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Recruitment of SMC by ParB-*parS* Organizes the Origin Region and Promotes Efficient Chromosome Segregation

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SUMMARY

Organization and segregation of replicated chromosomes are essential processes during cell division in all organisms. Similar to eukaryotes, bacteria possess centromere-like DNA sequences (*parS*) that cluster at the origin of replication and the structural maintenance of chromosomes (SMC) complexes for faithful chromosome segregation. In *Bacillus subtilis*, *parS* sites are bound by the partitioning protein Spo0J (ParB), and we show here that Spo0J recruits the SMC complex to the origin. We demonstrate that the SMC complex colocalizes with Spo0J at the origin and that insertion of *parS* sites near the replication terminus targets SMC to this position leading to defects in chromosome organization and segregation. Consistent with these findings, the subcellular localization of the SMC complex is disrupted in the absence of Spo0J or the *parS* sites. We propose a model in which recruitment of SMC to the origin by Spo0J-*parS* organizes the origin region and promotes efficient chromosome segregation.

INTRODUCTION

A fundamental unsolved problem in the biology of bacteria is how chromosomes are organized and faithfully segregated during the cell cycle. Insights into these processes have emerged from cytological methods to visualize specific positions on the chromosome and their movement during growth and division. In *Bacillus subtilis*, the newly replicated origins move from mid-cell toward opposite cell poles (Webb et al., 1998). Moreover, the location of a particular region of the chromosome inside the cell correlates with its position in the genome (Nielsen et al., 2006; Niki et al., 2000; Teleman et al., 1998; Viollier et al., 2004; Wu and Errington, 1998). How this organization is achieved and how the factors responsible for its maintenance participate in chromosome segregation are still poorly understood.

Two of the most highly conserved factors implicated in both the organization and segregation of bacterial chromosomes are the structural maintenance of chromosomes (SMC) condensation complex and the chromosomally encoded plasmid partitioning system (Britton et al., 1998; Livny et al., 2007). SMC complexes are present in all eukaryotes and in most bacteria (Hirano, 2006; Nasmyth and Haering, 2005). In eukaryotes, they participate in mitotic chromosome condensation, sister chromatid cohesion, recombination, and X chromosome dosage compensation. In *B. subtilis*, the SMC complex (composed of SMC, ScpA [the kleisin subunit], and ScpB) is required for chromosome compaction and faithful DNA segregation (Britton et al., 1998; Hirano and Hirano, 2004; Mascarenhas et al., 2002; Soppa et al., 2002). In chromatin immunoprecipitation (ChIP) experiments, *B. subtilis* SMC can be crosslinked to all regions of the genome, suggesting that it acts throughout the chromosome (Lindow et al., 2002). However, subcellular localization of SMC indicates that it is also concentrated in discrete foci (Britton et al., 1998; Mascarenhas et al., 2002). The function of these foci remains unclear (Lindow et al., 2002; Volkov et al., 2003). The loss of chromosome condensation in the absence of the SMC complex suggests that bacterial SMC is most similar to eukaryotic condensin (Hirano, 2006; Nasmyth and Haering, 2005). How SMC complexes function to compact bacterial and eukaryotic chromosomes is not known.

The plasmid-encoded *par* locus consists of two genes often called *parA* and *parB* and a centromere-like sequence referred to as *parS*. All three elements are essential for faithful plasmid inheritance (Ebersbach and Gerdes, 2005). ParB binds to its cognate *parS* site and spreads along the DNA forming a nucleoprotein complex. ParA proteins are Walker-box ATPases that act on the ParB-*parS* complex to partition the plasmids toward opposite cell poles. Chromosomally encoded orthologs of ParA, ParB, and *parS* have been identified in >65% of all sequenced bacterial genomes (Livny et al., 2007). In almost all cases, the *parS* site is located in close proximity to the origin of replication. Moreover, most genomes have more than one origin-proximal *parS* (Livny et al., 2007). Work in several model organisms indicates that the chromosomal partitioning system performs a similar function to its plasmid counterpart. However, instead of segregating entire chromosomes, the chromosomal partitioning system participates in repositioning of the replicated origins toward opposite cell poles (Fogel and Waldor, 2006; Lee and Grossman, 2006; Toro et al., 2008; Wu and Errington, 2002, 2003).

In *Bacillus subtilis*, the ParA protein is called Soj and the ParB protein is referred to as Spo0J (Ireton et al., 1994). Ten *parS* sites

have been identified in the *B. subtilis* chromosome. Eight of these sites (with the highest affinity for Spo0J) are located in the origin-proximal 20% of the chromosome (Breier and Grossman, 2007; Lin and Grossman, 1998; Murray et al., 2006). Both Soj and Spo0J are required to maintain an unstable plasmid in which a *parS* site has been inserted (Lin and Grossman, 1998; Yamaichi and Niki, 2000). Moreover, both proteins are necessary for efficient repositioning of chromosomal origins (Lee and Grossman, 2006). Interestingly, a Soj (ParA) mutant has virtually no defect in chromosome segregation as assayed by the production of anucleate cells (Ireton et al., 1994). This result suggests that functionally redundant mechanisms ensure faithful chromosome segregation in the absence of efficient origin repositioning. Consistent with this idea, cells lacking Soj and the chromosome condensation protein SMC have a synthetic chromosome segregation defect (Lee and Grossman, 2006). Paradoxically, unlike Soj mutants, cells lacking Spo0J (ParB) are defective in chromosome segregation. In a Spo0J mutant, 1%–2% of the cells are anucleate, a frequency ~100-fold higher than wild-type (Ireton et al., 1994). It is unclear why Spo0J plays a more central role than Soj in faithful chromosome segregation. One possible explanation is that, in addition to its role in origin segregation, Spo0J has been implicated in chromosome organization.

Using assays to study chromosome organization during sporulation, it was observed that the origin region of the chromosome is disorganized in cells lacking both Soj and Spo0J (Lee et al., 2003; Sharpe and Errington, 1996; Wu and Errington, 2002). Spo0J mutants cannot enter sporulation but are suppressed by a mutation in *soj* (Ireton et al., 1994). Importantly, chromosome organization appears normal in the absence of Soj, suggesting that Spo0J alone is responsible for organizing the origin region. ChIP experiments indicate that Spo0J binds all eight origin-proximal *parS* sites in vivo (Breier and Grossman, 2007; Lin and Grossman, 1998; Murray et al., 2006), and fluorescence microscopy suggests that Spo0J localizes as a single focus per origin (Glaser et al., 1997; Lewis and Errington, 1997; Lin et al., 1997). These results have led to the current view that Spo0J organizes the origin region by gathering the dispersed origin-proximal *parS* sites into a single nucleoprotein complex.

Here, we investigate how Spo0J bound to *parS* organizes the origin region. Using a single-cell-based assay to quantitatively assess chromosome organization and deletions of the origin-proximal *parS* sites, we show that gathering dispersed *parS* sites is not the mechanism by which Spo0J organizes the origin region. These findings led us to the discovery that Spo0J bound to *parS* recruits the SMC condensation complex to the origin. We show that SMC foci are lost in the absence of Spo0J or the eight origin-proximal *parS* sites. Moreover, insertion of *parS* sites near the terminus targets the SMC complex to this ectopic position and causes gross perturbations to chromosome organization and segregation. Finally, we show that purified SMC binds Spo0J-coated DNA with higher affinity than naked DNA or DNA coated with an unrelated DNA-binding protein. All together, our data support a model in which recruitment of the SMC complex to the origin by Spo0J-*parS* organizes the origin region and promotes efficient chromosome segregation. These data link two of the most highly conserved factors in chromosome dynamics and suggest that targeting SMC complexes to the origin by ParB bound to *parS* is likely to be a feature of chromosome organization and segregation in many bacteria. In addition, interesting parallels exist between the recruitment of the *B. subtilis* SMC complex to the origin and the targeting of the SMC dosage compensation complex to the X chromosomes in *C. elegans*. Finally, these data highlight fundamental similarities and important differences in how chromosomes are faithfully segregated in bacteria and eukaryotes.

RESULTS

A Quantitative Single-Cell Assay to Analyze Chromosome Organization

To quantitatively assess the roles of Spo0J and the *parS* sites in organizing the chromosome, we modified an assay originally described by Wu and Errington (Wu and Errington, 1998) to monitor the organization of the replicated chromosomes during sporulation. Sporulating *B. subtilis* cells divide asymmetrically generating a large mother cell and a small forespore. Prior to polar division, the replicated chromosomes adopt an elongated structure that extends from one cell pole to the other (known as the axial filament). The origins reside at the extreme poles and the termini at mid-cell. As a result of axial filament formation, the polar division plane traps approximately one-third of the forespore chromosome in the small spore compartment. The rest of the chromosome is then pumped into the forespore by a DNA translocase called SpoIIIE (Wu and Errington, 1994). The original assay and our modified version take advantage of a mutant in the SpoIIIE translocase (*spoIIIE36*) that engages the forespore chromosome after polar division but is blocked in DNA transport. Using this mutant, the organization of the axial filament at the time of division can be assessed by monitoring which regions of DNA are trapped in the spore compartment by the polar septum. To do this, we fused *cfp* and *yfp* to a promoter (P_{spoIIQ}) that is recognized by a forespore-specific transcription factor. These two reporters were inserted at different positions on the *B. subtilis* chromosome (Figure 1B). Accordingly, depending on their location in the axial filament, the spore compartment contained one, both, or neither of the fluorescent reporters (Figure 1A). The original assay was a population-based assay using a *lacZ* reporter inserted at different chromosomal positions (Wu and Errington, 1998). The assay described here monitors every cell in the field and provides greater sensitivity allowing us to detect and quantify more subtle perturbations in chromosome organization.

Synchronous sporulation was induced and CFP and YFP fluorescence were analyzed 30–45 min after polar division was complete to allow for synthesis and maturation of the fluorescent proteins. Because DNA transport is blocked, the results provide a "snapshot" of the organization of the axial filament at the time of polar division. Assisted by imaging software, we assessed chromosome organization in 400–1000 sporulating cells per field (Figure S1 available online). Only small variations were observed in six independent experiments (Figure S2).

For our experiments, we placed one promoter fusion (*yfp*) at a site (−7°) close to the origin of replication. This chromosomal position is located near the cell pole during sporulation and is trapped in the forespore in 97%–99% of the cells (the sum of the first two classes in Figure 1B). This reporter served as our baseline site