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Analysis of the Role of Prespore Gene Expression in the Compartmentalization of Mother Cell-Specific Gene Expression during Sporulation of *Bacillus subtilis*

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A hallmark of sporulation of *Bacillus subtilis* is the formation of two distinct cells by an asymmetric division. The developmental programs in these two cells involve the compartmentalized activities of σ^E in the larger mother cell and of σ^F in the smaller prespore. Activation of σ^E requires expression of the σ^F-directed gene *spoIIR*. By immunofluorescence microscopy of a strain containing a *spoIIR-lacZ* fusion, we have shown that *spoIIR* is transcribed exclusively in the prespore. By placing *spoIIR* under the control of P_{spoIIE}, it was possible to express *spoIIR* before the spore septum was formed. Strains containing the P_{spoIIE}-*spoIIR* construct activated σ^E only in the mother cell in organisms that underwent the asymmetric sporulation division. Thus, compartmentalization of σ^E activity did not require the compartmentalization of *spoIIR* expression. Nor did the compartmentalization of σ^E require SpoIIAA, SpoIIAB, σ^F, or σ^F-dependent transcription, all of which are required for prespore-specific gene expression. It is inferred that although σ^F and σ^E direct compartmentalized gene expression, neither of these σ factors, nor the genes under their control, directs the process of compartmentalization.

During an early stage of spore formation in bacilli and clostridia, an asymmetric division occurs, yielding two distinct cell types, the mother cell and the prespore (also called the forespore). Upon the formation of these two cell types, two different genetic programs are initiated. These two programs are transcriptionally controlled by the compartmentalized activities of a set of sigma factors (4, 21). Early transcription occurs in the mother cell through the action of RNA polymerase containing σ^E (E-σ^E) and in the prespore through the action of E-σ^F. The structural genes for the two sigma factors are transcribed and translated before the asymmetric (sporulation) division (6, 26) occurs, but the sigma factors do not become active until the formation of the asymmetric septum (1, 17). Both the activation and compartmentalization of σ^F in the prespore can occur in the absence of σ^E (2, 22). In contrast, the activation of σ^E requires the activity of σ^F (12, 34) through the activity of the σ^F-directed gene *spoIIR* (15, 20). It has been suggested by Losick and Stragier (21) that *spoIIR*, which was originally called X, would be transcribed by E-σ^F in the prespore and act as a vectorial cell-cell signal that would activate σ^E only in the mother cell. Thus, compartmentalization of *spoIIR* expression in the prespore could in itself lead to the compartmentalization of σ^E activity in the mother cell.

Consistent with SpoIIR acting as a cell-cell signal, characterization of the *spoIIR* gene indicated that it encoded a protein with a secretion signal (15), and recent biochemical analysis has shown that SpoIIR is secreted (10). Once secreted, SpoIIR could then interact with SpoIIGA, a multispanning membrane protein that has been proposed to be the protease that cleaves the pro sequence from pro-σ^E to activate it (14, 23, 34). However, because SpoIIR is secreted, as opposed to it being membrane bound, it may not act vectorially across the sporulation septum from the prespore membrane to the mother cell membrane. Rather, SpoIIR may be free to diffuse in the space between the membranes and to interact with SpoIIGA or other factors that might be in both the prespore and mother cell membranes. Thus, although SpoIIR clearly acts as the prespore signal required to activate transcription in the mother cell, another factor(s) may be required to control the vectorial nature of that signal. In this study, we have used immunofluorescence microscopy to show that, as expected from the known location of σ^F activity (8), *spoIIR* is expressed solely in the prespore but that its compartmentalization is not required for the compartmentalization of σ^E activity. Furthermore, no other σ^F-directed gene is required for the compartmentalization of σ^E activity. Thus, we conclude that compartmentalization must be controlled by the asymmetric segregation of a preexisting factor, or its activity, to one of the two cell types when they are formed.

MATERIALS AND METHODS

Strains. BR151, *Bacillus subtilis* 168 *trpC2 metB*10 *lys*-3, was used as the parent strain for all experiments. *Escherichia coli* DH5α (GIBCO/BRL, Gaithersburg, Md.) was used to maintain all plasmids. MLK778 contains pMLK217 (*spoIIR-lacZ*) that has recombined by a single crossover event at *spoIIR*. MLK528 contains pMLK151 (*cotE-lacZ*) that has recombined by a single crossover event at *cotE*. MLK1039 was created by transforming MLK528 with MLK947 chromosomal DNA and selecting for the Neo^r P_{spoIIE}-*spoIIR* construct. MLK947 was created by transforming MLK351 (BR151 containing an *erm* cassette in the *amyE* gene) with pMLK230 (containing P_{spoIIE}-*spoIIR* and *neo* inserted in *amyE*), selecting for Neo^r, and screening for double crossover events by Erm sensitivity. The strains containing P_{spoIIE}-*spoIIR* and the various sporulation mutations were created by first transforming the sporulation mutants *spoIIGA*49 (SL5565), *spoIIE*20 (SL547), *spoIIAC*63 (SL5040), and *spoIIR*::*erm* (MLK933) with chromosomal DNA isolated from MLK947. Chromosomal DNA was isolated from the resulting strains and used to transform MLK528, with subsequent selection for Neo^r (P_{spoIIE}-*spoIIR*) and screening for the transformation of the sporulation mutation, by congression, as determined by a stage II sporulation phenotype. The resulting strains were as follows: MLK1040, *spoIIGA*69 *cotE-lacZ* P_{spoIIE}-*spoIIR*; MLK1041, *spoIIE*20 *cotE-lacZ* P_{spoIIE}-*spoIIR*; MLK1042, *spoIIAC*63 *cotE-lacZ* P_{spoIIE}-*spoIIR*; and MLK1043, *spoIIR*::*erm cotE-lacZ* P_{spoIIE}-*spoIIR*. The *spoIIAA*42 strain carrying *cotE-lacZ* and P_{spoIIE}-*spoIIR*, MLK1053, was made by transforming SL5041 (BR151 with *spoIIAA*42) with chromosomal DNA of both MLK528 and MLK947 and selecting for Cm^r and Neo^r. Strain SL6680 was made by transforming SL5037 (BR151 with *spoIIA*Δ*4*, a deletion including *dacF*, the *spoIIA* operon, and part of *spoVA*) with chromosomal DNA from MLK1039, selecting for Cm^r, and screening for congression of Neo^r. SL6709 was

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FIG. 1. Localization of *spoIIR-lacZ* and *cotE-lacZ* expression by immunofluorescence microscopy. (A) Localization of *spoIIR-lacZ* in BR151 (MLK778) at $t_{3.5}$ of sporulation. (B) Localization of *cotE-lacZ* in BR151 (MLK528) at t_4 of sporulation. (C) Localization of *cotE-lacZ* in strain MLK1039 carrying the P_{spoIIE}-*spoIIR* construct at t_4 of sporulation. Cells were immunostained with fluorescein (yellow or green color). The white arrows indicate an organism showing prespore-specific immunofluorescence (A) and organisms showing mother cell-specific immunofluorescence (B and C). The cells were counterstained with propidium iodide (red color) to visualize nucleoids and to identify cells not detected by immunofluorescence. No fluorescein fluorescence was detected in bacteria lacking the *lacZ* fusion. Bar, 1.5 μm.

a result of transforming MLK1039 chromosomal DNA into a BR151 derivative in which *spoIIAA* and *spoIIAB* had been replaced by double recombination of linearized pLZ71 in the *spoIIA* region, selecting for Cm^r, and screening for β-galactosidase activity on sporulation agar containing X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside), as well as for a stage II sporulation phenotype.

Media. Modified Schaeffer's sporulation medium (28, 30) was used as an exhaustion medium to induce sporulation. Time is indicated in hours after the end of exponential growth (t_1, 1 h; t_2, 2 h; etc.). Sporulation agar consisted of Schaeffer's sporulation medium (30) solidified with 1.5% Difco Bacto agar.

Plasmids. The *spoIIR-lacZ* fusion plasmid, pMLK217, contains the 900-bp *Not*I-*Eco*RV fragment (which includes the *spoIIR* promoter) cloned via an intermediate vector as an *Eco*RI-*Bam*HI fragment into pJM783 (5) which had been digested with *Eco*RI and *Bam*HI. The *cotE-lacZ* fusion plasmid, pMLK151, contains the 400-bp *Eco*RI-*Bam*HI fragment from pCotEΔp2-*lacZ* (a gift of B. Beall and C. P. Moran, Jr.) (3) cloned into *Eco*RI-*Bam*HI-digested pJM783. The P_{spoIIE}-*spoIIR* construct plasmid pMLK230 was made as follows: pMLK83 (16) was digested with *Cla*I, the ends were filled in with the Klenow fragment of *E. coli* DNA polymerase I, the product was digested with *Hin*dIII, and then it was ligated with the *Sma*I-*Hin*dIII fragment of pGV49 (a gift of K. York and P. Youngman) (36) containing the *spoIIE* promoter. The resulting plasmid, pMLK179, was digested with *Hin*dIII, the ends were filled in with the Klenow fragment of *E. coli* DNA polymerase I, the product was digested with *Bam*HI, and then it was ligated with an 860-bp *Bsa*JI (ends made blunt with Klenow fragment)-*Bam*HI fragment containing the *spoIIR* open reading frame but lacking its promoter, creating pMLK230. pMLK227 (*spoIIR*::*erm*) was made by inserting an *erm* cassette into the *Hin*dIII site in the *spoIIR* gene in plasmid pMLK198, which was derived from pDIA5329 (29) which had been digested with *Xho*I and intramolecularly ligated, leaving the *Not*I-*Xho*I fragment containing *spoIIR* and flanking DNA. An intermediate plasmid, pMLK207, containing a *neo* cassette in the *Hin*dIII site of pMLK198 was digested with *Eco*RI and *Sma*I to remove the *neo* cassette. This was ligated with the *erm* cassette isolated from a derivative of pJPM15 (24) that was digested with *Sal*I; the ends were made blunt with the Klenow fragment, and the product was digested with *Eco*RI.

A *spoIIAA spoIIAB* replacement plasmid, pLZ71, was made from pPP22. Plasmid pPP22 consists of the large *Hin*dIII fragment of pHM2 (19) which has self-ligated; it contains *dacF*, *spoIIAA*, and *spoIIAB*. It was digested with *Bst*XI, the ends were made blunt with the Klenow fragment, and the product was digested with *Bgl*II. This digestion removed the 3′ end of *dacF*, the *spoIIA* promoter, *spoIIAA*, and the 5′ portion of *spoIIAB*. The removed fragment was replaced with the *Sph*I-*Bam*HI-isolated *neo* cassette from pBEST502, with the *Sph*I end made blunt by the Klenow fragment. The *neo* gene was oriented in the direction opposite *spoIIAC*. The resulting plasmid, pLZ71, was linearized before being used to transform strain BR151 to Neo^r, causing a disruption of *spoIIAA* and *spoIIAB* by double recombination.

β-Galactosidase assays. β-Galactosidase assays were performed essentially as described by Nicholson and Setlow (25), using lysozyme to permeabilize the cells.

Immunofluorescence study. Immunofluorescence was attained as described by Harry et al. (8), using rabbit polyclonal anti-β-galactosidase antibody (5 Prime-3 Prime, Inc., Boulder, Colo.) that had been preabsorbed to wild-type BR151, as well as anti-rabbit fluorescein isothiocyanate-conjugated antibody (Jackson Laboratories, Bar Harbor, Maine). Propidium iodide was used to visualize nucleoids, and hence to determine the number of cells in a field, as well as to identify their stage of sporulation (8, 18, 32); organisms that have formed the sporulation division septum display an asymmetric nucleoid distribution. Other microscopy techniques were essentially as described by Bylund et al. (2). Quantitation was done by direct observation with a microscope. Photographs were obtained with Kodak Ektachrome ASA 400 film.

RESULTS AND DISCUSSION

Location of *spoIIR* expression. To determine the location of *spoIIR* expression, we have employed immunofluorescence microscopy (8) to detect β-galactosidase protein expressed from a *spoIIR-lacZ* transcriptional fusion inserted at the *spoIIR* locus. As shown in Fig. 1A, *spoIIR-lacZ* was expressed only in the prespore. In a similar study of a strain containing a *cotE-lacZ* fusion, the latter being an E-σ^E-transcribed gene (3), β-galactosidase was confined to the mother cell in all expressing organisms (Fig. 1B), which is in agreement with the results of Harry et al. for σ^E-transcribed genes (8). Since the *spoIIR* locus is transcribed only by Eσ^F (15, 20), the results for *spoIIR-lacZ* confirm the conclusion that σ^F is only active in the prespore (8, 18, 22) and that *spoIIR* acts from the prespore to activate σ^E in the mother cell (15, 20, 21).

The role of SpoIIR in the compartmentalization of σ^E activity. To test whether compartmentalized *spoIIR* expression was necessary for the compartmentalization of σ^E activity, we removed *spoIIR* from the control of σ^F. To do this, we placed *spoIIR* under the control of the *spoIIE* promoter. We chose the *spoIIE* promoter because it is expressed before septation (1, 6, 26) and its expression is not compartmentalized (our unpublished results). Furthermore, it is the latest-expressed promoter of the preseptation class (16); thus, the timing of *spoIIR* expression would be the least disturbed. The P_{spoIIE}-*spoIIR* construct was inserted at the *amyE* locus in both spo^+ and *spoIIR*::*erm* mutant bacteria containing *cotE-lacZ* fusions, creating strains MLK1039 and MLK1043, respectively. As shown in Fig. 2, *cotE-lacZ* is expressed in both strains in a pattern similar to *cotE-lacZ* expression in the parental strain, in which *spoIIR* is solely under the control of its endogenous promoter, although to a slightly higher level than in the parental strain. Furthermore, MLK1043 and MLK1039 produced similar numbers of heat-resistant spores, although at a lower frequency than the parental strain: 69, 58, and 56% sporulation (in different experiments) for the parental strain; 46, 37, and 21% for MLK1039; and 31, 29, and 20% for MLK1043. In the presence of a *spoIIGA* mutation, no *cotE-lacZ* expression was detected (Fig. 2), indicating that uncompartmentalized SpoIIR was acting in a wild-type manner, through SpoIIGA, to activate σ^E. These results indicated that the P_{spoIIE}-*spoIIR* construct produced a *spoIIR* product that was able to activate σ^E and efficiently complement *spoIIR*::*erm*.

To determine the effect of the P_{spoIIE}-*spoIIR* construct on the compartmentalization of σ^E activity, we assayed the localization of *cotE-lacZ* in strain MLK1039 by immunofluores-

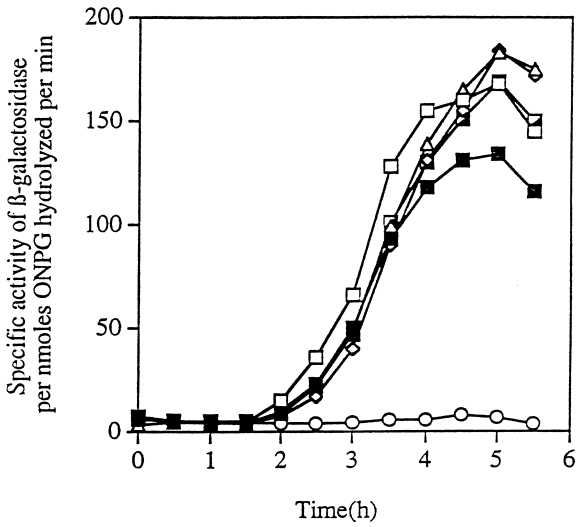

FIG. 2. Expression of *cotE-lacZ* in strains containing the P_{spoIIE}-*spoIIR* construct during sporulation. β-Galactosidase activities from the *cotE-lacZ* fusions are shown for the following strains: ■, wild-type BR151 (MLK528); □, BR151 with P_{spoIIE}-*spoIIR* (MLK1039); ◪, *spoIIR*::*erm* with P_{spoIIE}-*spoIIR* (MLK1043); ○, *spoIIGA*49 with P_{spoIIE}-*spoIIR* (MLK1040); ◇, *spoIIAC*63 with P_{spoIIE}-*spoIIR* (MLK1042); △, *spoIIE*20 with P_{spoIIE}-*spoIIR* (MLK1041). β-Galactosidase levels for MLK1040 were equal to those of BR151 without a *lacZ* fusion. ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

cence microscopy. The cells were also counterstained with propidium iodide to determine the nucleoid distribution and thus the stage of sporulation for each cell (8, 18, 32). β-Galactosidase-specific immunofluorescence was detected from t_2, and by t_5, 80% of the cells displayed *cotE-lacZ* expression (Table 1 and Fig. 1C). There were two populations exhibiting β-galactosidase expression: (i) organisms exhibiting an asymmetric nucleoid pattern typical of stages II to III of sporulation (8, 32) and mother cell-specific β-galactosidase expression, and (ii) organisms exhibiting a symmetric nucleoid pattern and whole-cell β-galactosidase expression. Similar results were obtained with MLK1043 (Table 1), as well as with another E-σ^E-transcribed gene, *spoIID* (data not shown). In contrast, only one type of *cotE-lacZ* expression pattern was seen in the parental strain, in which *spoIIR* is controlled by its endogenous σ^F promoter: an asymmetric nucleoid pattern with mother cell-specific expression (8) (Table 1 and Fig. 1B).

The P_{spoIIE}-*spoIIR* construct appears to cause a block in the asymmetric sporulation division in a portion of the cells, as indicated by the asymmetric nucleoid frequencies (Table 1). This block is most likely the consequence of activating σ^E prior to septation, as shown previously (7). The presence of two populations could indicate that *spoIIE* expression overlaps a critical time for the initiation of septation. If some cells in the population express *spoIIE*, and thus P_{spoIIE}-*spoIIR*, earlier than this critical time, then σ^E would be activated prematurely, causing a block in septation and hence in sporulation; however, if P_{spoIIE}-*spoIIR* is expressed in other cells in the population after this critical time, then the asymmetric septum can form and sporulation can proceed normally. This block in asymmetric septation in a portion of the *cotE*-expressing cells would explain why the strains containing the P_{spoIIE}-*spoIIR* construct, MLK1039 and MLK1043, produced fewer spores than did the parental strain that lacked the construct, MLK528. The results indicate that the compartmentalization of *spoIIR* expression is not required for the compartmentalization of σ^E activity. Rather, we propose that *spoIIR* is under the control of σ^F to ensure that it cannot activate σ^E before the formation of the asymmetric septum, not to determine the mother cell specificity of σ^E activity.

The role of prespore-specific gene expression, σ^F, SpoIIAA, and SpoIIAB in the compartmentalization of σ^E activity. To determine if a factor(s) required for the compartmentalization of σ^E activity is encoded by σ^F-directed genes, we introduced a point mutation in the *spoIIAC* gene, which encodes σ^F. We also introduced mutations in the *spoIIAA* and *spoIIAB* genes, which encode a positive and a negative regulator of σ^F-compartmentalized activation, respectively (31), to determine if the encoded proteins might have a role independent of σ^F in the activation and/or compartmentalization of σ^E. The *spoIIAC*63 and *spoIIAA*42 point mutations, as well as deletions covering both the *spoIIAA* and *spoIIAB* genes or the entire *spoIIA* operon, were analyzed in the MLK1039 background. All the strains showed behavior similar to that of MLK1039, although a smaller proportion of organisms had asymmetric nucleoids and the corresponding mother cell-specific β-galactosidase expression (representative data are shown in Table 1). Thus, mutational loss of σ^F, SpoIIAA, or SpoIIAB did not prevent the compartmentalization of σ^E activity into the mother cell of those organisms that were able to form sporulation septa. This indicates that none of these three proteins, nor any E-σ^F-transcribed gene, is required for σ^E compartmentalization. Since σ^G and σ^F have some overlapping activity, we introduced a deletion in the *spoIIIG* gene, which encodes σ^G, into the *spoIIA* deletion strains. The resulting strains maintained the same pattern of immunofluorescence as their parent strains (data not shown), which is again consistent with compartmentalization of σ^E activity occurring independently of prespore-specific gene expression.

The role of SpoIIE in σ^E activation. Finally, we attempted to determine the role of SpoIIE in σ^E activation and compartmentalization. SpoIIE is required for σ^E activation during sporulation (22) but not during exponential (vegetative)

TABLE 1. Immunofluorescence staining pattern of bacteria containing a *cotE-lacZ* fusion[a]

Strain	Relevant genotype[b]	Organisms with asymmetric nucleoids (%)[c]	Nucleoid class[d]	No. of organisms showing the indicated immunofluorescent pattern[e]			
				Whole cell	Mother cell	Pre-spore	No fluorescence
MLK528	*spo*$^+$	71	S	0	0	0	250
			A	0	148	0	6
MLK1039	*spo*$^+$	49	S	138	0	0	42
			A	0	125	0	25
MLK1041	*spoIIE20*	0	S	105	0	0	47
MLK1042	*spoIIAC63*	39	S	139	0	0	21
			A	0	114	0	14
MLK1043	*spoIIR*::*erm*	53	S	135	0	0	30
			A	0	125	0	17
SL6680	*spoIIA*Δ*4*	42	S	112	0	0	9
			A	0	98	0	12
SL6709	*spoIIA*::*neo*	40	S	121	0	0	13
			A	2	113	0	8

[a] Samples were taken at t_5. Similar results were obtained at t_4, although a smaller proportion of organisms displayed immunofluorescence and fewer had asymmetric nucleoids.

[b] All strains contained P_{spoIIE}-*spoIIR* except MLK528.

[c] The percentage was determined by counting at least 200 propidium iodide-stained cells.

[d] S, symmetric nucleoids; A, asymmetric nucleoids.

[e] The immunofluorescence patterns were analyzed separately for the A and S nucleoid classes, and the numbers of A and S organisms analyzed do not correspond exactly to their proportions in the population.

growth (20). The morphology of *spoIIE* mutants indicates that SpoIIE could play a role in determining the structure of the sporulation septum (11, 27) and thus could affect the ability of SpoIIR to act as a cell-to-cell signal for σ^E activation. Previously, it was not possible to determine SpoIIE's role in σ^E activation during sporulation, because SpoIIE is required for σ^F activation (22). However, with *spoIIR* expression being independent of σ^F, we could separate SpoIIE's role in σ^F activation from any potential role it played in σ^E activation. We found that the introduction of the *spoIIE20* mutation into MLK1039 had no effect on *cotE-lacZ* expression (strain MLK1041, Fig. 2), indicating that SpoIIE was not required for σ^E activation in sporulation conditions. Interestingly, none of the bacteria produced asymmetric nucleoids, and *cotE-lacZ* expression was not compartmentalized. We infer that the sporulation septum was not formed by MLK1041. This inference is supported by the observation that MLK1041 did not form abortively disporic cells, which are diagnostic of *spoIIE* and other *spoII* mutants that complete the sporulation division (27). Although this failure to septate prevented us from testing for a role in compartmentalization, it is potentially an interesting observation with regard to the function of SpoIIE in the formation of the sporulation septum. For example, it could indicate that SpoIIE is required for efficient initiation of sporulation septum formation; in the absence of SpoIIE, the P_{spoIIE}-*spoIIR* construct could activate σ^E prior to the critical time for septation in 100% of cells and thus block septation in 100% of cells.

In summary, we have shown that compartmentalization of σ^E activity does not require the compartmentalization of *spoIIR* expression. Nor does it require SpoIIAA, SpoIIAB, σ^F, or any E-σ^F-transcribed gene. Previously, we showed that σ^E itself is not required for the compartmentalization of σ^F activity (2). Thus, we can infer that although σ^F and σ^E direct compartmentalized gene expression, neither directs the process of compartmentalization. Rather, it appears that properties inherent to the two cell types must control the compartmentalization of σ^E activity. What those properties are is the subject of speculation (see, for example, references 9, 22, 33, and 35). A prespore-specific protease that degrades active σ^E could be activated upon prespore formation; activated σ^E has been shown to be particularly sensitive to proteolysis (13). A factor that is required for σ^E activation could be localized to the mother cell; for example, SpoIIGA might have a polar orientation within the sporulation septum (9) that confines its pro-σ^E processing activity to the mother cell.

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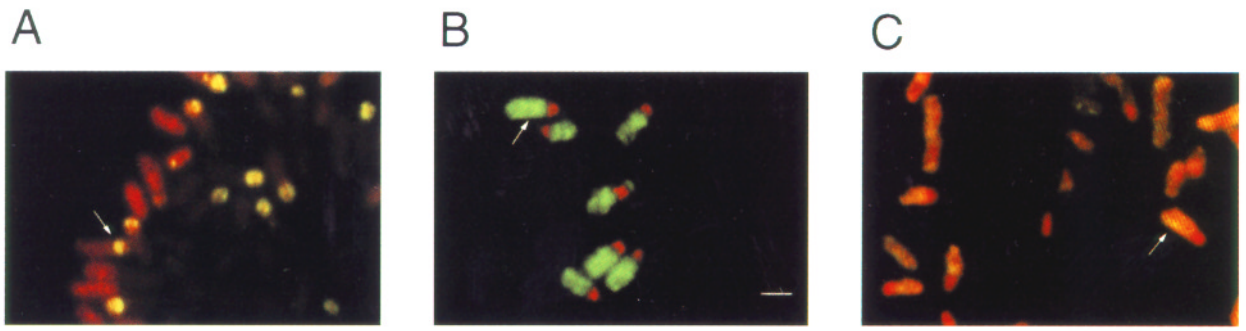
REFERENCES

1. **Beall, B., and J. Lutkenhaus.** 1991. FtsZ in *Bacillus subtilis* is required for vegetative septation and for asymmetric septation during sporulation. Genes Dev. **5:**447–455.
2. **Bylund, J. E., L. Zhang, M. A. Haines, M. L. Higgins, and P. J. Piggot.** 1994. Analysis by fluorescence microscopy of the development of compartment-specific gene expression during sporulation of *Bacillus subtilis*. J. Bacteriol. **176:**2898–2905.
3. **Diederich, B., K. M. Tatti, C. H. Jones, B. Beall, and C. P. Moran, Jr.** 1992. Genetic suppression analysis of σ^E interaction with three promoters in sporulating *Bacillus subtilis*. Gene **121:**63–69.
4. **Errington, J.** 1993. *Bacillus subtilis* sporulation: regulation of gene expression and control of morphogenesis. Microbiol. Rev. **57:**1–33.
5. **Ferrari, E., D. J. Henner, M. Perego, and J. A. Hoch.** 1988. Transcription of *Bacillus subtilis* subtilisin and expression of subtilisin in sporulation mutants. J. Bacteriol. **170:**289–295.
6. **Gholamhoseinian, A., and P. J. Piggot.** 1989. Timing of *spoII* gene expression relative to septum formation during sporulation of *Bacillus subtilis*. J. Bacteriol. **171:**5747–5749.
7. **Haldenwang, W. G.** 1995. The sigma factors of *Bacillus subtilis*. Microbiol. Rev. **59:**1–30.
8. **Harry, E. J., K. Pogliano, and R. Losick.** 1995. Use of immunofluorescence to visualize cell-specific gene expression during sporulation in *Bacillus subtilis*. J. Bacteriol. **177:**3386–3393.
9. **Higgins, M. L., and P. J. Piggot.** 1992. Septal membrane fusion—a pivotal event in bacterial spore formation? Mol. Microbiol. **6:**2565–2571.
10. **Hofmeister, A. E. M., A. Londoño-Vallejo, E. Harry, P. Stragier, and R. Losick.** 1995. Extracellular signal protein triggering the proteolytic activation of a developmental transcription factor in B. subtilis. Cell **83:**219–226.
11. **Illing, N., and J. Errington.** 1991. Genetic regulation of morphogenesis in *Bacillus subtilis*: roles of σ^E and σ^F in prespore engulfment. J. Bacteriol. **173:**3159–3169.
12. **Jonas, R. M., and W. G. Haldenwang.** 1989. Influence of *spo* mutations on σ^E synthesis in *Bacillus subtilis*. J. Bacteriol. **171:**5226–5228.
13. **Jonas, R. M., H. K. Peters III, and W. G. Haldenwang.** 1990. Phenotypes of *Bacillus subtilis* mutants altered in the precursor-specific region of σ^E. J. Bacteriol. **172:**4178–4186.
14. **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.
15. **Karow, M. L., P. Glaser, and P. J. Piggot.** 1995. Identification of a gene, *spoIIR*, that links the activation of σ^E to the transcriptional activity of σ^F during sporulation in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **92:**2012–2016.
16. **Karow, M. L., and P. J. Piggot.** 1995. Construction of *gusA* transcriptional fusion vectors for *Bacillus subtilis* and their utilization for studies of spore formation. Gene **163:**69–74.
17. **Levin, P. A., and R. Losick.** 1994. Characterization of a cell division gene from *Bacillus subtilis* that is required for vegetative and sporulation septum formation. J. Bacteriol. **176:**1451–1459.
18. **Lewis, P. J., S. R. Partridge, and J. Errington.** 1994. σ factors, asymmetry, and the determination of cell fate in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **91:**3849–3853.
19. **Liu, H. M., K. F. Chak, and P. J. Piggot.** 1982. Isolation and characterisation of a recombinant plasmid carrying a functional part of the *Bacillus subtilis spoIIA* locus. J. Gen. Microbiol. **128:**2805–2812.
20. **Londoño-Vallejo, J.-A., and P. Stragier.** 1995. Cell-cell signaling pathway activating a developmental transcription factor in *Bacillus subtilis*. Genes Dev. **9:**503–508.
21. **Losick, R., and P. Stragier.** 1992. Crisscross regulation of cell-type-specific gene expression during development in *B. subtilis*. Nature (London) **355:**601–604.
22. **Margolis, P., A. Driks, and R. Losick.** 1991. Establishment of cell type by compartmentalized activation of a transcription factor. Science **254:**562–565.
23. **Masuda, E. S., H. Anaguchi, T. Sato, M. Takeuchi, and Y. Kobayashi.** 1990. Nucleotide sequence of the sporulation gene *spoIIGA* from *Bacillus subtilis*. Nucleic Acids Res. **18:**657.
24. **Mueller, J. P., G. Bukusoglu, and A. L. Sonenshein.** 1992. Transcriptional regulation of *Bacillus subtilis* glucose starvation-inducible genes: control of *gsiA* by the ComP-ComA signal transduction system. J. Bacteriol. **174:**4361–4373.
25. **Nicholson, W. L., and P. Setlow.** 1990. Sporulation, germination and outgrowth, p. 391–429. *In* C. R. Harwood and S. M. Cutting (ed.), Molecular biological methods for *Bacillus*. John Wiley and Sons, Ltd., West Sussex, England.
26. **Partridge, S. R., and J. Errington.** 1993. The importance of morphological events and intercellular interactions in the regulation of prespore-specific gene expression during sporulation in *Bacillus subtilis*. Mol. Microbiol. **8:**945–955.
27. **Piggot, P. J., and J. G. Coote.** 1976. Genetic aspects of bacterial endospore formation. Bacteriol. Rev. **40:**908–962.
28. **Piggot, P. J., and C. A. M. Curtis.** 1987. Analysis of the regulation of gene expression during *Bacillus subtilis* sporulation by manipulation of the copy number of *spo-lacZ* fusions. J. Bacteriol. **169:**1260–1266.
29. **Santana, M., M. S. Ionescu, A. Vertes, R. Longin, F. Kunst, A. Danchin, and P. Glaser.** 1994. *Bacillus subtilis* F_0F_1 ATPase: DNA sequence of the *atp* operon and characterization of *atp* mutants. J. Bacteriol. **176:**6802–6811.
30. **Schaeffer, P., J. Millet, and J.-P. Aubert.** 1965. Catabolic repression of bacterial sporulation. Proc. Natl. Acad. Sci. USA **54:**704–711.
31. **Schmidt, R., P. Margolis, L. Duncan, R. Coppolecchia, C. P. Moran, Jr., and R. Losick.** 1990. Control of developmental transcription factor σ^F by sporulation regulatory proteins SpoIIAA and SpoIIAB in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **87:**9221–9225.
32. **Setlow, B., N. Magill, P. Febroriello, L. Nakhimovsky, D. E. Koppel, and P.**

Setlow. 1991. Condensation of the forespore nucleoid early in sporulation of *Bacillus* species. J. Bacteriol. **173:**6270–6278.

33. **Stragier, P.** 1989. Temporal and spatial control of gene expression during sporulation: from facts to speculations, p. 243–254. *In* I. Smith, R. A. Slepecky, and P. Setlow (ed.), Regulation of procaryotic development: structural and functional analysis of bacterial sporulation and germination. American Society for Microbiology, Washington, D.C.
34. **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.
35. **Stragier, P., P. Margolis, and R. Losick.** 1994. Establishment of compartment-specific gene expression during sporulation in *Bacillus subtilis*, p. 139–154. *In* P. J. Piggot, C. P. Moran, Jr., and P. Youngman (ed.), Regulation of bacterial differentiation. American Society for Microbiology, Washington, D.C.
36. **York, K., T. J. Kenney, S. Satola, C. P. Moran, Jr., H. Poth, and P. Youngman.** 1992. Spo0A controls the σ^A-dependent activation of *Bacillus subtilis* sporulation-specific transcription unit *spoIIE*. J. Bacteriol. **174:**2648–2658.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