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Dynamic Control of the DNA Replication Initiation Protein DnaA by Soj/ParA

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SUMMARY

Regulation of DNA replication and segregation is essential for all cells. Orthologs of the plasmid partitioning genes *parA, parB,* and *parS* are present in bacterial genomes throughout the prokaryotic evolutionary tree and are required for accurate chromosome segregation. However, the mechanism(s) by which *parABS* genes ensure proper DNA segregation have remained unclear. Here we report that the ParA ortholog in *B. subtilis* (Soj) controls the activity of the DNA replication initiator protein DnaA. Subcellular localization of several Soj mutants indicates that Soj acts as a spatially regulated molecular switch, capable of either inhibiting or activating DnaA. We show that the classical effect of Soj inhibiting sporulation is an indirect consequence of its action on DnaA through activation of the Sda DNA replication checkpoint. These results suggest that the pleiotropy manifested by chromosomal *parABS* mutations could be the indirect effects of a primary activity regulating DNA replication initiation.

INTRODUCTION

Proper transmission of genetic material is essential for the viability of all organisms. Nucleic acid replication and segregation must be precisely coordinated to ensure accurate genome inheritance. Eubacterial chromosomes contain a single origin of replication (*oriC*) that is recognized by the initiator protein DnaA. DnaA bound at *oriC* forms a homo-oligomer that mediates open complex formation and allows assembly of an initiation complex that loads the replicative helicase. Production of the initiation complex is followed by recruitment of the remaining replisome components, leading to replication of the bacterial chromosome (Kornberg and Baker, 1992; Messer et al., 2001; Mott and Berger, 2007). After duplication, daughter chromosomes are rapidly segregated toward opposite poles of the cell as part of a coordinated regulatory network to ensure accurate chromosome inheritance (Errington et al., 2005; Thanbichler and Shapiro, 2006a).

Orthologs of the plasmid partitioning proteins ParA and ParB are present on the chromosomes of bacteria found throughout all branches of the prokaryotic evolutionary tree and are most often located proximal to *oriC* (Gerdes et al., 2000; Livny et al., 2007). They have been shown to affect accurate chromosome segregation in several species, suggesting that they play an important and active role required for proper inheritance of bacterial genomes. However, in spite of being the focus of study for several decades, the molecular mechanism(s) underlying the activities of bacterial chromosomal partitioning genes are poorly understood.

The *par* operons derive their name from homology to partitioning systems of low copy number plasmids that ensure faithful pDNA segregation into daughter cells (Austin and Abeles, 1983; Gerdes et al., 1985; Ogura and Hiraga, 1983). The bacterial orthologs are required for chromosome replication and segregation, chromosome origin localization and separation, cell division, and developmental gene regulation in *Bacillus subtilis* (Hranueli et al., 1974; Ireton et al., 1994; Lee and Grossman, 2006; Lee et al., 2003; Ogura et al., 2003; Sharpe and Errington, 1996; Wu and Errington, 2003); cell-cycle progression and cell division in *Caulobacter crescentus* (Mohl et al., 2001); chromosome segregation and cell growth in *Mycobacterium smegmatis* (Jakimowicz et al., 2007a); chromosome organization and segregation, cell growth, and motility in *Pseudomonas aeruginosa* (Bartosik et al., 2004; Lasocki et al., 2007); chromosome segregation and cell morphology in *Pseudomonas putida* (Godfrin-Estevenon et al., 2002; Lewis et al., 2002); chromosome segregation and cell division in *Streptomyces coelicolor* (Jakimowicz et al., 2007b; Kim et al., 2000); and chromosome origin localization in *Vibrio cholerae* (Fogel and Waldor, 2006; Saint-Dic et al., 2006).

One of the best studied chromosomal partitioning systems is the *B. subtilis par* operon (referred to as *soj(parA), spo0J(parB),* and *parS*). *parS* is a specific DNA sequence motif that acts as the binding site for the DNA-binding protein Spo0J (Leonard et al., 2004; Lin and Grossman, 1998). In *B. subtilis* eight of the ten *parS* sites are located close to the origin of DNA replication, and the sites closest to *oriC* are most frequently bound by Spo0J (Breier and Grossman, 2007; Lin and Grossman, 1998). *parS* nucleates the spreading of Spo0J into flanking regions of DNA to create large nucleoprotein structures that extend for several kbp around a *parS* site (Breier and Grossman, 2007; Murray et al., 2006).

Soj is a Walker-type ATPase that interacts with Spo0J and is required for proper separation of sister origins and synchronous DNA replication, as well as for the regulation of sporulation

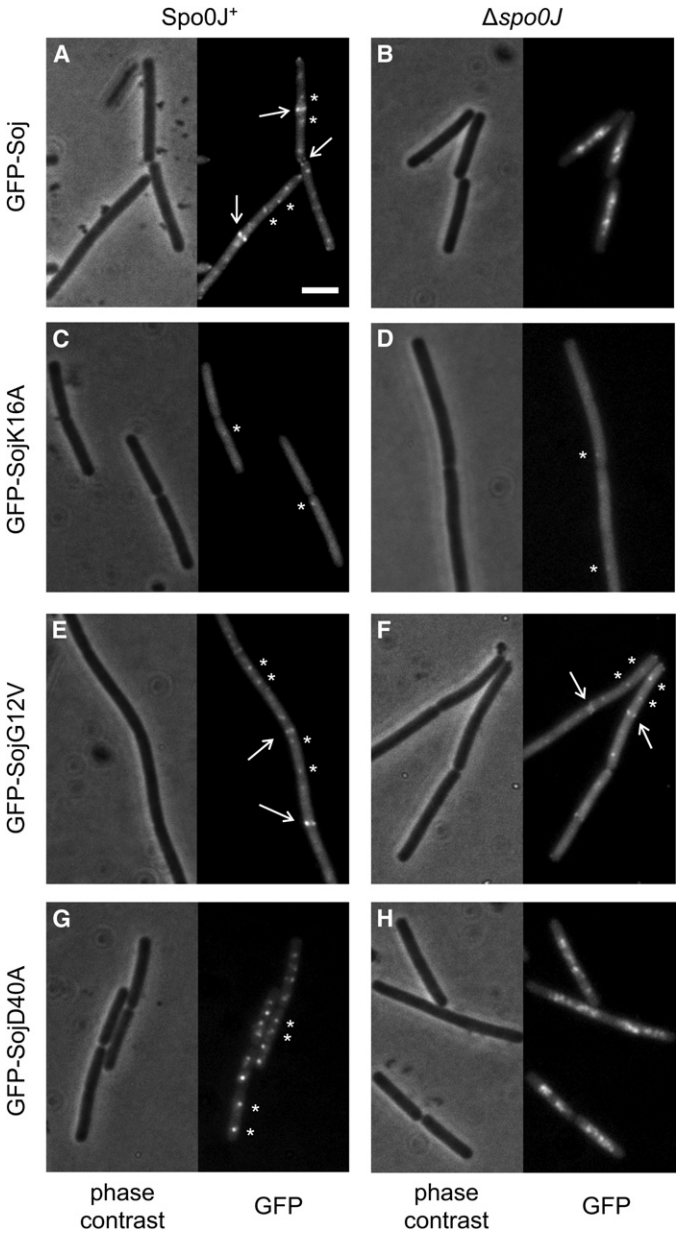

Figure 1. Localization of Wild-Type Soj and Soj Mutants in the Presence and Absence of Spo0J

The localization of GFP-Soj variants was observed using epifluorescence microscopy. Cells were grown in CH medium at 30°C. The left column shows results using a wild-type strain and the right column shows results using a *Δspo0J* mutant. An asterisk (*) denotes localization as a focus and an arrow (→) indicates localization at a septum. (A) HM4, (B) HM13, (C) HM7, (D) HM24, (E) HM14, (F) HM25, (G) HM5, (H) HM23. Scale bar: 3 μm.

(Ireton et al., 1994; Lee and Grossman, 2006; Leonard et al., 2005; Ogura et al., 2003). Biochemical and structural analysis of *Thermus thermophilis* Soj has shown that the protein is a dynamic molecular switch that is capable of forming an ATP-dependent "sandwich" dimer (Leonard et al., 2005). The ATP-bound dimer binds cooperatively to nonspecific DNA and contains ATP-hydrolysis activity (Hester and Lutkenhaus, 2007; Leonard et al., 2005; McLeod and Spiegelman, 2005). ATP hydrolysis by Soj leads to dissociation from DNA and resets the cycle.

We have investigated the activities of *B. subtilis* Soj in vivo by studying the effects of three mutations that alter the functions of Soj proteins in vitro. These mutations inhibit specific activities of Soj (ATP binding, cooperative DNA binding, or ATP hydrolysis) and lead to accumulation of different protein intermediates. We find that the Soj variants have distinct intracellular localization patterns and that they differentially regulate initiation of DNA replication. Both Soj localization and regulation of DNA replication initiation require the DNA replication initiator protein DnaA. Additionally, we show that Soj regulates sporulation by activating the DNA replication initiation checkpoint protein Sda.

RESULTS

Localization of Mutant Soj Proteins in Living Cells

Previous work describing the localization of wild-type Soj suggested that the protein either (1) localized mostly to cell poles or (2) localized dynamically to a subset of nucleoids within the cell (Marston and Errington, 1999; Quisel et al., 1999). To reconcile the differences between these observations, *soj* was replaced with *gfp-soj* (expressed from its native transcriptional and translational expression system at its endogenous location in the chromosome) and GFP-Soj localization was determined using epifluorescence microscopy. In this strain, GFP-Soj was observed to localize to septa (Figure 1A, arrows) and as relatively faint punctate foci within the cytoplasm (Figure 1A, asterisks) (similar results were obtained with a Soj-GFP fusion; data not shown). The localization pattern of GFP-Soj was dependent on Spo0J, and in a *Δspo0J* mutant GFP-Soj colocalized with the nucleoid as previously shown (Figure 1B) (Marston and Errington, 1999; Quisel et al., 1999).

The differences in the localization patterns reported for Soj could be due to the expression level of the GFP fusion. To test this the *gfp-soj* chimera was placed at an ectopic locus under the control of an inducible expression system (Figure S1A available online). At low expression levels GFP-Soj was observed to localize to septa and as foci within the cytoplasm. However, at higher expression levels the protein formed bright patches that colocalized with a subset of nucleoids similar to patterns previously reported. Thus, the localization of GFP-Soj depends on its expression level and the correct pattern is probably that observed at low expression levels. For the remainder of this work (unless noted) all *soj* alleles and fusions were expressed under native control (western blot analysis showed that all Soj proteins were expressed to approximately the same level as wild-type Soj; Figure S2).

To begin exploring the consequences of conformational changes in Soj, the subcellular distributions of various Soj mutants were compared with that of the wild-type. (N.B., for *B. subtilis:* SojK16A = ATP binding deficient, cooperative DNA