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 lyases [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 $\sigma^F$ and $\sigma^K$, which become active only in the forespore [4], and $\sigma^E$, which becomes active only in the mother cell [5] (Fig. 1b). As subunits of RNA polymerase that provide promoter specificity, $\sigma^F$ and $\sigma^K$ direct the expression of genes essential for establishing the developmental fates of the two compartments, including a second pair of sigma factors, $\sigma^G$ and $\sigma^R$, which act in the forespore and mother cell, respectively [3,6]. Because transcription of the genes encoding $\sigma^F$ and $\sigma^K$ occurs before septation, the mechanism responsible for restricting the activity of $\sigma^F$ and $\sigma^K$ to one compartment must act post-transcriptionally [7]. In fact, both $\sigma^F$ and $\sigma^K$ 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 $\sim$15 min) [16,17] is mediated by SpoIIE [18]. SpoIIE 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 $\sigma^F$ activation

To appreciate their idea, it is first necessary to describe the mechanism of $\sigma^F$ activation in more detail (Fig. 3a). $\sigma^F$ is held inactive by a stable interaction with a dimer of its
anti-sigma factor SpoIIAB [21–24]. Release of $\sigma^F$ from SpoIIAB occurs through the action of the phosphoprotein, SpoIIAA [25], which disrupts the binding of $\sigma^F$ with the SpoIIAB dimer, allowing $\sigma^F$ to interact with core RNA polymerase and direct the transcription of its target genes. SpoIIAA can only disrupt the $\sigma^F$–SpoIIAB$_2$ complex when it is dephosphorylated [25], but in the process of inducing the release of $\sigma^F$, 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 $\sigma^F$ [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 $\sigma^F$) from its original position to various chromosomal loci (Fig. 2c). In these strains, the gene encoding the SpoIIAB anti-$\sigma^F$ factor, *spoIIAB*, remained at its original position near the terminus. They found that strains carrying *spoIAC* 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 *spoIAC* 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 *spoIAC* was located near the origin, expression of *spoIAC* 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 $\sigma^F$ relative to its inhibitor, SpoIIAB, in the forespore, and consequently allow some $\sigma^F$ molecules to associate with core RNA polymerase and activate transcription. When *spoIAC* was located at a terminus-proximal position, there would never be an imbalance in synthesis of $\sigma^F$ and SpoIIAB and...
spoIIAB had only a modest effect on sporulation or on $\sigma^F$ 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 $\sigma^F$ activation [31] (Fig. 3b).

Specifically, when spoIIAB was located at positions in the origin-proximal third of the chromosome, sporulation and $\sigma^F$ activation were significantly reduced, but when it was at other positions, sporulation and $\sigma^F$ 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 SpoIIE would be expected to occur in both the mother cell and the forespore, but the absence of spoIIAB would prevent the anti-$\sigma^F$ factor from being replenished in the forespore. This would cause a decrease in the SpoIIE level in the forespore relative to its more stable partners, $\sigma^F$ and SpoIIE. Because the activation of $\sigma^F$ is highly sensitive to the level of the anti-sigma factor [30], this decrease would result in the preferential activation of $\sigma^F$ in the forespore. Consistent with this interpretation, strains carrying a mutant copy of spoIIE encoding a stable derivative of SpoIIE exhibit a reduction in sporulation and $\sigma^F$ activation [30].

Earlier studies of $\sigma^F$ activation reported that a mutation, spoIIE36, in the DNA translocase that blocks entry of the last two-thirds of the chromosome into the forespore, significantly increased the activation of certain $\sigma^F$-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 SpoIIE is unstable, $\sigma^F$ activation would progressively increase as SpoIIE 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 spoIIE36 mutation, the increased $\sigma^F$ 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 $\sigma^F$ [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 SpoIIE 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-$\sigma^F$ factor relative to $\sigma^F$. Given that $\sigma^F$ 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.

**Transgenic genetic asymmetry: $\sigma^E$ activation**

A second example of transient genetic asymmetry in sporulation occurs during the activation of $\sigma^E$, the first
mother-cell-specific sigma factor. \( \sigma^E \) is initially synthesized as an inactive pro-protein, pro-\( \sigma^E \), 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, \( \sigma^E \) is able to associate with core RNA polymerase and initiate transcription from its target promoters. The activity of SpoIIGA is regulated by the SpoIR protein [38], and the spoIIR gene is under control of \( \sigma^E \), 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 \( \sigma^E \) is essential because of its many downstream targets involved in the synthesis and assembly of the outer spore layers [2]. The mechanism that restricts \( \sigma^E \) activation to the mother cell appears to include proteolysis of pro-\( \sigma^E \) 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-\( \sigma^E \) and the SpoIIGA protease [42].

The proper activation of \( \sigma^E \) is also important because several \( \sigma^E \)-dependent genes prevent the formation of a second polar septum in the mother cell. The absence of \( \sigma^E \) 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 \( \sigma^E \) activation is reinforced by the observation that premature expression of the \( \sigma^E \)-dependent genes responsible for preventing formation of the second septum prevents the formation of even the first polar septum [46]. Thus, \( \sigma^E \) 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.

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 spoIAB gene suggests that \( \sigma^E \) activation precedes completion of translocation of the chromosome into the forespore, \( \sigma^E \)-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 \( \sigma^E \) 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 \( \sigma^E \) activation [17,47], as well as a delay and moderate block in pro-\( \sigma^E \) 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 \( \sigma^E \).

**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 spoIAB near the terminus is mostly, although not absolutely, conserved. Intriguingly, the position of spoIAB is less conserved than the position of spoIIR, suggesting that there could be some differences in the regulatory network underlying \( \sigma^E \) 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
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 [12,45–50]. 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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Table 1. Position of spoIIR and spoIAB in the genomes of endospore-forming bacteria

<table>
<thead>
<tr>
<th>Organism</th>
<th>Genome size (Mb)</th>
<th>Position of spoIIR</th>
<th>Position of spoIAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>4.21</td>
<td>3.79 (0.90)</td>
<td>2.44 (0.58)</td>
</tr>
<tr>
<td>Bacillus anthracis</td>
<td>5.23</td>
<td>5.06 (0.97)</td>
<td>3.92 (0.74)</td>
</tr>
<tr>
<td>Bacillus halodurans</td>
<td>4.02</td>
<td>3.89 (0.97)</td>
<td>1.61 (0.38)</td>
</tr>
<tr>
<td>Clostridium acetobutylicum</td>
<td>3.94</td>
<td>3.03 (0.77)</td>
<td>2.41 (0.61)</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>3.03</td>
<td>2.53 (0.83)</td>
<td>2.34 (0.77)</td>
</tr>
</tbody>
</table>

*All data were retrieved from http://www.ncbi.nlm.nih.gov/PMGifs/Genomes/eub_g.html

*Value in parenthesis refers to position of gene as a fraction of genome size.
pathway activating a developmental transcription factor in *Bacillus subtilis*. Genes Dev. 9, 503–508
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