formation of complex 1. Fractions eluting from heparin-Sepharose (Pharmacia) at 400 to 500 mM KCl were pooled, and batch incubations were done with ARRE-1 DNA-affinity beads in the presence of nonspecific competitor. After approximately 12 hours, the beads were poured into a small column and washed extensively with 100 mM KCl and 200 mM KCl in wash buffer (50 mM Hepes, 0.1 mM EDTA, 0.03% lauryl dimethylamine oxide, and 1 mM dithiothreitol). Activity was eluted with 600 mM KCl in wash buffer, and 5% of the final material was denatured with guanidine-HCl and reduced with 50 mM dithiothreitol. After heating the sample 5 min at 65°C, we loaded it onto a reverse-phase HPLC C_8 column and eluted it with a gradient of 20 to 60% acetonitrile in 0.1% trifluoroacetic acid. Fractions were collected, and after adding 25 μl of 100 mM KCl in wash buffer we dried the fractions down to 25 μl. The pH of each fraction was adjusted to pH 7 to 8, and the samples were left on ice for 12 hours. Each fraction (10 μl) was run on a 10% SDS-polyacrylamide gel. The fraction corresponding to ~40% acetonitrile is shown in Fig. 3C. To detect OAP^{40}-binding activity, we incubated 0.5 μL of this HPLC fraction with and without a DEAE fraction containing Oct-1. Approximately 0.6 μg of protein from a heparin-Sepharose 200 mM KCl wash was also included in each binding reaction as a source of nonspecific protein.

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Establishment of Cell Type by Compartmentalized Activation of a Transcription Factor

PETER MARGOLIS, ADAM DRIKS, RICHARD LOSICK*

Early in the process of spore formation in *Bacillus subtilis* a septum is formed that partitions the sporangium into daughter cells called the forespore and the mother cell. The daughter cells each have their own chromosome but follow dissimilar programs of gene expression. Differential gene expression in the forespore is now shown to be established by the compartmentalized activity of the transcription factor σ^F. The σ^F factor is produced prior to septation, but is active only in the forespore compartment of the post-septation sporangium. The σ^F factor is controlled by the products of sporulation operons *spoIIA* and *spoIIE*, which may be responsible for confining its activity to one of the daughter cells.

A FUNDAMENTAL PROBLEM IN DEvelopmental biology is the process by which a cell of one type gives rise to one or more dissimilar cell types as a consequence of cell division. A primitive developmental process that has been cultivated as an experimental system for the establishment of cell type is the formation of endospores by the Gram-positive soil bacterium *Bacillus subtilis* (*1*). During sporulation an asymmetrically positioned septum forms, which partitions the developing cell (sporangium) into daughter cells called the mother cell and the forespore. These daughter cells differ in developmental fate from each other and from their parent cell; the forespore eventually becomes the spore, whereas the mother cell is discarded by lysis when maturation of the spore is complete. Each daughter cell type follows a distinct program of gene expression. At late developmental stages, differential gene expression is controlled by the compartment-specific transcription factors σ^G, which is present in the forespore, and σ^K, which is present in the mother cell. We now investigate the earliest regulatory events that establish distinct programs of gene expression in the two compartments.

σ^F is encoded by the promoter-distal member (*spoIIAC*) of the three-cistron sporulation operon *spoIIA* (*2, 3*) (Table 1). Expression of *spoIIA* commences prior to septum formation (*4*). Yet, σ^F directs the transcription of genes expressed in the forespore, such as *spoIIIG*, which encodes σ^G (*5–7*), and *gpr*, which encodes a forespore protease (*8*). Transcription of *spoIIIG* and *gpr* are also under the control of σ^G (*8–10*). Thus, it has not been possible to distinguish whether forespore-specific gene expression commences with the action of σ^F or, subsequently, with the action of σ^G.

To distinguish between these possibilities, we performed immunoelectron microscopy with antibodies to β-galactosidase (anti-βgal) to visualize the site of expression of *lacZ*, fused to a gene under σ^F control, in mutant sporangia that lacked σ^G. Because expression of *spoIIIG-lacZ* and *gpr-lacZ* in the mutant cells that lacked σ^G was too low to be detected by immunoelectron microscopy, we made use of a system for obtaining high-level synthesis of β-galactosidase from a σ^F-controlled reporter gene (*5, 11*). The system is based on an altered promoter recognition specificity mutant of σ^F that bears a valine-to-alanine substitution at residue 233 (VA233). The mutant σ^F directs transcription of the strongly expressed *sspB* gene, which is normally under the exclusive control of σ^G (*3*). Strain PM73 contains the VA233 mutant σ^F and a single chromosomally integrated copy of a plasmid (pPS490) (*12*) bearing an *sspB-lacZ* fusion and the gene encoding chloramphenicol acetyl transferase. To enhance σ^F-directed synthesis of β-galactosidase, PM73 was subjected to successive rounds of growth in the presence of increasing concentrations of chloramphenicol to select for amplification of pPS490 (*13*).

PM73 cells bearing approximately ten copies of pPS490 per chromosome were harvested during sporulation and subjected to immunoelectron microscopy (*13, 14*). As expected for *spoIIIG* mutants in which sporulation is blocked at engulfment, the sporangia were arrested at the stage (III) at which the forespore is pinched off as a free protoplast within the mother cell (Fig. 1). Although the level of decoration varied among sporangia, in all cases gold particles were more prevalent in the forespore than in the mother cell (Fig. 1, A to E). Cells bearing tandem chromosomal arrays of *lacZ* fused to mother cell–specific genes showed decoration of the mother cell and not the forespore (*15, 16*). Little decoration was observed in sporangia that lacked the *sspB-lacZ* fusion (Fig. 1F).

These observations are consistent with the view that σ^F-directed gene expression is largely restricted to the forespore chamber of the sporangium. However, because expression of *sspB-lacZ* is normally confined to the forespore (*12, 14*), we did not know whether the compartmentalization of σ^F-directed gene expression was controlled at the level of σ^F or *sspB* (for example, by the presence in the mother cell of a repressor of *sspB* transcription). To distinguish between these possibilities, we used an additional altered specificity mutant of σ^F bearing a valine-to-methionine substitution at residue 233 (VM233) (*11*). The VM233 mutant σ^F recognizes the promoter for *ctc*, a gene that is normally under the control of the nonsporulation transcription factor σ^B (*17*). *ctc* is normally

Department of Cellular and Developmental Biology, The Biological Laboratories, Harvard University, Cambridge, MA 02138.

*To whom correspondence should be addressed.

Table 1. Preseptation regulatory proteins that participate in the establishment of cell type.

Operon	Gene	Product	Function
spoIIE	*spoIIEA*	SpoIIEA	Regulation of σ^F activity
	spoIIEB	SpoIIEB	
	spoIIEC	SpoIIEC	
spoIIA	*spoIIAA*	SpoIIAA	
	spoIIAB	SpoIIAB	
	spoIIAC	σ^F	Forespore gene expression
spoIIG	*spoIIGA*	SpoIIGA	Processing of pro-σ^E
	spoIIGB	Pro-σ^E	Mother cell gene expression

induced under conditions in which spore formation is prevented, but in cells (strain PM646) that bear the VM233 mutant σ^F and lack σ^B, expression of a *ctc-lacZ* fusion was switched on during the second hour of sporulation (Fig. 2A).

The compartmentalization of *ctc-lacZ* expression was investigated by subjecting to immunoelectron microscopy thin sections of sporangia of strain PM646 bearing tandem chromosomal copies of the *ctc-lacZ* fusion. Although expression of *sspB-lacZ* was two- to threefold higher than that for *ctc-lacZ* and, thus, less gold decoration was obtained with PM646, thin sections were readily observed in which the distribution of gold particles was biased to the forespore (*15*). Typically, such sporangia had 10 to 25 particles in the forespore versus one to three particles in the mother cell, reinforcing the view that σ^F-directed gene expression is restricted to the forespore. Moreover, as *ctc* is not normally subject to sporulation control (*17*), the finding of compartmentalized *ctc* expression when *ctc* is brought under the control of σ^F suggests that compartmentalization is controlled at the level of σ^F and not at the level of its target promoter(s).

How might the action of σ^F be restricted to the forespore? Because expression of the *spoIIA* operon commences shortly before septum formation (*4, 18*) (that is, in the predivisional sporangium), forespore-specific transcription of genes under the control of σ^F cannot be a result of differential transcription of *spoIIA*. Rather, a post-transcriptional mechanism must exist for limiting the presence or activity of σ^F to the forespore chamber. Such a mechanism could involve the products of the promoter-proximal members (*spoIIAA* and *spoIIAB*; Table 1) of *spoIIA* (*5, 6, 19*); SpoIIAB is an inhibitor of σ^F-directed gene expression and SpoIIAA antagonizes the action of SpoIIAB (*5*).

To identify genes involved in controlling σ^F, we used *lacZ* fusions to *sspB* and *ctc* (in conjunction with VA233 and VM233, respectively) and to *gpr* (in conjunction with wild-type σ^F) to monitor σ^F-directed gene expression in mutants blocked at the septation (II) and engulfment (III) stages of sporulation (Table 2 and Fig. 2) (*20*). Excluded from this analysis were mutants in which the transcription of the *spoIIA* operon is prevented (*21, 22*). In confirma-

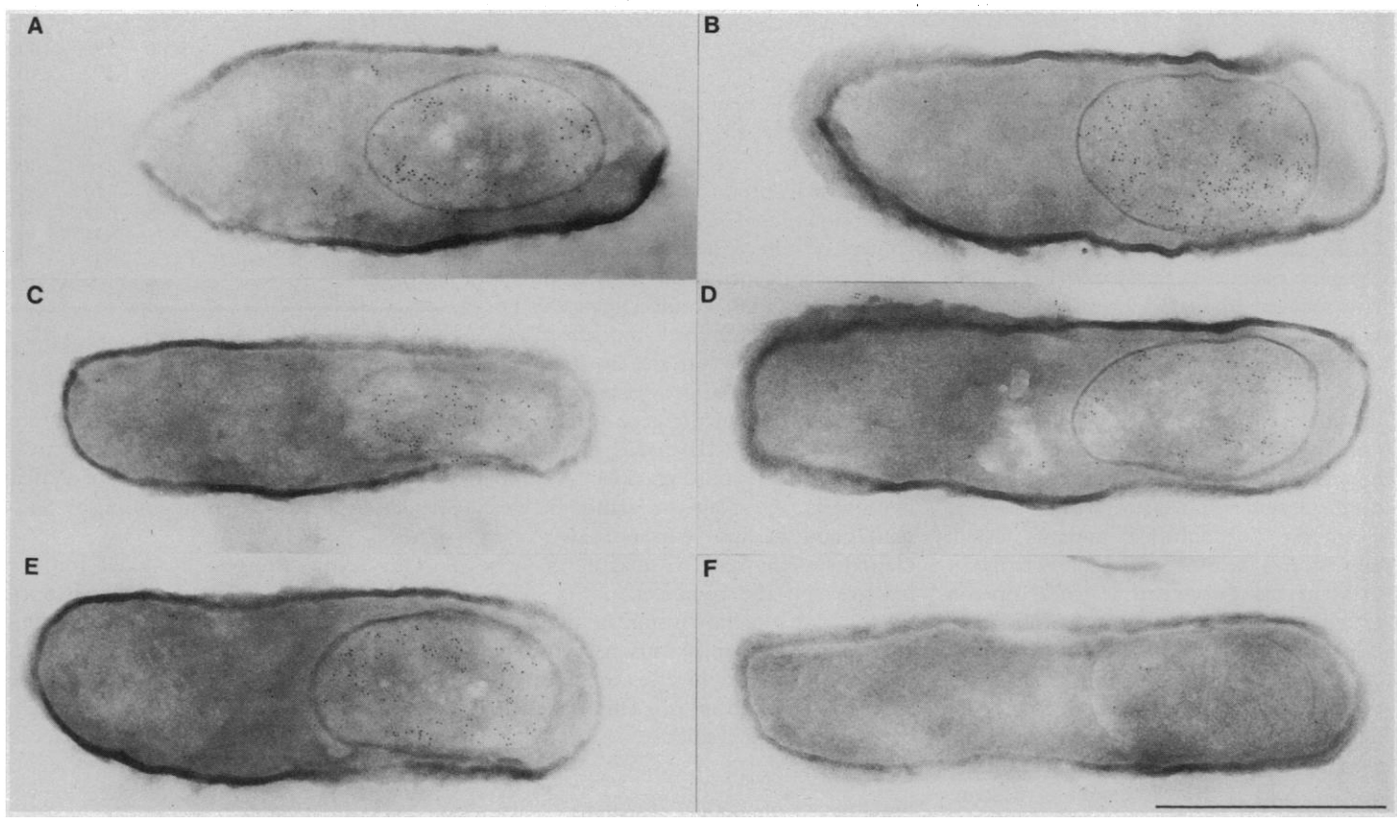


Fig. 1. Visualization of σ^F-directed gene expression by immunoelectron microscopy. Thin sections of fixed and embedded sporangia were treated first with rabbit anti-βgal and then with gold-conjugated antibody to rabbit IgG as described (*13*). The figure shows electron micrographs of the decorated thin sections. The dark specks are gold particles. (**A** to **E**) Sporangia of strain PM73 (*spoIIAC-VA233 spoIIIGΔ1 sspB-lacZ*) (*5*) that contain multiple chromosomal copies of the *sspB-lacZ*–bearing plasmid pPS490 are depicted. (**F**) A sporangium of the control strain SC500 (*spoIIIGΔ1*) is shown, which lacks pPS490. The scale bar represents 500 nm.

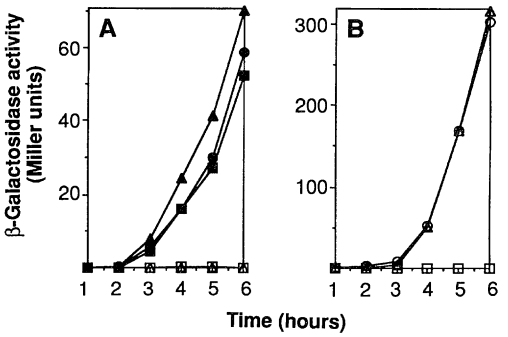


Fig. 2. Effect of *spo* mutations on the expression of a gene under the control of σ^F. Cells were grown and sporulated by nutrient depletion in DS medium and assayed for β-galactosidase activity at the indicated times after the end of the exponential phase of growth as described (*5, 13*). (**A**) The pattern of β-galactosidase synthesis in strain PM646 (filled triangles) and in congenic strains containing *spoIID::Tn917ΩHU298* (PM637; filled circles), *spoIIE48* (PM607; open squares), *spoIIE::Tn917ΩHU181* (PM638; open triangles), and *spoIIIA::Tn917ΩHU25* (PM641; filled squares) is shown. (**B**) The pattern of β-galactosidase synthesis in congenic derivatives of PM646 containing *spoIIE48* (PM607; open squares), *spoIIABΔ1* (PM599; open circles), and both *spoIIE48* and *spoIIABΔ1* (PM606; open triangles) is shown. PM646 contains a null mutation in the σ^B structural gene (*sigB::neo*) (*34*) the σ^F VM233 altered-specificity mutation (*spoIIAC561*) (*35*) and a *ctc-lacZ* fusion contained in the prophage of phage SPβ (*17*).

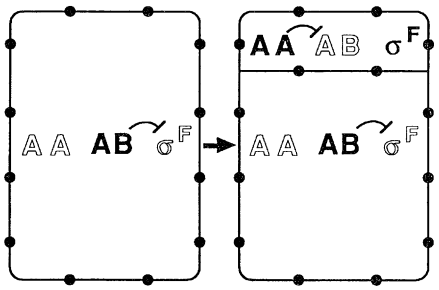


Fig. 3. Model for the compartmentalization of σ^F-directed gene expression. The figure depicts a predivisional sporangium and a (stage II) (*25*) sporangium that has been partitioned into mother-cell and forespore compartments by formation of the sporulation septum. All three products of the *spoIIA* operon are present in the predivisional cell and in both compartments of the post-septation sporangium, with AA representing SpoIIAA and AB representing SpoIIAB. Solid lettering indicates that the gene product is in an active state and shadow lettering indicates that the gene product is in an inactive state. Membrane-bound complexes of the SpoIIE proteins (filled circles) could be distributed throughout the membranes of both sporangia, as depicted, or they could be limited to the septum that separates the two compartments.

Table 2. Effect of *spo* mutations on σ^F-directed gene expression. Production of β-galactosidase from strains that contain the indicated gene fusions was determined from time course experiments carried out as described for Fig. 2. The values presented are the amounts of β-galactosidase accumulation observed in the presence of the indicated *spo* mutations as a percent of enzyme accumulation in corresponding congenic parent strains (~3, 120, and 50 Miller units for the *gpr-, sspB-*, and *ctc*-fusion–bearing parent strains, respectively; that is, the + row in the table). Except for those indicated below, the strains used had the following genotypes: *spoIIIGΔ1 gpr-lacZ, spoIIAC-VA233 spoIIIGΔ1 sspB-lacZ* (*5*), and *spoIIAC-VM233 sigB::neo ctc-lacZ* (see legend to Fig. 2) or were otherwise isogenic derivatives of these parent strains bearing the indicated *spo* mutations (from the laboratory strain collection). In some cases both the parent strain and the congenic derivative used to monitor *gpr-lacZ* and *sspB-lacZ* expression contained *spoIIIGΔ::neo* instead of *spoIIIGΔ1*. Also, the effect of *spoIIG*41 on *ctc-lacZ* expression was determined (in both the parent and its *spoIIG41*-bearing derivative) in the presence of *spoIIIGΔ::neo* and in the presence of *sigBΔ::erm* instead of *sigB::neo*.

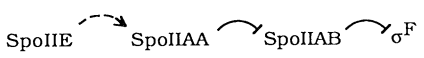
spo mutation	β-Galactosidase synthesis from gene fusions (%)		
	gpr-lacZ	*sspB-lacZ*	*ctc-lacZ*
+	100	100	100
spoIIAA69	<2	<2	2
spoIIABΔ1	5100	290	1000
spoIIAA69 spoIIABΔ1	1600	192	2400
spoIID::Tn917ΩHU298	53	ND	73
spoIIE::Tn917ΩHU181	<2	ND	<2
spoIIE48	<2	9	<2
spoIIE48 spoIIABΔ1	2900	320	1000
spoIIG41	106	40	84
spoIIM::Tn917ΩHU287	76	ND	41
spoIIIA::Tn917ΩHU25	96	ND	66
spoIIIDΔ::erm	106	ND	90
spoIIIE36	<2	8	<2

ND; not determined.

tion and extension of earlier results (*5*), a mutation in *spoIIAA* prevented expression of the *gpr-, sspB-*, and *ctc-lacZ* fusions, whereas a mutation in *spoIIAB* caused overexpression of these gene fusions (Table 2). In addition, the block in expression of the gene fusions by the *spoIIAA* mutation was relieved in *spoIIAA spoIIAB* double mutants. Finally, in support of the view that the inhibition by SpoIIAB is exerted at the level of σ^F and not its target promoters, the effect of the *spoIIAA* and *spoIIAB* mutations was observed with three promoters, including one (*ctc*) that is not normally under sporulation control.

Among the additional mutants tested, only strains bearing either of two mutations in the three-cistron *spoIIE* operon (*23*) or a mutation in the *spoIIIE* gene exhibited a block in σ^F-directed gene expression (Table 2 and Fig. 2A). Thus, the products of either or both loci could be involved in governing σ^F activity. However, *spoIIIE* is unlikely to have an important role in the expression of σ^F-controlled genes in that the effect of the *spoIIIE* mutation is strongly influenced by the position of the σ^F-recognized promoters in the chromosome (*24*). This leaves only *spoIIAA, spoIIAB*, and the members of the *spoIIE* operon (Table 1) as likely to be involved in governing the activity of σ^F.

Because its products are not required for expression of *spoIIA* (*21, 22*), *spoIIE* might control σ^F-directed gene expression indirectly by influencing the activity of SpoIIAA or SpoIIAB. To investigate this possibility, we determined the effect of a *spoIIAB* mutation on the *spoIIE*-dependence of σ^F-directed gene expression. Our results showed that the gene fusions were expressed in *spoIIAB spoIIE* double mutants (Table 2 and Fig. 2B). A simple hypothesis can explain the relation between *spoIIE* and the SpoIIAA-SpoIIAB-σ^F regulatory system; SpoIIAA may normally be inactive and the products of the *spoIIE* operon may somehow stimulate the activity of SpoIIAA, thereby causing it to antagonize (or otherwise reverse) the inhibitory effect of Spo-IIAB on σ^F. This is depicted by the following cascade:



The dashed arrow emphasizes that the SpoIIE proteins could influence the regulatory pathway indirectly (see below). Alternative possibilities to this linear pathway are that the SpoIIE proteins cause the inactivation of SpoIIAB or a modification of σ^F that renders it insensitive to inhibition by Spo-IIAB.

Our results suggest a model for the establishment of cell type in which Spo-IIAA is in an inactive state in the predivisional cell and mother cell, thereby allowing SpoIIAB to prevent σ^F-directed gene expression in these cell types (Fig. 3) (*25*). Some unknown feature of the forespore under the control of the SpoIIE proteins may activate SpoIIAA, thereby freeing σ^F from inhibition by Spo-IIAB and selectively turning on forespore-specific genes under the control of σ^F. A variation of the model is that SpoIIAA must be present in high concentration in order to antagonize

SpoIIAB and that the SpoIIE proteins cause SpoIIAA to concentrate in the forespore. SpoIIEA has features of an integral membrane protein (*23*), and the two compartments of the stage II sporangium differ markedly in their surface area to volume ratios. Thus, for example, if the SpoIIE proteins were a transport system (for SpoIIAA or for an ion or molecule to which SpoIIAA responds), transport into or out of the two cell types might occur with different efficiencies. Mutants in *spoIIE* produce sporangia with aberrant sporulation septa that contain a thick peptidoglycan layer (*26*). This observation is consistent with a view in which the forespore-specific activation of σ^F depends on (possibly SpoIIE-mediated) interactions between the two cell types (*26*).

The discovery that σ^F-directed gene expression is confined to the forespore has implications for the establishment of daughter cell–specific programs of gene expression. The earliest event in the establishment of the mother cell line of gene expression is the appearance of σ^E, which governs the appearance of the mother-cell regulatory proteins SpoIIID, σ^K, and GerE (*27*). σ^E is encoded by the two-cistron *spoIIG* operon as an inactive precursor called pro-σ^E, which is converted to the mature factor by the putative processing enzyme SpoIIGA (*28–30*) (Table 1). Both pro-σ^E and SpoIIGA are produced in the predivisional sporangium (*4*), but processing of pro-σ^E may only take place in the mother cell (*16, 31*). Because processing requires the action of σ^F (*29, 32*), our finding that σ^F is active in the forespore suggests that the forespore-specific product of a gene under the control of σ^F acts vectorially across the membrane barrier between the two cell types and causes selective processing of pro-σ^E in the mother cell. This is analogous to the way the forespore-specific product of a gene (*spoIVB*) under the control of σ^G stimulates the processing of pro-σ^K in the mother cell (*13*).

The forespore-specific transcription factor σ^G is encoded by *spoIIIG* (*3, 9*), whose promoter is controlled both by σ^F (*5–7, 33*) and, autogenously, by σ^G (*9, 10*). Appearance of σ^G in the forespore could result from selective transcription of *spoIIIG* in the forespore by σ^F (*7*). Evidence further suggests that σ^G is in an inactive state prior to stage III and that after engulfment some feature of the pinched-off forespore relieves this inhibition (*10*). In this view, σ^G-directed gene expression depends on the engulfment of the forespore by the mother cell, which, in turn, requires the expression of genes under the control of σ^E (*1*). Because pro-σ^E processing may depend on σ^F-directed gene expression, both the synthesis and the activation of σ^G may ultimately be determined by the compartmentalized action of σ^F.

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11. The creation of the altered specificity mutants of σ^F is based on the observation that σ^F, σ^G, and σ^B are highly related sigma factors; among residues in the helix-turn-helix structure of region 4 thought to contact bases in the −35 region of cognate promoters [T. Gardella, H. Moyle, M. M. Susskind, *J. Mol. Biol.* **206**, 579 (1989); D. A. Siegele, J. C. Hu, W. A. Walter, C. A. Gross, *ibid.*, p. 591], σ^F differs from the other two sigma factors simply by the presence of an alanine in σ^G and by the presence of a methionine in σ^B at the positions homologous to the valine at residue 233 in σ^F. Work with the VA233 (*5*) and VM233 (*34*) substitutions show that the replacement of the valine at residue 233 with alanine and methionine is sufficient to enable σ^F to use σ^G- and σ^B-controlled promoters, respectively. A mutation (*spoIIAC561*) (*35*) that creates a methionine codon at codon 233 was used to create VM233.
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18. The use of *lacZ* fusions to *spoIIA* indicated that the operon is induced at, or shortly before, the time of septation (*21*). However, more recent experiments with genetic mosaics show directly that expression of *spoIIA* must commence prior to septation; mosaic sporangia in which the predivisional sporangium bears a *spoIIAC* mutation in the chromosome that is to become segregated to the forespore (and a wild-type *spoIIAC* gene in the chromosome that is to become segregated to the mother cell) produce heat-resistant (but genotypically *spo*$^-$) spores (*4*). Hence *spoIIA* gene products produced prior to the formation of the forespore are sufficient to allow spore formation to occur, and the forespore can rely entirely on σ^F that it captures from the predivisional cell.
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24. *spoIIIE36* inhibits transcription from *spoIIIG* when the promoter is located at its normal chromosomal position, but does not prevent transcription from *spoIIIG* when the promoter has been transplanted to the *amyE* locus (*7*). Also, *spoIIIE36* impairs transcription from the *ctc* promoter (under the direction of the σ^F altered specificity mutant) when the promoter is contained within a SPβ prophage, but enhances transcription when the promoter is located at its normal chromosomal position (*34*). In contrast, the effect of *spoIIE* mutations on σ^F-directed gene expression is not dependent on chromosome position (*34*). Because of the position effect and because *spoIIIE* is a vegetatively expressed gene (*33*), the effect of the *spoIIIE* mutation is evidently adventitious and is probably not indicative of a role for the *spoIIIE* gene product in the control of σ^F-directed gene expression.
25. In keeping with the observation that genes under the control of σ^F are efficiently induced in certain mutants (for example, *spoIID* and *spoIIG*; Table 2) that block sporulation prior to engulfment (stage III), relief of σ^F from inhibition by SpoIIAB is assumed to occur during morphological stage II in the model. A prediction of the model is that the *spoIIABΔ1* mutation should permit σ^F-directed gene expression to occur in both sporangial compartments, but this cannot be tested at present; the high amount of σ^F activity in *spoIIABΔ1* mutant cells blocks sporulation prior to the formation of the sporulation septum (*15*).
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