Forespore-specific transcription of a gene in the signal transduction pathway that governs Pro- σ^K processing in *Bacillus subtilis*

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We present studies on the regulation of a developmental gene (*spoIVB*) whose product is required at a late stage of morphogenesis during the process of sporulation in *Bacillus subtilis*. Earlier work implicated the *spoIVB* gene product in a signal-transduction pathway that governs the conversion of pro- σ^K to the mature and active form of the mother cell σ factor, σ^K , in response to a signal generated within the forespore chamber of the sporangium. We now show that (1) *spoIVB* is induced at the engulfment stage of sporulation, (2) this transcription is restricted to the forespore, and (3) *spoIVB* is under the direct control of the forespore σ factor σ^G . The discovery that *spoIVB* is a forespore-expressed gene suggests that the *spoIVB* gene product, or a developmental event under its control, triggers the processing of pro- σ^K and thereby mediates the coupling of σ^K -directed gene expression in the mother cell to σ^G -directed gene expression in the forespore. We also show that *spoIVB* transcription is partially dependent on the action of the mother cell regulatory gene *spoIID*, a finding that suggests that the transcription of certain forespore-expressed genes is influenced by events in the mother cell.

[Key Words: *Bacillus subtilis*; sporulation; σ factor; signal transduction]

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The earliest morphological manifestation of the process of sporulation in the Gram-positive bacterium *Bacillus subtilis* is the formation of an asymmetrically positioned transverse septum, which partitions the developing cell into separate mother cell and forespore compartments. The two compartments, each of which receives a chromosome from the last round of vegetative DNA replication, follow separate pathways of cellular differentiation. Thus, the forespore metamorphoses into the dormant cell type of the endospore, while the mother cell, which participates in the formation of the spore, lyses when maturation of the spore is complete.

Gene expression in the two compartments is regulated differentially, with certain genes being expressed in the forespore and others in the mother cell (for review, see Stragier and Losick 1990). This differential gene expression is, in turn, controlled by the action of at least three compartment-specific transcriptional regulatory proteins. Forespore gene expression is controlled by the regulatory gene *spoIIIG*, whose product is the RNA polymerase σ factor σ^G , which is present in the forespore, where it directs the transcription of forespore-expressed

genes (Masuda et al. 1988; Karmazyn-Campelli et al. 1989; Sun et al. 1989). Gene expression in the mother cell, on the other hand, is controlled in part by the action of two mother-cell regulatory genes, *spoIID* and *sigK*. *spoIID* encodes a small, DNA-binding protein that (1) governs the creation of *sigK*, a composite of two truncated genes, by a DNA rearrangement in the mother cell chromosome, and (2) directs the subsequent transcription of the composite gene (Kroos et al. 1989; Kunkel et al. 1989, 1990; Stragier et al. 1989; Sato et al. 1990; Stevens and Errington 1990). The product of *sigK* is the mother-cell σ factor σ^K , which directs the transcription of genes involved in the formation of the cortex and coat compartments of the spore (Kroos et al. 1989).

Although separately controlled by different transcriptional regulatory proteins, the mother cell and forespore lines of gene expression are not entirely independent. Thus, mutations in the forespore regulatory gene *spoIIIG*, as well as mutations in two other loci (*spoIIIA* and *spoIIIE*) whose products are needed for σ^G -directed gene expression, prevent σ^K -directed gene expression in the mother cell (Sandman et al. 1988; Cutting et al. 1989, 1990; Zheng and Losick 1990). Recent work has shown that this intercompartmental coupling is mediated at the level of the conversion of an inactive pro-protein form

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(pro- σ^K) of σ^K to the mature and active σ factor (Kroos et al. 1989; Cutting et al. 1990; Lu et al. 1990). Genetic experiments identify the products of *spoIVF* (a two-cis-tron operon; S. Cutting, S. Roels, and R. Losick, unpubl.) as the pro- σ^K processing enzyme or as a regulator of the processing enzyme (Cutting et al. 1990). It is believed that a signal emanating from within the developing forespore stimulates the activity of the *spoIVF* gene products in the mother cell, thereby promoting the conversion of pro- σ^K to its active form (Cutting et al. 1990). This work also led to the identification of a fourth gene (*spoIVB*) of unknown function whose product evidently lies in the signal transduction pathway that leads from gene expression in the forespore to SpoIVF-mediated processing of pro- σ^K in the mother cell.

Here we report studies on the regulation of the *spoIVB* gene, whose product is a 46-kD polypeptide and whose transcription is known to commence at an intermediate stage of sporulation (Van Hoy and Hoch 1990). Using a previously constructed transcriptional fusion of *spoIVB* to the *lacZ* gene of *Escherichia coli* (Van Hoy and Hoch 1990), we show that *spoIVB* is induced at approximately the engulfment stage of sporulation and that this transcription, which is shown herein to be under the direct control of the forespore σ factor σ^G , is confined to the forespore compartment of the developing sporangium. On the basis of these results and those published previously (Cutting et al. 1990), we infer that SpoIVB lies between σ^G and SpoIVF in the signal transduction pathway that controls pro- σ^K processing in the mother cell. Unexpectedly, *spoIVB* transcription and, to a lesser extent, transcription of certain other forespore-expressed genes, is found to depend partially on the product of the mother cell regulatory gene *spoIIID*. This finding suggests that σ^G -directed gene expression in the forespore is influenced by events occurring within the mother cell.

Results

Timing and compartmentalization of *spoIVB-lacZ* expression

The time course experiment of Figure 1 shows that *spoIVB-lacZ* expression was induced slightly later than a *lacZ* fusion to the forespore-expressed gene *sspB* and significantly later than *lacZ* fusions to the forespore regulatory gene *spoIIIG* and the mother cell regulatory gene *spoIIID*. These results and other findings presented below indicate that *spoIVB* transcription commences during engulfment, the stage (III) at which the forespore becomes pinched off as a free protoplast within the mother cell.

Since *spoIVB* is induced after the sporangium has been partitioned into two compartments, we wondered whether its transcription was compartmentalized. We therefore compared the accessibility of fusion-encoded β -galactosidase to ONPG (*o*-nitro-phenyl- β -D-galactoside) substrate in cells treated with toluene to that observed in cells treated with lysozyme (Mason et al. 1988). β -Galactosidase sequestered in the forespore is partially

Figure 1. A comparison of the time course of expression of *spoIVB-lacZ* to that of other *spo-lacZ* fusions. Isogenic strains (Spo^+) containing different *lacZ* fusions were induced to sporulate by resuspension in SM medium, and samples were taken at the indicated times after the onset of sporulation (T_0). Cells were permeabilized by lysozyme treatment, and β -galactosidase activity was determined. Levels of β -galactosidase activity are shown as a percentage of the maximum levels obtained for each fusion. The fusions were *spoIIIG-lacZ* ($\circ$; PM30), *spoIIID-lacZ* ($\blacksquare$; BK595), *spoIVB-lacZ* ($\square$; SC1080), and *sspB-lacZ* ($\bullet$; SC780).

"masked" from the action of toluene but not from lysozyme because the forespore is somewhat resistant to the permeabilizing effect of the organic solvent, but not to the digestive effect of the cell wall-degrading enzyme. In contrast, because toluene readily permeabilizes the mother cell, fusion-encoded β -galactosidase produced in the mother cell is fully accessible to ONPG in cells that have been treated with either toluene or lysozyme. The time course experiment of Figure 2B shows that treatment of cells bearing a *lacZ* fusion to the forespore-expressed gene *sspB* with lysozyme revealed considerably more β -galactosidase activity (open squares) than did treatment of the cells with toluene (solid squares). As a further indication of sequestration, treatment of the cells with SDS, which inactivates enzyme in the mother cell, followed (after washing to remove the detergent) by lysozyme treatment to release the contents of the forespore, showed that after hour 6 of sporulation, at least some *sspB-lacZ* fusion-encoded β -galactosidase had been rendered inaccessible to the action of the detergent (open circles). In contrast, lysozyme and toluene treatment released similar levels of fusion-encoded β -galactosidase from cells harboring a *lacZ* fusion to the mother cell-expressed gene *gerE* (Fig. 2C). Furthermore, essentially all of the *gerE-lacZ*-produced enzyme was inactivated by treatment of the cells with SDS. As the patterns (Fig. 2A) of accessibility of β -galactosidase produced under the direction of the *spoIVB-lacZ* fusion closely mimicked those observed with the *sspB-lacZ* fusion, we infer that *spoIVB* is a forespore-expressed gene.

Use of immuno-electron microscopy to localize *spoIVB*-directed β -galactosidase synthesis

As an independent test of compartmentalization, we used immuno-electron microscopy to visualize the

Figure 2. Sequestration of β -galactosidase synthesized from *spoIVB-lacZ* during sporulation. The specific activity of β -galactosidase was determined after resuspension in SM medium at the indicated times after the onset of sporulation (T_0) in *spo*⁺ cells carrying either *spoIVB-lacZ* (A), *sspB-lacZ* (B), or *gerE-lacZ* (C). Each panel shows the amounts of β -galactosidase activity obtained after the cells had been permeabilized by toluene treatment (■), lysozyme treatment (□), or SDS extraction followed by lysozyme treatment (○).

location of *spoIVB*-directed β -galactosidase synthesis (Francesconi et al. 1988). Because the level of expression of *spoIVB-lacZ* (~100 Miller units) is too low to be detected by immuno-electron microscopy, we increased the copy number of the gene fusion by subjecting cells (strain SC1080) containing a chromosomally integrated copy of the *spoIVB-lacZ*-bearing plasmid pJB2026 to successive rounds of growth in the presence of increasing concentrations of chloramphenicol to select for amplification of the integrated plasmid. (pJB2026 contains the *cat* chloramphenicol-resistance gene.) Tandem chromosomal arrays selected in this manner are known to be relatively stable and are maintained for several generations of growth in the absence of drug selection (Janniére et al. 1985). At a level of resistance to 200 μ g/ml of drug, a strain was obtained that was capable of accumulating β -galactosidase to a sp. act. of 900 Miller units when grown and sporulated in the absence of chloramphenicol. Despite the increase in copy number, the temporal pattern of *spoIVB-lacZ* expression was similar to that of the parental strain SC1080 (data not shown).

Thin sections of cells of the amplified, drug-resistant strain that had been harvested at hour 8 of sporulation were prepared. The sections were treated with anti- β -galactosidase antibodies, and antibodies bound on the surface of the sections were then visualized by applying gold-conjugated secondary antibodies. The level of decoration varied from cell to cell, but among cells exhibit-

ing a significant level of decoration, gold granules were far more prevalent in the forespore than in the mother cell. Several examples of decorated sporangia are presented in Figure 3. As a control, little decoration was observed when cells lacking *spoIVB-lacZ* were subjected to immuno-electron microscopy (A. Driks, data not shown). Also, when cells containing *lacZ* fusions to genes (*cotD* and *gerE*) under the control of the mother cell transcription factor σ^K were subjected to immuno-electron microscopy at hour 8 of sporulation, decoration by gold granules was principally restricted to the mother cell (A. Driks, data not shown).

Dependence of spoIVB expression on the products of other spo genes

spoIVB-lacZ was introduced by transformation into a collection of isogenic strains, each containing a different sporulation mutation. The resulting fusion-bearing strains were induced to sporulate by suspension in SM (Sterlini-Mandelstam) medium. Samples were taken at various times throughout sporulation and assayed after lysozyme extraction for *spoIVB-lacZ*-directed β -galactosidase synthesis. Time course experiments were done in parallel with an otherwise isogenic *Spo*⁺ strain containing the *spoIVB-lacZ* fusion (strain SC1080). The pattern of *spoIVB* expression in all mutants is summarized in Table 1, and some representative time course experiments are shown in Figure 4. In addition to *spoIVB*, we determined the dependence patterns of the forespore-expressed genes *sspA*, *sspB*, and *sspE* (Table 1; Fig. 4).

Our results first show that the dependence pattern of *spoIVB* expression was similar to that of the other forespore-expressed genes. Second, three levels of dependence were observed for the mutations examined: those that caused a strong block in *spoIVB* and *ssp* expression (e.g., *spoIIG55*, *spoIIIA53*, *spoIIIE36*, *spoIIIGΔ1*); those that had no effect on expression (e.g., *spoIIIC94*, *spoIVCB23*); and those that caused a partial impairment of expression (i.e., *spoIIID83* and *spoIIIDA::erm*).

These results are generally consistent with previous reports on the regulation of *ssp* genes (Mason et al. 1988). Furthermore, the observed block in *spoIVB* (and *ssp*) expression caused by *spoIIIA*, *spoIIIG*, and *spoIIIE* mutations is consistent with the view that expression of this sporulation gene is confined to the forespore. Both the *spoIIIG* and *spoIIIE* gene products are known to be needed for efficient forespore-specific gene expression (Mason et al. 1988; Foulger and Errington 1989; Karmazyn-Campelli et al. 1989). A recent report indicates that the *spoIIIA* gene products are not important regulators of forespore gene expression (Illing and Errington 1990), but our results show that *spoIIIA* mutations substantially impaired the transcription of the known forespore-expressed *ssp* genes while also preventing *spoIVB* transcription.

The partial impairment of *spoIVB* and *ssp* expression caused by mutations in the mother cell regulatory gene *spoIIID* merits further comment. First, the extent of the

Figure 3. Localization of *spoIVB-lacZ*-directed β -galactosidase synthesis by immuno-electron microscopy. Thin sections of sporulating cells containing multiple copies of the *spoIVB-lacZ* fusion were prepared and treated with an anti- β -galactosidase antibody, followed by treatment with a gold-conjugated secondary antibody, as described in Materials and methods. The dark specks are gold particles. Forespores (FS) and mother cells (MC) are labeled. Bar, 100 nm.

impairment of *spoIVB* expression varied from experiment to experiment, ranging from a strong block (3%) to a partial block (31%). We do not understand the basis for this variability. Second, *spoIVB* expression was impaired more severely by *spoIIID* mutations than was *ssp* expression. Nevertheless, *spoIIID* mutations did cause a noteworthy (two- to threefold) inhibition of *sspA*, *sspB*, and *sspE* expression, the extent of inhibition being somewhat greater than that observed in previous dependence studies of *sspA* and *sspE* expression (Mason et al. 1988).

Use of mosaic sporangia to verify that the requirement for the spoIIID gene product is restricted to the mother cell

The partial dependence of *spoIVB* and *sspA*, *sspB*, and *sspE* expression on the *spoIIID* gene product was surpris-

ing in light of earlier genetic experiments, which indicated that expression of *spoIIID* is only required in the mother cell (De Lencastre and Piggot 1979). As a further test of whether the requirement for *spoIIID* expression is confined to the mother cell, we used the recently devised, integrational plasmid excision method of Illing et al. (1990) to create "mosaic sporangia" bearing a wild-type chromosome in one compartment and a chromosome with a disrupted *spoIIID* gene in the other compartment. An integrational plasmid (pBK56) containing an internal fragment (200 bp) of *spoIIID* was used to transform wild-type cells (Spo^+) of strain PY79 to Cm^R (pBK56 contains the *cat* gene). The resulting transformants were Spo^- due to integration of the plasmid into the chromosome at the *spoIIID* locus by a Campbell-like recombination. Two independent transformants were then grown and sporulated. In each case, the number of

Table 1. Effect of *spo* mutations on *spoIVB*, *sspA*, *sspB*, and *sspE*-directed β -galactosidase synthesis

Relevant mutation ^a	Synthesis of β -galactosidase ^b			
	<i>spoIVB</i>	<i>sspA</i>	<i>sspB</i>	<i>sspE</i>
<i>spoIIAC1</i>	4	<1	<1	<1
<i>spoIID66</i>	6	3	2	1
<i>spoIID298</i>	3	3	1	2
<i>spoIID :: Tn917ΩHU298</i>	2			
<i>spoIIE48</i>	7			
<i>spoIIG55</i>	10	<1		<1
<i>spoIIIA35</i>	9	9	5	5
<i>spoIIIA53</i>	6	5	3	4
<i>spoIIIA59</i>	4	7	3	5
<i>spoIIIA65</i>	5		2	
<i>spoIIIA :: Tn917ΩHU25</i>	8	11	6	8
<i>spoIIIC94</i>	103	105	95	102
<i>spoIIID83</i>	6–31		30–58	
<i>spoIIIDΔ :: erm</i>	3–19	31–35	31–46	33–72
<i>spoIIIE36</i>	2	<1	<1	<1
<i>spoIIIGΔ1</i>	3	<1	<1	<1
<i>spoIVA67</i>	98			
<i>spoIVA178</i>	128			
<i>spoIVB :: erm</i>	41			
<i>spoIVCA133</i>	123			
<i>spoIVCB23</i>	111	113		83
<i>spoIVF152</i>	108			

^aStrains carrying the indicated allele were transformed with chromosomal DNA containing either a *spoIVB-lacZ*, *sspA-lacZ*, *sspB-lacZ*, or *sspE-lacZ* fusion.

^bExpressed as a percentage of the level of β -galactosidase activity observed in wild-type cells at peak activity (~6–8 hr) after resuspension in SM medium. The values given are means of at least two independent experiments, with the exception of the *spoIIID* alleles in which case the range of values obtained are shown. Typical wild-type levels of β -galactosidase activity (in Miller units) for each *lacZ* fusion were *spoIVB*, 80–200; *sspA/E*, 400–500; and *sspB*, 500–5000.

Cm^R (Spo^-) heat-resistant cells/ml was determined 24 hr after the start of sporulation. The results of these experiments showed that the excision of pBK56 occurred at a very low frequency (1 excision per 10^3 to 10^4 cells) during growth and sporulation and generally resulted in the production of normal (Spo^+) heat-resistant cells, presumably due to recreation of the wild-type *spoIIID* gene (Table 2). However, a small but significant number of the heat-resistant spores were found to be both Cm^R and Spo^- . These presumably arose from mosaic sporangia bearing a wild-type copy of *spoIIID* in the mother-cell chromosome. Their existence indicates that expression of *spoIIID* is required only in the mother cell, a finding in agreement with the original experiments of De Lencastre and Piggot (1979). Thus, *spoIIID* action is confined to the mother cell, and the partial dependence of forespore gene expression on the *spoIIID* product is presumably an indirect effect, reflecting an influence of mother cell development on the level of gene transcription in the forespore.

spoIVB is under the direct control of the forespore σ factor σ^G

Despite the unexpected inhibitory effects of *spoIIID* mutations on forespore gene expression, the dependence studies indicate that *spoIVB* (like *sspA*, *sspB*, and *sspE*) is under the direct control of the forespore transcription factor σ^G . As an independent approach to testing whether *spoIVB* transcription is under σ^G control, we investigated whether σ^G is capable of directing *spoIVB* transcription in cells that have been engineered to produce the forespore transcription factor during vegetative growth. Accordingly, we introduced the autonomously replicating plasmid pDG298 into cells containing a *spoIIIG Δ 1* mutation and the *spoIVB-lacZ* fusion by selection for kanamycin resistance (10 $\mu\text{g}/\text{ml}$). pDG298 carries *lacI* and a modified *spoIIIG* gene that lacks its own promoter but is instead fused to the IPTG (isopropyl- β -D-thiogalactopyranoside)-inducible *spac* promoter (Sun et al. 1989), such that transcription of *spoIIIG* can be induced by addition of IPTG. Since *spoIIIG* is autoregulatory, the *spoIIIG Δ 1* mutation was included in our strain construction to ensure that no transcription of the chromosomal *spoIIIG* gene occurred. Figure 5 shows that addition of IPTG caused a rapid induction of *spoIVB*-directed β -galactosidase synthesis.

Consistent with the view that *spoIVB* is transcribed by σ^G -RNA polymerase is the similarity of the -10 and -35 sequences of its P1 promoter [the principal promoter governing *spoIVB* transcription (Van Hoy and Hoch 1990)] to the corresponding consensus sequences for σ^G -recognized promoters (Nicholson et al. 1989). Thus, the *spoIVB* -10 region (tATcCaA) matches the consensus -10 region (CATACTA) in four of seven po-

Figure 4. Expression of *spoIVB-lacZ* and *sspB-lacZ* in various *spo* mutants. The specific activity of β -galactosidase was determined after suspension in SM medium at the indicated times after the onset of sporulation (t_0) in *spo*⁺ cells (■) or isogenic strains containing the following mutations: *spoIIIC94* (●), *spoIIID Δ :: erm* (○), *spoIIIG Δ 1* (□), and *spoIIIA53* (▲). (A) Each strain contained an integrated copy of the *spoIVB-lacZ* fusion; (B) an *sspB-lacZ* fusion (see Materials and methods).

Table 2. Frequencies and phenotypes of spores arising from excision of an integrational plasmid (pBK56) in the *spoIIID* gene

Strain ^a (experiment no.)	CFU/ml ^b		Cm ^R (Spo ⁻) cells (%)
	without chlor- amphenicol ^c	with chlor- amphenicol ^d	
PY79 :: pBK56-transformant A			
1	4 × 10 ⁴	1 × 10 ³	2.5
2	8 × 10 ⁴	1 × 10 ³	1.3
3	4 × 10 ⁴	4 × 10 ²	1.0
PY79 :: pBK56-transformant B			
1	4 × 10 ⁴	6 × 10 ²	1.5
2	8 × 10 ⁴	6 × 10 ³	7.5
3	8 × 10 ⁴	1 × 10 ³	1.3

^aPY79 :: pBK56-transformant A and PY79 :: pBK56-transformant B are two independently isolated transformants. Each was obtained by transforming strain PY79 (Spo⁺) with the integrational plasmid pBK56 and selecting for Cm^R. Transformants (Spo⁻) resulted from integration of pBK56 into *spoIIID*. pBK56 contains an internal (200 bp) fragment of the *spoIIID* gene in addition to the *cat* gene.

^bThe indicated strains were induced to sporulate, and samples were taken 24 hr after the onset of sporulation (T₂₄). Each sample was heat-treated (82°C, 15 min), and appropriate dilutions were plated on DS agar plates with and without chloramphenicol (5 µg/ml) to determine the proportion of cells that still contained an integrated plasmid. Sporulation was induced in experiments 1 and 2 by resuspension in SM medium containing chloramphenicol (5 µg/ml) and in experiment 3 by exhaustion in DS medium with no antibiotic selection.

^cFor comparison, the parent strain PY79 produced 1 × 10⁸ heat-resistant CFU/ml.

^dAs a demonstration that the Cm^R (Spo⁻) cells did not arise from leakiness in the requirement for the *spoIIID* gene product, a strain bearing a nonreversible (nonexcisable) *spoIIID* mutation (*spoIIIDΔerm*; Kunkel et al. 1989) produced <10 heat-resistant CFU/ml in parallel experiments.

sitions, and the *spoIVB* -35 region (TGcAcA) matches the consensus -35 region (TGAATA) in four of six positions.

The *spoIVB* gene product is required for normal cortex and coat formation

Having established that *spoIVB* is a forespore-expressed gene, we were interested in investigating the nature of the requirement for its product in morphogenesis. The structure of a *spoIVB* :: *ermG* mutant sporangium (Fig. 6B) is compared with that of wild-type (Fig. 6A) and *spoIVCB23* mutant sporangia (Fig. 6C). [*spoIVCB23* is a mutation in the gene for the amino-terminal portion of σ^K.] The electron micrographs show that like the *spoIVCB* mutant, the *spoIVB* mutant was defective in cortex and coat formation. We also examined the block caused by the original *spoIVB* mutation of Coote (1972), who did not observe a defect in cortex formation in *spoIVB165* cells. Our results showed that like the null

mutant, *spoIVB165* cells are defective in cortex as well as coat formation (data not shown).

Discussion

We have studied temporal, spatial, and genetic aspects of the regulation of the stage-IV sporulation gene *spoIVB*. Our principal conclusion is that transcription of *spoIVB* is induced in the forespore chamber of the sporangium. This conclusion rests on the following evidence: (1) Transcription of *spoIVB* is turned on at the engulfment stage of sporulation; (2) β-galactosidase produced by a *spoIVB-lacZ* fusion is sequestered in the forespore; and (3) *spoIVB* transcription depends on, and is under the direct control of, the forespore σ factor σ^G. Thus, *spoIVB* is to be added to the growing list of genes and operons [e.g., *spoIIIG*, *spoVA*, *sspA-E*, *gdh*, *gerA*, and 0.3 kb (Errington and Mandelstam 1986b; Mason et al. 1988; Rather and Moran 1988; Karmazyn-Campelli et al. 1989; Panzer et al. 1989; Feavers et al. 1990)], whose transcriptions are confined to the forespore. However, *spoIVB* is only the third example [the other two being *spoIIIG* (Karmazyn-Campelli et al. 1989) and *spoVA* (Errington and Mandelstam 1986b)] of a forespore-expressed gene whose product is required for spore formation (i.e., *spoIVB* mutations are Spo⁻).

The discovery that *spoIVB* is a forespore-expressed gene provides an important insight into the signal transduction pathway that couples σ^K-directed gene expression in the mother cell to σ^G-directed gene expression in the forespore. Earlier work showed that the mother-cell σ factor σ^K is initially produced as an inactive pro-protein, pro-σ^K, which is then converted to the mature and active transcription factor (Kroos et al. 1989; Lu et al.

Figure 5. IPTG-dependent induction of *spoIVB-lacZ*-directed β-galactosidase synthesis. *spoIIIGΔ1* cells containing the *spoIVB-lacZ* fusion and the *Pspac-IIIIG* plasmid pDG298 (strain RS208) were grown in 2XYT medium (with kanamycin 10 µg/ml). Plasmid pDG298 carries the *spoIIIG* gene under the control of the *spac* promoter. At an OD₆₀₀ of 0.3, the culture was split, and IPTG (to a final concentration of 1 mM) was added to one portion and growth was continued. Samples were removed and assayed for β-galactosidase at the indicated times after IPTG induction. Symbols: (●) With the addition of IPTG; (○) without the addition of IPTG.

Figure 6. Morphology of wild-type and mutant sporangia. Shown are electron micrographs of sporangia from the wild-type strain (PY79; A) and from otherwise isogenic strains bearing the mutations *spoIVB::ermG* (strain BK750; B) and *spoIVCB23* (strain BK556; C). (FS) Forespore; (FM) forespore membrane; (C) cortex; (IC) inner coats; (OC) outer coats; (MC) mother cell. Bar, 100 nm.

1990). Processing of pro- σ^K depends on the products of five stage III–IV loci, *spoIIIA*, *spoIIIE*, *spoIIIG*, *spoIVB*, and *spoIVF* (Cutting et al. 1990; Lu et al. 1990). Based in part on the discovery of change-of-function mutations (*bofB*; Cutting et al. 1990) in *spoIVF* that bypass the requirement for the other four gene products in σ^K -directed gene expression, we inferred that *spoIVF* encodes the pro- σ^K protease or a regulator of the protease and that the *spoIIIA*, *spoIIIE*, and *spoIIIG* gene products generate a signal within the forespore that is transduced to the mother cell where it stimulates the activity of the *spoIVF* gene products.

The present finding that the transcription of *spoIVB* depends on the products of *spoIIIA*, *spoIIIE*, and *spoIIIG* and is under the control of σ^G indicates that SpoIVB acts after SpoIIIA, SpoIIIE, and SpoIIIG (σ^G) in the pathway leading to the stimulation of the *spoIVF* gene products. Likewise, as noted above, the finding (Cutting et al. 1990) that the requirement for SpoIVB in σ^K -directed gene expression can be eliminated by *bofB* mutations in *spoIVF* indicates that the action of SpoIVB precedes that of the *spoIVF* gene products. Thus, in our model (Fig. 7), the chain of events leading to the processing of pro- σ^K is as follows: First, SpoIIIA, SpoIIIE, and SpoIIIG turn on the transcription of *spoIVB* in the forespore. Next, the product of *spoIVB*, or a developmental event under its control, stimulates the activity of the *spoIVF* gene products, thereby promoting the processing of pro- σ^K . An important feature of our model is that SpoIVB or an event

under its control is the signal that stimulates pro- σ^K processing and thereby links σ^G -directed gene expression in the forespore to σ^K -directed gene expression in the mother cell. In support of our model, *spoIVB* is the only σ^G -controlled gene known whose product is required for pro- σ^K processing. Also, in agreement with the view that an important aspect of SpoIVB function is to stimulate pro- σ^K processing, the phenotype of a *spoIVB* mutant (Fig. 6B; impaired in cortex and coat formation) is similar to that of mutants in mother cell genes, such as *spoIVCB* (Fig. 6C); *spoIIIC*, *spoIVCA*, and *spoIVF* (Piggot and Coote 1976), that are directly involved in the production of σ^K . [However, stimulation of pro- σ^K processing is not the only function of SpoIVB, since the block in spore formation caused by *spoIVB165* or *spoIVB::ermG* is not relieved by mutations that bypass the requirement for SpoIVB in σ^K -directed gene expression (Cutting et al. 1990; S. Cutting, unpubl.).]

A remaining mystery is the question of exactly how SpoIVB stimulates the activity of the *spoIVF* gene products. No clue is provided by computer analysis of the 425-amino-acid-long predicted product of *spoIVB*, which does not resemble a known bacterial protein (Van Hoy and Hoch 1990). A simple hypothesis is that SpoIVB interacts directly with SpoIVF across the membranes separating the two compartments, with SpoIVB being localized in the forespore membrane and SpoIVF being integrated into the mother cell membrane that engulfs the forespore.

Figure 7. Proposed signal transduction pathway for the control of pro- σ^K processing. Shown is the proposed sequence of events leading to the processing of pro- σ^K in the mother cell. The stippled region represents the membrane partition between the forespore and the mother cell, across which SpoIVB stimulates the activity of SpoIVF. The diagram is not meant to imply that *spoIIIA* and *spoIIIE* are expressed in the forespore, but rather that the *spoIIIA* and *spoIIIE* gene products are required for σ^G -directed transcription of *spoIVB* in the forespore.

Although its precise role in the pro- σ^K signal transduction pathway is not known, *spoIVB* is to be added to the list of genes whose products are intimately involved in the production of σ^K . These genes are *spoIVCB* and *spoIIIC*, the truncated coding elements for σ^K (Stragier et al. 1989); *spoIVCA*, the recombinase gene that governs the construction of the composite σ^K -coding sequence (Kunkel et al. 1990; Sato et al. 1990); *spoIIID*, the regulatory gene that governs both the rearrangement and the transcription of the rearranged gene (Kunkel et al. 1989, 1990); and the two members (A and B) of the *spoIVF* operon, which govern the processing of pro- σ^K (Cutting et al. 1990; S. Cutting, S. Roels, and R. Losick, unpubl.). Thus, the activation of cortex and coat genes in the mother cell may depend on the action of as many as seven gene products that participate in the production of the mother cell σ factor.

A further aspect of the present work that bears on the issue of interactions between the mother cell and the forespore is the unexpected finding that *spoIVB* transcription is significantly dependent on the product (SpoIIID) of the mother cell regulatory gene *spoIIID*. Indeed, the transcription of several forespore-expressed genes (e.g., *sspA*, *sspB*, and *sspE*) was found to be partially impaired in *spoIIID* mutant cells, although not as severely as *spoIVB* transcription. Earlier experiments of various kinds indicate that *spoIIID* is a mother cell-expressed gene and that its product is required exclusively in the mother cell (De Lencastre and Piggot 1979; Errington and Mandelstam 1986b; Kunkel et al. 1989; Stevens and Errington 1990). The most important of these are the experiments of De Lencastre and Piggot (1979), which showed that mosaic sporangia in which the forespore chromosome harbors a *spoIIID* mutation produce heat-resistant (but genotypically *spo*⁻) spores, a finding that we have confirmed by use of the integrational plasmid, gene disruption method of Illing et al. (1990). We therefore infer that the partial requirement of SpoIIID in forespore gene expression is an indirect effect, being the consequence of SpoIIID action in the mother cell. Since the forespore at engulfment and later stages is entirely

contained within the mother cell, it is logical to suppose that normal forespore metabolism (and, hence, efficient forespore gene transcription) depends on the normal functioning of the mother cell. Thus, the block in mother cell development caused by *spoIIID* mutations could indirectly hamper macromolecular synthesis in the forespore, which may depend on the mother cell for energy and metabolites. In this view, *spoIVB* transcription is impaired more severely by *spoIIID* mutations than is transcription of *ssp* genes simply because *spoIVB* is induced slightly later than these other forespore-expressed genes. Alternatively, SpoIIID or the product of a gene under its control may be part of a specific signal transduction pathway that somehow influences the transcription of certain forespore-expressed genes.

spoIIID is not the only example of a mother cell gene whose product is required for efficient forespore gene expression. Genetic mosaic experiments indicate that expression of the *spoIIIA* operon is exclusively required in the mother cell (De Lencastre and Piggot 1979; Illing et al. 1990). Yet, as observed here and reported previously (Mason et al. 1988), *spoIIIA* mutations substantially inhibit *sspA*, *sspB*, and *sspE* transcription as well as transcription of *spoIVB*. Once again, the requirement for *spoIIIA* gene products in efficient transcription of forespore gene expression may reflect the dependence of the forespore on the overall metabolism of the surrounding mother cell or on a specific regulatory mechanism that links σ^G -directed gene expression to events occurring within the mother cell (Illing and Errington 1990).

In summary, our study of *spoIVB* expression and regulation has provided evidence for reciprocal pathways of interaction between the mother cell and the forespore chambers of the sporangium: On the one hand, the action of SpoIVB in the forespore promotes σ^K -directed gene expression in the mother cell; on the other hand, the action of SpoIIID in the mother cell influences the level of expression of certain σ^G -transcribed genes in the forespore. Thus, the mother cell and forespore lines of gene expression are not independent; rather, they are coordinated by at least two distinct intercellular interac-

tions that may be responsible for keeping the course of development in the sister cell types of the sporangium in register.

Materials and methods

Bacterial strains

Strains of *B. subtilis* are listed in Table 3. All strains used in this study were isogenic to the prototrophic wild-type (*Spo*⁺) strain PY79 and were constructed previously or by congression into

strain PY78 (Sandman et al. 1988); PY79 is the prototrophic derivative of PY78.

General methods

Competent cells were prepared and transformed by the method of Dubnau and Davidoff-Abelson (1971). Selection for *Cm*^R was made on agar plates containing 5 µg/ml of chloramphenicol.

Sporulation was induced on solid medium using DS (Difco sporulation) agar and in liquid by either resuspension in SM medium, using the method of Sterlini and Mandelstam (1969),

Table 3. *Bacillus subtilis* strains

Strain	Genotype	Source or reference
PY79	Prototrophic	lab stock (Youngman et al. 1984)
PY78	<i>glnA100</i>	lab stock
SC1080 ^a	<i>spoIVB</i> :: <i>pJB2026 (spoIVB-lacZ)</i>	this work
SC262	<i>sspA</i> :: <i>sspA-lacZ trpC2</i>	P. Setlow (Mason et al. 1988)
SC263	<i>sspB</i> :: <i>sspB-lacZ trpC2</i>	P. Setlow (Mason et al. 1988)
SC264	<i>sspE</i> :: <i>sspE-lacZ trpC2</i>	P. Setlow (Mason et al. 1988)
SC780 ^a	<i>sspB</i> :: <i>sspB-lacZ</i>	from SC263 into PY79
SC433 ^a	<i>SPβ</i> :: <i>gerE-lacZ</i>	lab stock (Cutting et al. 1989)
RS208 ^a	<i>spoIIIGΔ1 spoIVB-lacZ (pDG298)</i>	this work
BK595 ^a	<i>spoIIID</i> :: <i>pBK45 (spoIIID-lacZ)</i>	lab stock (Kunkel et al. 1989)
PM30 ^a	<i>amyE</i> :: <i>spoIIIG-lacZ</i>	P. Stragier (Karmazyn-Campelli et al. 1989)
1.5	<i>spoIIAC1 trpC2</i>	J. Errington (Errington and Mandelstam 1986a)
SC1159 ^a	<i>spoIIAC1</i>	from 1.5 into PY78 ^b
1S33 ^c	<i>spoIID66 trpC2 rpoB2</i>	BGSC ^d
SC1158 ^{a,c}	<i>spoIID66</i>	from 1S33 into PY78 ^b
298.4 ^c	<i>spoIID298 trpC2</i>	J. Errington (Errington and Mandelstam 1986a)
SC1160 ^{a,c}	<i>spoIID298</i>	from 298.4 into PY78 ^b
KS298 ^a	<i>spoIID</i> :: <i>Tn917ΩHU298</i>	lab stock (Sandman et al. 1987)
48.7	<i>spoIIIE48 trpC2</i>	J. Errington (Errington and Mandelstam 1986a)
SC1161 ^a	<i>spoIIIE48</i>	from 48.7 into PY78 ^b
55.3	<i>spoIIIG55 trpC2</i>	J. Errington (Errington and Mandelstam 1986a)
SC1163 ^a	<i>spoIIIG55</i>	from 55.3 into PY78 ^b
SC614 ^{a,c}	<i>spoIIIA35</i>	lab stock (Cutting et al. 1989)
SC615 ^{a,c}	<i>spoIIIA53</i>	lab stock (Cutting et al. 1990)
SC620 ^a	<i>spoIIIA59</i>	lab stock (Cutting et al. 1989)
SC616 ^{a,c}	<i>spoIIIA65</i>	lab stock (Cutting et al. 1989)
KS25 ^{a,c}	<i>spoIIIA</i> :: <i>Tn917ΩHU25</i>	lab stock (Sandman et al. 1987)
BK410 ^a	<i>spoIIIC94</i>	lab stock (Kunkel et al. 1988)
BK395 ^a	<i>spoIIID83</i>	lab stock (Kunkel et al. 1988)
BK541 ^a	<i>spoIIIDΔ</i> :: <i>erm</i>	lab stock (Kunkel et al. 1989)
SC622 ^a	<i>spoIIIE36</i>	lab stock (Cutting et al. 1989)
SC500 ^a	<i>spoIIIGΔ1</i>	lab stock (Cutting et al. 1990)
67	<i>spoIVA67 trpC2</i>	J. Errington (Errington and Mandelstam 1986a)
SC1164 ^a	<i>spoIVA67</i>	from 67 into PY78 ^b
1S46	<i>spoIVA178 pheA12</i>	BGSC
SC1165 ^a	<i>spoIVA178</i>	from 1S46 into PY78 ^b
SL765	<i>spoIVB165 trpC2</i>	J. Hoch (Van Hoy and Hoch 1990)
BK754 ^a	<i>spoIVB165</i>	from SL765 into PY78 ^b
JH12719	<i>spoIVB</i> :: <i>ermG trpC2 phe-1</i>	J. Hoch (Van Hoy and Hoch, 1990)
BK750 ^a	<i>spoIVB</i> :: <i>ermG</i>	from JH12719 into PY79
BK558 ^a	<i>spoIVCA133</i>	lab stock (Kunkel et al. 1989)
BK556 ^a	<i>spoIVCB23</i>	lab stock (Kunkel et al. 1989)
SC834 ^a	<i>spoIVF152</i>	lab stock (Cutting et al. 1990)

^aIsogenic with PY79.

^bConstructed in the present work using the congression procedure of Sandman et al. (1988) using PY78, a *gln*⁻ derivative of PY79.

^cThe *spo* allele was complemented, in *trans*, by the *spoIID*⁺ bacteriophage φ105J85 (Errington and Jones 1987).

^d*Bacillus* Genetic Stock Center.

^eThe *spo* allele was complemented, in *trans*, by the *spoIIIA*⁺ bacteriophages, φ105J83 and/or φ105J112 (Errington and Jones 1987; J. Errington, pers. comm.).

or by growth and exhaustion in DS medium, as described by Sandman et al. (1988).

Introduction of *lacZ* fusions into isogenic strains

An integrational plasmid (pJB2026) containing the *spoIVB-lacZ* transcriptional fusion (Van Hoy and Hoch 1990) was used to transform wild-type (*Spo*⁺) cells of strain PY79, followed by selection for Cm^R (pJB2026 carries the *cat* gene). One Cm^R transformant was isolated, purified, and used to prepare chromosomal *spoIVB-lacZ* DNA. This DNA was used to transform various mutant strains with selection for Cm^R. In addition, we transformed the wild-type strain (PY79) with this DNA and used the resulting derivative (strain SC1080) as an isogenic, *Spo*⁺ control in time course experiments. Likewise, strains SC262, SC263, and SC264 were used to prepare chromosomal DNA containing the *sspA-lacZ*, *sspB-lacZ*, and *sspE-lacZ* fusions, respectively. As with *spoIVB-lacZ*, these DNAs were used to transform both mutant strains and the isogenic wild-type strain PY79.

Measurement of β -galactosidase activity

Samples (1.0 ml) to be analyzed for β -galactosidase activity were collected at appropriate times and stored at -70°C until the time of assay. Cells were permeabilized either by treatment with toluene (Miller 1972), lysozyme (Mason et al. 1988), or with SDS followed by lysozyme treatment (Mason et al. 1988). β -Galactosidase activity was assayed using the substrate ONPG as described by Miller (1972). One unit of enzyme hydrolyzes 1 μmole of ONPG/min per A_{595} unit.

Immuno-electron microscopy

Strain SC1080 was subjected to increasing concentrations of chloramphenicol by growth on Luria broth agar plates containing progressively higher concentrations of chloramphenicol in increments of 10–25 $\mu\text{g/ml}$ to a final concentration of 200 $\mu\text{g/ml}$. A single colony of the high drug-resistance strain was dispersed in DS medium and spread on a DS plate containing 200 $\mu\text{g/ml}$ of drug. After overnight growth, the resulting lawn of cells was scraped into 5 ml of DS medium without chloramphenicol and used to inoculate 50 ml of DS medium. After incubation for 16 hr at 37°C without the drug, the culture was used to inoculate a second culture of DS medium, which was allowed to enter sporulation by growth and nutrient exhaustion at 37°C .

Cells (15 ml) at hour 8 of sporulation were collected by centrifugation and suspended in 1.0 ml of phosphate-buffered Karnofsky's fixative (Karnofsky 1965) and left at 4°C for 18 hr. The fixed cells were washed once in 0.5 M NH_4Cl , suspended in 2% agarose in water, and dehydrated by a progressive lowering of temperature as follows. Cells were sequentially maintained in 30% ethanol at 4°C for 30 min, 50% ethanol at -20°C for 2 hr, 70% ethanol at -35°C for 1 hr, and 100% ethanol for 1 hr, Lowicryl K4M/ethanol (1 : 1) at -35°C for 1 hr, and finally, twice in 100% Lowicryl K4M at -35°C for 1 hr. After adding fresh resin, polymerization was carried out with UV irradiation at -35°C in flat embedding molds covered with Saran Wrap overnight. After polymerization, the blocks were thin-sectioned (gold-silver sections) with a glass knife, and sections were placed on Formvar-coated nickel grids. The grids were placed on droplets of 1% glycine, 1% gelatin, for 30 min, and then onto a 1 : 25,000 dilution of rabbit anti- β -galactosidase antibodies (U.S. Biochemical) overnight in a hydrated chamber. Grids were washed five times by floating on droplets of 10 mM

Tris-HCl (pH 8.0), 0.1 mM EDTA, for 10 min, and treated with a 1 : 50 dilution of goat anti-rabbit antibodies conjugated to 5 nm gold particles (Bio-Rad) for 3 hr. After a second wash, the grids were stained as described previously (Francesconi et al. 1988).

Electron microscopy

Wild-type cells (strain PY79) and otherwise isogenic derivatives of PY79 bearing the mutations *spoIVCB23* (BK556) and *spoIVB::ermG* (BK750) were harvested 12 hr after suspension in SM medium. The cells were fixed and embedded as described previously (Rosenbluh et al. 1981), except that Spurr's resin was substituted for Vestopal and phosphate buffer (pH 6.7) was used throughout. Staining was by treatment with 1% uranyl acetate for 5 min and Reynold's lead (Hayat 1972) for 1 min.

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References

- 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.
- 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.
- 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.
- De Lencastre, H. and P. Piggot. 1979. Identification of different sites of expression for *spo* loci by transformation of *Bacillus subtilis*. *J. Gen. Microbiol.* **114**: 377–389.
- Dubnau, D. and R. Davidoff-Abelson. 1971. Fate of transforming DNA following uptake by competent *B. subtilis*. *J. Mol. Biol.* **56**: 209–221.
- Errington, J. and D. Jones. 1987. Cloning of *Bacillus subtilis* by transfection with bacteriophage vector $\phi 105J27$: Isolation and preliminary characterization of transducing phages for 23 sporulation loci. *J. Gen. Microbiol.* **133**: 493–502.
- Errington, J. and J. Mandelstam. 1986a. Use of a *lacZ* gene fusion to determine the dependence pattern of sporulation operon *spoIIA* in *spo* mutants of *Bacillus subtilis*. *J. Gen. Microbiol.* **132**: 2967–2976.
- . 1986b. Use of a *lacZ* fusion to determine the dependence pattern and the spore compartment expression of the sporulation operon *spoVA* in *spo* mutants of *Bacillus subtilis*. *J. Gen. Microbiol.* **132**: 2977–2985.
- 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.

- 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.
- 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.
- Hayat, M.A. 1972. *Basic electron microscopy techniques*. Van Nostrand Reinhold Co., New York.
- Illing, N. and J. Errington. 1990. The *spoIIA* locus is not a major determinant of prespore-specific gene expression during sporulation in *Bacillus subtilis*. *J. Bacteriol.* **172**: 6930–6936.
- Illing, N., M. Young, and J. Errington. 1990. Use of integrational plasmid excision to identify the cellular localization of gene expression during sporulation in *Bacillus subtilis*. *J. Bacteriol.* **172**: 6937–6941.
- Janniere, L., B. Niaudet, F. Pierre, and S.D. Ehrlich. 1985. Stable gene amplification in the chromosome of *Bacillus subtilis*. *Gene* **40**: 47–55.
- Karmazyn-Campelli, C., C. Bonamy, B. Savelli, and P. Stragier. 1989. Tandem genes encoding σ -factors for consecutive steps of development in *Bacillus subtilis*. *Genes & Dev.* **3**: 150–157.
- Karnofsky, M.J. 1965. A formaldehyde-glutaraldehyde fixative with high osmolarity for use in electron microscopy. *J. Cell Biol.* **27**: 137A–138A.
- 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.
- 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.
- 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.
- 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.
- 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.* **87**: 9722–9726.
- 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.
- Masuda, E.S., H. Anaguchi, K. Yamada, and Y. Kobayashi. 1988. Two developmental genes encoding sigma factor homologs are arranged in tandem in *Bacillus subtilis*. *Proc. Natl. Acad. Sci.* **85**: 7637–7641.
- Miller, J.H. 1972. *Experiments in molecular genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Nicholson, W.L., D. Sun, B. Setlow, and P. Setlow. 1989. Promoter specificity of σ^C -containing RNA polymerase from sporulating cells of *Bacillus subtilis*: Identification of a group of forespore-specific promoters. *J. Bacteriol.* **171**: 2708–2718.
- Panzer, S., R. Losick, D. Sun, and P. Setlow. 1989. Evidence for an additional temporal class of gene expression in the forespore compartment of sporulating *Bacillus subtilis*. *J. Bacteriol.* **171**: 561–564.
- Piggot, P.J. and J.G. Coote. 1976. Genetic aspects of bacterial endospore formation. *Bacteriol. Rev.* **40**: 908–962.
- Rather, P.N. and C.P. Moran Jr. 1988. Compartment-specific transcription in *Bacillus subtilis*: Identification of the promoter for *gdh*. *J. Bacteriol.* **170**: 5086–5092.
- Rosenbluh, A., C.D. Banner, R. Losick, and P.C. Fitz-James. 1981. Identification of a new developmental locus in *Bacillus subtilis* by construction of a deletion mutation in a cloned gene under sporulation control. *J. Bacteriol.* **148**: 341–351.
- 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.
- 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.
- Sato, T., Y. Samori, and Y. Kobayashi. 1990. DNA rearrangement during sporulation in *Bacillus subtilis*. *J. Bacteriol.* **172**: 1092–1098.
- Sterlini, J.M. and J. Mandelstam. 1969. Commitment to sporulation in *Bacillus subtilis* and its relationship to development of actinomycin resistance. *Biochem. J.* **113**: 29–37.
- Stevens, C.M. and J. Errington. 1990. Differential gene expression during sporulation in *Bacillus subtilis*: Structure and regulation of the *spoIIID* gene. *Mol. Microbiol.* **4**: 543–552.
- Stragier, P. and R. Losick. 1990. Cascades of sigma factors revisited. *Mol. Microbiol.* **4**: 1801–1806.
- 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.
- 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.
- Van Hoy, B.E. and J.A. Hoch. 1990. Characterization of the *spoIVB* and *recN* loci of *Bacillus subtilis*. *J. Bacteriol.* **172**: 1306–1311.
- Youngman, P.J., J.B. Perkins, and R. Losick. 1984. A novel method for rapid cloning in *Escherichia coli* of *Bacillus subtilis* chromosomal DNA adjacent to Tn917 insertions. *Mol. Gen. Genet.* **195**: 424–433.
- Zheng, L. and R. Losick. 1990. Cascade regulation of spore coat gene expression in *Bacillus subtilis*. *J. Mol. Biol.* **212**: 645–660.

Forespore-specific transcription of a gene in the signal transduction pathway that governs Pro-sigma K processing in *Bacillus subtilis*.

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