Timing of *spoII* Gene Expression Relative to Septum Formation during Sporulation of *Bacillus subtilis*

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***spoII* mutants formed heat-resistant spores when transformed with *spo*⁺ DNA near the start of sporulation. Many of the spores formed remained genetically *spoII*. It is deduced from this result and previous epistasis experiments that the *spoII* loci are transcribed before the spore septum is formed.**

Formation of the asymmetrically sited sporulation septum is a crucial event in spore formation. A series of genetic loci, the *spoII* loci, are defined by mutations that permit formation of the spore septum but prevent any further morphological development (11). These loci are clearly essential for spore formation, but their exact relation to formation of the septum is unclear; in all cases the appearance of a *spoII* mutant septum is different from that of the wild-type sporulation septum. Two *spoII* loci, *spoIIA* and *spoIIG*, include genes coding for RNA polymerase sigma factors (9). σ^E , the product of *spoIIGB* (a gene within the *spoIIG* locus), is first made as an inactive precursor, P³¹ (7). Appearance of P³¹ coincides with transcription of *spoIIG*; processing of P³¹ to σ^E begins about 1 h after P³¹ formation (7). Processing depends on the activity of *spoIIA* (the first gene of the *spoIIG* locus) and of the polycistronic *spoIIA* and *spoIIE* loci (7, 13). It has been proposed that the processing of P³¹ by the product of *spoIIA* is triggered by correct formation of the spore septum (7, 13); *spoIIA* and *spoIIE* are presumed to be required for correct septum formation and hence for processing (13). A major attraction of this hypothesis is that it ties the complex pattern of gene transcription during spore formation to a critical morphological event. Determination of the time of transcription of *spoIIA*, *spoIIE*, and *spoIIG* relative to the formation of the septum would clearly provide a test for the hypothesis.

The two cells that result from correct sporulation septum formation are of unequal size, and their developmental fates are radically different: the forespore becomes the heat-resistant spore, whereas the mother cell ultimately lyses. Both cells are necessary for spore formation. We had previously devised a genetic method to demonstrate compartmentalization of late *spo* locus expression during sporulation (8). The method utilized the following facts: shortly before spore septum formation, two copies of the chromosome are present in one cell; and one chromosome is destined for the mother cell, and the other is destined for the forespore. By transforming *spo* mutants as they started to sporulate, it was possible to obtain conditions in which only one of the two copies of the chromosome was likely to have been transformed with the *spo*⁺ marker. (Bacteria that are already sporulating are refractory to transformation.) The *spo*⁺ chromosome could presumably end up in the mother cell or in the forespore. If correct expression in the mother cell is sufficient for spore formation, then it should be possible to obtain heat-resistant spores that are genetically asporogenous (*spo*). However, if transcription in the fore-

spore is required, then all the heat-resistant spores formed would be genetically *spo*⁺. By using these criteria, it was concluded that *spoVA* had to be transcribed in the forespore, whereas *spoVB* and *spoIIA*, for example, need be transcribed only in the mother cell in order to obtain heat-resistant spores (8). In the study presented here, we adapted the sporulation-transformation strategy to determine the time of *spoII* locus transcription relative to septum formation.

Strains were induced to grow in the exhaustion medium of Lencastre and Piggot (8) and transformed with *spo*⁺ DNA towards (i.e., less than one generation from) the end of exponential growth. Cultures were incubated for 16 to 20 h after the end of exponential growth, and then spores were selected by heating at 85°C for 20 min (8). (In this medium, *spo*⁺ strains form heat-resistant spores within 12 h of the end of exponential growth.) Survivors were plated on Schaeffer sporulation agar (12) and incubated for 48 h at 42°C. Phenotypically, Spo⁺ and Spo⁻ colonies were distinguished by opacity and pigmentation. The distinction was always confirmed for representative colonies from each cross by phase-contrast microscopy and by testing heat resistance. The phenotypes of the colonies are presumed to reflect the genotypes of the heat-resistant spores from which they were derived; these are referred to as *spo*⁺ spores and *spo* spores, respectively. (Up to about 5% of colonies were sectored for spore pigment. They were presumed to result from either heteroduplex or diploid spores and were scored as *spo*⁺.) All the *spoIIA*, *spoIIE*, and *spoIIG* mutants tested gave a substantial proportion of *spo* spores as a result of transformation (Table 1). In contrast, strains harboring mutations in loci known to be expressed in the forespore, *spoVA* (4, 8) and *spoIIG* (5) in strains SL17 and SL4007, respectively, gave exclusively *spo*⁺ spores. In confirmation of previous results (8), strain SL11 gave about 60% *spo* spores; this strain harbored a mutation in the mother cell locus *spoVB*.

We consider the appearance of *spo* spores the result of DNA-mediated transformation (8) (Table 1) of the mother cell genome. Abortive transformation of the forespore is formally also possible. Abortive transformation, in the sense of unilinear inheritance of unincorporated DNA, has not been reported for *B. subtilis*, however, and is considered unlikely here, for the following reasons: the *spoIIA* and *spoVA* loci are closely linked by transformation (10), yet they give opposite results in the sporulation-transformation experiment (Table 1); no evidence for abortive transformation was found during testing for auxotrophic markers in this system (8); hydroxyphenylazo uracil (HPUra) treatment would not be expected to increase the proportion of *spo*

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TABLE 1. Transformation of *spo* mutants for the ability to form heat-resistant spores^a

Strain	Relevant genotype	No. of heat-resistant survivors				% of heat-resistant transformants that were <i>spo</i>
		<i>spo</i> ⁺ DNA		No DNA		
		<i>spo</i> ⁺	<i>spo</i>	<i>spo</i> ⁺	<i>spo</i>	
SL650	<i>spoIIAA69</i>	608	328	0	0	35
SL1013 ^b	<i>spoIIAA69</i>	572	356	0	0	38
SL401	<i>spoIIAC1</i>	528	572	0	344	30 ^c
SL547	<i>spoIIIE20</i>	1,156	252	0	0	18
SL635	<i>spoIIIE64</i>	502	305	0	150	24 ^c
SL658	<i>spoIIIE71</i>	845	315	0	0	27
SL617	<i>spoIIIGB55</i>	1,216	244	0	0	17
SL4007	<i>spoIIIGΔ1</i> ^d	310	0	0	0	0
SL17 ^b	<i>spoVA89</i>	315	0	0	0	0
SL11 ^b	<i>spoVB91</i>	712	1,268	0	0	64

^a Strains were transformed with 5 μg of DNA per ml within one generation of the end of exponential growth in the exhaustion sporulation medium of Lencastre and Piggot (8). Spores were selected 16 to 20 h after the end of exponential growth by heating at 85°C for 20 min. Survivors were scored as *spo*⁺ or *spo* as described in the text. Numbers of transformants are the numbers of colonies obtained from 0.2-ml samples.

^b These strains were not isogenic with MB24.

^c Percentage corrected for heat-resistant survivors obtained with no DNA.

^d Strain containing this mutation was kindly provided by Peter Setlow. The *spoIIIGΔ1* mutation was transferred into our standard strain MB24 by congression so as to compare it with isogenic *spoII* mutants.

spores (7) (Table 2 and see below); congression frequencies for unlinked auxotrophic markers were similar for *spo*⁺ spores and *spo* spores (8; unpublished observations).

σ^E is present in both forespore and mother cell (2). In addition, epistasis experiments have indicated that transcription of *spoIIA*, *spoIIIE*, and *spoIIIG* is required for the transcription of *spo* loci whose transcription is localized in the forespore (4, 5) and also of *spo* loci whose transcription is localized in the mother cell (6). The opposed membranes separating the mother cell from the forespore form an effective barrier between the two cell types (14). Consequently, if the *spoII* loci were transcribed after septation, they would presumably have to be transcribed in both cell types in order to control transcription in both cell types, and the sporulation-transformation experiment would have yielded exclusively *spo*⁺ spores. However, if they were transcribed before septation, then, because the *spoII* mutations are recessive to *spo*⁺, *spo* spores could result from a

sporulation-transformation experiment. The data fit the hypothesis of transcription before septum formation; they do not rule out continued transcription or functioning of the gene product after the septum is formed.

It remains formally possible that the *spoII* loci are transcribed in the mother cell after septation and that they control expression of forespore genes by somehow overcoming the permeability barrier between the two cell types. Dependence of *cot* genes and *gerE* in the mother cell on expression of the forespore locus *spoIIIG* (3) indicates that in certain circumstances the barrier can be overcome. Against barrier breakdown and in support of expression before septation is the observation that the proportion of *spo* spores obtained with *spoII* mutants was considerably smaller than the proportion obtained with a *spoVB* mutant and with strains mutated in other mother cell loci (8) (Table 1). This difference was still present when the extent of DNA replication after transformation, and hence the chance of two transformed chromosomes being present, was reduced by the addition of HPURa (1). A replacement sporulation system (8) was used to provide an easy operational definition of the start of sporulation. With *spoVB91* the proportion of heat-resistant spores that were *spo* was 80% when HPURa was added 3 h after transformation at the start of sporulation (8), consistent with mother cell expression. In contrast, the proportion of *spo* spores did not exceed 50% with a *spoIIIGB55* strain, SL617 (Table 2); similar results were obtained with a *spoIIAA69* strain, SL650 (data not shown). A maximum of 50% *spo* spores would be expected in these experiments if the loci were transcribed before septation and subsequent partitioning of the chromosomes (one *spo*⁺, one *spo*) was random. It is still possible that the septum presents no barrier in either direction and that the *spoII* loci can be expressed after septation in either cell type in order to effect correct gene expression in both cell types; we consider this unlikely. Rather, we conclude that *spoIIA*, *spoIIIE*, and *spoIIIG* are transcribed before the spore septum separates the mother cell from the forespore. This is consistent with the involvement of these loci in septum formation. In particular, it is consistent with, but does not prove, the Stragier-Haldenwang model (7, 13) for coupling gene expression to morphological development; a contrary result would have contradicted the model.

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TABLE 2. Effect of restricting DNA replication with HPURa on the proportion of *spo* transformant spores obtained with SL617^a

Time (h) of HPURa addition	No. of heat-resistant transformants		% of heat-resistant transformants that were <i>spo</i>
	<i>spo</i>	<i>spo</i> ⁺	
1¼	1	0	
2½	28	46	38
4	370	1,101	25
5	780	2,296	25
6	972	3,168	23
24	1,740	9,040	16

^a SL617 (*spoIIIGB55*) was induced to sporulate in the replacement medium of Lencastre and Piggot (8). *spo*⁺ DNA (5 μg/ml) was present in the sporulation medium at the time of transfer of SL617 to it (no heat-resistant transformants were obtained in the absence of DNA). At different times thereafter, HPURa was added to 66.6 μM (8). Cultures were incubated 24 h after the time of transfer and then heated at 85°C for 20 min. Survivors were scored on Schaeffer sporulation agar (12). Numbers of transformants are the numbers of colonies obtained from 0.2-ml samples.

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