFΔAB::cat* mutation blocks processing of chromosomally encoded pro- σ^K as well as σ^K -directed gene expression when pro- σ^K is produced at the normal level (data not shown).

Table 2 shows that overproducing pro- σ^K from pSL1 greatly enhanced the ability of all three *spoIVF* mutants to form heat-resistant spores. This distinguishes the *spoIVF* mutants from the mutants blocked in forespore-specific gene expression and suggests that *spoIVF* gene products have no essential function in sporulation other than in the production of σ^K . The *spoIVF* mutants, like the forespore gene expression mutants, produced a low level of a polypeptide that comigrates with σ^K during sporulation (Fig. 3, lanes 1 and 2; Fig. 4A) and expressed *cotD-lacZ* (Fig. 4B). Overproducing pro- σ^K in the *spoIVF* mutants appeared to allow a small amount of σ^K to be made, an amount sufficient for substantial sporulation and *cotD-lacZ* expression. These results were surprising given the critical role proposed for *spoIVF* gene products in pro- σ^K processing (5, 7, 27) and suggest that there is a *spoIVF*-independent mechanism for converting pro- σ^K to σ^K .

Production of active σ^E is critical for pro- σ^K processing. To explore the requirements for *spoIVF*-independent pro- σ^K processing, we examined the effects of overproducing pro- σ^K in earlier-blocked sporulation mutants. Mutants in this group all fail to make pro- σ^K (27, 28). Of particular interest was a *sigE* mutant, since *sigE* encodes σ^E (40, 42), a sigma factor that directs early mother cell gene expression (8). A *sigE* mutant fails to express *spoIVF* (7) as well as many other mother cell-specific genes, so we wanted to determine whether *spoIVF*-independent pro- σ^K processing would occur in this mutant. Overproducing pro- σ^K in the *sigE* mutant did not result in accumulation of a polypeptide that comigrates with σ^K (Fig. 3, lane 7; note that enhanced accumulation of presumed pro- σ^K breakdown products makes it difficult to see the σ^K region in

FIG. 4. Rescue of σ^K accumulation and *cotD-lacZ* expression in *spoIVF* mutants overproducing pro- σ^K . (A) Levels of pro- σ^K and σ^K in a pSL1-containing *spoIVFΔAB::cat* mutant during sporulation. Western blot analysis using anti-pro- σ^K antibodies and detection method 2 was performed as described in Materials and Methods except that 15 μ g of total protein was loaded in each lane. Lane numbers correspond to the number of hours after the initiation of sporulation. The unmarked lane contained 5 μ g of total protein from wild-type PY79 cells harvested at T_6 during sporulation, a time when both pro- σ^K and σ^K are present and could serve as markers. (B) *cotD*-directed β -galactosidase activity during sporulation of pSL1-containing PY79 (Δ ; same data as shown in Fig. 1), *spoIVFA::Tn917ΩHU179* ($\bullet$), *spoIVFB152* ($\blacksquare$), and *spoIVFΔAB::cat* ($\blacktriangle$) strains. Points are the average of three determinations, and error bars indicate 1 standard deviation of the data. The *spoIVFΔAB::cat* mutant containing pDG148 ($\square$) served as a negative control.

the photograph, but several different exposures of each of two separate Western blots revealed no evidence for a polypeptide that comigrates with σ^K). Furthermore, *cotD-lacZ* expression in the *sigE* mutant overproducing pro- σ^K increased to less than half of the already low level observed in the *spoIVF* null mutant (Table 3). These results indicate that active σ^E is critical for *spoIVF*-independent pro- σ^K processing. Not surprisingly, overproduction of pro- σ^K did not enhance the ability of the *sigE* mutant to form heat-resistant spores (data not shown).

Other mutants that fail to make active σ^E showed similar results as the *sigE* mutant when pro- σ^K was overproduced (Table 3 and data not shown). These included *spoOA::cat* and *spoOH::cat* mutants, which are blocked very early in sporulation at the initiation step (14), and *spoIIAC1* and *spoIIIGΔ5* mutants, which accumulate pro- σ^E but fail to process it to active σ^E (18). The only differences that we noted among this group of mutants were that the *spoOA* mutant accumulated less of the presumed pro- σ^K breakdown products (Fig. 3, lane 6) and expressed even less *cotD*-directed β -galactosidase activity (Table 3). This finding suggests that pro- σ^K breakdown products smaller than σ^K may account for the residual *cotD-lacZ* expression observed in mutants (except the *spoOA* mutant) unable to make active σ^E .

Effects of overproducing pro- σ^K on *gerE* and *cotA* expres-

TABLE 3. Summary of *cotD-lacZ*, *gerE-lacZ*, and *cotA-lacZ* expression in strains overproducing pro- σ^K

Relevant genotype	Activity ^a		
	<i>cotD-lacZ</i>	<i>gerE-lacZ</i>	<i>cotA-lacZ</i>
<i>spo</i> ⁺	114 (100)	222 (100)	238 (100)
<i>spoIIA53</i>	59 (52)	138 (62)	17 (7)
<i>spoIIIE36</i>	20 (18)	52 (23)	16 (7)
<i>spoIIIGΔ1</i>	72 (63)	174 (78)	10 (4)
<i>spoIVB165</i>	52 (46)	75 (34)	18 (8)
<i>spoIVFA::Tn917ΩHU179</i>	74 (65)	61 (27)	24 (10)
<i>spoIVFB152</i>	56 (49)	200 (90)	15 (6)
<i>spoIVFΔAB::cat</i>	18 (16)	46 (21)	3 (1)
<i>spoΔOA::cat</i>	2 (2)	3 (1)	ND
<i>spoOH::cat</i>	6 (5)	23 (10)	ND
<i>spoIIAC1</i>	8 (7)	36 (16)	11 (5)
<i>spoIIIGAΔ5</i>	7 (6)	63 (28)	ND
<i>ΔsigE::erm</i>	7 (6)	39 (18)	3 (1)
<i>spoIIIDΔerm</i>	14 (12)	104 (47)	17 (7)
<i>spoIIID83 bofA::cat</i>	93 (82)	174 (78)	128 (54)

^a Maximum β -galactosidase activity of the fusion in the indicated wild-type or mutant pSL1-containing strain during an 8-h time course of sporulation, expressed as a percentage of the peak activity of the corresponding fusion in the wild-type strain without plasmid. Average peak levels of β -galactosidase activity were 190, 120, and 20 Miller units for the *cotD*, *gerE*, and *cotA* fusions, respectively. The values in parentheses are normalized with respect to the corresponding fusion in the wild-type strain with pSL1.

^b ND, not determined.

sion. *gerE* and *cotA*, like *cotD*, are transcribed by σ^K -containing RNA polymerase (6, 36, 45). To determine the generality of our results, we tested the effects of overproducing pro- σ^K on expression of *gerE-lacZ* and *cotA-lacZ* fusions in wild-type and mutant strains.

Overproducing pro- σ^K in wild-type cells resulted in *gerE*-directed β -galactosidase activity beginning at T_3 , about 2 h earlier than in wild-type cells without plasmid, and reached a maximum level that was about twofold higher than in the strain without plasmid (Table 3). *cotA*-directed β -galactosidase activity in the wild-type strain overproducing pro- σ^K began at the same time as in the wild-type strain without plasmid (T_4) but like *gerE-lacZ* expression reached about twice the level observed for the strain without plasmid (Table 3).

In general, results for the *gerE-lacZ* fusion were similar to those for *cotD-lacZ*, showing that pro- σ^K overproduction substantially uncouples expression of both of these σ^K -dependent genes from their normal dependence on forespore events and *spoIVF* function. One difference was that *gerE-lacZ* expression was somewhat higher than *cotD-lacZ* expression in *spoOH*, *spoIIAC*, *spoIIIGA*, and *sigE* mutants overproducing pro- σ^K . Perhaps the presumed pro- σ^K breakdown products observed in these mutants when pro- σ^K is overproduced (Fig. 3, lane 7, and data not shown) support a higher level of *gerE* than *cotD* transcription. This interpretation could also explain the earlier induction of *gerE-lacZ* expression in wild-type cells and all of the mutants that we tested when pro- σ^K was overproduced (data not shown). Consistent with this idea is the observation that *gerE* failed to be expressed in a *spoOA* mutant (Table 3), which accumulated less of the presumed pro- σ^K breakdown products (Fig. 3, lane 6). Uncoupling of *cotA-lacZ* expression from dependence on forespore events and *spoIVF* function was not as apparent in mutants overproducing pro- σ^K . Perhaps the *cotA* promoter requires a threshold concentration of σ^K (or active pro- σ^K breakdown products) higher than that required for *cotD* and *gerE* transcription (33). In addition, production of GerE is expected to repress *cotA* transcription (36, 45).

A

B

FIG. 5. A *bofA* mutation bypasses the requirement for *spoIIID* in pro- σ^K processing. (A) *cotD*-directed β -galactosidase activity during sporulation of pSL1-containing PY79 (Δ ; same data as shown in Fig. 1), *spoIIIDΔerm* ($\blacksquare$), and *spoIIID83 bofA::cat* ($\bullet$) strains. Points are averages of three determinations, and error bars indicate 1 standard deviation of the data. The *spoIIIDΔerm* mutant containing pDG148 ($\square$) served as a negative control. (B) Levels of pro- σ^K and σ^K during sporulation in a pSL1-containing *spoIIID83 bofA::cat lacZ* double mutant, as determined by Western blot analysis performed as described in Materials and Methods, using anti-pro- σ^K antibodies and detection method 1.

***spoIIID* is required for efficient pro- σ^K processing, and *bof* mutations bypass this requirement.** SpoIIID is a small DNA-binding protein (11, 22) that is required for the DNA rearrangement that generates *sigK* (41) and is also needed for transcription of *sigK* (11, 21, 24). Therefore, a *spoIIID* mutant fails to make pro- σ^K (27). The ability to overproduce pro- σ^K from a plasmid enabled us to bypass the need for SpoIIID in making pro- σ^K , so that we could examine the effect of a *spoIIID* mutation on pro- σ^K processing.

Overproduction of pro- σ^K in a *spoIIIDΔerm* mutant did not enhance its ability to make heat-resistant spores (data not shown). However, a low level of a polypeptide that comigrates with σ^K was detected during sporulation (Fig. 3, lane 8), and expression of *cotD-lacZ* increased, to a small extent, late in sporulation (Fig. 5A; Table 3); expression of *gerE-lacZ* increased substantially (Table 3). These results show that *spoIIID* is required for efficient processing of pro- σ^K but suggest that a small amount of σ^K can accumulate in the absence of *spoIIID* when pro- σ^K is overproduced.

Two factors might account for inefficient pro- σ^K processing in the *spoIIID* mutant. First, the *spoIVB* gene is expressed at only 20% of its normal level in a *spoIIID* mutant, presumably causing decreased production in the forespore of SpoIVB, a component of the signal transduction pathway that activates pro- σ^K processing (4). Second, a negative regulator of pro- σ^K processing, BofA, is overproduced at least sevenfold in the *spoIIID* mutant (16). Both of these effects could be eliminated with a *bofA* null mutation because the negative regulator (BofA) would not be produced and the need for a signal from the forespore would be bypassed (5, 35). Therefore, we constructed a double mutant (*spoIIID83 bofA::cat*) in which both *spoIIID* and *bofA* were inactivated (see Materials and Methods).

Overproducing pro- σ^K in the double mutant restored σ^K accumulation (Fig. 5B) and *cotD-lacZ* expression (Fig. 5A) to about the normal levels during sporulation. To determine whether this effect is specific to *bofA*, we also tested a *spoIIID83 bofB8* double mutant. The *bofB8* mutation lies near the 3' end of *spoIVFA* and appears to remove the inhibitory effect of SpoIVFA on SpoIVFB, so that pro- σ^K processing occurs in the absence of a signal from the forespore (7). Overproducing pro- σ^K in the *spoIIID83 bofB8* double mutant resulted in normal processing of pro- σ^K (data not shown), despite the fact that the negative regulator, BofA, should be overproduced in this double mutant. Thus, *bof* mutations can bypass the requirement for *spoIIID* in pro- σ^K processing. However, overproducing pro- σ^K did not enhance the ability of either double mutant to form heat-resistant spores (data not shown), suggesting that SpoIIID plays another role(s) in sporulation in addition to its multiple roles in generating active σ^K .

DISCUSSION

We have shown that overproducing pro- σ^K substantially uncouples σ^K -dependent, mother cell gene expression from dependence on a signal from the forespore. Furthermore, *spoIVF* gene products, which are made in the mother cell and appear to transduce the forespore signal that normally governs pro- σ^K processing (7), become largely dispensable for sporulation when pro- σ^K is overproduced. In *spoIVF* mutant cells overproducing pro- σ^K , a small amount of σ^K appears to accumulate beginning at T_4 during sporulation (Fig. 4). This increase in the σ^K level probably results from increased processing of pro- σ^K rather than increased stability of σ^K , because σ^K stably accumulates in growing cells and cells in the early stages of sporulation when it is produced from a truncated *sigK* gene fused to an IPTG-inducible promoter (12, 13, 33). Thus, sporulating cells appear to acquire the ability to process pro- σ^K to σ^K in a *spoIVF*-independent manner. The apparent temporal regulation of *spoIVF*-independent processing during sporulation is probably due to its dependence on σ^E . Presumably, σ^E RNA polymerase transcribes one or more genes whose products are required for *spoIVF*-independent processing of pro- σ^K .

Does *spoIVF*-independent processing occur when pro- σ^K is made at its normal level during sporulation? A *spoIVF* null mutant fails to produce a level of σ^K detectable in our Western blot experiments (data not shown); however, the mutant (*spoIVF Δ AB::cat*; Table 2) forms heat-resistant spores about 30-fold more efficiently than does a *sigK* null mutant (*spoIIIC94*; data not shown). This observation suggests that a small amount of σ^K activity is produced by a *spoIVF*-independent mechanism even when pro- σ^K is not overproduced.

What is the relationship between the *spoIVF*-independent and *spoIVF*-dependent mechanisms for pro- σ^K processing? The answer is likely to depend on how *spoIVF* gene products normally function in processing. Previous genetic studies suggest that SpoIVFA and BofA negatively regulate the activity of SpoIVFB, the product of the downstream gene in the *spoIVF* operon, until a signal is received from the forespore (5, 7, 35). SpoIVFB has been proposed to be either the protease that processes pro- σ^K or a regulator of the processing reaction (7). If SpoIVFB is a protease that processes pro- σ^K , *spoIVF*-independent processing probably involves another protease(s) substituting weakly for SpoIVFB. Alternatively, if SpoIVFB regulates processing (e.g., by stimulating a protease or interacting with pro- σ^K to make it a better substrate for a protease), *spoIVF*-independent processing may represent weak activity of the protease normally responsible for pro- σ^K processing. To explore the mechanism of *spoIVF*-independent processing

further, we have isolated mutants that suppress the sporulation defect of a *spoIVF* null mutant (28).

Several observations suggest that *spoIVF* gene products retain partial function in some of the mutants that we examined. The *spoIVF* null mutant (*spoIVF Δ AB::cat*) exhibited a more severe sporulation defect than did the *spoIVFA::Tn917 Ω* *HU179* and *spoIVFB152* mutants (Table 2), suggesting that the latter mutants are not null mutants. Consistent with this idea is the observation that these mutants expressed *cotD* at a higher level than did the null mutant when pro- σ^K was overproduced (Fig. 4B). The level of *cotD* expression in *spoIIIA*, *spoIIIG*, and *spoIVB* mutants overproducing pro- σ^K was similar to that in the non-null *spoIVF* mutants, whereas *cotD* expression in a *spoIIIE* mutant was comparable to that in the *spoIVF* null mutant (Table 3). Perhaps the *spoIIIE* mutant is capable only of *spoIVF*-independent processing, while the other forespore regulatory gene mutants, in addition, exhibit feeble, *spoIVF*-dependent processing of overproduced pro- σ^K .

A *spoIIID* mutation produces a severe pro- σ^K processing defect. A *spoIIID* mutant overproducing pro- σ^K expressed *cotD* at a level comparable to that produced by the *spoIVF* null mutant (Table 3), suggesting that only *spoIVF*-independent processing was operative. *bof* mutations bypassed the requirement for *spoIIID* for efficient processing of overproduced pro- σ^K (Fig. 5B) and high-level expression of σ^K -dependent genes (Table 3). The *bof* mutations restore pro- σ^K processing and σ^K -dependent gene expression in forespore regulatory gene mutants (5). Hence, the *spoIIID* mutant behaves like a forespore regulatory gene mutant with respect to its ability to be bypassed by *bofA* or *bofB* mutations. This finding suggests that SpoIIID, like the products of the forespore regulatory genes, is less directly involved in pro- σ^K processing than *spoIVF* gene products, since the requirement for *spoIVF* is not bypassed by *bofA* mutations, and *bofB* mutations lie in *spoIVF* (5, 7). Indeed, it has been shown that a *spoIIID* mutation impairs expression of several forespore-specific genes, including *spoIVB*, whose product lies on a signal transduction pathway governing pro- σ^K processing (4). Thus, SpoIIID may affect *spoIVF*-dependent pro- σ^K processing indirectly, as a result of its influence on forespore-specific gene expression. Alternatively or in addition, SpoIIID may influence pro- σ^K processing directly or through an effect on mother cell gene expression, and *bof* mutations may render the processing reaction insensitive to the loss of SpoIIID. In the mother cell, SpoIIID positively and negatively regulates expression of both σ^E - and σ^K -transcribed genes (10, 15, 16, 21, 22, 24). Defective mother cell and/or forespore gene expression presumably accounts for the failure of *bof spoIIID* double mutants to sporulate even when pro- σ^K is overproduced and σ^K production is restored. Clearly, SpoIIID must serve another essential role(s) in sporulation in addition to its roles in generating the rearranged *sigK* gene (41), activating *sigK* transcription (11, 21, 24), and permitting efficient processing of pro- σ^K .

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REFERENCES

1. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248-254.

2. Coote, J. G. 1972. Sporulation in *Bacillus subtilis*. Characterization of oligosporogenous mutants and comparison of their phenotypes with those of asporogenous mutants. *J. Gen. Microbiol.* **71**:1–15.
3. Cutting, S. (University of Pennsylvania School of Medicine). Personal communication.
4. Cutting, S., A. Driks, R. Schmidt, B. Kunkel, and R. Losick. 1991. Forespore-specific transcription of a gene in the signal transduction pathway that governs pro- σ^K processing in *Bacillus subtilis*. *Genes Dev.* **5**:456–466.
5. Cutting, S., V. Oke, A. Driks, R. Losick, S. Lu, and L. Kroos. 1990. A forespore checkpoint for mother-cell gene expression during development in *Bacillus subtilis*. *Cell* **62**:239–250.
6. Cutting, S., S. Panzer, and R. Losick. 1989. Regulatory studies on the promoter for a gene governing synthesis and assembly of the spore coat in *Bacillus subtilis*. *J. Mol. Biol.* **207**:393–404.
7. Cutting, S., S. Roels, and R. Losick. 1991. Sporulation operon *spoIVF* and the characterization of mutations that uncouple mother-cell from forespore gene expression in *Bacillus subtilis*. *J. Mol. Biol.* **221**:1237–1256.
8. Driks, A., and R. Losick. 1991. Compartmentalized expression of a gene under the control of sporulation transcription factor σ^E in *Bacillus subtilis*. *Proc. Natl. Acad. Sci. USA* **88**:9934–9938.
9. Dubnau, D., and R. Davidoff-Abelson. 1971. Fate of transforming DNA following uptake by competent *B. subtilis*. *J. Mol. Biol.* **56**:209–221.
10. Errington, J., L. Wootten, J. C. Dunkerley, and D. Foulger. 1989. Differential gene expression during sporulation in *Bacillus subtilis*: regulation of the *spoIV* gene. *Mol. Microbiol.* **3**:1053–1060.
11. Halberg, R., and L. Kroos. Unpublished data.
12. Halberg, R., and L. Kroos. 1992. Fate of the SpoIIID switch protein during *Bacillus subtilis* sporulation depends on the mother-cell sigma factor, σ^K . *J. Mol. Biol.* **228**:840–849.
13. Halberg, R., V. Oke, and L. Kroos. Unpublished data.
14. Hoch, J. A., M. A. Shiflett, J. Trowsdale, and S. M. Chen. 1978. Stage O genes and their products, p. 131–135. In G. Chambliss and J. C. Vary (ed.), *Spores VII*. American Society for Microbiology, Washington, D.C.
15. Illing, N., and J. Errington. 1991. The *spoIIIA* operon of *Bacillus subtilis* defines a new temporal class of mother-cell-specific sporulation genes under the control of the σ^E form of RNA polymerase. *Mol. Microbiol.* **5**:1927–1940.
16. Ireton, K., and A. Gossman. 1992. Interaction among mutations that caused altered timing of gene expression during sporulation in *Bacillus subtilis*. *J. Bacteriol.* **174**:3185–3195.
17. Jaacks, K. J., J. Healy, R. Losick, and A. D. Grossman. 1989. Identification and characterization of genes controlled by the sporulation regulatory gene *spoOH* in *Bacillus subtilis*. *J. Bacteriol.* **171**:4121–4129.
18. Jonas, R. M., and W. G. Haldenwang. 1989. Influence of *spo* mutations on σ^E synthesis in *Bacillus subtilis*. *J. Bacteriol.* **171**:5226–5228.
19. Jonas, R. M., E. A. Weaver, T. J. Kenney, C. P. Moran, Jr., and W. G. Haldenwang. 1988. The *Bacillus subtilis* *spoIIG* operon encodes both σ^E and a gene necessary for σ^E activation. *J. Bacteriol.* **170**:507–511.
20. Kenney, T. J., and C. P. Moran, Jr. 1987. Organization and regulation of an operon that encodes a sporulation-essential sigma factor in *Bacillus subtilis*. *J. Bacteriol.* **169**:3329–3339.
21. Kroos, L., B. Kunkel, and R. Losick. 1989. Switch protein alters specificity of RNA polymerase containing a compartment-specific sigma factor. *Science* **243**:526–529.
22. Kunkel, B., L. Kroos, H. Poth, P. Youngman, and R. Losick. 1989. Temporal and spatial control of the mother-cell regulatory gene *spoIIID* of *Bacillus subtilis*. *Genes Dev.* **3**:1735–1744.
23. Kunkel, B., R. Losick, and P. Stragier. 1990. The *Bacillus subtilis* gene for the developmental transcription factor σ^K is generated by excision of a dispensable DNA element containing a sporulation recombinase gene. *Genes Dev.* **4**:525–535.
24. 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.
25. Losick, R., and P. Stragier. 1992. Crisscross regulation of cell-type-specific gene expression during development in *B. subtilis*. *Nature (London)* **355**:601–604.
26. Losick, R., P. Youngman, and P. J. Piggot. 1986. Genetics of endospore formation in *Bacillus subtilis*. *Annu. Rev. Genet.* **20**:625–669.
27. Lu, S., R. Halberg, and L. Kroos. 1990. Processing of the mother-cell σ factor, σ^K , may depend on events occurring in the forespore during *Bacillus subtilis* development. *Proc. Natl. Acad. Sci. USA* **87**:9722–9726.
28. Lu, S., and L. Kroos. Unpublished data.
29. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. 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.
31. Matsudaira, P. 1987. Sequence from picomole quantities of proteins electroblotted onto polyvinylidene difluoride membranes. *J. Biol. Chem.* **262**:10035–10038.
32. Miller, J. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. Oke, V., and R. Losick. 1993. Studies on multilevel regulation of the sporulation transcription factor, σ^K . *J. Bacteriol.* **175**:7341–7347.
34. Popham, D. L., and P. Stragier. 1992. Binding of the *Bacillus subtilis* *spoIVCA* product to the recombination sites of the element interrupting the σ^K -encoding gene. *Proc. Natl. Acad. Sci. USA* **89**:5991–5995.
35. Ricca, E., S. Cutting, and R. Losick. 1992. Characterization of *bofA*, a gene involved in intercompartmental regulation of pro- σ^K processing during sporulation in *Bacillus subtilis*. *J. Bacteriol.* **174**:3177–3184.
36. Sandman, K., L. Kroos, S. Cutting, P. Youngman, and R. Losick. 1988. Identification of the promoter for a spore coat protein gene in *Bacillus subtilis* and studies on the regulation of its induction at a late stage of sporulation. *J. Mol. Biol.* **200**:461–473.
37. Sandman, K., R. Losick, and P. Youngman. 1987. Genetic analysis of *Bacillus subtilis* *spo* mutations generated by Tn917-mediated insertional mutagenesis. *Genetics* **117**:603–617.
38. Sato, T., Y. Samori, and Y. Kobayashi. 1990. The *cisA* cistron of *Bacillus subtilis* sporulation gene *spoIVC* encodes a protein homologous to a site-specific recombinase. *J. Bacteriol.* **172**:1092–1098.
39. Stragier, P., C. Bonamy, and C. Karmazyn-Campelli. 1988. Processing of a sporulation sigma factor in *Bacillus subtilis*: how morphological structure could control gene expression. *Cell* **52**:697–704.
40. Stragier, P., J. Bouvier, C. Bonamy, and J. Szulmajster. 1984. A developmental gene product of *Bacillus subtilis* homologous to the sigma factor of *Escherichia coli*. *Nature (London)* **312**:376–378.
41. Stragier, P., B. Kunkel, L. Kroos, and R. Losick. 1989. Chromosomal rearrangement generating a composite gene for a developmental transcription factor. *Science* **243**:507–512.
42. Trempey, J. E., C. Bonamy, J. Szulmajster, and W. G. Haldenwang. 1985. *Bacillus subtilis* sigma factor, σ^{29} , is the product of the sporulation-essential gene *spoIIG*. *Proc. Natl. Acad. Sci. USA* **82**:4189–4192.
43. Youngman, P., J. B. Perkins, and R. Losick. 1983. Genetic transposition and insertional mutagenesis in *Bacillus subtilis* with the *Streptococcus faecalis* transposon Tn917. *Proc. Natl. Acad. Sci. USA* **80**:2305–2309.
44. Youngman, P., J. B. Perkins, and R. Losick. 1984. Construction of a cloning site near one end of Tn917 into which foreign DNA may be inserted without affecting transposition in *Bacillus subtilis* or expression of the transposon-borne *erm* gene. *Plasmid* **12**:1–9.
45. Zheng, L., R. Halberg, S. Roels, H. Ichikawa, L. Kroos, and R. Losick. 1992. Sporulation regulatory protein GerE from *Bacillus subtilis* binds to and can activate or repress transcription from promoters for mother-cell-specific genes. *J. Mol. Biol.* **226**:1037–1050.
46. Zheng, L., and R. Losick. 1990. Cascade regulation of spore coat gene expression in *Bacillus subtilis*. *J. Mol. Biol.* **212**:645–660.