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# Control of Development by Altered Localization of a Transcription Factor in *B. subtilis*

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## Summary

In *B. subtilis*, the chromosome partitioning proteins Soj (ParA) and Spo0J (ParB) regulate the initiation of sporulation. Soj is a negative regulator of sporulation gene expression, and Spo0J antagonizes Soj function. Using fusions of Soj to green fluorescent protein, we found that Soj localized near the cell poles and upon entry into stationary phase oscillated from pole to pole. In the absence of Spo0J, Soj was associated predominantly with DNA. By *in vivo* cross-linking and immunoprecipitation, we found that Soj physically associates with developmentally regulated promoters, and this association increased in the absence of Spo0J. These results show that Soj switches localization and function depending on the chromosome partitioning protein Spo0J. We further show that mutations in the Soj ATPase domain disrupt localization and function and render Soj insensitive to regulation by Spo0J.

## Introduction

Many organisms have mechanisms to modulate gene expression in response to cell cycle events. In *Bacillus subtilis*, gene expression during the initiation of sporulation is affected by DNA replication and damage, and by proteins involved in chromosome partitioning (Ireton and Grossman, 1992, 1994; Ireton et al., 1994; Grossman, 1995). *B. subtilis* Soj and Spo0J, members of the ParA and ParB families of proteins, respectively, are involved in chromosome partitioning and also regulate the initiation of sporulation. ParA and ParB family members are required for accurate partitioning of many low-copy number plasmids (Nordström and Austin, 1989; Williams and Thomas, 1992), and chromosome-encoded ParA and ParB homologs from *Caulobacter crescentus* and *B. subtilis* function in chromosome partitioning (Ireton et al., 1994; Mohl and Gober, 1997). Chromosomal genes coding for ParA and ParB homologs have now been identified in many Gram-negative and Gram-positive bacteria, suggesting a high degree of evolutionary conservation in the components involved in chromosome segregation.

Our knowledge of ParA- and ParB-type proteins is based primarily on studies of the plasmid-encoded proteins, particularly those from the P1 prophage (ParA and ParB) and the F plasmid (SopA and SopB) of *Escherichia coli*. *parA* and *parB* are typically together in an operon. ParA is an ATPase, interacts with ParB, and can also

repress transcription of the *parAB* operon (Friedman and Austin, 1988; Mori et al., 1989; Davis et al., 1992). ParB binds to specific sites in the plasmid (termed *parS* sites) (Hayakawa et al., 1985; Davis et al., 1990), and formation of the ParB-*parS* complex is a critical step in plasmid partitioning. ParB interacts with ParA, modulating both the ATPase and the transcriptional repressor activities of ParA (Friedman and Austin, 1988; Mori et al., 1989; Davis et al., 1992; Bouet and Funnell, 1999). The chromosome-encoded ParA and ParB family members share many of the same properties as the plasmid-encoded family members (Mohl and Gober, 1997; Cervin et al., 1998; Lin and Grossman, 1998), although no chromosome-encoded ParA has yet been shown to have ATPase activity.

In *B. subtilis*, the chromosome-encoded ParA and ParB homologs, Soj and Spo0J, respectively, function both in chromosome partitioning and in sporulation (Ireton et al., 1994; Sharpe and Errington, 1996; Lewis and Errington, 1997; Lin and Grossman, 1998). Spo0J binds to a series of sites (*parS* sites) in the origin-proximal 20% of the chromosome and organizes the origin region into a structure that facilitates efficient partitioning (Lin and Grossman, 1998). Cells lacking functional Spo0J are partially defective in chromosome partitioning, as evidenced by an increase in the number of anucleate cells produced during exponential growth (Ireton et al., 1994). The role for Soj in chromosome partitioning is less clear. Deletion of *soj* causes little or no increase in the production of anucleate cells (Ireton et al., 1994). However, when a *ParS* site is placed on an unstable plasmid, that plasmid is stabilized only if both Soj and Spo0J are present (Lin and Grossman, 1998), indicating that Soj does function in *ParS*- and Spo0J-mediated partitioning of a plasmid. Soj may also participate in the *ParS*- and Spo0J-mediated partitioning of the chromosome, perhaps in combination with other proteins with overlapping functions.

In addition to their roles in chromosome partitioning, Spo0J and Soj regulate sporulation. *spo0J* null mutants are defective in sporulation, and this defect is relieved by null mutations in *soj* (suppressor of *spo0J*) (Ireton et al., 1994), indicating that Soj is a negative regulator of sporulation that is antagonized by Spo0J. Although the  $\Delta(\textit{soj-spo0J})$  double mutant can sporulate at wild-type levels, it is still defective for chromosome partitioning, indicating that the primary role of Spo0J in sporulation is regulatory. This may represent a signaling pathway that controls sporulation when chromosome organization or partitioning is defective.

We have investigated the mechanisms by which Spo0J and Soj regulate spore formation. Soj is known to inhibit expression of sporulation genes that are activated by the phosphorylated form of the transcription factor Spo0A (Ireton et al., 1994). At least part of this effect appears to be direct, as purified Soj inhibits transcription *in vitro* of one of the sporulation promoters (Cervin et al., 1998). Using cross-linking and immunoprecipitation, we have found that Soj is associated with the promoter regions of several sporulation genes *in vivo*,

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and this association is increased in the absence of Spo0J. Furthermore, using fusions of Soj to GFP, we found that in *spo0J*<sup>+</sup> cells, Soj is localized predominantly near the cell poles. Upon entry into stationary phase, Soj oscillated from pole to pole. In *spo0J* mutant cells, Soj was predominantly associated with DNA, consistent with the cross-linking and immunoprecipitation results. These findings indicate that Soj functions as a regulatory switch controlled by Spo0J. When Spo0J is functional, Soj associates with cell poles. When Spo0J is absent, Soj can relocate to the chromosome, binding to and inhibiting transcription from the sporulation promoters. Mutations in the conserved ATPase domain of Soj alter localization and render Soj insensitive to Spo0J. Spo0J probably regulates Soj by affecting ATP binding and hydrolysis.

## Results

### Soj Cross-Links to the *spolIA*, *spolIE*, and *spolIG* Promoter Regions in a Spo0J-Regulated Manner

Previous work has shown that expression of the *spolIA*, *spolIE*, and *spolIG* operons is inhibited in a *spo0J* null mutant (Ireton et al., 1994). All three of these loci are required for the establishment of cell type-specific gene expression and are directly activated by the transcription factor Spo0A (Wu et al., 1989; Satola et al., 1991; York et al., 1992). Soj might affect transcription of *spolIA*, *spolIE*, and *spolIG* by functioning as a direct transcriptional repressor of each promoter. Other members of the ParA family are known to act as transcriptional repressors (Friedman and Austin, 1988; Mori et al., 1989), and it was recently shown that Soj could disrupt transcription from the *spolIG* promoter in vitro (Cervin et al., 1998).

We tested for association of Soj with the *spolIA*, *spolIE*, and *spolIG* promoter regions in vivo by using a cross-linking/immunoprecipitation technique (Solomon and Varshavsky, 1985; Dedon et al., 1991; Strahl-Bolsinger et al., 1997; Lin and Grossman, 1998) (see Experimental Procedures). In brief, formaldehyde was added to cultures to cross-link proteins and DNA. Cells were lysed, and the DNA was sheared into fragments with an average size of 500–1000 bp. We used affinity-purified polyclonal antibodies to immunoprecipitate Soj and DNA associated with Soj. After thorough washing, the cross-links were reversed, and the presence or absence of a particular region of the chromosome in the immunoprecipitate was analyzed by PCR.

We found that Soj was associated with the promoter regions of the *spolIA*, *spolIE*, and *spolIG* operons in vivo, in both wild-type cells and in a *spo0J* null mutant (Figures 1a–1c). Cross-linking was done 2 hr after the end of stationary phase, a time when the effect of Soj on transcription of sporulation genes is readily apparent. In the experiments shown (Figure 1), dilutions of total DNA (before immunoprecipitation) and dilutions of DNA from the immunoprecipitates were used for PCR. This allows for comparison of relative amounts of specific regions in the immunoprecipitate from wild-type and *spo0J* mutant cells. There was approximately 4-fold more specific DNA in the immunoprecipitates for the *spo0J* null mutant than from wild-type cells (Figure 1). This difference was not caused by an increase in the

amount of DNA present in the immunoprecipitation nor in the amount of Soj present in the cells. All the immunoprecipitations were done from an equal amount of sheared DNA (Figure 1, right-hand panels), and there is slightly less Soj protein present in *spo0J* null mutants than in wild-type cells (S. Venkatasubramanyam and A. D. G., unpublished results). The presence of DNA from these sporulation genes in the immunoprecipitate was specific to Soj, as little or no DNA was detected in immunoprecipitates from an *soj spo0J* double mutant (Figure 1). These results indicate that Soj specifically associates with DNA near the *spolIA*, *spolIE*, and *spolIG* operons and that this association increases in the absence of Spo0J. Given the effects on gene expression in vivo (Ireton et al., 1994) and in vitro (Cervin et al., 1998), it seems most likely that Soj is directly associated with the promoter regions of these sporulation genes.

### Soj Localizes Near the Cell Poles in a Spo0J-Regulated Manner

We localized Soj in living cells by visualizing the fluorescence of both N- and C-terminal fusions of Soj to GFP (Soj-GFP and GFP-Soj). *gfp-soj* was expressed from the xylose-inducible promoter P<sub>xyl</sub>, and *soj-gfp* was expressed from a functional portion of the native *soj* promoter (see Experimental Procedures). The amount of GFP-Soj expressed from P<sub>xyl</sub> (0.1% xylose) was similar to the amount of wild-type Soj, and the GFP-Soj fully complemented an *soj* null mutation. The amount of the Soj-GFP fusion protein was ~25% of that of wild-type Soj and only partly complemented an *soj* null mutation. Soj-GFP allowed approximately 1% of cells to sporulate in a  $\Delta$ *spo0J* background versus wild-type Soj, which allows 0.1% sporulation. Approximately 50% of *spo0J*<sup>+</sup> cells sporulate. The partial complementation might be due to the reduced amount of the fusion protein. Nonetheless, like the wild-type Soj, the Soj-GFP was able to cross-link to the sporulation promoter regions in a *spo0J*-regulated manner, indicating that Soj-GFP was functional.

In *spo0J*<sup>+</sup> cells, both fusions localized to the extreme poles of the cells, typically as a band or two adjacent foci that may represent a ring structure (Figures 2a–2h). A low level of fluorescence was also detected throughout the cell. Localization of Soj-GFP in cells stained with the membrane dye FM4-64 demonstrated that Soj is closely appressed to cell septa (Figures 2c–2f). Incomplete septa can be visualized as broken lines crossing the cell (Pogliano et al., 1999). Soj-GFP is only rarely associated with incomplete septa, suggesting that it arrives late to newly forming cell poles, usually after septation is complete (Figures 2e and 2f). The localization pattern of Soj is clearly distinct from that of Spo0J. Spo0J typically localizes as foci at the 1/4 and 3/4 positions in the cell (halfway between midcell and each cell pole) (Glaser et al., 1997; Lin et al., 1997) (D. C.-H. L. and A. D. G., unpublished data).

Strikingly, in the absence of Spo0J, Soj-GFP and GFP-Soj were distributed across the center of the cell (Figures 2i–2m), in a pattern nearly identical to nucleoid localization (compare Figures 2j and 2k). These results show that Spo0J is needed to maintain Soj at the poles. In the absence of Spo0J, Soj moves to the nucleoid, where, as shown above by cross-linking in vivo, it associates with the promoter regions of several sporulation genes.

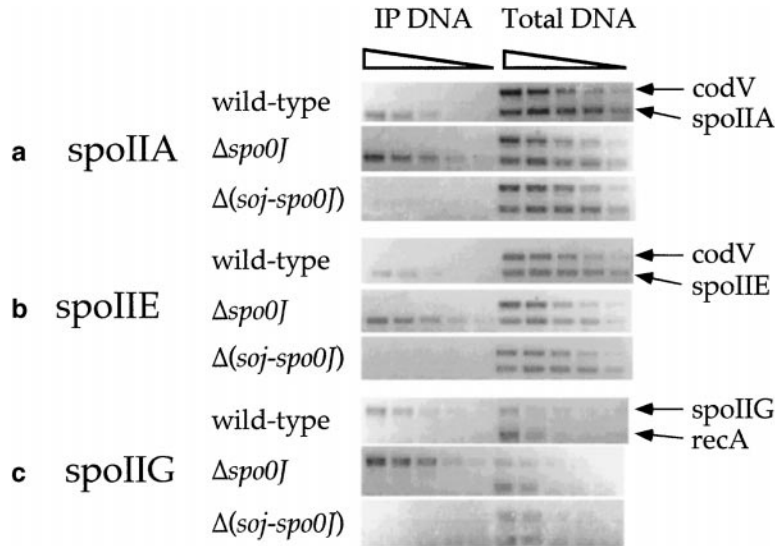


Figure 1. Association of Soj with the Promoter Regions of Sporulation Genes In Vivo Wild-type (JH642),  $\Delta spo0J$  (AG1468), and  $\Delta(soj-spo0J)$  (AG1505) strains were grown in 2xSG medium at 37°C. Two hours after the end of exponential growth, formaldehyde was added to cross-link protein and DNA. Protein-DNA complexes were immunoprecipitated using anti-Soj antibodies. The presence of a given promoter region was assayed by PCR amplification with primers designed to amplify the promoter regions of *spoIIA* (a), *spoIIE* (b), *spoIIG* (c), and *codV* and *recA* as controls. IP DNA lanes, DNA products from PCR assays performed on a dilution series (4, 2, 1, 0.5, and 0.25  $\mu$ l) of the immunoprecipitated material. Total DNA lanes, DNA products from PCR assays performed on a dilution series (the equivalent of 1/250, 1/500, 1/1000, 1/2000, and 1/4000  $\mu$ l) of sample DNA taken prior to immunoprecipitation.

The fact that both GFP fusions exhibit Spo0J-dependent localization strongly suggests that the fusion proteins accurately reflect localization of the wild-type Soj protein. We were unable to localize Soj by immunofluorescence. Fixation of cells disrupts localization of Soj-GFP and GFP-Soj fusions and Soj. Other labs have reported similar difficulties localizing MinD (a protein similar to Soj) by immunofluorescence (Marston et al., 1998).

#### GFP-Soj Moves from Pole to Pole in *spo0J*<sup>+</sup> Cells

As cells entered stationary phase, the polar bands of GFP-Soj became larger foci, and these foci moved from pole to pole. Time lapse photography revealed that on a time scale of minutes, a focus of GFP-Soj at a pole dispersed and then reformed at the opposite pole of the cell (Figures 3a and 3b). Foci may repeat this activity several times over a 20 min time course (Figure 3b). In cells that had this movement, foci tended to dwell at poles from 5 to 20 min and took as little as 1 min to as much as 15 min to reassemble, almost always at the opposite pole. In a few cases (approximately 1%), a focus disassembled and then apparently reassembled at the same pole. This may represent a full back-and-forth oscillation during the time lapse interval, or there

may be some exceptions to the generally back-and-forth motion.

We observed this dynamic behavior of GFP-Soj in approximately one third of the *spo0J*<sup>+</sup> cells over a 20 min period. In contrast, GFP-Soj was not dynamic in the absence of Spo0J; it remained nucleoid associated and static. It is not clear why movement of Soj was observed in one third of *spo0J*<sup>+</sup> cells and not in the other two thirds. After multiple light exposures during the time course, most cells no longer had clear foci of GFP-Soj, making it difficult to acquire additional images. Perhaps asynchronous cell cycle events and damage due to the multiple exposures contribute to the heterogeneity.

#### Soj Localization and Mobility Require the Conserved ATP-Binding Domain

Mutational analysis of the Soj Walker boxes indicated that Soj localization and mobility require these conserved motifs. We constructed three mutations in the Walker boxes. Lysine to glutamine at amino acid 16 (K16Q) and aspartate to alanine at amino acid 125 (D125A) are predicted to abolish nucleotide binding. Lysine 16 (or its equivalent) is nearly invariant across all Walker A boxes (Walker et al., 1982; Traut, 1994) and

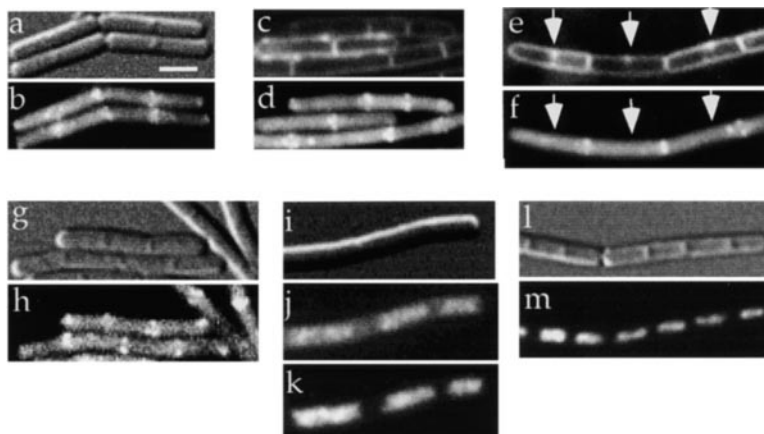


Figure 2. Subcellular Localization of Soj as Visualized with Fusions to GFP

(a) DIC and (b) autofluorescence of *spo0J*<sup>+</sup> cells with a C-terminal Soj-GFP fusion (DL554). (c-f) Fluorescent membrane dye FM4-64 (c and e) and Soj-GFP (d and f). Arrows (e and f) indicate partial septa. (g) DIC and (h) GFP-Soj N-terminal fusion in *spo0J*<sup>+</sup> cells (JQ436). In  $\Delta spo0J$  cells (i-m), Soj-GFP (j) and GFP-Soj (m) localize to the cell center, coincident with the nucleoid. (i) DIC, (j) Soj-GFP, and (k) DAPI in *spo0J* null mutant cells (DL555). (l) DIC and (m) GFP-Soj in *spo0J* null mutant cells (JQ437). All samples were taken from exponentially growing cultures. Scale bar is 2  $\mu$ m.

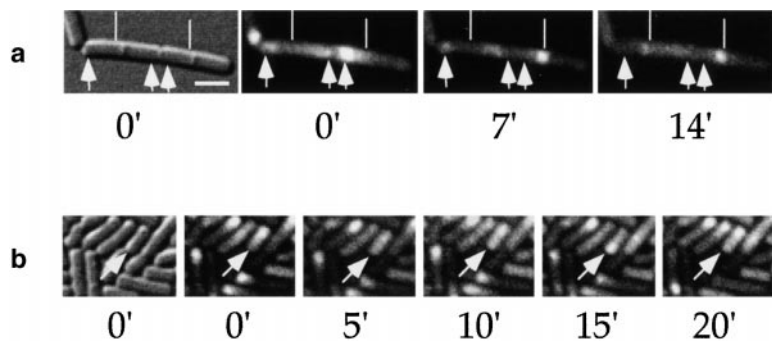


Figure 3. Movement of Soj from Pole to Pole in Cells in Stationary Phase

Cells expressing GFP-Soj in a *spo0J*<sup>+</sup> background (JQ436) were grown in 2xSG medium at 25°C and sampled 1 hr after exit from exponential growth. Far left panels are DIC images; other panels are GFP autofluorescence images. Scale bar is 2  $\mu$ .

(a) Fluorescence images were captured at 7 min intervals. Arrows indicate the starting positions of GFP-Soj foci. Lines indicate the final positions.

(b) Fluorescence images were captured at 5 min intervals. The arrow indicates a cell in which the GFP-Soj focus undergoes a full oscillation cycle, ending at the same pole from which it started.

interacts with the  $\alpha$ - and  $\beta$ -phosphates of the bound nucleotide (Pai et al., 1989; Story and Steitz, 1992). Mutations in the conserved lysine typically disrupt nucleotide binding (Pause and Sonenberg, 1992; Seefeldt et al., 1992; Weng et al., 1996; Yamauchi and Baker, 1998). Aspartate 125 indirectly coordinates the magnesium ion in the ATP-binding pocket (Pai et al., 1989; Story and Steitz, 1992), and in nitrogenase, an enzyme with an ATPase domain closely related to Soj, aspartate 125 also makes contacts with lysine 16 (Georgiadis et al., 1992). Western blot analysis indicated that the level of SojK16Q mutant protein was similar to that of wild-type Soj (data not shown). The level of the SojD125A protein was  $\sim$ 20% of wild type, suggesting that this mutation destabilizes the protein (data not shown).

The third mutation, glycine to valine at amino acid 12 (G12V), should alter nucleotide hydrolysis. The equivalent glycine-to-valine mutation in Ha-*ras* p21 results in a Ras protein that can bind GTP but has a greatly reduced rate of hydrolysis (Vogel et al., 1988). The SojG12V mutant protein accumulates to near wild-type levels (data not shown).

We tested the function and localization of each of the mutant proteins. All three behaved genetically like null mutations. That is, they bypassed the need for *spo0J* for sporulation, indicating that the ATPase mutants were unable to function to inhibit sporulation. Visualization of the intracellular localization of SojK16Q and SojD125A with C-terminal GFP fusions indicated that the mutant proteins were distributed throughout the cell (Figures 4a–4d). The presence or absence of Spo0J had no apparent effect on the localization of these mutant proteins

(Figures 4a–4d), in contrast to the effect on the wild-type Soj-GFP (Figures 2i–2m). Since SojK16Q and SojD125A are probably defective in nucleotide binding, these results indicate that binding of Soj to either ATP or ADP may be required for polar localization and/or DNA binding. Of course, with loss-of-function mutations, other interpretations are possible.

Localization of the SojG12V mutant was much different than that of the other two mutants and indicates that ATP hydrolysis is important for Soj function and localization. Like wild-type Soj, the SojG12V mutant localized to the cell poles (as visualized with a GFP fusion) in *spo0J*<sup>+</sup> cells (Figure 4e). Remarkably, in a *spo0J* null mutant, SojG12V still localized to the cell poles (Figure 4f). This contrasts sharply with wild-type Soj-GFP, which associates with the nucleoid in the absence of *spo0J* (compare Figures 2i–2k with Figure 4f). We suspect that SojG12V can bind to ATP but has a decreased rate of hydrolysis, causing it to remain predominantly in the ATP-bound form. If this is the case, then Soj-ATP localizes to the cell poles and Soj-ADP binds DNA (see Discussion).

The *sojG12V* mutation also abolished pole-to-pole movement. When cells expressing wild-type GFP-Soj entered stationary phase, the narrow polar bands of GFP-Soj changed into large polar foci that became mobile (Figures 3a and 3b). GFP-SojG12V did not form large polar foci. Instead, it remained in narrow polar bands that neither moved nor showed any tendency to disassemble and reassemble (data not shown).

Despite considerable effort, ATP binding and hydrolysis have not been detected with purified Soj in vitro

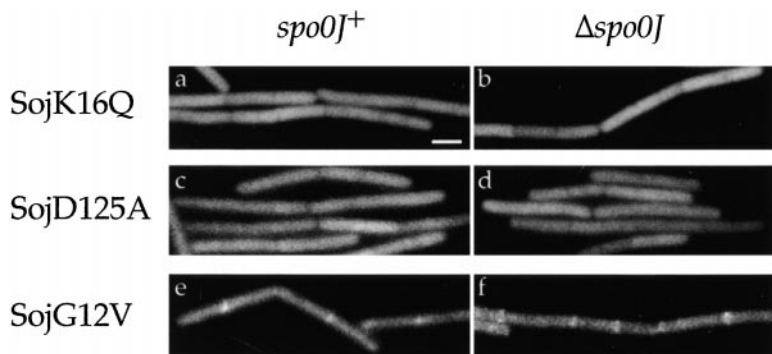


Figure 4. Mutations in the Conserved ATPase Domains Perturb Localization of Soj

Autofluorescence of mutant forms of Soj-GFP was detected in living cells. (a) SojK16Q-GFP in *spo0J*<sup>+</sup> (JQ510) and (b) *spo0J* mutant (JQ511) cells. (c) SojD125A-GFP in *spo0J*<sup>+</sup> (JQ512) and (d) *spo0J* mutant (JQ513) cells. (e and f) SojG12V-GFP localizes to cell poles in (e) *spo0J*<sup>+</sup> (DL643) and (f) *spo0J* mutant (DL647) cells. All samples were taken from exponentially growing cultures. Scale bar is 2  $\mu$ .

(Cervin et al., 1998) (J. D. Q. and A. D. G., unpublished results). Therefore, we have not been able to determine the effects of the mutations on ATP binding and hydrolysis in vitro.

## Discussion

We have shown that Soj is a surprisingly mobile protein within the cell, exhibiting dynamic localization that correlates with its role as a developmental regulator. In *spo0J*<sup>+</sup> cells, Soj associates with cell poles, undergoing rapid, pole-to-pole oscillation as cells enter stationary phase. Soj responds to a defect in the Spo0J chromosome partitioning protein by relocating to the nucleoid and ceasing any dynamic behavior. Concurrent with nucleoid localization, Soj increases its association with sporulation promoters and inhibits transcription. In addition, both localization and pole-to-pole movement of Soj are probably regulated by ATP binding and hydrolysis.

### Soj Associates with and Regulates Sporulation Promoters

Using in vivo formaldehyde cross-linking, we have shown that Soj is associated with DNA near three developmentally regulated promoters: *spollA*, *spollE*, and *spollG*. In addition, we have found that Soj inhibits synthesis of the transcription factor Spo0A, which is itself an activator of these three sporulation loci (J. D. Q. and A. D. G., unpublished data). In a *spo0J* null mutant, Soj associates more strongly with these loci, and expression of the genes is repressed in a Soj-dependent manner. Most likely, Soj is a direct transcriptional repressor that binds to these promoters and inhibits transcription. It is known that other ParA family members act directly as transcriptional repressors (Friedman and Austin, 1988; Mori et al., 1989). Additionally, Soj is known to inhibit *spollG* transcription in vitro (Cervin et al., 1998). At least part of the defect in sporulation caused by a *spo0J* null mutation is due to the effect of Soj on expression of *spo0A* (J. D. Q. and A. D. G., unpublished data). We suspect that the increased binding to these and perhaps other sporulation promoters is sufficient to cause the defect in sporulation.

Visualization of the subcellular localization of Soj, with GFP fusions, indicated that most of the Soj protein is near the cell poles and that Spo0J is required to maintain Soj at the poles. In the absence of Spo0J, Soj dissociates from the cell poles and colocalizes with the nucleoid. Soj localization to the nucleoid correlates with the increased association of Soj with the sporulation promoters and the inhibition of sporulation.

### Spo0J and the Soj ATPase Domain Regulate Soj Localization

Spo0J regulates Soj localization even though, for the most part, the two proteins do not colocalize. This contrasts with bacterial cell division proteins that assemble sequentially into a ring at midcell and are retained through stable interactions with other colocalizing proteins (Lutkenhaus and Addinall, 1997; Chen et al., 1999; Weiss et al., 1999).

We propose that Spo0J regulates Soj through transient interactions that influence the ratio of Soj bound

to ATP versus ADP and that it is the bound nucleotide that determines whether Soj localizes to the pole or to the nucleoid. Spo0J is required to maintain wild-type Soj at the cell pole but has no effect on localization of Soj proteins carrying mutations in the ATPase domain. The SojG12V mutant protein localizes to the cell pole even in  $\Delta spo0J$  cells. We suspect that the SojG12V mutant binds ATP but is defective in hydrolysis, causing it to remain primarily in the ATP-bound state. Together these data suggest that Soj-ATP localizes to the poles, while Soj-ADP, like ParA-ADP (Bouet and Funnell, 1999), binds to DNA. Perhaps Spo0J acts to increase the ratio of Soj-ATP to Soj-ADP. Spo0J could accomplish this by either inhibiting Soj-ATP hydrolysis or by acting as a nucleotide exchange factor to rapidly convert Soj-ADP back to Soj-ATP.

It is also possible that SojG12V is a hyperactive ATPase. In this case, it would be Soj-ATP that is bound to DNA and Soj-ADP that is at the cell poles. Spo0J would then stimulate ATP hydrolysis or stabilize the ADP-bound form.

Based on analogy to the plasmid partitioning protein ParA, we favor the model in which Soj-ADP binds DNA. ParA-ADP binds DNA and represses transcription of its own operon, while ParA-ATP interacts with the partitioning complex. ParB (a Spo0J homolog) transiently contacts ParA and affects its function by modulating the ATPase activity of ParA (Bouet and Funnell, 1999). We predict that the plasmid ParB (SopB) proteins stimulate ATP hydrolysis by stimulating exchange of ADP for ATP.

Some Soj protein is found throughout *spo0J*<sup>+</sup> cells, consistent with the model that Soj exists in an equilibrium between a nucleoid-associated state and a pole-associated state, with a small fraction in transit between locations. The function of Spo0J may be to continually convert Soj from the nucleoid-associated state to the pole-associated state, preventing Soj from accumulating at sporulation promoters. Soj localization would be sensitive to changes in Spo0J activity, and a failure in the chromosome partitioning process that disrupted Spo0J function could quickly lead to relocation of Soj to the nucleoid and repression of target promoters.

### Oscillation of Soj from Pole to Pole

Surprisingly, we have found that GFP-Soj foci move from pole to pole in early stationary phase cells. This motion requires Spo0J and happens on a time scale of minutes. In a *spo0J* null mutant, GFP-Soj loses mobility and adheres to the nucleoid. In *E. coli*, the ATPase and cell division regulator MinD, an Soj homolog, exhibits similar oscillatory motion on a time scale of seconds (Raskin and de Boer, 1999).

The pole-to-pole oscillation of Soj appears to require functional ATP binding and hydrolysis. The SojG12V mutant protein is fully capable of localizing to the cell pole but cannot undergo the coordinated disassembly and reassembly necessary for pole-to-pole movement. Given the similarity of Soj to MinD, we suspect that MinD also requires ATPase activity to shift back and forth across the cell.

What is the purpose of this motion? We propose that it is a mechanism to allow Soj to sample the nucleoid.

Table 1. *B. subtilis* Strains Used

Strain	Relevant Genotype
JH642	Wild-type ( <i>trpC2 pheA1</i> )
AG1468	$\Delta spo0J::spc$
AG1505	$\Delta(soj-spo0J)::spc$
DL554	$\Delta(soj-spo0J)::spc thr::(spo0J^+ erm) amyE::(soj-gfp neo)$
DL555	$\Delta(soj-spo0J)::spc amyE::(soj-gfp neo)$
DL643	$\Delta(soj-spo0J)::spc thr::(spo0J^+ erm) amyE::(sojG12V-gfp neo)$
DL647	$\Delta(soj-spo0J)::spc amyE::(sojG12V-gfp neo)$
JQ416	$\Delta(soj-spo0J)::spc amyE::(sojK16Q-gfp neo)$
JQ417	$\Delta(soj-spo0J)::spc thr::(spo0J^+ erm) amyE::(sojK16Q-gfp neo)$
JQ420	$\Delta(soj-spo0J)::spc amyE::(sojD125A-gfp neo)$
JQ421	$\Delta(soj-spo0J)::spc thr::(spo0J^+ erm) amyE::(sojD125A-gfp neo)$
JQ436	$\Delta(soj-spo0J)::spc thr::(spo0J^+ erm) amyE::(Pxyl-gfp-soj cat)$
JQ437	$\Delta(soj-spo0J)::spc amyE::(Pxyl-gfp-soj cat)$
JQ508	$\Delta(soj-spo0J)::spc thr::(spo0J^+ erm) amyE::(Pxyl-gfp-sojG12V cat)$
JQ509	$\Delta(soj-spo0J)::spc amyE::(Pxyl-gfp-sojG12V cat)$
JQ510	$\Delta(soj-spo0J)::spc thr::(spo0J^+ erm) amyE::(Pxyl-gfp-sojK16Q cat)$
JQ511	$\Delta(soj-spo0J)::spc amyE::(Pxyl-gfp-sojK16Q cat)$
JQ512	$\Delta(soj-spo0J)::spc thr::(spo0J^+ erm) amyE::(Pxyl-gfp-sojD125A cat)$
JQ513	$\Delta(soj-spo0J)::spc amyE::(Pxyl-gfp-sojD125A cat)$

Perhaps as Soj foci disassemble and move across the cell (in an ADP-bound form?), Soj transiently binds to the nucleoid. In the absence of Spo0J, Soj may be unable to release from the nucleoid. In this situation, Soj would associate with and repress sporulation promoters. Perhaps other chromosomal defects might also cause Soj to adhere to the nucleoid and inhibit sporulation. This nucleoid sampling may occur primarily in cells about to enter sporulation, as this is the time when regulatory pathways affecting the decision to sporulate are of particular importance. During exponential growth, the effect of Soj on developmental gene expression may be minimal because many other regulatory effects prevent sporulation in unstarved, growing cells (Grossman, 1995).

Soj is a developmental regulator that exhibits dynamic localization that correlates with its regulatory function. In *B. subtilis*, localization of the sporulation sigma factor of RNA polymerase, sigma-E, is also regulated during development. The precursor form of sigma-E is associated with the cytoplasmic membrane, then with the polar sporulation septum, and after processing, it associates with RNA polymerase to promote transcription (Hofmeister, 1998). In eukaryotes, many transcription factors switch from the cytoplasm to the nucleus when they are activated. Bacteria do not have a nuclear membrane to exclude transcriptional regulators from the chromatin but could conceivably sequester DNA-binding proteins to the cell poles to prevent inappropriate gene activation or repression. We suspect that this type of regulation may be widespread in prokaryotes.

#### Experimental Procedures

##### Strains and Media

The *B. subtilis* strains used are listed in Table 1. All strains are derivatives of JH642 (Perego et al., 1988), which is referred to as wild type. Standard procedures were used for transformations and strain construction (Harwood and Cutting, 1990). The  $\Delta spo0J::spc$  and  $\Delta(soj-spo0J)::spc$  mutations are deletion insertions (Iretton et al., 1994).

Routine growth and maintenance of *E. coli* and *B. subtilis* was done in Luria-Bertani (LB) medium. All experiments using *B. subtilis*

were carried out in the rich sporulation broth, 2xSG (Leighton and Doi, 1971). Media in plates were solidified with 15 g of agar (Difco Laboratories) per liter. Xylose was used at 0.1% to induce expression of *gfp-soj* from Pxyl. Antibiotics were used at the following concentrations: ampicillin at 100  $\mu$ g/ml, chloramphenicol (*cat*) at 5  $\mu$ g/ml, spectinomycin (*spc*) at 100  $\mu$ g/ml, neomycin (*neo*) at 5  $\mu$ g/ml, and erythromycin and lincomycin together (*erm*) at 0.5 and 12.5  $\mu$ g/ml, respectively.

##### Formaldehyde Cross-Linking and Immunoprecipitations

Cells were grown in 2xSG until 2 hr after the end of exponential growth, at which point 10 ml samples of culture were taken for analysis. Generally, cross-linking and sample preparations were performed as described (Lin and Grossman, 1998) and based on previously published chromatin immunoprecipitation assays (Solomon and Varshavsky, 1985; Dedon et al., 1991; Strahl-Bolsinger et al., 1997). Samples were treated with NaPO<sub>4</sub> (10 mM final concentration) and formaldehyde (1% final concentration) for 3 min at room temperature. Cells were pelleted and washed twice with 10 ml of phosphate-buffered saline (pH 7.3) (Ausubel et al., 1990). Cells were resuspended in 500  $\mu$ l of solution A (10 mM Tris [pH 8], 20% sucrose, 50 mM NaCl, 10 mM EDTA) containing 20 mg/ml lysozyme and incubated at 37°C for 30 min. Five hundred microliters of 2 $\times$  IP buffer (100 mM Tris [pH 7], 300 mM NaCl, 2% Triton X-100), and PMSF (final concentration of 1 mM) were added, and the cells were incubated another 10 min at 37°C. DNA was sheared to an average size of 500–1000 bp by sonication. Insoluble debris was removed by centrifugation, and the supernatant was transferred to a fresh microfuge tube. To determine the amount of DNA immunoprecipitated relative to the amount of total DNA prior to immunoprecipitation, 100  $\mu$ l of supernatant was removed and saved for later analysis ("total" DNA control).

Protein and protein-DNA complexes were immunoprecipitated (overnight, 4°C) with affinity-purified polyclonal anti-Soj antibodies followed by incubation with 30  $\mu$ l of a 50% protein A-Sepharose slurry (1 hr, room temperature). Complexes were collected by centrifugation and washed seven times with 1 $\times$  IP buffer and twice with 1 ml TE (10 mM Tris [pH 8], 0.1 mM EDTA). The slurry was resuspended in 100  $\mu$ l of TE. The 100  $\mu$ l "total" DNA control was mixed with 100  $\mu$ l TE and brought to a final concentration of 0.1% SDS. Formaldehyde cross-links of both immunoprecipitated and total DNA samples were reversed by incubation at 65°C for 6 hr. Samples were used for PCR without further treatment.

PCR was performed with Vent DNA polymerase (New England Biolabs) using serial dilutions of the immunoprecipitate and the total DNA as the template. Oligonucleotide primers were typically 20–25 bases in length and amplified fragments ranging from ~250–550 bp in size. Sequences of all primers are available upon request. Relative

amounts of Soj-DNA complexes were determined by comparing the intensity of bands in the linear range of the PCR from both the immunoprecipitate and the "total" DNA control. Gels were photographed onto Polaroid 665 film, and the negatives were scanned using Adobe Photoshop software.

#### GFP Fusions

C-terminal GFP fusions were constructed in essentially the same manner as the previously reported *spo0J-gfp* fusion (Lin et al., 1997). In brief, *soj*, including a functional portion of the native promoter (224 bp upstream of the start codon) (Ireton et al., 1994), was amplified by PCR with primers designed to replace the stop codon with an XhoI site and ligated into pGEMcat (Youngman et al., 1989). *gfp* (variant with the mutations S65T V163A) was amplified by PCR from pJK19-1 (Kahana and Silver, 1996) so as to introduce an in-frame XhoI site immediately upstream of the start codon, and this fragment was cloned into the XhoI site at the 3' end of *soj*, introducing a two-amino acid linker, leu-glu, between *soj* and *gfp*. The *soj-gfp* fusion was then excised from pGEMcat, cloned into pAS23, an *amyE* insertion vector (A. Srivastava and A. D. G., unpublished data), to create pDL132. *soj-gfp* was introduced into the *B. subtilis* chromosome by double crossover at the *amyE* locus. N-terminal GFP fusions were constructed in pEA18, an *amyE* insertion vector that places expression of the N-terminal GFP fusion under control of the xylose inducible promoter, P<sub>xyI</sub>. pEA18 contains GFP variant *mut2*, S65T V68L S72A (Cormack et al., 1996). *soj* was amplified by PCR so as to introduce an in-frame NotI site upstream of the initiation codon and cloned into pEA18 at the NotI and BamHI sites to generate pJQ94. This introduces a linker, ala-gly-gly-arg, between GFP and Soj.

#### Fluorescence Microscopy

Cells were grown in 2xSG medium at 27°C and collected by brief (8 s) centrifugation in a microcentrifuge. The supernatant was aspirated, and cells were resuspended in defined minimal medium (Vasantha and Freese, 1980; Jaacks et al., 1989). Initial control experiments showed that centrifugation does not affect Soj localization. Agarose (0.8%) (in phosphate-buffered salts with 1 mM MgSO<sub>4</sub>) was applied to a microscope slide and allowed to cool, and resuspended cells were added on top of the agarose pad. After 2–5 min, excess media was aspirated, and a glass cover slip was placed on top. Cell boundaries were visualized using the membrane stain FM4-64 (400 ng/ml, mixed with cells just prior to placement on agarose pads; Molecular Probes) (Pogliano et al., 1999).

Microscopy was performed on a Nikon E800 fluorescence microscope with a 100× DIC objective. Images were captured with an ORCA CCD camera (Hamamatsu) and Openlabs 1.7.8 software. GFP and FM4-64 were visualized using Chroma filter set #41012 and #41002c, respectively. Image processing was done in Adobe Photoshop 3.0.

#### Site-Directed Mutagenesis

ATPase mutants were generated by site-directed mutagenesis (G12V, K16Q) (Ausubel et al., 1990), or by PCR-based mutagenesis using splicing by overlap extension (D125A) (Vallejo et al., 1995). Mutated clones were sequenced, amplified by PCR, and cloned into pDL132 or pJQ94, replacing the wild-type copy of *soj*. GFP fusion vectors were sequenced and then transformed into *B. subtilis*.

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