Tandem genes encoding σ -factors for consecutive steps of development in *Bacillus subtilis*

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During sporulation, *Bacillus subtilis* undergoes successive morphological changes that can be arrested at various stages by mutations in many genes. One of these, *spoIIGB*, encodes a transcriptional factor, σ^E , which is necessary to proceed beyond stage II and to differentiate the cell in two compartments, the forespore and the mother cell. Mutations were introduced in an open reading frame located immediately downstream of *spoIIGB*. They block sporulation at stage III and define a new gene, *spoIIIG*, encoding a 260-amino-acid polypeptide highly similar to bacterial σ -factors. A promoter was identified in the *spoIIGB*–*spoIIIG* interval by transcriptional fusion to *lacZ*. It is turned on 1 hr after the start of σ^E synthesis and is specifically activated in the forespore. The tandemly arranged *spoIIGB* and *spoIIIG* genes appear to encode homologous proteins that modulate transcription in a sequential fashion during sporulation.

[Key Words: σ -Factor; transcription; compartmentalization; sporulation; development; *Bacillus subtilis*]

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Genetic organization in higher organisms sometimes reflects the temporal or spatial role of related gene products during development. For instance, in most mammals, the α -globin and β -globin genes are arranged in order of their sequential expression during development (Maniatis et al. 1980). In the Bithorax Complex of *Drosophila*, mutations are clustered in a series of phenotypic classes and aligned on the chromosome in the exact order of the body segments that they affect (for review, see Duncan 1987). Nothing equivalent has been described in *Bacillus subtilis*, a bacterium that elaborates a multiresistant endospore, according to a temporal and spatial sequence of gene activation, in response to nutrient depletion (for review, see Losick et al. 1986). Sporulation proceeds through several morphological stages (O, II, III, ..., VII), each one involving the products of 5–10 different operons. These *spo* genes are scattered all around the chromosome without any noticeable organization.

Early steps of sporulation are dependent on the synthesis and activation of new σ -factors that modify RNA polymerase transcription specificity, namely the *spoOH*, *spoIIAC*, and *spoIIGB* gene products (see Losick et al. 1986). The latter, σ^E (formerly σ^{29}), plays a key role in the temporal program of transcription. Its synthesis in active form appears to be coupled to the formation of the asymmetric septum characteristic of stage II (Stragier et al. 1988) and is needed to allow the subsequent morphological modifications that lead to a compartmentalized system at stage III. Thereafter, differential gene expression occurs in the two subcellular compartments, the

forespore and the mother cell. For instance, the *ssp* genes, encoding small acid-soluble proteins accumulated in the spore, are turned on only in the forespore (Mason et al. 1988); conversely, the *cot* genes encoding the spore coat proteins are expressed only in the mother cell (cited by Panzer et al. 1989). Although the existence of additional σ -factors has been conjectured (Kunkel et al. 1988; Mason et al. 1988), the molecular mechanisms that control compartmentalized gene expression are still elusive.

In this paper we report the existence of a new gene, *spoIIIG*, tandemly arranged with *spoIIGB*, which potentially encodes a late σ -factor. In the accompanying paper, Sun et al. (1989) demonstrate that the *spoIIIG* product is actually a σ factor and that it is involved in forespore-specific gene expression.

Results

spoIIIG, a new stage III locus

The *spoIIG* locus (Fig. 1) was initially cloned as a 3.7-kb *Bam*HI fragment, and the sequence of the region covering the *spoIIG41* and *spoIIG55* mutations revealed the existence of an open reading frame encoding a σ -like polypeptide (Stragier et al. 1984). This polypeptide was identified as the inactive precursor of σ^E (Trempey et al. 1985; LaBell et al. 1987). Further characterization of the *spoIIG* locus indicated that it actually comprised two genes (Kenney and Moran 1987) and that the product of the first gene of the operon, *spoIIGA*, was likely to be the pro- σ^E processing enzyme (Stragier et al. 1988). The

Figure 1. Characterization of the *spoIIIG* locus. (Top) The DNA fragments that were cloned in the integrative vector pJH101 and the associated Spo phenotype after transformation of strain 168 or JH642. (Middle) The hatched and dotted boxes indicate the open reading frames as determined by DNA sequencing. (Bottom) The interrupted arrow starts from the fragment that was used to walk up to the *Cla*I and *Eco*RV sites (see text). The site of insertion of a chloramphenicol resistance cartridge (not on scale) that inactivates the *spoIIIG* gene is indicated. The DNA fragments that were used to create the *spoIIIG* Δ 1 mutation are shown; they bracket a 70-bp region that was replaced by 40 bp provided by pUC and Bluescript vectors. The restriction sites are abbreviated as follows: *Bam*HI (B); *Cla*I (C); *Dde*I (D); *Eco*RI (E); *Bgl*II (G); *Hind*III (H); *Pst*I (P); *Rsa*I (R); *Sca*I (S); *Eco*RV (V). The leftmost *Bam*HI site was created when the *spoIIIG* locus was cloned after partial *Sau*3A digestion (Stragier et al. 1984) and does not exist on the chromosome.

presence of that other gene immediately upstream of the pro- σ^E -coding sequence (henceforth called *spoIIGB*) had first been pointed out by Kobayashi and Anaguchi (1985). They also indicated that a third open reading frame started 139 bp downstream of *spoIIGB*. We wondered whether that gene was also involved in sporulation.

From the data published by Kobayashi and Anaguchi (1985) it could be predicted that the rightmost *Hind*III–*Bam*HI fragment carried by our original insert (see Fig. 1) was entirely within that third open reading frame. This DNA fragment was subcloned into a nonreplicative plasmid carrying an antibiotic resistance marker selectable in *B. subtilis*. Transformation of a wild-type *B. subtilis* strain with this recombinant plasmid led to disruption of the gene located downstream of *spoIIGB* by homologous recombination. The transformed clones remained unpigmented on nutrient agar plates, a phenotype associated with asporogeny. Electron microscopic examination of these clones grown in liquid sporulation medium confirmed this diagnosis and indicated that the cells displayed a classic stage III phenotype, the development being arrested after engulfment of the forespore by the mother cell (Fig. 2). Thus, the *spoIIGB* gene is followed by a *spo* gene, unidentified as yet, that we designate as *spoIIIG*, the next available name (Losick et al. 1986).

Two other mutations were introduced by marker replacement in the *spoIIIG* gene when its sequence became available (see Fig. 1 and Experimental procedures). The first one, *spoIIIG* Δ 1, was intended to be an in-frame

deletion between codons 18 and 42. However, nucleotide sequencing revealed that an extra 18-bp *Hae*III fragment from pUC8 had inadvertently been ligated at the junction and introduced a stop codon at the very beginning of *spoIIIG*. The second mutation, *spoIIIG* :: *cat*, was created by insertion of a chloramphenicol cartridge between codons 166 and 167 of *spoIIIG* so that it could easily be moved to various genetic backgrounds. Both mutations display the same stage III phenotype as the original *spoIIIG* mutant (Fig. 2).

We anticipated that *spoIIIG* was not the third gene of the *spoIIIG* operon because no *spo* operon to date has been described to contain genes in which mutations block sporulation at different stages. A restriction fragment overlapping the distal part of *spoIIGB* and the proximal part of *spoIIIG* (see Fig. 1) was used to integrate a plasmid at that location into the chromosome. Both open reading frames were reconstituted, but the *spoIIIG* gene was moved away from the *spoIIIG* operon by ~5 kb. A similar plasmid integration into the *spoIIA* operon leads to a Spo[−] phenotype by separating the distal *spoIIAC* gene from its promoter (Piggot et al. 1984). Conversely, clones obtained by integration of a plasmid between the *spoIIGB* and *spoIIIG* cistrons were brown on nutrient agar plates and full of bright refractile spores, as judged by phase-contrast microscopy. This result confirms a similar observation by Kenney and Moran (1987) and indicates that the 427-bp DNA fragment used in that experiment contains a promoter for *spoIIIG*.

The distal part of the *spoIIIG* gene was cloned by the

Figure 2. Electron micrograph of a *spoIIIG* mutant. The MO428 strain was grown in DS medium and cells were harvested 5 hr after the end of exponential growth (kindly provided by A. Ryter).

'walking' technique devised by Youngman et al. (1984). A plasmid that could be selected for and replicated in *Escherichia coli* was integrated into the *B. subtilis* chromosome at the distal border of our original insert (Fig. 1). Chromosomal DNA of that strain was purified, cut with various restriction enzymes, ligated at low DNA concentration, and introduced by transformation in *E. coli*. Clones were obtained that carried overlapping DNA fragments, the largest one extending 2.9 kb downstream of our initial insert.

spoIIIG encodes a σ -like polypeptide

Nucleotide sequence of the *spoIIIG* locus (Fig. 3) was assembled from various overlapping restriction fragments and extended our previously published sequence of the *spoIIGB* locus (Stragier et al. 1984). An open reading frame encoding a 260-residue, 30,055-m.w. polypeptide was found at the location indicated by Kobayashi and Anaguchi (1985). The translational start was assigned to a GTG codon preceded by a good ribosome binding site; it has been confirmed by analysis of the *spoIIIG* polypeptide, the f-Met residue being post-translationally removed (Sun et al. 1989). The stop codon is immediately followed by an inverted repeat that could be a transcription termination signal.

To localize the distal border of the *spoIIIG* operon, we followed the same strategy as described above. A restriction fragment carrying the last 94 codons of *spoIIIG* and extending 91-bp downstream (see Fig. 1) was used to integrate a plasmid at that location into the chromosome. The resulting clones displayed a Spo⁺ phenotype, indicating that there is no other *spo* gene farther downstream, depending on transcription initiated at the *spoIIIG* promoter.

Close examination of the predicted sequence of the *spoIIIG* product revealed motifs that were familiar to us (Stragier et al. 1985; Stragier 1986), because they are shared by most bacterial σ -factors. The sequence of the *spoIIIG* polypeptide can be aligned on its entire length with the sequences of eight other bacterial σ -factors (Fig. 4). Three domains of σ -factors play important functional roles. One is involved in binding to core RNA polymerase and has been predicted to be the most conserved region in bacterial σ -factors (Stragier et al. 1985; Gribskov and Burgess 1986). Extension of the alignments to nine polypeptides reinforces this proposal (see Fig. 4). The two other domains should contact specific nucleotides in the -10 and -35 regions of promoters and have now been localized (for review, see Helmann and Chamberlin 1988). A common framework is ensured by the presence of some conserved residues interspersed among variable amino acids that mediate recognition specificity. It is striking to observe (Fig. 4) that both in the -10 and the -35 region, the *spoIIAC* and *spoIIIG* polypeptides share almost identical sequences, with only one difference in each case (the F and Y residues in their -10 regions are functionally equivalent). It can be expected that if these polypeptides are actually σ -factors, their cognate promoters will be very similar.

Temporal and spatial expression of *spoIIIG*

Determination of the upstream border of the *spoIIIG* operon has indicated the presence of a promoter for *spoIIIG* in the 427-bp *HindIII*-*PstI* fragment that overlaps *spoIIGB* and *spoIIIG* (Fig. 1). This fragment was cloned upstream of a promoterless *lacZ* gene and recombined as a single copy into the *amy* region of the chromosome (see Experimental procedures). β -Galactosidase was assayed during growth and sporulation (Fig. 5). It

Figure 3. Structure of the *spoIIIG* gene. The nucleotide sequence was determined on both strands from overlapping restriction fragments cloned in the Bluescript phagemid (Short et al. 1988) and subjected to the dideoxy sequencing procedure of Sanger et al. (1977), with the modifications of Biggin et al. (1983). Only the nontranscribed DNA strand is shown, starting from the *spoIIGB* stop codon, with the predicted amino acid sequence. Initiation and stop codons are boxed. The ribosome-binding site is underlined. A putative transcription termination signal is shown by arrows.

started to be synthesized 2.5 hr after the end of exponential growth ($t_{2.5}$) and was accumulated during the next 2 hr. Such a timing of expression is in good accordance with the developmental stage at which the *spoIIIG* product is needed. Transcription of *spoIIIG* is turned on 1 hr later than *spoIID*, a gene activated by σ^E (Stragier et al. 1988), and 2 hr later than *spoIIG* itself (Kenney and Moran 1987).

The dependence of *spoIIIG* expression on the products of other *spo* genes was analyzed by introducing the *spoIIIG-lacZ* fusion into various asporogenous strains. Results given in Table 1 indicate that *spoIIIG* transcription is shut off by all stage II mutations (with two exceptions discussed later), as well as by mutation in *spoIIIA*, and severely impaired by a *spoIIIF* mutation. Conversely, *spoIIIG* expression is almost normal or even enhanced in *spoIIIC*, *spoIIID*, and *spoIIIE* backgrounds. Interestingly *spoIIIG* transcription is also abolished in a strain mutated in *spoIIIG* itself. This result will be analyzed later (see Discussion).

With the sole exception of the *spoIIIE* mutation, such a dependence pattern is typical of genes specifically expressed in the forespore, as *spoVA* (Errington and Mandelstam 1986b), *gdh*, and *ssp* genes (Mason et al. 1988). Fractionation methods have been devised that give a good estimation of the compartmentalization of a gene product during late stages of sporulation (Errington and Mandelstam 1986b). Following a similar approach, β -galactosidase (under the control of the *spoIIIG* promoter) was analyzed and compared to glucose dehydrogenase,

the product of the *gdh* gene. After fractionation of a sample of sporulating cells (harvested around t_5), 92% of glucose dehydrogenase and β -galactosidase were found in the forespore, their specific activities being 20-fold higher in forespore than in mother cell. Moreover, essentially all of the β -galactosidase and glucose dehydrogenase synthesized during sporulation were found to end up in mature spores (data not shown).

Discussion

spoIIIG, a new member of a multigene family

The *spoIIIG* gene has been identified by reverse genetics and found to encode a σ -like polypeptide. A similar situation has been encountered with the *spoIIAC* gene, which was already defined by standard genetic analysis and, despite the absence of supporting biochemical evidence, proposed to code for a developmental σ -factor (Errington et al. 1985; Stragier 1986). In the accompanying paper, Sun et al. (1989) demonstrate that both *spoIIAC* and *spoIIIG* gene products are actually new σ -factors, σ^F and σ^G . In accordance with the rules proposed by Losick et al. (1986), the *spoIIAC* and *spoIIIG* genes should alternatively be named *sigF* and *sigG*.

The tandem arrangement of *spoIIIG* with *spoIIGB*, the gene encoding pro- σ^E , suggests that these two genes resulted from a duplication of an ancestral gene, as previously proposed for two genes of the *spoVA* operon (Errington et al. 1985). Seven *B. subtilis* genes encoding σ -factors have now been cloned. From their location on

the chromosome (Fig. 6), it can be speculated that this multigene family arose by successive duplications, some of these reflecting duplications of the ancestral chromosome, as suggested by Binnie et al. (1986). The latter hypothesis would be strengthened if additional *sig* genes were to be found in the 300° region of the chromosome.

Expression of *spoIIIG* is compartmentalized

spoIIIG expression is controlled by its own promoter located in the 323-bp preceding its ribosome-binding site. This promoter is activated at $t_{2.5}$ and is under the dependence of most stage II gene products, as well as products of the *spoIIIA*, *spoIIIF*, and *spoIIIG* genes. The presence of β -galactosidase in a *spoIIIF* strain carrying a *spoIIIG-lacZ* fusion can be correlated with the oligosporogenous phenotype of that strain, which produces 10–30% of the wild-type amount of spores. The striking difference observed between the two *spoIID* alleles was also reported by Mason et al. (1988) in their study of *gdh* and *ssp* genes. It indicates that the mutations (each of which completely prevents spore production) must affect the *spoIID* product in a very different way, the *spoIID298* mutation allowing expression of some post-stage II genes.

Altogether these results suggest that forespore-specific gene expression is a consequence of activation of the *spoIIIG* promoter exclusively in the forespore, which could be controlled by the *spoIIIA* and *spoIIIF* products. However, the absolute dependence of *spoIIIG* transcription on σ^G itself implies that some other promoter must be involved in *spoIIIG* expression. Our preliminary results indicate that *spoIIIG* is expressed at an early time by read through from the *spoIIIG* operon (C. Karmazyn-Campelli and P. Stragier, unpubl.). The synthesis of σ^G before septation, when no forespore-specific promoter is expressed, suggests that σ^G activity might require some special environment that would be achieved only in the forespore (and be dependent on the *spoIIIA* and *spoIIIF* products). σ^G would then enhance its own synthesis only in the forespore by activation of a promoter located between *spoIIIGB* and *spoIIIG*. This burst of σ^G synthesis would, in turn, displace σ^E and allow forespore-specific gene expression. Thus, autoregulation of *spoIIIG* could play a major role in compartmentalization of gene expression.

Genes expressed specifically in the forespore or the mother cell follow opposite patterns for their dependence on stage III gene products (Errington and Mandelstam 1986b; Kunkel et al. 1988; Mason et al. 1988). Ex-

Figure 5. Time course of *spoIIG-lacZ* expression. The specific activity of β -galactosidase was measured during growth and sporulation in DS medium of strain MO464, a JH642 derivative containing a *spoIIG-lacZ* fusion inserted by a double recombination event at the *amy* locus. The background level found in a strain harboring the same *lacZ* insertion without any promoter was subtracted.

pression of *spoIIG* shows a forespore-type dependence pattern, and fractionation experiments confirm that *spoIIG* is transcribed predominantly, if not only, in the forespore. These data strongly suggest that the *spoIIG* product is a σ -factor for genes expressed specifically in the forespore, a hypothesis confirmed in the accompanying paper by Sun et al. However, because a *spoIIG-lacZ* fusion is transcribed actively in a *spoIIIE* mutant

Table 1. Effect of *spo* mutations on expression of a *spoIIG-lacZ* fusion

<i>spo</i> allele ^a	β -Galactosidase activity ^b
<i>spoOJ87</i>	17
<i>spoIIAA69</i>	5
<i>spoIIAC1</i>	6
<i>spoIIB131</i>	8
<i>spoIID66</i>	19
<i>spoIID298</i>	90
<i>spoIIE48</i>	6
<i>spoIIF96</i>	57
<i>spoIIIGAΔ17</i>	6
<i>spoIIIGBΔ66</i>	5
<i>spoIIIA53</i>	12
<i>spoIIIC94</i>	49
<i>spoIIID83</i>	157
<i>spoIIIE36</i>	244
<i>spoIIF590</i>	21
<i>spoIIGΔ1</i>	6

^a The *spoOJ87* mutation blocks sporulation at stage III (Stragier et al. 1988); contradictory results were obtained with the *spoIIB2* mutation, depending on the origin of the strain used.

^b Activity is expressed as a percentage of the maximum level of β -galactosidase observed in wild-type cells between t_4 and t_5 .

Figure 6. Genetic map of *B. subtilis* *sig* genes. Locations of genes encoding σ -factors on the genetic map of *B. subtilis* are indicated with their alternate names. The *sigC* gene encoding σ^{32} has not yet been cloned. The *sigD* location was determined by Helmann et al. (1988). Other references are listed in Losick et al. (1986).

while other forespore specific genes are turned off, it follows that the *spoIIIE* gene product(s) must somehow be required for σ^G activity.

Cascades of σ -factors for switching between developmental stages

Before any genetic evidence was available, Losick and Pero (1981) proposed that a cascade of σ -factors governs the sequential expression of gene sets during bacterial sporulation. Four *spo* genes encoding σ -factors have now been identified in *B. subtilis* and have been found to control successive steps of development. The *spoOH* product (σ^H) acts at the very beginning of sporulation, presumably in response to metabolic changes occurring at the end of exponential growth (Carter and Moran 1986; Dubnau et al. 1988). σ^H is needed for expression of the *spoIIA* and *spoIIG* operons (Errington and Madelstam 1986a; Kenney and Moran 1987). The *spoIIAC* product (σ^F) is necessary for proper completion of the asymmetric septum (Piggot and Coote 1976) which, in turn, apparently activates processing of pro- σ^E , the *spoIIGB* product (Stragier et al. 1988). σ^E binds strongly to core RNA polymerase, leading to a major switch of the transcriptional pattern (Haldenwang et al. 1981). σ^E is needed for expression of *spoIIG* (Table 1), which encodes σ^G , the σ -factor activating most, if not all, forespore-specific genes (Sun et al. 1989). Additional σ -factor(s) may be expected to control mother-cell-specific genes (Kunkel et al. 1988).

Identification of a σ -factor dedicated to the transcription of genes in the forespore paves the way to elucidation of the control of compartmentalization of gene expression during sporulation. Our results point the pivotal role of the *spoIIIA* and *spoIIF* products in activation of forespore genes. Analyzing their own synthesis and functional role should lead us one step backwards, where the two genomes of the sporulating cell differentiate from each other.

Experimental procedures

Strains and media

B. subtilis strains are derivatives of strain 168trpC2 or JH642 trpC2 pheA1. Sporulation mutant strains were provided by S. Cutting, P. Setlow, and J. Errington. The *spoIIIG* mutations have been described [Stragier et al. 1988]. The $\Delta 1$ deletion in the *spoIIIG* locus was created in vitro on cloned DNA fragments and introduced into the *B. subtilis* chromosome by spontaneous exchange with a replicative plasmid carrying the mutated allele, screening for Spo⁻ colonies [Chak et al. 1982]. Growth in the absence of antibiotics led to subsequent loss of the plasmid. A *cat* insertion was introduced into the *spoIIIG* locus by transformation of *B. subtilis* with a linearized plasmid carrying a 1.5-kb *cat* fragment from pMI1101 [Donovan et al. 1987] in the *Bam*HI site of the *spoIIIG* gene. Transformation of *B. subtilis* was made according to Anagnostopoulos and Spizizen [1961]. For all sporulation experiments, *B. subtilis* strains were grown in DS medium [Schaeffer et al. 1965], and chloramphenicol (5 μ g/ml) was added if necessary.

Plasmid constructions were performed using the *E. coli* strains DH5 α and JM107. *E. coli* bacteria were grown in LB medium [Miller 1972] in the presence of the appropriate antibiotics (50 μ g/ml ampicillin, 10 μ g/ml tetracycline).

Identification and cloning of the *spoIIIG* locus

Fragments of the *spoIIG*–*spoIIIG* locus were cloned in the integrative vector pJH101 [Ferrari et al. 1983], usually after a subcloning step in the pUC or Bluescript vectors that introduced convenient restriction sites on the borders of the insert. Homologous recombination by a Campbell insertion inactivated the locus if the fragment did not carry one end of the operon [Piggot et al. 1984].

The rightmost 242-bp *Dde*I–*Bam*HI fragment from our initial insert [Stragier et al. 1984] was cloned in pUC18 (after filling in the *Dde*I site), recovered as a *Sph*I–*Bam*HI fragment, and cloned in pJH101. The resulting plasmid was introduced into *B. subtilis* and recombined at the *spoIIIG* locus. Cleaving the chromosomal DNA after this insertion with restriction enzymes and using some of the cloning sites of the original pJH101 vector allowed us to recover the adjacent downstream region of the *spoIIIG* locus in *E. coli*, after ligation and transformation (see Youngman et al. 1984).

Construction and analysis of a transcriptional *spoIIIG*–*lacZ* fusion

A 427-bp *Hind*III–*Pst*I fragment containing the *spoIIIG* promoter was cloned in pUC8, recovered as a *Hind*III–*Bam*HI fragment, and cloned in pDG268, a derivative of ptpBG1 [Shimotsu and Henner 1986], upstream of a promoterless *lacZ* gene using the translational signals of the *B. subtilis* *spoVG* gene. After linearization of the plasmid, this fusion was introduced into the *B. subtilis* chromosome of various strains by marker replacement through the adjacent *amy* regions. All clones selected for chloramphenicol resistance were found to be Amy⁻ on plates. Bacteria harboring this fusion were grown in DS medium. Ten-milliliter samples of culture were harvested at regular intervals, washed with Z buffer [Miller 1972], and kept at –20°C. After resuspension in 0.5 ml of Z buffer, the samples were sonicated and clarified by centrifugation. β -Galactosidase-specific activity was measured according to Miller [1972] and expressed as nmoles of 2-nitrophenyl- β -D-galactopyranoside (ONPG) hydrolyzed per minute per milligram of protein.

Fractionation experiments

The Spo⁺ *B. subtilis* strain harboring the integrated *spoIIIG*–*lacZ* fusion was grown and treated as described previously [Mason et al. 1988]. Sporulating cells were harvested around t_5 and separated into mother cell and forespore fractions according to Fujita et al. [1977]. Another sample was harvested around t_{28} , and the mature spores were treated with lysozyme after detergent sensitization. Extracts were assayed for β -galactosidase and glucose dehydrogenase, as described by Mason et al. [1988] and Fujita et al. [1977], respectively.

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Note added in proof

While this paper was under review, the sequence of the reading frame located downstream of the *spoIIGB* gene was reported by E.S. Masuda, H. Anaguchi, K. Yamada, and Y. Kobayashi [Proc. Natl. Acad. Sci. 85: 7637–7641]. Their data agree completely with our sequence of the gene identified herein as *spoIIIG*.

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