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Differential Gene Expression Governed by Chromosomal Spatial Asymmetry

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Summary

The activity of the transcription factor σ^F is confined to one (the forespore) of two cells created by asymmetric division during sporulation in *B. subtilis*. We show that σ^F activation is partly governed by the position of the gene for the unstable anti-σ^F factor SpoIIAB. Because cytokinesis precedes chromosome segregation, most of the chromosome is translocated into the forespore after division. We hypothesize that because *spoIIAB* enters the forespore late, SpoIIAB lost to proteolysis is temporarily not replenished. Thus, chromosome asymmetry would be translated into the asymmetric distribution of SpoIIAB. Supporting this idea, transposition of *spoIIAB* to sites present in the forespore at the time of division impaired sporulation when a second pathway that participates in σ^F activation was disabled.

Introduction

A major challenge in developmental biology is the problem of understanding how transcription factors are activated in a cell-specific manner during differentiation (Horvitz and Herskowitz, 1992). A paradigm for this problem is the cell-specific activation of the sporulation transcription factor σ^F in *B. subtilis*. Asymmetric division during sporulation generates dissimilarly sized progeny called the forespore (the smaller cell) and the mother cell, which exhibit differential gene transcription (Errington, 1996; Losick, 1996). The σ^F factor is produced at the start of sporulation (in the predivisional cell or sporangium) but does not become active in directing transcription until after asymmetric division when its action is confined to the forespore (Margolis et al., 1991). The activation of σ^F initiates a cascade of cell-specific regulatory proteins that drives subsequent differentiation of the forespore and the mother cell (Losick and Stragier, 1992). How is σ^F-directed transcription restricted to one cell? Activation of σ^F is governed by three proteins: the anti-σ^F factor SpoIIAB, its antagonist, the phosphoprotein SpoIIAA, and the membrane-bound phosphatase SpoIIE (Duncan et al., 1995; Schmidt et al., 1990). Here we present evidence that cell-specific activation of σ^F is achieved by two partially redundant pathways, one involving the subcellular localization of the SpoIIE phosphatase and another based on the chromosomal location of the gene (*spoIIAB*) for the anti-σ^F factor SpoIIAB.

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The σ^F factor is held in an inactive complex in the predivisional sporangium and in the mother cell by the antisigma factor SpoIIAB (Duncan and Losick, 1993). SpoIIAA mediates escape from the complex by reacting with SpoIIAB-σ^F in the forespore to cause its dissociation into free SpoIIAB and free σ^F, which can then bind to RNA polymerase and direct transcription of genes under its control. The ability of SpoIIAA to disrupt the SpoIIAB-σ^F complex is determined by its phosphorylation state; only dephosphorylated SpoIIAA can effect the release of σ^F from the complex (Duncan et al., 1996). The phosphorylation state of SpoIIAA is governed by SpoIIAB itself, which is both a protein kinase and an antisigma factor (Duncan and Losick, 1993; Garsin et al., 1998; Min et al., 1993), and by the phosphatase SpoIIE (Arigoni et al., 1996; Duncan et al., 1995; Feucht et al., 1996).

A clue to the nature of the mechanism that restricts the activity of σ^F to the forespore came from the discovery that the SpoIIE phosphatase, an integral membrane protein with ten membrane spanning segments in its N-terminal region (Arigoni et al., 1999), localizes specifically to the polar septum (Arigoni et al., 1995). The septal localization of the SpoIIE phosphatase and the possible dependence of its function on its proper localization could be, in principle, a mechanism to delay σ^F activation until asymmetric division. Also, sequestration of SpoIIE to the septum would be expected to increase the ratio of phosphatase to kinase in the forespore (due to the smaller volume of the forespore) and thereby bias σ^F activation to the small chamber of the sporangium. However, cells producing a truncated form of SpoIIE that lacks the membrane spanning segments (SpoIIEΔmem) are capable of sporulation and of σ^F activation in a cell-specific manner, although not as efficiently as in the wild-type (Arigoni et al., 1999). The truncated SpoIIE protein is uniformly distributed throughout the cytoplasm of the sporangium, suggesting that although SpoIIE localization may contribute to the proper temporal and spatial regulation of σ^F, it is evidently not essential for it. If so, then some other mechanism(s) must contribute to the cell-specific activation of σ^F.

A candidate for an additional regulatory mechanism emerged from the recent discovery that free SpoIIAB (but not SpoIIAB bound in a complex with σ^F) is proteolytically unstable with a half-life of approximately 25 min and that this instability is important for the activation of σ^F (Pan et al., 2001). Proteolysis of SpoIIAB requires a determinant located at the extreme C terminus of the protein and degradation is dependent on the ClpCP protease (Pan et al., 2001). These findings raise the possibility that degradation could lead to reduced levels of the SpoIIAB anti-σ^F factor in the forespore and hence to cell-specific activation of σ^F. If so, how could a compartment-specific reduction in SpoIIAB levels be achieved? A simple mechanism would be transient genetic asymmetry of the *spoIIAB* gene. Because asymmetric division occurs before chromosome segregation is complete, the developing cell experiences a period in which only the replication origin-proximal one-third

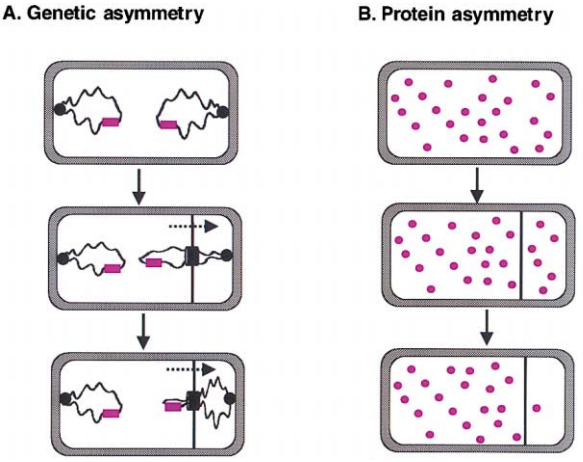


Figure 1. Schematic Diagram of Gene and Protein Asymmetry during Sporulation

(A) Following initiation of DNA replication, the *oriC* region (black dot) of each chromosome moves to the cell poles. Formation of the polar septum generates two cellular compartments, the forespore and the mother cell, and results in a bisection of the chromosome destined for the forespore. The forespore copy of *spoIIAB* (purple box) remains in the mother cell until SpoIIIE (black ring) mediated chromosome segregation is largely complete.

(B) In the predivisional cell, SpoIIAB protein is located diffusely in the cytoplasm. Formation of the polar septum transiently excludes the *spoIIAB* gene from the forespore as described in (A), resulting in lower SpoIIAB levels in the forespore.

of the chromosome is present in the forespore (Wu and Errington, 1994, 1998). The remainder of the chromosome is subsequently pumped into the forespore across the polar septum by the DNA translocase SpoIIIE (not to be confused with the SpoIIE phosphatase) (Wu et al., 1995). Thus, as pointed out by Frandsen et al. (1999), genes located outside this origin-proximal region are transiently excluded from the forespore (Figure 1A). Because the entire *spoIIA* operon comprising the genes encoding SpoIIAA, SpoIIAB, and σ^F is located in one of the last regions of the chromosome to enter the forespore, the gene for the SpoIIAB anti-σ^F factor is initially not present in the forespore. Whereas degradation of SpoIIAB would be expected to occur in both the mother cell and the forespore, the absence of *spoIIAB* would prevent the anti-σ^F factor from being replenished in the forespore. This would cause a decrease in SpoIIAB levels in the forespore relative to its more stable partners, σ^F and SpoIIAA (Figure 1B). Because the activation of σ^F is highly sensitive to the level of the antisigma factor (Pan et al., 2001), this decrease would result in the preferential activation of σ^F in the forespore.

Our model for the regulation of σ^F predicts that the chromosomal position of the *spoIIA* operon and, in particular, of the *spoIIAB* gene, participates in the activation of the forespore-specific transcription factor. Molecular genetic manipulations that transpose *spoIIAB* to origin-proximal positions would therefore be expected to inhibit σ^F activation because at this location *spoIIAB* would not be transiently excluded from the forespore compartment. Here we show by transposing *spoIIAB* and the *spoIIA* operon to various positions around the chromosome that the position of the gene for the anti-sigma factor helps to govern the activation of σ^F. We also show that permanent exclusion of *spoIIAB* from the forespore through the use of a mutant of the SpoIIIE DNA translocase causes hyperactivation of the forespore transcription factor. *In toto*, our results provide evidence for a novel mechanism for the establishment of cell-specific gene activation in which the spatial asymmetry of a chromosome is translated into the asymmetric distribution of an unstable regulatory protein.

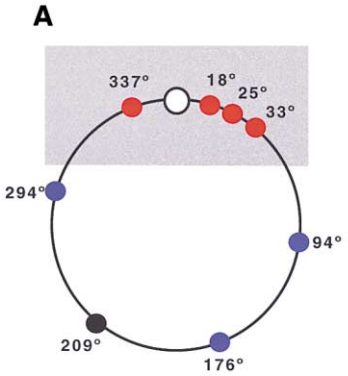
Results

Effects of Chromosomal Position of *spoIIAB* on Sporulation

A series of strains were constructed that contained a single copy of *spoIIAB* under control of its native promoter at seven different chromosomal positions (Figure 2A). Sporulation was only mildly impaired in these strains (40%–80% of wild-type) and likewise σ^F activation was not significantly different in any of these strains as compared to a wild-type strain (data not shown). If, as we hypothesize, the subcellular localization of the SpoIIE phosphatase and the chromosomal location of the *spoIIAB* gene independently contribute to the cell-specific activation of σ^F, then cells producing delocalized SpoIIE (SpoIIEΔmem) and harboring a mislocalized *spoIIAB* gene should exhibit a strong defect in sporulation. Indeed, doubly mutant strains producing SpoIIEΔmem and containing a single copy of *spoIIAB* at origin-proximal positions were markedly impaired in sporulation (Figure 2B). This was a synergistic effect because the level of sporulation for the double mutants was much lower than that expected from the simple product of the sporulation efficiencies of the SpoIIEΔmem mutant and of cells harboring a single copy of *spoIIAB* at an origin-proximal position (Table 2). Importantly, this synergistic effect depended upon the chromosomal location of *spoIIAB* in that doubly mutant cells harboring a single copy of *spoIIAB* at any of four origin-proximal positions (337°, 18°, 25°, 33°) were more impaired in sporulation than SpoIIEΔmem mutant cells containing *spoIIAB* at its native position in the *spoIIA* operon (209°) or at three other origin-distal locations (94°, 176°, 294°).

In the experiments described above, *spoIIAB* was transposed to various positions around the chromosome whereas the two other genes (*spoIIAA* and *spoIIAC*) of the *spoIIA* operon remained at their original position. To test whether the chromosome position effect was due to the position of *spoIIAB* per se and not to its position relative to *spoIIAA* and *spoIIAC,* strains were constructed in which the *spoIIA* operon was deleted from its native position and a copy of the entire *spoIIA* operon was transposed to a variety of positions (Figure 2B). As we observed for transposing *spoIIAB* alone, strains producing SpoIIEΔmem and containing a single copy of the *spoIIA* operon at origin-proximal locations (337°, 18°, 25°) were markedly more impaired in sporulation than cells containing a single copy of *spoIIA* at origin-distal locations (176°, 294°).

Because the relative concentrations of SpoIIAB, SpoIIAA, and σ^F are critical for σ^F activation, an alternative explanation for the *spoIIAB* position effect on sporulation in the *spoIIEΔmem* background would be that



B

chromosomal position	sporulation of a *spoIIEΔmem* mutant	
	spoIIAB	*spoIIA* operon
209°	1.0	1.0
337°	.01 2	.00 70
18°	.00 95	.00 14
25°	.00 75	.00 47
33°	.01 5	ND
94°	.45	ND
176°	.33	.37
294°	.41	.42

Figure 2. Position Effect of *spoIIAB* and the *spoIIA* Operon on Sporulation

(A) Chromosomal positions of transposed copies of *spoIIAB* and *spoIIA* are illustrated. The normal position for the *spoIIA* locus is at 209°. The open circle represents the location of *oriC*. The gray box delineates the approximate *oriC* proximal region of the chromosome that is initially confined to the forespore following polar septation (Wu and Errington, 1998).

(B) Congenic strains containing *spoIIEΔmem* as well as single copies of *spoIIAB* or the *spoIIA* operon at various chromosomal positions were assayed for the production of heat-resistant spores by growth for 24 hr in DS medium followed by heat treatment. Sporulation is expressed relative to that of a strain containing a wild-type *spoIIA* locus, which is set at 1.0. Sporulation values reported are the means of at least three experiments. For sporulation efficiencies >0.1, the variability was within 50% for all measurements; for sporulation efficiencies <0.1, the variability was less than 3-fold.

spoIIAB expression differs systematically between origin-proximal and origin-distal positions. We think that this is not likely for two reasons. First, multiple, independent immunoblot experiments revealed no consistent variation in whole cell SpoIIAB levels among strains harboring a single copy of *spoIIAB* at different positions, at least not in a manner that correlated with chromosomal position (data not shown). For example, when SpoIIAB and σ^F were visualized with ^{125}I-labeled, secondary antibodies, the relative levels of the two proteins in strains containing *spoIIAB* at six positions were essentially constant, exhibiting a variance of only about 12%. Second, as indicated above, we observed a similar position effect when the entire *spoIIA* operon, which includes the genes for both SpoIIAB and σ^F, was transposed to multiple positions around the chromosome (Figure 2B).

Effect of Chromosomal Position of *spoIIAB* on σ^F Activity

To investigate whether the sporulation defects observed were due to impaired σ^F activation, a *lacZ* reporter gene fused to a gene (*spoIIQ*) under the control of σ^F was introduced into strains producing SpoIIEΔmem and containing a single copy of *spoIIAB* at various chromosomal positions. (Figure 3A). When a single copy of *spoIIAB* was located at origin-proximal positions (337°, 18°, 25°, 33°), β-galactosidase activity was markedly lower than when a single copy of *spoIIAB* was located at positions distal from the origin (176°, 294°), including its native position (209°). Thus, σ^F activation is dependent on the position of the *spoIIAB* gene when the SpoIIE phosphatase has been delocalized from the polar septum.

Effect of a Protease-Resistant Mutant of SpoIIAB

A key feature of the transient genetic asymmetry model is that the anti-σ^F factor SpoIIAB is partially depleted from the forespore by degradation. A prediction of the model is that cells producing a mutant SpoIIAB, such as SpoIIAB-C145E (harboring an amino acid substitution at the penultimate residue), that is resistant to proteolysis (Pan et al., 2001) should exhibit a sporulation defect similar to that observed for strains harboring a single copy of *spoIIAB* at replication-origin proximal locations. The results showed that cells producing the mutant protein were modestly impaired in sporulation (~30%, Table 1). However, and in accord with the prediction of the model, cells producing both delocalized phosphatase (SpoIIEΔmem) and protease-resistant SpoIIAB (SpoIIAB-C145E) sporulated ~400-fold less efficiently than did the wild-type. Moreover, the sporulation efficiency of the

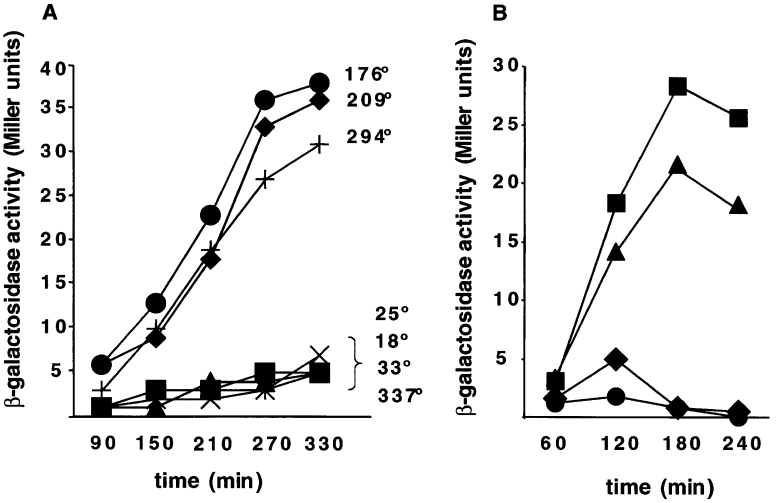


Figure 3. Position Effect of *spoIIAB* on σ^F Activation

Cells were grown and sporulated in DS medium and assayed for β-galactosidase activity at the indicated times after the end of the exponential phase of growth. Shown in each panel is a typical experiment; repeat experiments found similar patterns of β-galactosidase activity. (A) The panel shows the pattern of β-galactosidase activity in congenic strains containing *spoIIQ-lacZ* and a single copy of *spoIIAB* at various chromosomal positions (circles, 176°; plus signs, 294°; times signs, 25°; squares, 18°; open diamonds, 33°; triangles 337°) as well as the wild-type position (filled diamonds, 209°). (B) The pattern of β-galactosidase activity in a wild-type strain containing *spoIIR-lacZ* (diamonds) and in *spoIIR-lacZ*-containing strains that harbored *spoIIIE36* and *spoIIAB* at its normal position (squares), at 176° (triangles) or at 18° (circles).

Table 1. Synergistic Effects of *spoIIEΔmem* and *spoIIAB* Mutations on Sporulation

Strain	Relevant Genotype	Sporulation
PY79	wild-type	1.0
JDB111	*spoIIEΔmem*	.20
JDB480	*amyE::spoIIAB*	.40
JDB483	*amyE::spoIIAB spoIIEΔmem*	.008
JDB494	*spoIIAB-C145E*	.29
JDB495	*spoIIAB-C145E spoIIEΔmem*	.0023
JDB455	*spoIVFA^{bofB8}*	.25
JDB457	*spoIVFA^{bofB8} spoIIEΔmem*	.04
JDB567	*ΔspoVG*	.05
JDB568	*ΔspoVG spoIIEΔmem*	.003

Sporulation was assayed in congenic strains by growth for 24 hr in DS sporulation medium. Sporulation values reported are the means of at least three experiments. For sporulation efficiencies >0.1, the variability was within 50% for all measurements; for sporulation efficiencies <0.1, the variability was within 3-fold. The *amyE* locus is located at 25°.

double mutant was far lower (25-fold) than that expected from the simple product of the sporulation efficiencies of the two single mutants (Table 1). Thus, the *spoIIEΔmem* mutation and the *spoIIAB-C145E* mutation exhibited a pronounced synergistic effect on sporulation, which was similar to that observed between *spoIIEΔmem* and wild-type *spoIIAB* that had been transposed to origin-proximal positions.

Specificity of Synergy

If the synergy observed between *spoIIEΔmem* and *spoIIAB-C145E* or mislocalized *spoIIAB* is specific to the activation of σ^F, then synergy should not be observed between *spoIIEΔmem* and mutations in sporulation genes that are not involved in the activation of σ^F. The *spoIVFA^{bofB8}* mutation affects the activation of a late-stage mother cell-specific transcription factor (σ^K) and results in a mild sporulation defect (Cutting et al., 1990). A double mutant harboring *spoIIEΔmem* and *spoIVFA^{bofB8}* exhibited a greater defect in sporulation than strains singly mutant for *spoIIEΔmem* or *spoIVFA^{bofB8}*, but this defect was approximately the same as would be expected from the product of the effect of the two single mutations (Table 1). Similarly, the *ΔspoVG* mutation appears to affect the proper timing of asymmetric septation and by itself causes a moderate defect in sporulation (Matsuno and Sonenshein, 1999). The double mutant *spoIIEΔmem ΔspoVG* was more impaired than either single mutant, but the level of sporulation of the double mutant was approximately the same as that expected from the product of the sporulation efficiencies of the two single mutants (Table 1). Thus, synergy with *spoIIEΔmem* was observed only with mutations in genes involved in σ^F activation.

Permanent Genetic Asymmetry

Chromosome translocation into the forespore requires the activity of the SpoIIIE translocase, an ATP-dependent DNA tracking protein that is located in the polar septum (Bath et al., 2000; Wu and Errington, 1994, 1997). The *spoIIIE* missense mutation *spoIIIE36* blocks chromosome translocation but does not prevent selective activation of σ^F in the forespore (Pogliano et al., 1997). In fact, as has been known for some time, promoters under the control of σ^F are much more active in a *spoIIIE* DNA translocase mutant than in the wild-type (Londono-Vallejo and Stragier, 1995; Schuch and Piggot, 1994; Sun et al., 1991a). A possible explanation for this mysterious phenomenon is that *spoIIAB* is permanently excluded from the forespore when chromosome translocation is blocked. Thus, not only would the level of SpoIIAB in the forespore of a *spoIIIE* mutant be expected to fall, but it would do so to a greater extent than in the wild-type because the *spoIIAB* gene would never enter the forespore and hence SpoIIAB could not be replenished. As a test of this explanation, we investigated the effect of the chromosomal position of *spoIIAB* on the level of expression of *lacZ* fused to the σ^F-controlled *spoIIR* gene in a *spoIIIE* DNA translocase mutant. (The *spoIIR-lacZ* fusion was located in the origin of replication and hence it would have been present in the forespore in the presence or absence of DNA translocation.) As observed previously, the level of expression of *spoIIR-lacZ* was much higher in a DNA translocase mutant than in the wild-type. As expected, transposing *spoIIAB* from its normal position (209°) to another origin-distal position (176°) had little effect on expression of *spoIIR-lacZ* in the DNA translocase mutant (Figure 3B). However, transposition of *spoIIAB* to an origin-proximal position (18°) resulted in a strong inhibition of *spoIIR-lacZ* expression in the *spoIIIE* mutant, lowering the expression of the fusion to a level similar to that observed in the wild-type (Figure 3B). While this inhibition could conceivably be due to differential synthesis of SpoIIAB and σ^F in the forespore, this appears not to be the case because translocation of the entire *spoIIA* operon to this origin-proximal position also resulted in a strong inhibition of *spoIIR-lacZ* expression (data not shown). We conclude that the chromosomal position of *spoIIAB* contributes to the activation of σ^F and that this effect is exaggerated in a mutant in which *spoIIAB* is permanently excluded from the forespore.

Discussion

The σ^F factor plays a central role in development in that it is responsible for initiating both the forespore and the mother-cell programs of gene expression. It directs the transcription of the gene (*spoIIR*) that governs the appearance of the earliest-acting regulatory protein (σ^E) in the mother-cell line of gene expression (Karow et al., 1995; Londono-Vallejo and Stragier, 1995), and it directs the transcription of the gene (*spoIIIG*) for the next transcription factor (σ^G) to appear in the forespore (Sun et al., 1991b). Given this importance of the forespore regulatory protein, it stands to reason that σ^F is subject to tight temporal and spatial regulation. Here we have presented evidence indicating that two partially redundant pathways help to achieve this tight regulation: a previously known pathway that acts through the localization of the SpoIIE phosphatase to the polar septum and a newly discovered pathway that acts through the transient exclusion of the *spoIIAB* gene from the forespore (Figure 4). If either pathway is disabled, sporulation and σ^F activation are only mildly impaired; if, however, both systems are disrupted, then the activation of the fore-

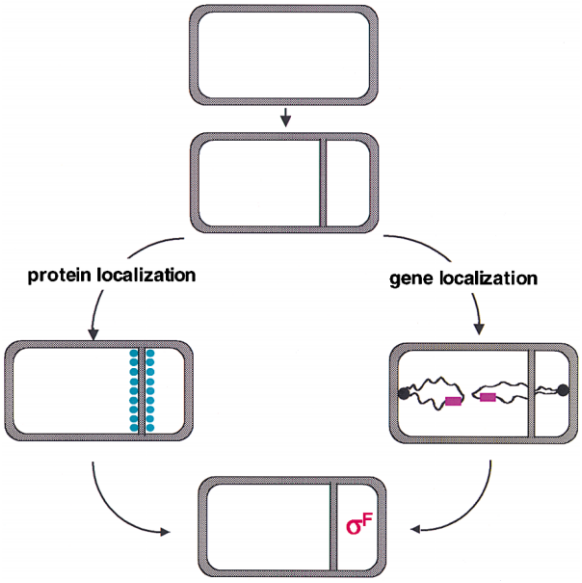


Figure 4. Two Partially Redundant Pathways Contribute to the Cell-Specific Activation of σ^F

One pathway involves the localization of the SpoIIE phosphatase (green dot) to the polar septum where it may have increased activity or concentration in the forespore. The second pathway involves the chromosomal position of the *spoIIAB* gene (purple box) relative to *oriC* (black dot), which is temporarily excluded from the forespore at the time of polar septation, and the instability of the SpoIIAB protein.

spore transcription factor and development are markedly inhibited.

According to our model, SpoIIAB, which is proteolytically unstable, is partially depleted from the forespore during the interval when the *spoIIAB* gene is excluded from the forespore. The time required for DNA translocation has not been measured directly but could be as short as 15 min (Lewis et al., 1994; Pogliano et al., 1999). If so, *spoIIAB* would be excluded from the forespore for a period shorter than the half-life for SpoIIAB (25 min; Pan et al., 2001) and the anti-σ^F factor would undergo only a modest decrease in concentration in the forespore. Nonetheless, even a modest decrease in the level of SpoIIAB, which is required in stoichiometric amounts to inhibit σ^F, could trigger a self-reinforcing cycle that would amplify the activation of σ^F. Thus, as proposed previously, degradation of SpoIIAB would increase the ratio of phosphatase (SpoIIE) to kinase (SpoIIAB), leading to an increased level of dephosphorylated SpoIIAA (Pan et al., 2001). This dephosphorylated SpoIIAA would react with additional SpoIIAB-σ^F to liberate additional free SpoIIAB molecules, which would in turn become potentially subject to proteolysis, thereby driving the ratio of phosphatase to kinase yet higher.

Transient genetic asymmetry of the *spoIIAB* gene may explain a previously mysterious aspect of σ^F regulation. It was known that activation of the forespore transcription factor depends on the formation of the polar septum (Levin and Losick, 1994). Yet recent work has shown that SpoIIAA-P is dephosphorylated to a substantial extent (in a manner that depends on the SpoIIE phosphatase) under conditions in which polar septation is prevented and σ^F is inactive (Feucht et al., 1999; King et al., 1999). These findings indicated that the accumulation of dephosphorylated SpoIIAA is insufficient to trigger the activation of σ^F. However, our present results and those of Pan et al. (2001) argue that degradation of SpoIIAB also contributes to the activation of the forespore transcription factor. Under conditions in which polar septation is prevented, transient genetic asymmetry would not be possible and hence SpoIIAB would remain at high levels and block the activation of σ^F even in the presence of dephosphorylated SpoIIAA. Of course, additional pathways could contribute to the activation of σ^F, such as a decrease in the ratio of ATP to ADP in the forespore (or the entire sporangium), which, as hypothesized previously, would favor the release of σ^F from SpoIIAB (Alper et al., 1994).

One final point about our transient genetic asymmetry experiments is that they may reveal a subtle but interesting feature of the chromosome segregation process in sporulation. We note that transpositions of *spoIIAB* close to but on the mother-cell side of the boundary between the two cells at the time of asymmetric division seemed to work as well in activating σ^F as did transpositions located closer to the terminus (Figure 2). A similar lack of evidence for a gradient that correlated with chromosomal position can be seen in the data of Stragier and coworkers (Frandsen et al., 1999). We therefore hypothesize that chromosomal translocation is itself relatively rapid and that there is a substantial pause between the time the polar septum is formed, or at least matures enough to become a partial barrier to diffusion of SpoIIAB, and the time that DNA translocation commences.

The temporary absence of *spoIIAB* from the forespore may be part of a broad strategy by which the *B. subtilis* chromosome is organized to take advantage of transient genetic asymmetry. Frandsen et al. (1999) showed that a strain in which the gene (*spoIIAC*) for σ^F was transposed to an origin-proximal position could sporulate with moderate efficiency in the absence of SpoIIAA and SpoIIE. The explanation for this remarkable finding was that during the period of chromosome translocation, the presence of *spoIIAC* in the forespore allowed for continued synthesis of σ^F without the corresponding synthesis of SpoIIAB. This was, of course, an artificial situation as *spoIIAC* is normally located far from the origin of replication and SpoIIAA and SpoIIE are normally indispensable for development. However, Frandsen et al. (1999) envisioned the existence of an unstable inhibitor of the SpoIIE phosphatase encoded by a gene located in the origin-distal region of the chromosome. In the spirit of this model, we have now shown that the origin-distal position of the *spoIIAB* gene in combination with the proteolytic instability of the SpoIIAB anti-σ^F factor is used by *B. subtilis* to help achieve cell-specific activation of σ^F.

Meanwhile, Piggot and coworkers (Khvorova et al., 2000) and Hofmeister and coworkers (Zupancic et al., 2001) have uncovered a complementary circumstance in which the origin-*proximal* position of a sporulation gene contributes to efficient sporulation. These workers have discovered that transposition of the *spoIIR* gene from its normal, origin-proximal position to origin-distal positions results in a delay of σ^E-directed gene expres-

Table 2. *B. subtilis* Strains Used

Strain	Genotype
PY79	prototroph
JDB111	*spoIIEΔ34-320*
JDB143	*spoIIEΔ34-320 amyE::spoIIAA-AC kan ΔspoIIAA-AC::spc*
JDB356	*spoIIEΔ34-320 amyE::spoIIAAΔAB+ kan spoIIAA+ ABΔAC+ mls*
JDB384	*thrC::spoIIQ-lacZ spc spoIIEΔ34-320*
JDB402	*spoIIEΔ34-320 zej-82::spoIIAAΔAB+ kan spoIIAA+ ABΔAC+ mls*
JDB403	*spoIIEΔ34-320 zib-82::spoIIAAΔAB+ kan spoIIAA+ ABΔAC+ mls*
JDB404	*spoIIEΔ34-320 zae-86::spoIIAAΔAB+ kan spoIIAA+ ABΔAC+ mls*
JDB405	*spoIIEΔ34-320 zba-88::spoIIAAΔAB+ kan spoIIAA+ ABΔAC+ mls*
JDB406	*spoIIEΔ34-320 zjd-89::spoIIAAΔAB+ kan spoIIAA+ ABΔAC+ mls*
JDB407	*spoIIEΔ34-320 zce-82::spoIIAAΔAB+ kan spoIIAA+ABΔAC+ mls*
JDB436	*spoIIEΔ34-320 zej-82::spoIIAA-AC kan ΔspoIIAA-AC::spc*
JDB437	*spoIIEΔ34-320 zib-82::spoIIAA-AC kan ΔspoIIAA-AC::spc*
JDB438	*spoIIEΔ34-320 zae-86::spoIIAA-AC kan ΔspoIIAA-AC::spc*
JDB439	*spoIIEΔ34-320 zba-8::spoIIAA-AC kan ΔspoIIAA-AC::spc*
JDB440	*spoIIEΔ34-320 zjd-89::spoIIAA-AC kan ΔspoIIAA-AC::spc*
JDB455	*bofB8 spoIVFBβΩpdr24a(spoIVFB wt) tet*
JDB457	*spoIIEΔ34-320 bofB8 spoIVFBβΩpdr24a(spoIVFB wt) tet*
JDB470	*thrC::spoIIQ-lacZ spc spoIIEΔ34-320 zej-82::spoIIAAΔAB+ kan spoIIAA+ ABΔAC+ mls*
JDB472	*thrC::spoIIQ-lacZ spc spoIIEΔ34-320 zib-82::spoIIAAΔAB+ kan spoIIAA+ ABΔAC+ mls*
JDB474	*thrC::spoIIQ-lacZ spc spoIIEΔ34-320 zae-86::spoIIAAΔAB+ kan spoIIAA+ ABΔAC+ mls*
JDB476	*thrC::spoIIQ-lacZ spc spoIIEΔ34-320 zba-88::spoIIAAΔAB+ kan spoIIAA+ ABΔAC+ mls*
JDB478	*thrC::spoIIQ-lacZ spc spoIIEΔ34-320 zjd-89::spoIIAAΔAB+ kan spoIIAA+ ABΔAC+ mls*
JDB480	*thrC::spoIIQ-lacZ spc amyE::AAΔAB+ kan AA+ABΔAC+ mls*
JDB483	*thrC::spoIIQ-lacZ spc spoIIEΔ34-320 amyE::AAΔAB+ kan AA+ ABΔAC+ mls*
JDB494	*dacF::spec spoIIAB(C145E)*
JDB495	*spoIIEΔ34-320 dacF::spec spoIIAB(C145E)*
JDB567	*ΔspoVG::tet*
JDB568	*spoIIEΔ34-320 ΔspoVG::tet*
JDB613	*amyE::spoIIR-lacZ cm*
JDB621	*spoIII36 amyE::spoIIR-lacZ cm*
JDB628	*spoIIIE36 amyE::spoIIR-lacZ cm zej-82::spoIIAAΔAB+ kan spoIIAA+ ABΔAC+ mls*
JDB629	*spoIIIE36 amyE::spoIIR-lacZ cm zae-86::spoIIAAΔAB+ kan spoIIAA+ ABΔAC+ mls*

sion and in the formation of abnormal sporangia with septa near both poles. The *spoIIR* gene, which, as indicated above, is under the control of σ^F, encodes a secreted intercellular signaling protein that triggers the activation of σ^E in the mother cell (Hofmeister et al., 1995; Karow et al., 1995). Evidently, the origin-proximal location of *spoIIR* helps to ensure that the SpoIIR signaling protein is produced as quickly as possible after polar septation and hence rapidly triggers the activation of σ^E. Whereas in the case of *spoIIAB* an origin-distal location facilitates the activation of σ^F, in the case of *spoIIR*, close proximity to the origin favors efficient activation of σ^E. Thus, the chromosomal position of the gene for a regulatory protein can act as a timing device (*spoIIR*) or as a device for activating a transcription factor in a spatially restricted manner (*spoIIAB*).

In light of these findings, it is tempting to imagine that the function of additional genes involved in sporulation is optimized by their chromosomal position. Three appealing examples are *spoIIE*, *clpC*, and *clpP*, which are all located in the origin-proximal region of the chromosome and whose products contribute positively to σ^F activation. The phosphatase product of *spoIIE* activates σ^F by dephosphorylating the phosphorylated form of SpoIIAA (Duncan et al., 1995) and the *clpC* and *clpP* products direct degradation of the anti-σ^F factor SpoIIAB (Pan et al., 2001). The presence of *spoIIE*, *clpC*, and *clpP* in the origin-proximal region of the chromosome might help to ensure the uninterrupted transcription of these genes following septation and enhance the accumulation of their products in the forespore, thereby maximizing the efficiency of σ^F activation. It will be interesting to investigate whether the chromosomal positions of *spoIIE*, *clpC*, *clpP*, and additional yet-to-be-recognized genes contribute to the efficiency of the sporulation process.

Transient genetic asymmetry is unique to sporulation due to its idiosyncratic mode of asymmetric division in which cytokinesis precedes chromosome segregation. We note, however, that chromosomes in a variety of bacteria that divide by binary fission are known to have a stereotyped organization and orientation within the cell (Niki et al., 2000; Teleman et al., 1998). Conceivably, chromosomal position contributes to optimal gene function in these bacteria as well. In particular, the localization of some membrane proteins could be determined by the location of their genes as a consequence of coupled transcription, translation, and insertion into the membrane (Lynch and Wang, 1993).

Asymmetric division is a common feature of cell differentiation in microorganisms and higher cells. However, the mechanisms by which the establishment of cell type is linked to the asymmetry of the division process vary widely. Here we have presented evidence that the spatial regulation of a transcription factor is linked to the asymmetry of the division process through the resulting spatial asymmetry of the chromosome. Whereas many precedents exist for the asymmetric distribution of proteins (e.g., CckA of *Caulobacter crescentus*; Jacobs et al., 1999) or of mRNAs involved in the establishment of

cell fate (e.g., ASH1 of *Saccharomyces cerevisiae*; Long et al., 1997; Takizawa et al., 1997), *spoIIAB* is an example of a gene whose asymmetric localization contributes to the cell-specific activation of a developmental transcription factor.

Experimental Procedures

Growth Conditions

To monitor sporulation efficiency, cells were grown for 24 hr in Difco sporulation (DS) medium (Schaeffer et al., 1965), and the number of spores was determined by heat killing (10 min at 80°). To measure *lacZ* expression, strains harboring the reporter were grown in DS medium, and culture samples were collected at intervals following the end of the exponential phase of growth. Measurement of β-galactosidase activity was as described previously (Duncan et al., 1996).

Strain Constructions

The *B. subtilis* strains used are all congenic derivatives of PY79 and are listed in Table 2. Standard techniques were used for strain construction (Harwood and Cutting, 1990). Strain JDB111 was constructed by transforming JDB1 (Δ*spoIIE*::*kan*, lab collection) with DNA from strain MO3431 (JH642 *spoIIEΔ34-320;* courtesy of P. Stragier) and selecting for sporulation+ transformants. Transfer of the *spoIIEΔ34-320* mutation was confirmed by PCR analysis. Strain JDB143 was constructed by transforming DNA from strain JDB139 (*amyE*::*spoIIAA-AC kan*) into strain JDB111 followed by transformation with DNA from strain MO1928 (JH642 Δ*(spoIIAA-AC) spc*; courtesy P. Stragier). Strain JDB139 was constructed by transformation with plasmid pCB11 containing the entire *spoIIA* operon in an *amyE* integration vector. Strain JDB356 was constructed by first transforming JDB111 with DNA from strain RL1222 (*amyE*::*spoIIAAΔ1 spoIIAB*+ *kan*, lab collection) and then by transformation with plasmid pCB9, a derivative of pPM23 (Schmidt et al., 1990) containing a wild-type copy of *spoIIAC* from pPM3 (Schmidt et al., 1990). Strain JDB384 was constructed by transformation of DNA from strain MO3416 (JH642 *thrC*::*spoIIQ-lacZ spc*, courtesy P. Stragier). To construct strains with *spoIIAB* at various chromosomal positions, plasmid pCB15, a derivative of pLD30 (Garsin et al., 1998) with a chloramphenicol gene between NarI and NcoI, was transformed by double-cross over integration into a series of strains containing pTV21Δ2 (Youngman et al., 1984) integrated at different Tn*917* loci. These strains were then transformed with the pER82 derivative pCB16 containing the BamHI *spoIIAAΔ1 spoIIAB* fragment from pDAG4 (Garsin et al., 1998) in the opposite orientation from the spectinomycin gene. Strain JDB111 was transformed by DNA from strains from our laboratory collection containing specific Tn*917* insertions carrying the *spoIIAB* gene as described above and DNA from strain JDB356, to yield strains JDB402-JDB407. Strains JDB436-JDB440 were constructed similarly to strain JDB143 except that plasmid pCB11 was transformed into the same series of strains containing *amyE* sequences at various Tn*917* loci as described above for pCB16. Strain JDB111 was then transformed by DNA from these strains followed by transformation with DNA from MO1928. Strains JDB455 and JDB457 were created by transformation of DNA from strain BDR432 (*bofB8 spoIVFBΩpdr24a(spoIVFB* wt*) tet;* courtesy D. Rudner) into PY79 and JDB111, respectively. Strains JDB470, JDB472, JDB474, JDB476, JDB478, and JDB483 were created by transformation of strains JDB402, JDB403, JDB404, JDB405, JDB406, and JDB356 with DNA from strain MO3416 (JH642 *thrC*::*spoIIQ-lacZ spc*, courtesy P. Stragier). Strains JDB494 and JDB495 were created by transformation of DNA from strain QPB275 (*thrC*::*spoIIQ-lacZ erm dacF*::*spec spoIIAB*(C145E)) into PY79 and JDB111, respectively. Strains JDB567 and JDB568 were created by transformation of DNA from strain RL1996 (Δ*spoVG*::*tet* Δ*spoIIB*::*kan*) into PY79 and JDB111, respectively, selecting for tetracycline resistance. Strains JDB613 and JDB621 were created by transformation of DNA from strain MO2029 (JH642 *amyE*::*spoIIR-lacZ cat*, courtesy of P. Stragier) into strains PY79 and RL76 (*spoIIIE36,* lab collection). Strains JDB628 and JDB629 were created by transformation of strain JDB621 with DNA from JDB402 and JDB404, respectively.

Immunoblot Analysis

Immunoblot analysis was conducted as described previously (Pan et al., 2001) except that ^{125}I-labeled donkey anti-rabbit (5 μCi/μl; Amersham) was used as secondary antibody. Membranes were exposed to a Hitachi BioMax screen for ~4 hr and quantified using MacBase (v2.5).

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