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Transient genetic asymmetry and cell fate in a bacterium

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Certain species of Gram-positive bacteria can initiate a developmental program that results in the formation of two daughter cells with different fates. One cell develops into a spore and the other cell undergoes programmed lysis, with each process being mediated by a cascade of cell-type-specific transcription factors. An early and critical step in this developmental pathway is the formation of a division septum near one pole, creating two compartments of different sizes. But how is this morphological asymmetry translated into the transcriptional asymmetry of the two compartments? Recent results suggest that the chromosomal position of the genes encoding several key components of the transcriptional regulatory network has an important role in this process.

The establishment of cellular asymmetry is fundamental to many developmental processes [1]. One well-studied example occurs during sporulation of the Gram-positive bacterium *Bacillus subtilis*, when conditions of nutrient limitation and high population density result in the initiation of a developmental pathway that culminates in the formation of a heat- and desiccation-resistant spore. Most bacteria (including vegetative *B. subtilis*) normally couple cell division with completion of DNA segregation, and place a septum between the two newly replicated chromosomes, generating two daughter cells of equal size. By contrast, sporulating *B. subtilis* places a septum near to one pole of the cell, initially over one of the chromosomes, generating a larger cell (the mother cell) and a smaller cell (the forespore) (Fig. 1a). The forespore is engulfed by the mother cell, and each cell subsequently follows a unique developmental program with the forespore developing into a mature spore within the mother cell, which eventually lyses [2].

Sporulating cells are asymmetric not just because they select a polar division site, but also because they activate different transcription factors in the forespore and mother cell [3]. The first compartment-specific transcription factors are σ^F, which becomes active only in the forespore [4], and σ^E, which becomes active only in the mother cell [5] (Fig. 1b). As subunits of RNA polymerase that provide promoter specificity, σ^F and σ^E direct the expression of genes essential for establishing the developmental fates of the two compartments, including a second pair of sigma factors, σ^G and σ^K, which act in the forespore and mother cell, respectively [3,6]. Because transcription of the genes encoding σ^F and σ^E occurs before septation, the mechanism responsible for restricting the activity of σ^F and σ^E to one compartment must act post-transcriptionally [7]. In fact, both σ^F and σ^E are inactive until completion of the polar septum, thus creating a developmental checkpoint that couples formation of an asymmetric morphological structure to the differential activation of genes in each compartment [8]. Although it is clear that such a checkpoint exists, the underlying molecular mechanism has remained mysterious.

Chromosome segregation in sporulation

In a remarkably insightful paper, Frandsen *et al.* [9] suggested that the unique pattern of chromosome segregation in sporulation offered a possible solution to this problem. In normal symmetric division, chromosomes typically inhibit septation through the mechanism of nucleoid occlusion, so septa rarely form over chromosomes [10]. In sporulation, however, the chromosomes have an extended, rod-like structure, the axial filament [11], and the polar septum forms over one of the two daughter chromosomes, such that only a third of the chromosome is initially contained within the forespore [12] (Fig. 2a). Similar to vegetative cells, the orientation of the newly replicated chromosomes in sporulation is fixed with the origins of replication (*oriC*) located near the cell poles, although in sporulation the *oriC* regions move to the extreme polar positions [13–15]. Thus, it is always the third of the chromosome proximal to *oriC* that is initially located in the forespore, and segregation of the remaining two-thirds of the chromosome (over a period of ~15 min) [16,17] is mediated by SpoIIIE [18]. SpoIIIE is a DNA tracking protein with ATPase activity [19] and a member of a large family of bacterial proteins involved in DNA translocation [20]. Frandsen *et al.* noted that genes located far from *oriC* were transiently excluded from the forespore until chromosome segregation was complete (Fig. 2b). Thus, they argued that the cells experienced a period of 'transient genetic asymmetry' that might allow for differential gene expression in the forespore and mother cell.

Regulation of σ^F activation

To appreciate their idea, it is first necessary to describe the mechanism of σ^F activation in more detail (Fig. 3a). σ^F is held inactive by a stable interaction with a dimer of its

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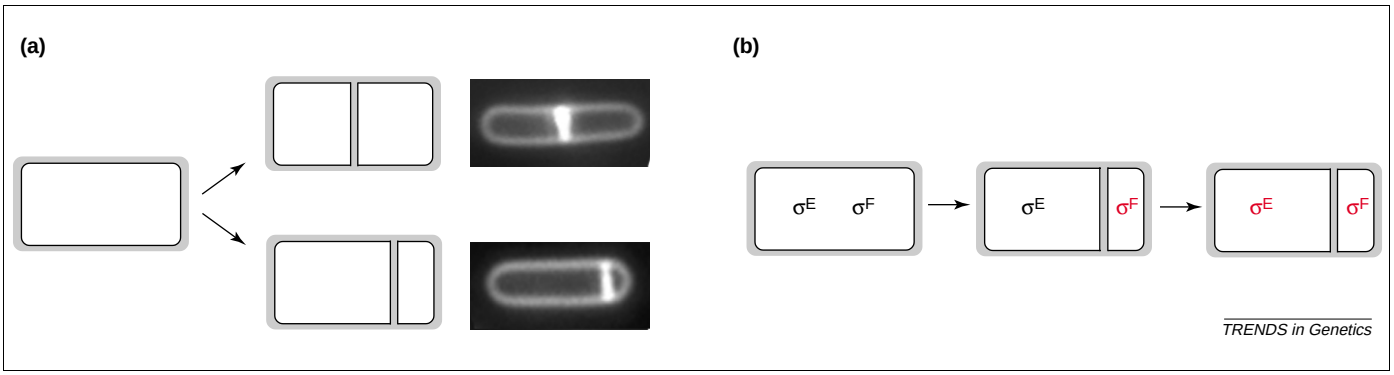

Fig. 1. Establishment of morphological and genetic asymmetry during sporulation. (a) *Bacillus subtilis* normally undergoes medial division (top), but under conditions of nutrient limitation and high population density, the division site is shifted to near one pole of the cell (bottom). Photomicrographs at right are of a vegetative cell (top) or of a sporulating cell (bottom) labeled with a membrane fluorescent dye (FM4–64). (b) The sporulation-specific sigma factors σ^E and σ^F are synthesized before polar division, but are inactive. Following septation, σ^F is activated (red) in the smaller (forespore) of the two compartments generated by the asymmetric division, followed by activation of σ^E (red) in the larger compartment (mother cell).

anti-sigma factor SpoIIAB [21–24]. Release of σ^F from SpoIIAB occurs through the action of the phosphoprotein, SpoIIAA [25], which disrupts the binding of σ^F with the SpoIIAB dimer, allowing σ^F to interact with core RNA polymerase and direct the transcription of its target genes. SpoIIAA can only disrupt the σ^F–SpoIIAB$_2$ complex when it is dephosphorylated [25], but in the process of inducing the release of σ^F, SpoIIAA is itself phosphorylated by SpoIIAB [21]. SpoIIAA is recharged through dephosphorylation by the SpoIIE membrane phosphatase, which is localized to the polar septum [26,27]. Although the precise nature of these interactions remains mysterious, it is clear that the regulation of SpoIIE activity is important for the compartment-specific activation of σ^F [28,29].

Transient genetic asymmetry

To test their idea of transient genetic asymmetry, Frandsen *et al*. constructed a series of *B. subtilis* strains lacking the genes for both SpoIIAA and SpoIIE. In addition, they moved a copy of *spoIIAC* (which encodes σ^F) from its original position to various chromosomal loci (Fig. 2c). In these strains, the gene encoding the SpoIIAB anti-σ^F factor, *spoIIAB*, remained at its original position near the terminus. They found that strains carrying *spoIIAC* at its wild-type position next to *spoIIAB* at the terminus (or at other origin-distal loci) sporulated at a severely reduced level, as might be expected from the lack of SpoIIAA and SpoIIE. Remarkably, however, strains carrying a copy of *spoIIAC* at origin-proximal positions (i.e. at chromosomal loci contained within the forespore following formation of the polar septum) were able to sporulate, albeit at a reduced level compared with wild type. They hypothesized that when *spoIIAC* was located near the origin, expression of *spoIIAC* would occur in the forespore immediately following septation, but *spoIIAB* expression would be restricted to the mother cell until the terminus region of the chromosome containing *spoIIAB* entered the forespore. Thus, this period of genetic asymmetry would result in an increased synthesis of σ^F relative to its inhibitor, SpoIIAB, in the forespore, and consequently allow some σ^F molecules to associate with core RNA polymerase and activate transcription. When *spoIIAC* was located at a terminus-proximal position, there would never be an imbalance in synthesis of σ^F and SpoIIAB and

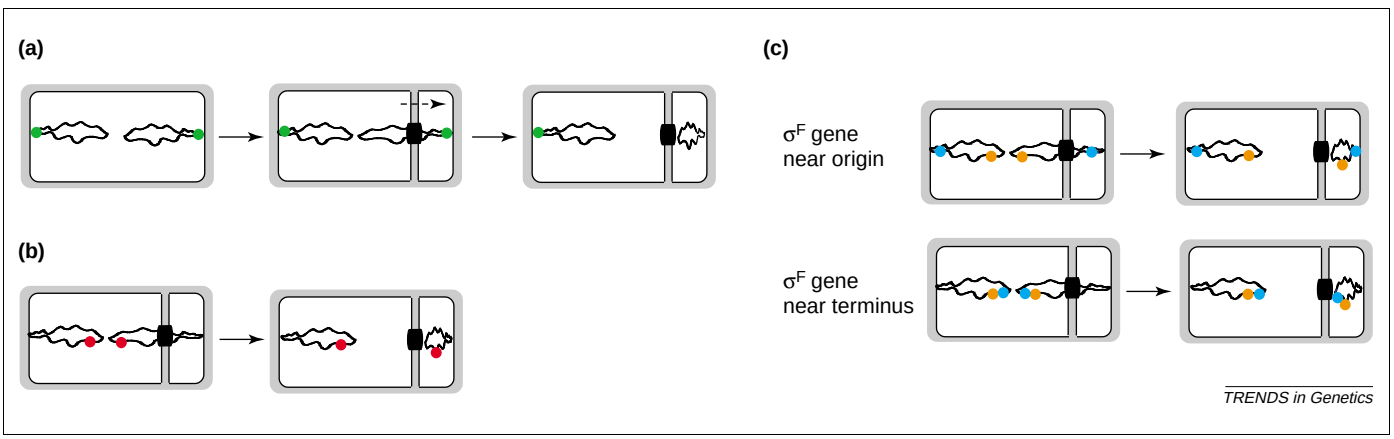

Fig. 2. Chromosome organization in sporulation and transient genetic asymmetry. (a) During sporulation, the polar septum forms over one of the two chromosomes, such that only the third of the chromosome surrounding *oriC* (green) is located within the forespore. DNA translocation of the remaining two-thirds of the chromosome is mediated by the SpoIIIE protein (black cylinder) over a period of ~15 min. (b) A gene (red) located near the terminus is present in two copies in the mother cell and excluded from the forespore, until chromosome translocation is nearly complete. (c) When the gene encoding σ^F (blue) is located near the origin and the gene encoding the anti-σ^F factor (orange) is located near the terminus, then partial sporulation occurs (top). By contrast, when the σ^F gene (blue) is moved to near the terminus, sporulation is inhibited (bottom).

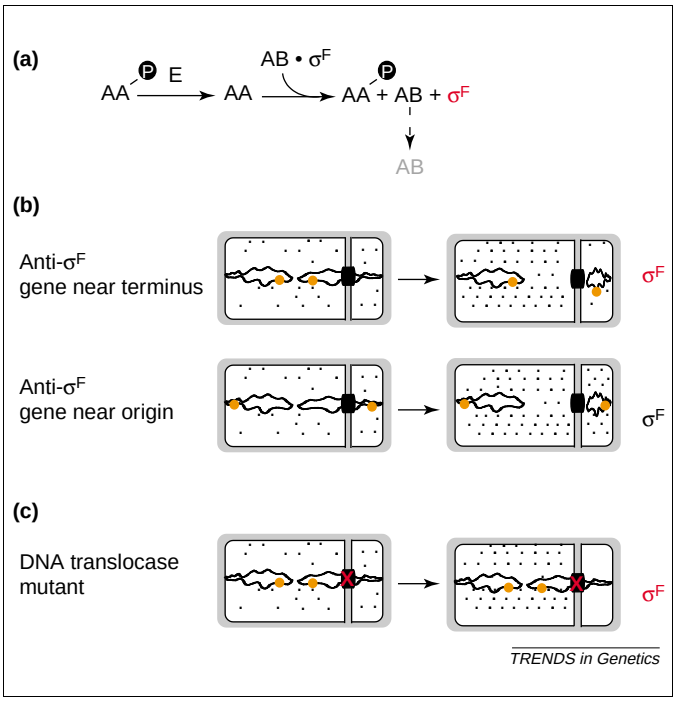

Fig. 3. Forespore-specific activation of σ^F and transient genetic asymmetry of the *spoIIAB* gene. (a) Dephosphorylation of the SpoIIAA phosphoprotein (AA) by the SpoIIE phosphatase (E) allows AA to disrupt the interaction of the anti-σ^F factor SpoIIAB (AB) and σ^F, resulting in the release of free σ^F (red) and SpoIIAB and phosphorylation of SpoIIAA. Degradation of free SpoIIAB (gray) is mediated by the ClpCP protease. (b) When the *spoIIAB* gene (orange) encoding the anti-σ^F factor is at its normal position near the terminus (upper panel), it is present in two copies in the mother cell and absent from the forespore during the period of chromosome translocation. During this interval, the level of SpoIIAB protein (black dots) falls selectively in the forespore because of ongoing degradation by ClpCP and the lack of new *spoIIAB* transcription and SpoIIAB synthesis, resulting in σ^F activation (red σ^F). By contrast, when *spoIIAB* is located near the origin (lower panel), one copy of the gene is located in each compartment, so no differential decrease in the level of SpoIIAB in the forespore occurs, thereby inhibiting σ^F activation (black σ^F). (c) A mutation in the DNA translocase blocks entry of the remainder of the chromosome including the *spoIIAB* gene into the forespore. The permanent exclusion of *spoIIAB* from the forespore combined with the continual degradation of SpoIIAB protein results in a precipitous drop in the level of SpoIIAB, and consequently elevated σ^F activation in the forespore (red σ^F).

thus, no activation of σ^F-dependent genes and a severely reduced ability to sporulate.

These experiments demonstrated clearly that transient genetic asymmetry could be used to activate σ^F in an artificial system that was lacking several factors. A key issue arising from these experiments was whether transient genetic asymmetry has a role in the actual activation pathway of σ^F. Frandsen *et al.* proposed that if an unstable inhibitor of σ^F were encoded by a gene located near the terminus, then following formation of the polar septum, the level of this inhibitor would fall in the forespore relative to σ^F, allowing activation of σ^F [9].

Transient genetic asymmetry: σ^F activation

The existence of such an inhibitor remained hypothetical until the discovery that, unlike its partners SpoIIAA and σ^F, free SpoIIAB is unstable, with a half-life of ~25 min *in vivo* [30]. The *spoIIAB* gene is located near the terminus as part of the *spoIIA* operon, so the obvious next question to test was whether the position of the *spoIIAB* gene has a role in σ^F activation. To do this, a series of *B. subtilis* strains carrying *spoIIAB* at various chromosomal positions was constructed [31]. Surprisingly, the position of *spoIIAB* had only a modest effect on sporulation or on σ^F activation. However, in a strain carrying a mutation in the *spoIIE* gene resulting in the synthesis of a SpoIIE phosphatase that fails to localize to its normal position at the polar septum [32], the position of *spoIIAB* had a strong effect on both sporulation and σ^F activation [31] (Fig. 3b). Specifically, when *spoIIAB* was located at positions in the origin-proximal third of the chromosome, sporulation and σ^F activation were significantly reduced, but when it was at other positions, sporulation and σ^F activation were similar to that observed when *spoIIAB* was at its original position near the terminus. Thus, under conditions of transient genetic asymmetry, degradation of SpoIIAB would be expected to occur in both the mother cell and the forespore, but the absence of *spoIIAB* would prevent the anti-σ^F factor from being replenished in the forespore. This would cause a decrease in the SpoIIAB level in the forespore relative to its more stable partners, σ^F and SpoIIAA. Because the activation of σ^F is highly sensitive to the level of the anti-sigma factor [30], this decrease would result in the preferential activation of σ^F in the forespore. Consistent with this interpretation, strains carrying a mutant copy of *spoIIAB* encoding a stable derivative of SpoIIAB exhibit a reduction in sporulation and σ^F activation [30].

Earlier studies of σ^F activation reported that a mutation, *spoIIIE36*, in the DNA translocase that blocks entry of the last two-thirds of the chromosome into the forespore, significantly increased the activation of certain σ^F-dependent genes [12,33–35]. Following from the concept of 'transient genetic asymmetry', this mutation would result in the permanent exclusion of the *spoIIAB* gene from the forespore ('permanent genetic asymmetry'), and because SpoIIAB is unstable, σ^F activation would progressively increase as SpoIIAB was degraded and not replaced [31] (Fig. 3c). In confirmation of this hypothesis, when the position of *spoIIAB* was moved from its wild-type position at the terminus to an origin-proximal position in a strain carrying the *spoIIIE36* mutation, the increased σ^F activation was abolished [31].

As noted above, the position effect of *spoIIAB* was only observed in a mutant background where SpoIIE was no longer localized to the polar septum. Thus, it is likely that two partially redundant mechanisms act in parallel to activate σ^F [31]. The first mechanism involves localization of the SpoIIE phosphatase to the polar septum where the localization of SpoIIE to the septum would increase the relative concentration of dephosphorylated SpoIIAA in the forespore (through an as yet uncharacterized mechanism). The second mechanism involves the transient genetic asymmetry of *spoIIAB*, where exclusion of the gene from the forespore during the period of chromosome translocation in combination with proteolysis of the SpoIIAB protein would reduce the level of the anti-σ^F factor relative to σ^F. Given that σ^F is the first compartment-specific transcription factor and therefore lies at the top of a hierarchy of the developmental transcription factors, its complex regulation is perhaps not surprising.

Transient genetic asymmetry: σ^E activation

A second example of transient genetic asymmetry in sporulation occurs during the activation of σ^E, the first

mother-cell-specific sigma factor. σ^E is initially synthesized as an inactive pro-protein, pro-σ^E, with an N-terminal extension of 27 amino acids [36] that is removed, presumably, by the SpoIIGA membrane-associated protease [37] (Fig. 4a). Following this cleavage, σ^E is able to associate with core RNA polymerase and initiate transcription from its target promoters. The activity of SpoIIGA is regulated by the SpoIIR protein [38], and the *spoIIR* gene is under control of σ^F, so it is only transcribed in the forespore [35,39]. It is thought that SpoIIR traverses the septum and interacts with SpoIIGA located on the mother-cell face of the sporulation septum [38]. The proper spatial activation of σ^E is essential because of its many downstream targets involved in the synthesis and assembly of the outer spore layers [2]. The mechanism that restricts σ^E activation to the mother cell appears to include proteolysis of pro-σ^E in the forespore before activation [40,41], as well as selective post-septational transcription in the mother cell of the *spoIIG* operon, which includes the genes encoding for pro-σ^E and the SpoIIGA protease [42].

The proper activation of σ^E is also important because several σ^E-dependent genes prevent the formation of a second polar septum in the mother cell. The absence of σ^E results in the formation of terminally arrested 'disporic' cells that contain septa at both poles, creating two small forespore compartments, each with one chromosome, and a large compartment in the middle that is devoid of DNA [43–45]. This regulation has a critical temporal component, because time-lapse microscopy shows that the two septa form in rapid succession [16]. The idea that septum formation is exquisitely sensitive to the timing of σ^E activation is reinforced by the observation that premature expression of the σ^E-dependent genes responsible for preventing formation of the second septum prevents the formation of even the first polar septum [46]. Thus, σ^E has to be activated early enough to prevent formation of the second septum, but not so early as to prevent formation of the first septum.

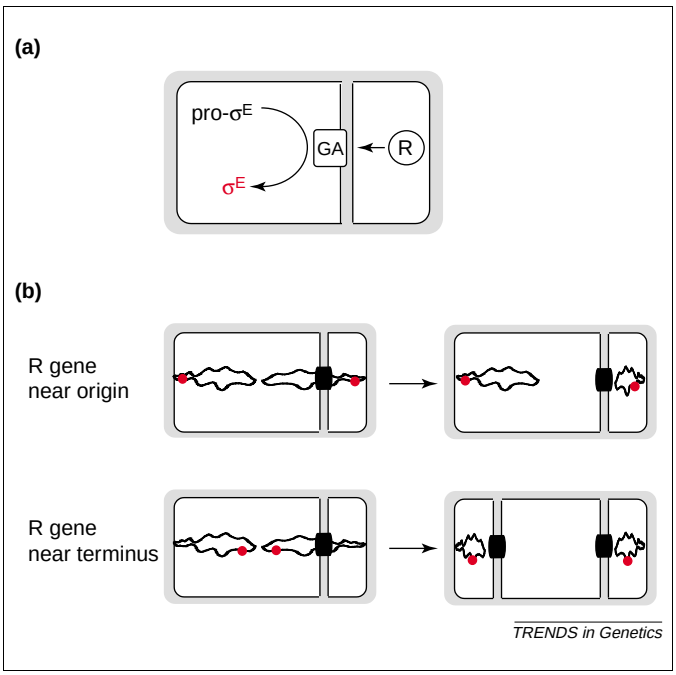

Fig. 4. Forespore-specific activation of σ^E and transient genetic asymmetry of the *spoIIR* gene. (a) Processing of σ^E from its inactive form (pro-σ^E) to its active form, (σ^E) is mediated by the SpoIIGA membrane protease (GA) localized at the polar septum. The activity of SpoIIGA is regulated by the SpoIIR protein (R) whose gene is under control of σ^F. SpoIIR synthesized in the forespore presumably traverses the septum and interacts with SpoIIGA in the mother cell, stimulating its proteolytic activity. (b) The normal position of the gene encoding SpoIIR, *spoIIR* (red) is near *oriC* so it is transcribed quickly following σ^F activation (top). When *spoIIR* is moved to positions nearer the terminus (bottom), expression of *spoIIR* is delayed, resulting in the terminal phenotype of a disporic cell with two forespore compartments, each containing a chromosome, and an empty, central compartment.

Making a single septum is important not just because disporic cells are terminally differentiated, but also because a single polar septum breaks the symmetry of the cell. Like a cell with a single medial septum, a cell with two polar septa is symmetric. The nature of the timing device that allows the formation of only a single polar septum was mysterious until the Piggot and Hofmeister laboratories showed independently that the position of the *spoIIR* gene that encodes the signal from the forespore had such a role.

Because the transient genetic asymmetry observed with the position of the *spoIIAB* gene suggests that σ^F activation precedes completion of translocation of the chromosome into the forespore, σ^F-dependent genes located in the origin-proximal third of the chromosome are likely to be transcribed before completion of translocation. One of these genes is *spoIIR*, so it seemed reasonable to propose that the position of *spoIIR* near the origin allowed it to be transcribed as early as possible following σ^F activation [17,47]. If so, then one would expect that moving *spoIIR* to positions away from the origin would impair sporulation because *spoIIR* expression would be delayed. The Piggot and Hofmeister laboratories moved the *spoIIR* gene to different chromosomal positions, finding that only when *spoIIR* was located at positions outside the origin-proximal region, was sporulation inhibited [17,47]. Consistent with this effect, *spoIIR* genes at origin-distal positions resulted in a delay and a reduction in σ^E activation [17,47], as well as a delay and moderate block in pro-σ^E processing [47]. Finally, the position of *spoIIR* also greatly affected the formation of disporic cells, with origin-proximal position (including the wild type) producing $<1\%$ disporic cells, and origin-distal positions producing 25–95% disporic cells [17,47] (Fig. 4b). The magnitude of this position effect appeared to correlate with distance away from the origin, because *spoIIR* located at the most origin-distal positions resulted in the strongest disporic phenotype [47]. Thus, the transient genetic asymmetry of a single gene, *spoIIR*, appears to regulate the proper temporal activation of σ^E.

Concluding remarks

A survey of the endospore-forming bacteria whose genomes have been completely sequenced (Table 1) shows that the position of *spoIIR* near the origin and *spoIIAB* near the terminus is mostly, although not absolutely, conserved. Intriguingly, the position of *spoIIAB* is less conserved than the position of *spoIIR*, suggesting that there could be some differences in the regulatory network underlying σ^F activation between closely related species. As more related genomes are sequenced, the extent of the conservation will presumably become clearer.

In conclusion, transient genetic asymmetry is used by *B. subtilis* in two key developmental checkpoints. To

Table 1. Position of *spoIIR* and *spoIIAB* in the genomes of endospore-forming bacteria

Organism[a]	Genome size (Mb)	Position of *spoIIR*[b]	Position of *spoIIAB*[b]
Bacillus subtilis	4.21	3.79 (0.90)	2.44 (0.58)
Bacillus anthracis	5.23	5.06 (0.97)	3.92 (0.74)
Bacillus halodurans	4.02	3.89 (0.97)	1.61 (0.38)
Clostridium acetobutylicum	3.94	3.03 (0.77)	2.41 (0.61)
Clostridium perfringens	3.03	2.53 (0.83)	2.34 (0.77)

[a]All data were retrieved from
http://www.ncbi.nlm.nih.gov/PMGifs/Genomes/eub_g.html
[b]Value in parenthesis refers to position of gene as a fraction of genome size.

enable the translation from morphological asymmetry into transcriptional asymmetry (and ultimately developmental asymmetry), the bacterium takes advantage of the organization of the chromosome. Although the unique nature of chromosome segregation in sporulation presumably allows the chromosomal position of a gene to have such an important role in the activity of its encoded protein, bacterial chromosomes in general have a stereotyped organization and orientation within the cell [12,48–50]. Thus, the localization of slowly diffusing membrane proteins could be determined by the location of their genes as a consequence of coupled transcription, translation and insertion in the membrane [51]. Many different proteins in bacteria show discrete patterns of localization that are important for their function, but an understanding of the mechanistic basis for these patterns remains incomplete [52]. The use of methods to move genes to different loci within bacterial chromosomes should allow an evaluation of position effects as a regulatory mechanism for protein localization and activity.

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