Structure and function of the *spoIIIJ* gene of *Bacillus subtilis*: a vegetatively expressed gene that is essential for σ^G activity at an intermediate stage of sporulation

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The *spo-87* mutation is one of two sporulation mutations originally used to define the *spo0J* locus of *Bacillus subtilis*. We now show that it blocks sporulation after completion of prespore engulfment (stage III). Surprisingly, the operon is expressed vegetatively, probably from a σ^A -dependent promoter, and its expression is shut down at the transcriptional level at about the onset of sporulation. DNA sequencing reveals that the locus defined by *spo-87*, which we now designate *spoIIIJ*, consists of a bicistronic operon. However, only the first gene is essential for sporulation; the function of the second cistron is cryptic. The predicted SpoIIIJ product has an M_r of 29409. It probably forms a lipoprotein and is rich in basic and hydrophobic amino acids. Mutations in *spoIIIJ* abolish the transcription of prespore-specific genes transcribed by the σ^G form of RNA polymerase but not transcription of the *spoIIIG* gene encoding σ^G . The SpoIIIJ product could be involved in a signal transduction pathway coupling gene expression in the prespore to events in the mother cell, or it could be necessary for essential metabolic interactions between the two cells.

Introduction

Sporulation in *Bacillus subtilis* is a simple developmental system involving the differentiation of two cells known as the prespore and the mother cell. About 50 genes involved in the regulation of sporulation have been defined by *spo* mutations, which block sporulation but have no observable effect on vegetative growth (Piggot & Coote, 1976; Losick *et al.*, 1986). Most of the *spo* genes have now been cloned and characterized, and their patterns of interaction have been partially elucidated (reviewed by Errington, 1992). It is clear that different programmes of gene expression occur in the prespore and mother cell from the time of their separation onwards (Errington & Illing, 1992; Losick & Stragier, 1992).

The *spo-87* mutation abolishes the expression of genes

in both the prespore and the mother cell from about the second hour of sporulation (Kunkel *et al.*, 1988; Sandman *et al.*, 1988; see below). The prespore-specific genes that are blocked are dependent on the σ^G form of RNA polymerase, which is synthesized specifically in the prespore from the *spoIIIG* gene (Sun *et al.*, 1989; Karmazyn-Campelli *et al.*, 1989). The mother-cell-specific genes that are blocked are dependent on the σ^K form of RNA polymerase (Kroos *et al.*, 1989). The effects of *spo-87* on σ^K -dependent gene expression are probably indirect, being mediated by a mechanism that couples the formation of active σ^K to gene expression in the prespore (Cutting *et al.*, 1990, 1991). In contrast, events associated with the first two hours of sporulation, up to about the stage of prespore engulfment (stage III), are unperturbed by the *spo-87* mutation.

It was originally thought that *spo-87* was a *spo0* mutation, both because it appeared to affect the early stages of morphological development and because genetic mapping seemed to place it in the same locus, *spo0J*, as the *spo-93* mutation (Hranueli *et al.*, 1974), which, on the basis of its effects on gene expression, is clearly a *spo0* mutation (e.g. Errington & Mandelstam,

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The nucleotide sequence data reported in this paper have been submitted to the EMBL Data Library and have been assigned the accession number Z14225.

Table 1. *Bacterial strains, plasmids and bacteriophages*

Strain	Description	Source or reference
<i>B. subtilis</i>		
168	<i>trpC2</i> Spo ⁺	Laboratory stock
CU267	<i>trpC2 ilvB2 leuB16</i> Spo ⁺	S. A. Zahler*
SG38	<i>trpC2</i> Spo ⁺	Errington & Mandelstam (1986a)
1.5	<i>trpC2 spoIIAC1</i>	Errington & Mandelstam (1986a)
17.4	<i>trpC2 spo0H17†</i>	Errington & Mandelstam (1986a)
43.6	<i>trpC2 spo0A43</i>	Errington & Mandelstam (1986a)
55.3	<i>trpC2 spoIIGB55</i>	Errington & Mandelstam (1986a)
87.4	<i>trpC2 spoIIJ87†</i>	Hranueli <i>et al.</i> (1974)
713	<i>trpC2</i> Ω (<i>spoIVA'</i> - <i>lacZ cat</i>)713	Stevens <i>et al.</i> (1992)
727	<i>trpC2</i> Ω (<i>spoIIJ::ermC</i>)727	This paper
729	<i>trpC2</i> Ω (<i>jag::ermC</i>)729	This paper
Plasmids		
pE194	<i>ermC</i>	Horinouchi & Weisblum (1982)
pSG2	<i>bla cat</i>	Fort & Errington (1985)
pSG28	<i>bla cat lacZ</i>	Errington (1986)
pSG139	<i>bla cat spoIIIG'-lacZ</i>	Foulger & Errington (1989)
pSG250	<i>bla ermC</i>	D. Foulger, unpublished
pUC18	<i>bla</i>	Yanisch-Peron <i>et al.</i> (1985)
Bacteriophages		
ϕ 105J20	<i>spoVA'-lacZ cat</i>	Errington & Mandelstam (1986b)
ϕ 105J49	<i>spoIIAA'-lacZ cat</i>	S.R.P. & J.E., unpublished
ϕ 105J93	<i>spoIIJ</i> ⁺	Errington & Jones (1987)

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† Strain made isogenic with SG38 by transformation of strain CU267 as described by Errington & Mandelstam (1986a).

1986a; Guzman *et al.*, 1988). More recently, however, cloning and complementation experiments have shown that *spo-87* and *spo-93* lie in separate loci (Errington & Jones, 1987). Conflicting results have been obtained by electron microscopical studies of *spo-87* mutants. Hranueli *et al.* (1974) and Clarke & Mandelstam (1987) concluded that the mutant was blocked before the formation of the spore septum (i.e. at stage 0), whereas Ryter (unpublished results cited by Stragier *et al.*, 1988) stated that it was blocked at stage III. Here we confirm that the *spoIIJ* mutation allows prespore engulfment to occur, in accordance with the results of Ryter, and hence propose that the gene defined by *spo-87* be redesignated *spoIIJ*, to distinguish it from the *spo0J* gene defined by *spo-93*.

We have also physically characterized the *spoIIJ* locus. Disruption of the gene blocks the expression of late sporulation genes but has no observable effect on vegetative growth. A downstream gene, which is probably coordinately regulated, is not essential for either vegetative growth or sporulation. Surprisingly, although *spoIIJ* is required only after engulfment, apparently for the appearance of σ^G activity, the gene is expressed in vegetative cells and turned off at the onset of sporulation.

Methods

Bacterial strains, plasmids and bacteriophages. These are listed in Table 1, except for newly constructed plasmids, which are illustrated in Fig. 3. Plasmids pP5, pH27, pH28 and pSX3 contain fragments of DNA from the insert in phage ϕ 105J93 subcloned in pSG2. Plasmids pSG173 and pSG1020 were constructed as follows. The 543 bp *Bcl*I-*Sst*I fragment from ϕ 105J93 was subcloned into pUC18 as an *Sst*I fragment, there being an *Sst*I site in the polylinker of ϕ 105J93 close to the *Bam*HI/*Bcl*I hybrid site at the end of the insertion in the phage. This resulted in a plasmid pUC18BS from which the same fragment of *spoIIJ* DNA could be removed by digestion with *Sma*I (there being a *Sma*I site adjacent to the left end of the insert in ϕ 105J93, and one in the polylinker of pUC18). This *Sma*I fragment was then cloned into pSG28 to construct a transcriptional *lacZ* fusion. Plasmid pSG173 has the insertion in the opposite orientation. Plasmid pSG250 contains the *Sst*I-*Cla*I fragment of pE194, containing the *ermC* gene cloned into *Sst*I/*Acc*I-digested pUC18. The insertion occurred in an unusual manner such that the *ermC* fragment was inverted and is flanked by almost completely duplicated polylinker sequences. The *ermC* gene can therefore be released by digestion with several enzymes that cut in the polylinker: from *Sst*I to *Xba*I inclusive (D. Foulger, unpublished results).

Construction of erm insertions in the spoIIJ and jag genes. Plasmids pUC18BS, pP5 and pSG250 were digested with *Sst*I to completion, then extracted with phenol and precipitated with ethanol. The redissolved DNA samples were mixed in the ratio 3:3:1, to give a total

of 0.5 µg DNA and added to a 10 µl ligation reaction. The ligation products were transformed into *B. subtilis* strain 168 with selection for the *erm* determinant from pSG250, on Oxoid nutrient agar containing 1 µg erythromycin ml⁻¹ and 25 µg lincomycin ml⁻¹. *Spo*⁻ transformants that were stable during repeated subculture in the absence of selection, and complemented by phage ϕ105J93, were checked by Southern hybridization for the expected chromosomal insertion of the *erm* gene. One such strain was isolated and designated strain 727.

To make an *erm* insertion in the *jag* gene, the *Taq*I fragment containing the *erm* gene from pE194 (see Horinouchi & Weisblum, 1982) was cloned into the unique *Pst*I site of pH28, after first blunting the ends of both DNA fragments by treatment with the Klenow fragment of DNA polymerase. The resultant plasmid was transformed into strain 168 with selection for erythromycin resistance as described above. A chloramphenicol sensitive transformant was checked for the expected chromosomal insertion by Southern hybridization, and designated strain 729.

In both cases the orientation of the *erm* gene is the same as that of *spoIIIJ* and *jag*.

Media and induction of sporulation. Cells were induced to sporulate by the resuspension method of Sterlini & Mandelstam (1969). Nutrient exhaustion experiments were done by growth in Difco sporulation medium (DSM; Schaeffer *et al.*, 1965).

Electron microscopy. Cells were fixed, sectioned and visualized as described previously (Illing & Errington, 1991*b*).

DNA sequencing. Fragments of the DNA originally cloned in ϕ105J93 were subcloned and sequenced (both strands) by the chain-termination method (Sanger *et al.*, 1977).

Isolation of RNA and primer extension analysis. RNA was isolated by the rapid procedure described previously (Foulger & Errington, 1989). An oligonucleotide, 5'-TCACACTCGAGCATCCAGCC-3', complementary to the *spoIIIJ* mRNA was used for the primer extension reaction, which was carried out with AMV reverse transcriptase (Pharmacia) essentially as described by Moran (1990). The sequencing ladder was generated by an oligonucleotide complementary to a segment of DNA from the *murE* gene of *B. subtilis* (R.D. & J.E., unpublished results).

***lacZ* fusion analysis.** Plasmids pSG173 and pSG1020 were transformed into *B. subtilis* by selection for chloramphenicol resistance. Purified transformants were induced to sporulate and assayed for β-galactosidase activity as described previously (Errington & Mandelstam, 1986*a*), except that lysozyme was used to permeabilize the cells (Mason *et al.*, 1988).

Results and Discussion

Ultrastructure of the *spo-87* mutant

In view of the conflicting reports on the phenotype of the *spo-87* mutant we induced strain 87.4 to sporulate and harvested the cells at *t*₅ for examination by electron microscopy. The majority of the cells (>60%) appeared to have completed engulfment (stage III) at this time. Other cells contained completed asymmetric septa or were in the process of engulfment. The prespores in cells that had reached stage III had a very indistinct appearance, similar to that seen transiently in wild-type cells that have just completed engulfment but not yet

initiated cortex synthesis (J.E., unpublished observations). Examples of cells in these various states are shown in Fig. 1. The indistinct appearance of the prespores of the *spo-87* mutant probably accounts for its earlier classification as a *spo0* mutant (Hranueli *et al.*, 1974).

We conclude that the *spo-87* mutation causes sporulation to be arrested at stage III, in accordance with the results of Ryter (cited by Stragier *et al.*, 1988). In view of the fact that the mutation is now known to lie in a distinct locus from the *spo-93* mutation (Mysliwiec *et al.*, 1991), we propose to redesignate the locus defined by *spo-87* as *spoIIIJ*, whilst retaining the *spo0J* designation for *spo-93*. We have passed over the next available *spoIII* designation *spoIIIH* in order to retain the suffix 'J'.

Effect of a null mutation in the *spoIIIJ* gene on expression of other sporulation genes

To define the role of the *spoIIIJ* gene in the expression of other sporulation genes, we introduced several *spo-lacZ* fusions into a newly constructed *spoIIIJ::ermC* insertional mutant (strain 727). We then induced the strains to sporulate and compared the levels of β-galactosidase activity in isogenic mutant and wild-type strains (Fig. 2). We also measured alkaline phosphatase activity, which is a marker for early mother-cell-specific gene expression (Akrigg & Mandelstam, 1978; Errington & Illing, 1992). As expected (Clarke & Mandelstam, 1987), alkaline phosphatase synthesis was not blocked by the *spoIIIJ::ermC* mutation, so the enzyme activity provided a control showing that sporulation was induced normally in each of the strains. Inactivation of *spoIIIJ* had little effect on expression of *spoIIAA-lacZ* (Fig. 2*a*) or *spoIVA-lacZ* fusions (not shown), as expected for genes expressed before stage III. It reduced expression of the prespore-specific genes *spoIIIG* and *spoVA* about twofold and 10-fold respectively (Fig. 2*b* and *c*). Similar results have been obtained with the *spoIIIJ87* mutation (S. Maguire & J. Errington, unpublished results). Thus, *spoIIIJ* mutations seem to affect the expression of prespore-specific genes but not early mother-cell-specific genes (see below).

Structure of the *spoIIIJ* operon

We have previously reported the isolation of phages ϕ105J92 and ϕ105J93 that complement the *spoIIIJ87* mutation (Errington & Jones, 1987). Restriction maps of the two recombinants indicated that they contained overlapping insertions. Fragments of the insertion from phage ϕ105J93 were subcloned into plasmid pSG2 (Fig. 3) and the derivative plasmids were tested for insertional

Fig. 1. Ultrastructural phenotype of strain 87.4, containing a *spo-87* mutation. Strain 87.4 was resuspended in sporulation medium and at t_4 a sample of cells was prepared for electron microscopy. Cells at all stages of development up to and including stage III were prevalent in the population. Representative cells that had attained stage IIii (a), stage IIiii (b) and stage III (c) are shown (see Illing & Errington, 1991b). Bar, 0.5 μ m.

inactivation of the *spoIIIJ* operon or for their ability to rescue the *spoIIIJ87* mutation. None of the plasmids obtained gave an observable phenotypic effect when integrated into the wild-type strain SG38, indicating that their insertions do not lie wholly within the *spoIIIJ* locus (see Piggot *et al.*, 1984). However, plasmids pH28 and pP5 gave a mixture of Spo⁺ and Spo⁻ colonies when

transformed into strain 87.4. This indicated that the insertions overlapped the site of the *spoIIIJ87* mutation and that one end of each insertion probably lay within the *spoIIIJ* transcription unit.

A region of 1830 bp from the left-hand part of the insertion in ϕ 105J93 as shown in Fig. 3 was sequenced (Fig. 4). Three major open reading frames (ORFs) were

Fig. 2. Effect of the *spoIIIJ*::*ermC* insertional mutation on expression of: (a) *spoIIAA'*-*lacZ*; (b) *spoIIIG'*-*lacZ*; (c) *spoVAA'*-*lacZ*. Wild-type (filled symbols) and *spoIIIJ* mutant strains (open symbols) were induced to sporulate and at intervals assayed for β -galactosidase (circles) and alkaline phosphatase (squares; a and c only).

found, all reading left to right as shown in Fig. 3. ORF1 began at the *Bcl*I site marking one end of the insert originally cloned in phage ϕ 105J93. This partial ORF was homologous to the *rnpA* gene of *Escherichia coli*. ORF2 began after a space of about 150 bp, and was capable of encoding a protein of 260 amino acid residues (*M*_r 29409). This ORF ended about 20 bp before the *Pst*I site at one end of the insert in plasmid pP5, suggesting strongly that this ORF corresponded to the *spoIIIJ* gene. It contains an unusually larger proportion of basic residues compared with acidic residues (K + R, 27; D + E, 13), and has several extensive regions containing mainly hydrophobic side chains. The N-terminal region

contains three basic amino acids, followed by a sequence of 20 mainly hydrophobic residues containing the sequence LAGC, suggesting that this is likely to form a lipoprotein (Pugsley & Schwartz, 1985).

Immediately adjacent to the putative *spoIIIJ* ORF was another ORF (ORF3) capable of encoding a polypeptide of 208 residues, again containing an excess of basic amino acids (K + R, 33; D + E, 23) but no extensive clusters of hydrophobic residues. Neither predicted protein showed significant homologies to heterologous proteins in the SWISSPROT database or to the translation products of GenBank/EMBL databases.

ORF2 and ORF3 were both preceded by reasonable ribosome-binding site (RBS) sequences (ΔG -16.6 and -14.4 kcal mol⁻¹ [-69.4 and -60.2 kJ mol⁻¹], respectively). ORF2 has adjacent ATG and TTG codons near the RBS sequence: the latter is probably the major initiation site, given its distance from the RBS (Mountain, 1989). ORF3 has a likely translation initiation site at a GTG codon that overlaps the end of ORF2 in the sequence GTGA. This sequence could allow translational coupling. Since plasmids pP5 and pH28 could integrate into the wild-type without generating an obvious phenotypic effect on either vegetative growth or sporulation, it seemed probable that ORF3 does not encode a function required for either of these activities. However, it was also possible that ORF3 could be expressed from a promoter within ORF2 or from a plasmid promoter.

To distinguish between these possibilities we inserted an erythromycin resistance gene into each ORF, generating strains 727 and 729 (Fig. 3). Both strains were constructed without difficulty and showed no obvious phenotype during growth (results not shown). Strain 729, with the insertion in ORF3, sporulated normally, but strain 727 was asporogenous and had a similar phenotype to that of the *spoIIIJ*87 mutant, as described above. These results confirmed that only ORF2 was essential for sporulation. We propose to designate ORFs 2 and 3 *spoIIIJ* and *jag* (*spoIIIJ* associated gene) respectively.

During the preparation of this manuscript, the sequence of this region of the chromosome was described by another group (Ogasawara & Yoshikawa, 1992). Our sequence differs from theirs at one position: our G at position 541 is A in the sequence of Ogasawara & Yoshikawa (1992). We are confident that our assignment is correct, because this base lies in a *Sst*I restriction site that we used for some of the plasmid constructions. The change would not affect the translation product, so it is possible that the discrepancy is due to natural strain variation. As pointed out by Ogasawara & Yoshikawa (1992) the *spoIIIJ* gene product exhibits strong sequence similarity to proteins in the replication origins of both *Pseudomonas putida* and *E. coli*. Unfortunately, the

Fig. 3. Physical map of the *spoIIIJ* region of the *B. subtilis* chromosome. The central part of the figure shows a physical map of the segment of DNA cloned in phage $\phi 105J93$, with abbreviations for restriction enzymes as follows: H, *Hind*III; P, *Pst*I; S, *Sst*I. The locations of the ORFs found by DNA sequence analysis (see Fig. 4) are also given. Above the physical map are shown the locations of the insertions of an erythromycin resistance cartridge inactivating ORF2 and ORF3 in the *B. subtilis* chromosome. Below are shown the insertions in various plasmids derived from $\phi 105J93$.

functions of these genes in the non-spore-forming organisms are unknown; this raises the possibility that *SpoIIIJ* may not only function during sporulation.

Regulation of *spoIIIJ* expression

To examine the regulation of the *spoIIIJ* locus we constructed plasmid pSG1020, which, on integration into the *B. subtilis* chromosome, would generate a *spoIIIJ'*-*lacZ* fusion. Plasmid pSG173 was similar to pSG1020 but with the promoter fragment in the reverse orientation (Fig. 3). The two plasmids were transformed into *B. subtilis* SG38 with selection for chloramphenicol resistance. The resultant strains were induced to sporulate by the resuspension method (Fig. 5a). As expected, β -galactosidase was produced by the strain containing pSG1020 but not by that containing pSG173 (apart from the low level of endogenous activity; Errington & Vogt, 1990). Surprisingly, the peak β -galactosidase activity in SG38::pSG1020 was found at t_0 , in the first sample taken. Thereafter, the activity fell steadily, until sampling was stopped at t_5 .

These results suggested that *spoIIIJ* expression occurs predominantly in vegetative cells. We therefore measured β -galactosidase production during growth and

sporulation in DSM, in which sporulation is initiated by exhaustion of a limiting amount of glucose. Again, substantial β -galactosidase synthesis occurred in the vegetative state and the activity fell from about t_0 onwards (Fig. 5b). The double peak of β -galactosidase activity in Fig. 5(b) was reproducible but its significance is unclear.

Genes expressed predominantly in vegetative cells would be expected to have promoters recognized by the major vegetative form of RNA polymerase, $E\sigma^A$, and thus should be unaffected by mutations affecting gene expression during sporulation. To test this, we introduced the *spoIIIJ'*-*lacZ* fusion plasmids into strains containing mutations in various genes encoding regulators of sporulation gene expression: *spo0A*, *spo0H*, *spoIIA* and *spoIIG*. Each derivative strain was induced to sporulate by resuspension and then assayed for β -galactosidase. In every case, the pattern of β -galactosidase synthesis was indistinguishable from the isogenic derivative with no *spo* mutation (results not shown). Thus, the *spoIIIJ* gene seems not only to be expressed predominantly in vegetative cells, but also to be unresponsive to sporulation-associated regulatory effectors.

To confirm that the *spoIIIJ* gene is transcribed by the σ^A form of RNA polymerase, we used primer extension

Fig. 4. DNA sequence of the *spoIIIJ* region. The sequence shown begins at the *Bcl*I site that marks one end of the insertion in phage ϕ 105J93 (see Fig. 3). The translation products corresponding to the C-terminal portion of the *rnpA* gene, *spoIIIJ* and *jag* are shown in single-letter code above the sequence. Putative ribosome-binding sites for *spoIIIJ* and *jag* are underlined. The direct repeats in the *spoIIIJ* coding sequence are indicated in bold-face type. The restriction endonuclease sites shown in Fig. 3 are given below the sequence. The transcription start site determined by primer extension analysis (see Fig. 6) is shown in bold-face type. A likely '-10' consensus sequence for σ^A -RNA polymerase is overlined.

analysis to determine its transcription start site *in vivo* (Fig. 6). A single predominant extension product was observed with RNA from vegetative cells, corresponding to initiation of transcription at nucleotide 352 in the sequence shown in Fig. 4. A specific transcript was barely detectable in the RNA from sporulating cells. As expected, the transcription start site lies just downstream from sequences that are quite similar to many promoters recognized by $E\sigma^A$ (Fig. 4), particularly in the '-10' and

'-16' regions (Moran *et al.*, 1982; Henkin & Sonenshein, 1987), though there is no obvious '-35' sequence in the appropriate position. This could indicate that transcription is facilitated by a positive regulator acting in conjunction with $E\sigma^A$. The location of this promoter within the region cloned in phage ϕ 105J93 would be consistent with the ability of this phage to complement mutations in the *spoIIIJ* gene. However, there is no clear transcriptional termination signal

Fig. 5. Expression of *spoIIIG'*-*lacZ* during growth and sporulation. Strains SG38::pSG173 (○) and SG38::pSG1020 (●) were induced to sporulate by resuspension (a) or nutrient exhaustion (b; the latter strain only) and assayed for β -galactosidase activity. The onset of sporulation was defined by the time of resuspension in sporulation medium (a) or by the time at which the growth rate (■) deviated from exponential (b).

between the *rnpA* and *spoIIIG* genes, so the possibility that some transcription of *spoIIIG* occurs from a promoter upstream of *rnpA* cannot be excluded. Indeed weak higher M_r bands were observed in some of the primer extension experiments (data not shown).

Conclusions

The *spoIIIG* locus probably consists of a bicistronic operon, which is expressed in vegetative cells and then turned off early in sporulation. The first gene but not the second is needed for sporulation. In the absence of *spoIIIG*, sporulation is arrested at stage III: prespore engulfment is completed but expression of prespore-specific genes, such as *spoVA*, is blocked, indicating that the prespore sigma factor σ^G (Sun *et al.*, 1989) is not made or is inactive. Since substantial expression of a *spoIIIG'*-*lacZ* translational fusion occurs in a *spoIIIG* mutant (Fig. 2b), it seems likely that the mutation affects the activity rather than the synthesis of σ^G (Stragier, 1992). The subsequent absence of late mother cell gene expression (Kunkel *et al.*, 1988; Sandman *et al.*, 1988) is

Fig. 6. Analysis of the *in vivo* *spoIIIG* transcription start site by primer extension. RNA was extracted from a culture of strain SG38 growing in DSM in the mid-exponential growth phase (veg) or 4 h after the onset of sporulation (t_4). An end-labelled oligonucleotide complementary in sequence to the *spoIIIG* mRNA was used as a primer for cDNA synthesis by AMV reverse transcriptase. The products of the reaction were run on a DNA sequencing gel alongside a DNA sequence ladder (lanes labelled A, C, G and T). The size of the product of the DNA sequencing ladder corresponding to the major reverse transcriptase extension product (detected by autoradiography) is marked on the left.

probably due to the requirement for expression of one or more prespore genes, such as *spoIVB*, which somehow control the proteolytic activation of the late mother cell sigma factor σ^K (Cutting *et al.*, 1990, 1991).

The apparent role of SpoIIIG in σ^G activation is interesting. Mutations in several other genes – *spoIIB*, *spoIID*, *spoIIM*, *spoIIIA* and *spoIIIG* – cause substantial reductions in prespore-specific gene expression but have relatively minor effects on a *spoIIIG'*-*lacZ* fusion

(Foulger & Errington, 1989; Karmazyn-Campelli *et al.*, 1989; Mason *et al.*, 1988; C. Karmazyn-Campelli & P. Stragier, unpublished results, cited in Stragier, 1992; S.R.P. & J.E., unpublished results). This could mean that their gene products act at the level of σ^G activity, as suggested previously (Stragier, 1992), or its stability. At least two of these genes are expressed in the mother cell, as a result of transcription by the σ^E form of RNA polymerase (Driks & Losick, 1991; Illing & Errington, 1991a). Stragier (1992) has suggested that the SpoIID product is required for engulfment, and that the SpoIIIA proteins are required for a signal transduction pathway that couples prespore gene expression (via σ^G activity) to mother cell development. An alternative possibility would be that loss of the later phase of prespore gene expression is due to the sensitivity of the engulfed prespore to the metabolic state of the mother cell, or to metabolic communication between the two cells (Illing & Errington, 1990). In either case, the probable membrane location of the putative SpoIIIJ protein would be compatible with a function in some form of communication between the prespore and mother cell. Elucidating the mechanisms whereby the developmental programmes of the prespore and mother cell are coupled is likely to be a challenging and rewarding problem.

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References

- AKRIGG, A. & MANDELSTAM, J. (1978). Extracellular manganese-stimulated deoxyribonuclease as a marker event in sporulation of *Bacillus subtilis*. *Biochemical Journal* **172**, 63–67.
- CLARKE, S. & MANDELSTAM, J. (1987). Regulation of stage II of sporulation in *Bacillus subtilis*. *Journal of General Microbiology* **133**, 2371–2380.
- CUTTING, S., OKE, V., DRIKS, A., LOSICK, R., LU, S. & KROOS, L. (1990). A forespore checkpoint for mother cell gene expression during development in *B. subtilis*. *Cell* **62**, 239–250.
- CUTTING, S. M., DRIKS, A., SCHMIDT, R., KUNKEL, B. & LOSICK, R. (1991). Forespore-specific transcription of a gene in the signal transduction pathway that governs Pro- σ^K processing in *Bacillus subtilis*. *Genes and Development* **5**, 456–466.
- DRIKS, A. & LOSICK, R. (1991). Compartmentalized expression of a gene under the control of sporulation transcription factor σ^E in *Bacillus subtilis*. *Proceedings of the National Academy of Sciences of the United States of America* **88**, 9934–9938.
- ERRINGTON, J. (1986). A general method for fusion of the *Escherichia coli* *lacZ* gene to chromosomal genes in *Bacillus subtilis*. *Journal of General Microbiology* **132**, 2953–2966.
- ERRINGTON, J. (1992). *Bacillus subtilis* sporulation: a paradigm for the temporal and spatial control of gene expression. In *Development – the Molecular Genetic Approach*, pp. 28–44. Edited by V. E. A. Russo, S. Brody, D. Cove & S. Ottolenghi. Heidelberg: Springer-Verlag.
- ERRINGTON, J. & ILLING, N. (1992). Establishment of cell-specific transcription during sporulation in *Bacillus subtilis*. *Molecular Microbiology* **6**, 689–695.
- ERRINGTON, J. & JONES, D. (1987). Cloning in *Bacillus subtilis* by transfection with bacteriophage vector ϕ 105J27: isolation and preliminary characterization of transducing phages for 23 sporulation loci. *Journal of General Microbiology* **133**, 493–502.
- ERRINGTON, J. & MANDELSTAM, J. (1986a). Use of a *lacZ* gene fusion to determine the dependence pattern of sporulation operon *spoIIA* in *spo* mutants of *Bacillus subtilis*. *Journal of General Microbiology* **132**, 2967–2976.
- ERRINGTON, J. & MANDELSTAM, J. (1986b). Use of a *lacZ* gene fusion to determine the dependence pattern and the spore compartment expression of sporulation operon *spoVA* in *spo* mutants of *Bacillus subtilis*. *Journal of General Microbiology* **132**, 2977–2985.
- ERRINGTON, J. & VOGT, C. H. (1990). Isolation and characterization of mutations in the gene encoding an endogenous *Bacillus subtilis* β -galactosidase and its regulator. *Journal of Bacteriology* **172**, 488–490.
- FORT, P. & ERRINGTON, J. (1985). Nucleotide sequence and complementation analysis of a polycistronic sporulation operon, *spoVA*, in *Bacillus subtilis*. *Journal of General Microbiology* **131**, 1091–1105.
- FOULGER, D. & ERRINGTON, J. (1989). The role of the sporulation gene *spoIIIE* in the regulation of prespore-specific gene expression in *Bacillus subtilis*. *Molecular Microbiology* **3**, 1247–1255.
- GUZMAN, P., WESTPHLING, J. & YOUNGMAN, P. (1988). Characterization of the promoter region of the *Bacillus subtilis* *spoIIIE* operon. *Journal of Bacteriology* **170**, 1598–1609.
- HENKIN, T. M. & SONENSHEIN, A. L. (1987). Mutations of the *Escherichia coli* *lacUV5* promoter resulting in increased expression in *Bacillus subtilis*. *Molecular and General Genetics* **209**, 467–474.
- HORINOCHI, S. & WEISBLUM, B. (1982). Nucleotide sequence and functional map of pE194, a plasmid that specifies inducible resistance to macrolide, lincosamide, and streptogramin type B antibiotics. *Journal of Bacteriology* **150**, 804–814.
- HRANUELI, D., PIGGOT, P. J. & MANDELSTAM, J. (1974). Statistical estimate of the total number of operons specific for *Bacillus subtilis* sporulation. *Journal of Bacteriology* **119**, 684–690.
- ILLING, N. & ERRINGTON, J. (1990). The *spoIIIA* locus is not a major determinant of prespore-specific gene expression during sporulation in *Bacillus subtilis*. *Journal of Bacteriology* **172**, 6930–6936.
- ILLING, N. & ERRINGTON, J. (1991a). 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. *Molecular Microbiology* **5**, 1927–1940.
- ILLING, N. & ERRINGTON, J. (1991b). Genetic regulation of morphogenesis in *Bacillus subtilis*: roles of σ^E and σ^F in prespore engulfment. *Journal of Bacteriology* **173**, 3159–3169.
- KARMAZYN-CAMPELLI, C., BONAMY, C., SAVELLI, B. & STRAGIER, P. (1989). Tandem genes encoding σ -factors for consecutive steps of development in *Bacillus subtilis*. *Genes and Development* **3**, 150–157.
- KROOS, L., KUNKEL, B. & LOSICK, R. (1989). Switch protein alters specificity of RNA polymerase containing a compartment-specific sigma factor. *Science* **243**, 526–529.
- KUNKEL, B., SANDMAN, K., PANZER, S., YOUNGMAN, P. & LOSICK, R. (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. *Journal of Bacteriology* **170**, 3513–3522.
- LOSICK, R. & STRAGIER, P. (1992). Crisscross regulation of cell-type-specific gene expression during development in *B. subtilis*. *Nature, London* **355**, 601–604.
- LOSICK, R., YOUNGMAN, P. & PIGGOT, P. J. (1986). Genetics of endospore formation in *Bacillus subtilis*. *Annual Review of Genetics* **20**, 625–669.
- MASON, J. M., HACKETT, R. H. & SETLOW, P. (1988). Regulation of expression of genes coding for small, acid-soluble proteins of *Bacillus subtilis* spores: studies using *lacZ* gene fusions. *Journal of Bacteriology* **170**, 239–244.
- MORAN, C. P., JR (1990). Measuring gene expression in *Bacillus*. In *Molecular Biological Methods for Bacillus*, pp. 267–293. Edited by C. R. Harwood & S. M. Cutting. Chichester: Wiley.
- MORAN, C. P., JR, LANG, N., LEGRICE, S. F. J., LEE, G., STEPHENS, M., SONENSHEIN, A. L., PERO, J. & LOSICK, R. (1982). Nucleotide sequences that signal the initiation of transcription and translation in *Bacillus subtilis*. *Molecular and General Genetics* **186**, 339–346.

- MOUNTAIN, A. (1989). Gene expression systems for *Bacillus subtilis*. In *Bacillus*, pp. 73–114. Edited by C. R. Harwood. New York: Plenum Press.
- MYSLIWIEC, T. H., ERRINGTON, J., VAIDYA, A. B. & BRAMUCCI, M. G. (1991). The *Bacillus subtilis* *spoJ* gene: evidence for involvement in catabolite repression of sporulation. *Journal of Bacteriology* **173**, 1911–1919.
- OGASAWARA, N. & YOSHIKAWA, H. (1992). Genes and their organization in the replication origin region of the bacterial chromosome. *Molecular Microbiology* **6**, 629–634.
- PIGGOT, P. J. & COOTE, J. G. (1976). Genetic aspects of bacterial endospore formation. *Bacteriological Reviews* **40**, 908–962.
- PIGGOT, P. J., CURTIS, C. A. M. & DE LENCASTRE, H. (1984). Use of integrational plasmid vectors to demonstrate the polycistronic nature of a transcriptional unit (*spoIIA*) required for sporulation of *Bacillus subtilis*. *Journal of General Microbiology* **130**, 2123–2136.
- PUGSLEY, A. P. & SCHWARTZ, M. (1985). Protein export and secretion by bacteria. *FEMS Microbiology Reviews* **1**, 3–39.
- SANDMAN, K., KROOS, L., CUTTING, S., YOUNGMAN, P. & LOSICK, R. (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. *Journal of Molecular Biology* **200**, 461–473.
- SANGER, F., NICKLEN, S. & COULSEN, A. R. (1977). DNA sequencing with chain terminating inhibitors. *Proceedings of the National Academy of Sciences of the United States of America* **74**, 5463–5467.
- SCHAEFFER, P., MILLET, J. & AUBERT, J.-P. (1965). Catabolic repression of bacterial sporulation. *Proceedings of the National Academy of Sciences of the United States of America* **54**, 704–711.
- STERLINI, J. M. & MANDELSTAM, J. (1969). Commitment to sporulation in *Bacillus subtilis* and its relationship to the development of actinomycin resistance. *Biochemical Journal* **113**, 29–37.
- STEVENS, C. M., DANIEL, R., ILLING, N. & ERRINGTON, J. (1992). Characterization of a sporulation gene, *spoIVA*, involved in spore coat morphogenesis in *Bacillus subtilis*. *Journal of Bacteriology* **174**, 586–594.
- STRAGIER, P. (1992). Establishment of forespore-specific gene expression during sporulation of *Bacillus subtilis*. In *Prokaryotic Structure and Function: a New Perspective*, pp. 297–310. Edited by S. Mohan, C. Dow & J. A. Cole. Cambridge: Cambridge University Press.
- STRAGIER, P., BONAMY, C. & KARMAZYN-CAMPELLI, C. (1988). Processing of a sporulation sigma factor in *Bacillus subtilis*: how morphological structure could control gene expression. *Cell* **52**, 697–704.
- SUN, D., STRAGIER, P. & SETLOW, P. (1989). Identification of a new σ -factor involved in compartmentalized gene expression during sporulation of *Bacillus subtilis*. *Genes and Development* **3**, 141–149.
- YANISCH-PERON, C., VIEIRA, J. & MESSING, J. (1985). Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* **33**, 103–119.