# Bacterial sporulation: **Pole-to-pole protein oscillation**

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**Sporulating bacteria need to temporally coordinate DNA replication, chromosome partitioning and sporulation initiation. Recent work has shown that one aspect of this coordination lies with the interdependent subcellular localization of two proteins, Spo0J and Soj, and in the Spo0J-dependent spatial oscillation of Soj.**

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All living organisms require the ability to adapt to changes in the environment. For many microorganisms, survival in extreme conditions involves the formation of heat resistant spores. In *Bacillus subtilis*, the vegetative growth cycle involves the replication of the chromosome, segregation of the daughter chromosomes to the opposite poles of the cell, and symmetrical cell division (Figure 1; reviewed in [1]). In unfavorable conditions, the replicated chromosomes are still segregated to the opposite poles of the cells, but the cell division is unequal and the chromosome of the smaller

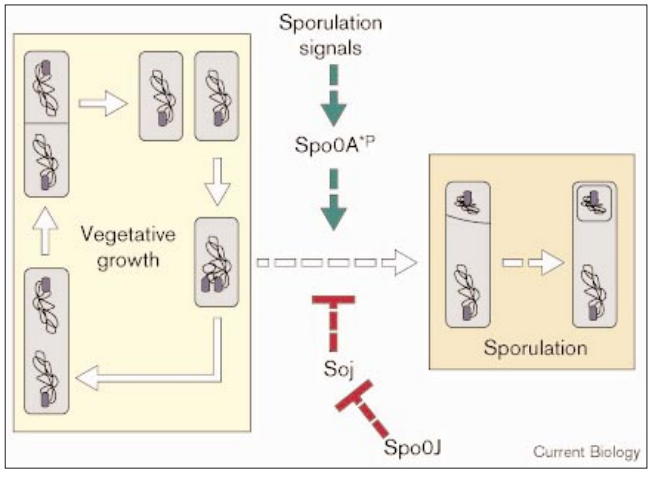


There is a clear link between DNA replication, chromosome partitioning, and initiation of sporulation in *B. subtilis* [2]. This cellular checkpoint is necessary to ensure that the chromosomes are appropriately replicated and sequestered to their respective cell poles prior to the asymmetric cell division. How is this remarkable coordination between gene expression and changes in cell physiology achieved? One answer lies in the coordinated movement of proteins involved in partitioning of chromosomes, and the coupling of this movement with the transcriptional control of sets of linked, coordinately regulated genes — 'regulons' — that encode proteins involved in sporulation. Two recent studies [3,4] have provided evidence that the cellular localization of the proteins Spo0J and Soj, and more significantly the Spo0J-dependent movement of Soj, are critical to the coordination of chromosome replication and sporulation in *B. subtilis*.

The chromosome of *B. subtilis* is oriented such that the origin of replication is found preferentially near a cell pole [5–7]. Moreover, the origin is associated with a protein, Spo0J [6,8,9], that is required for chromosome partitioning and sporulation [2]. Spo0J is a member of the ParB family of partitioning proteins and, like its *Escherichia coli* homologue ParB, Spo0J binds *in vivo* to specific DNA sequences, called *parS* sites, which are located near the site of initiation of chromosomal replication [9]. Observation of cells producing fusion proteins in which Spo0J was linked to the green fluorescent protein (GFP) revealed that Spo0J colocalizes with the chromosomal origin, forming discrete polar foci in both growing and sporulating cells [5,6,8].

Deletion of the *spo0J* locus in vegetative cells was found to result in an increase in the number of cells lacking a chromosome, indicating that proper partitioning of the daughter chromosomes did not occur during division of these cells [2]. Furthermore, cells lacking Spo0J are unable to activate transcription from sporulation-specific genes [2] and also display partitioning defects during sporulation [10]. These observations suggest that Spo0J plays a role in ensuring that chromosomes are replicated and partitioned prior to the onset of sporulation. How does Spo0J achieve this coordination? The answer, in part, lies in the association between Spo0J and another protein, Soj.

Soj is member of the ParA family of partitioning proteins, and is thought to bind ATP [2]. Soj was originally




Chromosome replication and cell division in *B. subtilis*. The vegetative growth (left) and sporulation (right) pathways of *B. subtilis* are diagrammed with reference to chromosome replication and cell division (reviewed in [1]). Chromosomes (black) in vegetative cells are replicated from *oriC* (blue) and segregated to opposite cell poles. Cells then divide symmetrically. When sporulation signals are received by the cells, one of the chromosomes condenses and the cells divide asymmetrically with the condensed chromosome in the smaller prespore cell. Phosphorylated Spo0A ($Spo0A^{*P}$) activates transcription of sporulation-specific genes upon receipt of sporulation signals. Spo0A activation is antagonized by Soj, which is in turn repressed by Spo0J.

identified genetically as a suppressor of a *spo0J* mutation; deletion of *soj* restores the ability to form spores to a *spo0J* null mutant [2]. Deletion of *soj* in *spo0J*$^-$ cells restores normal expression of sporulation-specific genes [2]. Soj directly binds to the promoter sequences of these genes [4] and disrupts transcription of at least one of these loci [11]. Surprisingly, Soj is not absolutely required for normal chromosome partitioning or sporulation, as deletion of *soj* alone results in cells that sporulate and partition their chromosomes normally [10]. But Soj is necessary for partitioning of plasmids, as Spo0J is capable of stabilizing plasmids that contain a *parS* site only in the presence of Soj [9].

Taken together, these data suggest that Spo0J and Soj coordinately mediate a checkpoint between DNA replication and the initiation of sporulation. How is the coordination between Spo0J and Soj orchestrated? The answer seems to lie in the location and movements of these proteins within the cell. Recent work from two laboratories [3,4] has demonstrated that, like Spo0J, Soj is found at the polar or polar-proximal regions of the cell, and accurate localization of each protein requires the presence of the other. Strikingly, Soj is capable of a Spo0J-dependent, dynamic oscillation between the poles of the cell [3,4].

In wild-type cells, although Spo0J binds eight separate *parS* sites, it is visualized as a single focus at the cell pole [3,5–9]. Chromosome replication results in a duplication of this focus, and the individual foci move to opposite poles of the cell [5,8]. In the absence of Soj, however, Spo0J exists in multiple clusters of smaller polar foci [3]. This suggests that Spo0J binds to *parS* sites independently of Soj, but that Soj is required for the aggregation of the Spo0J–*parS* complexes.

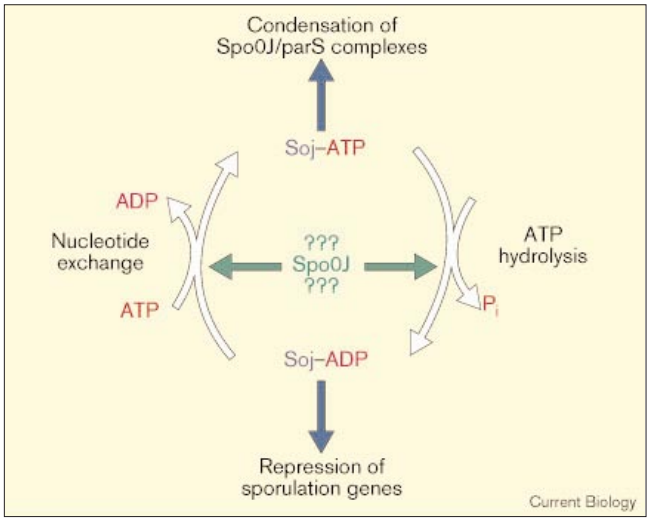
In order to understand the role of Soj in Spo0J aggregation, it was necessary to determine the intracellular location of Soj. This was elegantly achieved by Marston and Errington [3] and Quisel *et al.* [4] using Soj–GFP fusion proteins. Both groups found that, in normal vegetative cells, Soj–GFP concentrated in foci at or near the poles of the cell, though some of the protein remained diffused over the length of the cell. This observation suggests that, while the majority of the Soj is aggregated, there is also free Soj protein in the cell. There are some interesting differences between the images obtained by the two groups. Quisel *et al.* [4] observed tightly focused Soj foci distributed near the poles in all cells. Marston and Errington [3], observed somewhat larger Soj foci associated with the polar-proximal end of the nucleoid in only one of any given pair of sister cells. Quisel *et al.* [4] found more diffuse, nucleoid-proximal foci, similar to those seen by Marston and Errington [3], only in cells grown in sporulation medium.

Most significantly, however, both groups observed a dynamic, cooperative movement of Soj foci from pole to pole of the cell. Soj foci disassemble and reassemble on the order of minutes, frequently switching poles of the cell. Marston and Errington [3] detected this phenomenon in vegetative cells, whereas Quisel *et al.* [4] observed it only in stationary phase cells. Regardless of this discrepancy, however, only foci distributed over the polar ends of the chromosome were seen to oscillate. Interestingly, not all cells display this pole-to-pole movement of Soj; it was seen in approximately two-thirds of the vegetative cells and one-third of the stationary cells. Remarkably, the movement of Soj is Spo0J-dependent, as the formation of Soj foci depends on the presence of Spo0J in both vegetative and sporulating cells. Soj is distributed evenly across the nucleoid in cells lacking Spo0J. Clearly Spo0J is required for the aggregation, polar-proximal localization and pole-to-pole oscillation of Soj in *B. subtilis* [3,4].

Soj plays roles in both the repression of sporulation-specific genes and the aggregation of Spo0J–*parS* complexes, and it seems likely that the oscillation of Soj is somehow related to its dual function. What is responsible for the oscillation of Soj? Occupancy of its nucleotide-binding site seems to play some role. Although adenine nucleotide binding by Soj has not been formally demonstrated *in vitro*, the effects of mutations thought likely to affect nucleotide binding and hydrolysis by Soj were examined [4]. Amino acid changes predicted to abolish adenine-nucleotide binding by Soj disrupted both its polar localization and its chromosome association, indicating that nucleotide binding is likely to be required in some way for both of these features. This is consistent with the finding that the nucleotide-free form of the related protein ParA cannot bind DNA [12]. A mutation predicted to decrease the rate of hydrolysis of ATP resulted in localization of Soj to the poles in both *spo0J*$^+$ and *spo0J*$^-$ cells, and loss of its ability to oscillate from pole to pole. This suggests that the nucleotide-bound state of Soj, not just the presence of Spo0J, determines its polar localization.

Another factor that potentially contributes to control of the localization and oscillation of Soj, as well as to the localization of Spo0J, is the association of these proteins with the cell pole. To investigate this connection, Marston and Errington [3] assessed the localization of both Soj and Spo0J in cells rendered incapable of dividing by depletion of the cell-division protein FtsZ. In these elongated cells, Soj was found in a single, polar-proximal, nucleoid-associated focus. In non-dividing cells in the absence of Spo0J, however, Soj was distributed evenly over all — eight or more — of the cell's nucleoids. Soj foci in the elongated cells maintained some ability to dissociate and reassociate, but lost the ability to oscillate between the poles. This loss of movement in the context of remote poles suggests that the pole-to-pole movement of Soj depends on the proximity of the opposite cell pole. In the absence of FtsZ, Spo0J is predominantly localized

**Figure 2**




Model for the role of adenine nucleotides in the dual function of Soj. Soj is proposed to oscillate between ADP-bound and ATP-bound states. Soj–ATP is formed by the exchange of ADP for ATP, and Soj–ADP is formed by the hydrolysis of Soj-bound ATP. Soj–ADP is thought to bind to specific promoters and repress sporulation-specific gene expression and Soj–ATP is thought to facilitate aggregation of Spo0J–*parS* complexes. Spo0J is thought to function as either an adenine nucleotide exchange factor or an hydrolysis activating factor.

in small, fragmented foci. But at polar-proximal positions likely to be coincident with Soj aggregates, sharp, discrete Spo0J foci are observed. These observations indicate that localization of Soj and condensation of Spo0J foci are strongly influenced by the proximity of the cell poles, perhaps by as yet undefined polar-proximal proteins.

Why does Soj oscillate and what role does Spo0J play in Soj movement? Soj is both a partitioning protein and a transcriptional repressor of sporulation-specific genes. Perhaps the Spo0J-dependent oscillation of Soj is a means of coordinating its dual functions. The aggregation of Soj clearly serves to condense Spo0J–*parS* partitioning complexes into a single unit. The purpose of Spo0J aggregation itself remains unclear, although condensed Spo0J is known to be required for oscillation of Soj foci. Perhaps the dissociation of Soj aggregates is a means of freeing Soj to move from the pole to the chromosome. The results described by Quisel *et al.* [4], in conjunction with the work on ParA [12], suggest that ADP-bound Soj is the form that binds sporulation promoters and that the ATP-bound form of Soj associates with Spo0J (Figure 2). Spo0J may regulate Soj's pole-to-pole oscillation by altering its nucleotide bound state, by modulating either the exchange or hydrolysis rate of Soj-bound nucleotide.

It is clear from these reports that Spo0J and Soj display reciprocal control and interdependence, and that the polar localization of both proteins and remarkable pole-to-pole oscillation of Soj are critical to their functions. The precise functional relationship between Soj and Spo0J, and the impact of Soj oscillation on chromosome partitioning and sporulation remain to be explained. Intriguingly, the pole-to-pole movement of two *E. coli* proteins involved in septation-site selection also has been observed [13,14], indicating that the polar sequestration of protein complexes and their subsequent release may be a widespread mechanism of control in bacteria.

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