

The SMC Condensin Complex Is Required for Origin Segregation in *Bacillus subtilis*

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Summary

SMC condensin complexes play a central role in organizing and compacting chromosomes in all domains of life [1, 2]. In the bacterium *Bacillus subtilis*, cells lacking SMC are viable only during slow growth and display decondensed chromosomes, suggesting that SMC complexes function throughout the genome [3, 4]. Here, we show that rapid inactivation of SMC or its partner protein ScpB during fast growth leads to a failure to resolve newly replicated origins and a complete block to chromosome segregation. Importantly, the loss of origin segregation is not due to an inability to unlink precatenated sister chromosomes by Topoisomerase IV. In support of the idea that ParB-mediated recruitment of SMC complexes to the origin is important for their segregation, cells with reduced levels of SMC that lack ParB are severely impaired in origin resolution. Finally, we demonstrate that origin segregation is a task shared by the condensin complex and the *parABS* partitioning system. We propose that origin-localized SMC constrains adjacent DNA segments along their lengths, drawing replicated origins in on themselves and away from each other. This SMC-mediated lengthwise condensation, bolstered by the *parABS* system, drives origin segregation.

Results and Discussion

The SMC condensin complex plays a central role in chromosome condensation and segregation in all eukaryotes and virtually all bacteria [1, 2]. In vitro, these highly conserved complexes constrain DNA [5–7], but how they function to organize and segregate chromosomes in vivo remains poorly understood. In the bacterium *Bacillus subtilis*, the role of the SMC complex has been inferred by the phenotypes associated with loss-of-function mutants [3, 4, 8, 9]. Cells lacking SMC or its partner proteins ScpA and ScpB are inviable during fast growth. However, under slow growth conditions, the null mutants have decondensed chromosomes and an increased frequency of anucleate cells. These findings have led to the idea that SMC complexes function throughout the *B. subtilis* chromosome. Interestingly, SMC complexes are enriched adjacent to the origin of replication in *B. subtilis* in a manner that depends, in part, on the partitioning protein ParB (called Spo0J) bound to its centromere-like sequences called *parS* [10, 11]. The functional relevance of this enrichment has remained unclear. Here, we demonstrate a critical role for origin-localized SMC in resolving and segregating origins during fast growth. We further show that during slow growth,

the *parABS* partitioning system provides enough origin segregation activity to support chromosome segregation in the absence of SMC. Thus, ParB bound to *parS* serves as a central hub in origin segregation. This nucleoprotein complex recruits condensin complexes to the origin and is acted upon by ParA, resulting in efficient origin resolution and segregation.

Origin Segregation Is Lost upon Rapid Inactivation of Condensin Subunits

To investigate how SMC mutants impact chromosome organization and segregation in *B. subtilis*, we visualized chromosomes (called nucleoids) and replication origins in cells lacking SMC under permissive growth conditions, in which growth rates were reduced in minimal medium and/or at reduced temperature. As reported previously, a proportion of the cells lacked DNA [3, 4, 12] (Figure S1A available online). However, we also observed a striking heterogeneity in nucleoid morphology and cell size under all conditions tested (Figures 1A and S1A), precluding quantitative analysis of chromosome organization. To circumvent this problem, we isolated temperature-sensitive mutants (Figures 1B and S1B; see also Supplemental Experimental Procedures) and constructed degradable alleles [13] of *smc* and *scpB* to assess chromosome organization and segregation upon rapid inactivation of the condensin complex.

We first monitored the replication origins (using TetR-CFP bound to a *tetO* operator array [14]) upon rapid inactivation of a temperature-sensitive SMC mutant. Under permissive growth conditions (rich medium at 30°C, doubling time $\tau = 56 \pm 2$ min), the morphology of the nucleoid and the subcellular localization of the origins were similar to wild-type (Figures 1C and 1D). At the restrictive temperature (42°C), the cells continued to grow ($\tau = 29 \pm 1$ min) and divide for the duration of the experiment but contained larger and more extended nucleoids (Figures 1E–1G). Strikingly, in most cells, a single bright origin focus or a cluster of foci was present at or near the center of these extended nucleoids. In some cases, a division septum guillotined the DNA mass (Figures 1F and 1G). As a result of nucleoid bisection and DNA degradation [3], anucleate cells accumulated in the population. Similar phenotypes were observed using the ScpB(ts) mutant and in strains in which SMC or ScpB contained an SsrA tag, and the *Escherichia coli* adaptor protein SspB that targets SsrA-tagged proteins to the ClpXP proteasome was expressed [13] (Figures 1HI and S1C–S1E). Consistent with the idea that the elongated nucleoid was composed of multiple chromosomes and that the bright origin focus was made up of several unsegregated origins, the cells contained multiple replisome foci (Figure 1J), and the origin foci at 1.5 hr had, on average, a 4- to 8-fold increase in fluorescence intensity compared to the cells grown at the permissive temperature. These results indicate that during rapid growth the condensin complex plays an essential role in origin individualization and segregation. Reexamination of origin foci in the SMC null mutant (Figure 1A) clearly shows that origin segregation is also impaired under permissive growth conditions.

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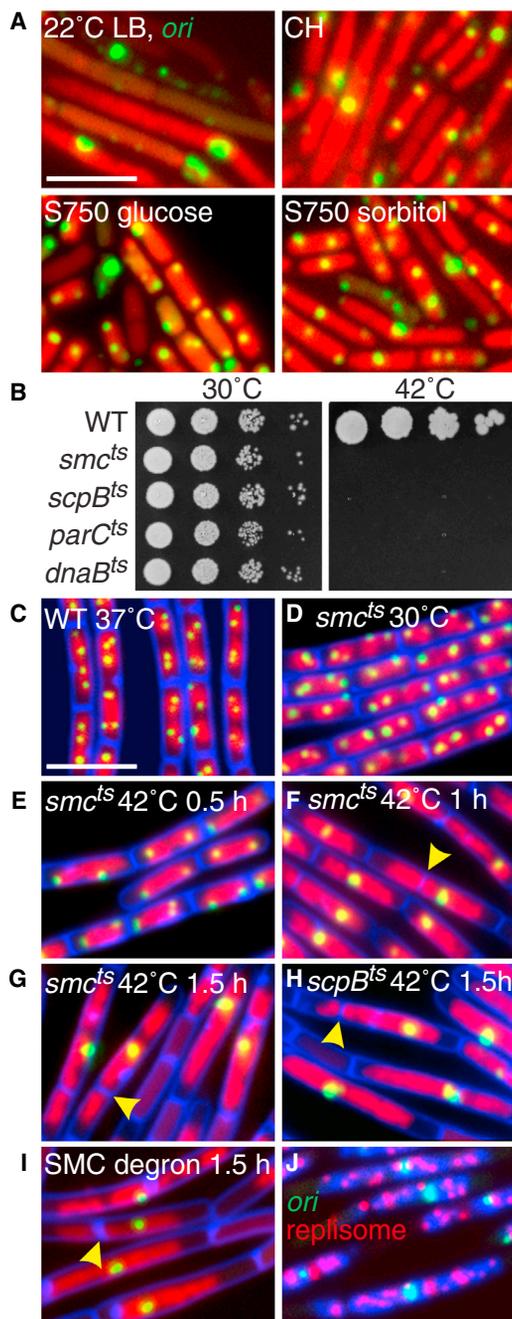


Figure 1. SMC Complexes Are Required for Origin Segregation

(A) Heterogeneous nucleoid morphologies and cell sizes in the SMC null mutant grown under permissive conditions. Representative images of Δsmc (strain BWX2208) grown at 22°C in Luria Broth (LB), casein hydrolysates (CH), and minimal medium (S750) supplemented with glucose or sorbitol. The nucleoids (red) and the origins (green) were visualized with HBsu-GFP and TetR-mCherry bound to a *tetO* array inserted adjacent to the origin.

(B) Spot dilutions of indicated temperature-sensitive mutants grown on LB-agar plates at permissive (30°C) and restrictive (42°C) temperatures.

(C–I) Representative images of DAPI-stained nucleoids (red) and origin foci (green) in wild-type cells (BWX811) grown in CH medium at 37°C (C); *smc*^{ts} (BWX2090) grown in CH medium at 30°C (D) and after shifting to 42°C (E–G); *scpB*^{ts} (BWX2092) grown in CH medium for 1.5 hr at 42°C (H); *smc-ssrA* (BWX1497) 1.5 hr after induction of SspB grown in CH medium at 37°C (I). At 1.5 hr, 85% (n = 1273) of the *smc*^{ts} cells, 81% (n = 1336) of the *scpB*^{ts} cells, and 85% (n = 1392) of *smc-ssrA* cells had a single origin focus or cluster of foci. Membranes (false-colored blue) were stained with FM4-64. Yellow arrowheads highlight septum formation on top of the nucleoid.

Global Chromosome Segregation Is Blocked upon Rapid Inactivation of Condensin

Next, we investigated the organization and segregation of loci on the left and right arms of the chromosome upon inactivation of SMC. We began by monitoring the replication origin and a locus at -87° . After shifting the cells to the nonpermissive temperature, the origins localized in a single bright focus close to the middle of the nucleoid while the -87° marker formed clusters of foci (Figures 2A, S2A, and S2B). In a strain in which we visualized the left and right arms using operator arrays inserted at $\pm 87^\circ$, clusters of -87° foci and separately $+87^\circ$ foci were observed in opposite cell halves (Figures 2A, S2A, and S2B). Finally, a strain containing two arrays on the same chromosome arm (at -87° and -120°) had clusters of foci in the same cell half (Figures 2A, S2A, and S2B). Similar results were obtained using the *ScpB*(ts) mutant (Figure S2C). These data indicate that upon condensin inactivation, DNA synthesis persists, but replicated chromosomes fail to segregate. Furthermore, the presence of clusters of foci at origin-distal positions compared to the single unresolved focus at the origin (Figures 1G–1I) suggests that an unidentified factor helps maintain origin cohesion [12]. Finally, these data indicate that the replicated chromosomes in cells lacking SMC lie on top of each other (or are intermingled) in a left-ori-right configuration, similar to what has been observed in *E. coli* during slow growth [15, 16].

ParB-Mediated Enrichment of Condensin at Replicated Origins Promotes Their Segregation

The failure to segregate replicated origins upon inactivation of SMC suggests that condensin acts at the origin. SMC is recruited to this site by the partitioning protein ParB [10, 11]. In the absence of ParB, origin-localized SMC complexes are significantly reduced, and loci in the origin region are less well organized [10, 11]; however, origin segregation is not significantly impaired [12] (Figure S2D). We reasoned that if ParB-mediated recruitment of SMC was important for origin individualization, then cells in which SMC levels were reduced would be more sensitive to the loss of ParB. To test this, we used the SMC-SsrA fusion under conditions in which the adaptor protein SspB was not induced, resulting in $\sim 2.5\times$ lower levels of SMC-SsrA compared to wild-type (Figure 2C). Importantly, origin resolution and segregation were similar to wild-type in this background (Figures 2B and S2D). Strikingly, deletion of *parB* resulted in a significant proportion of cells with large nucleoids in which the replicated origins failed to resolve (Figures 2B and S2D). Importantly, these phenotypes were principally due to the absence of ParB-mediated recruitment of SMC rather than the loss of the *parABS* partitioning system, because cells lacking ParA that are not impaired in SMC recruitment [11] displayed mild origin segregation defects in the SMC-SsrA background (Figures 2B and S2D). Thus, ParB-mediated recruitment of condensin complexes to the origin promotes origin individualization and efficient chromosome segregation.

(J) Origin (green), replisome foci (red, DnaX-YFP), and DAPI-stained nucleoids (blue) in the *smc-ssrA* strain (BWX1771) 1.5 hr after induction of SspB in CH medium at 37°C.

Scale bars represent 4 μm . Strains harboring wild-type copies of *smc*, *scpB*, or *parC* with a linked antibiotic resistance gene displayed normal chromosome organization and segregation when grown at 42°C (Figure S1F). See also Figure S1.

SMC Promotes Origin Resolution and Segregation

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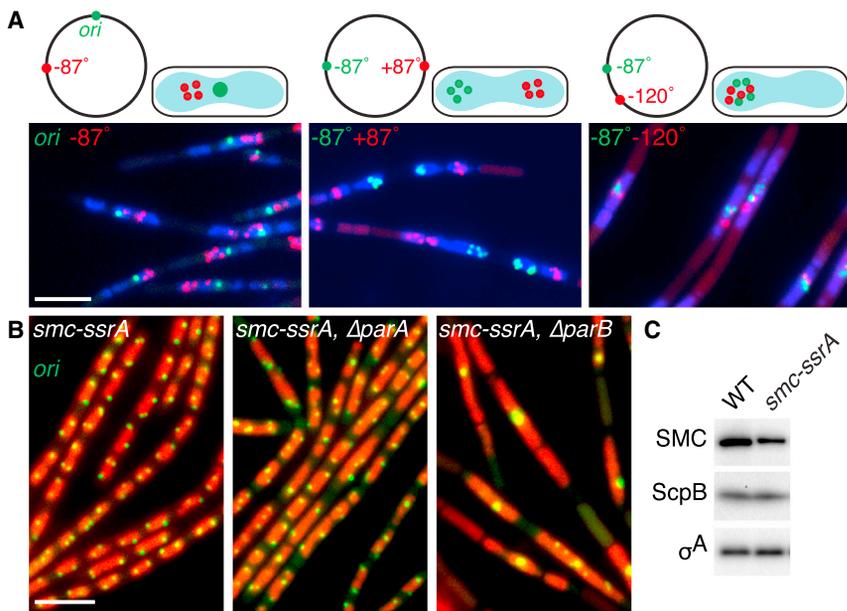


Figure 2. ParB-Mediated Recruitment of SMC Promotes Efficient Chromosome Segregation

(A) Bulk chromosome segregation is blocked upon SMC inactivation. Representative images of DAPI-stained nucleoids (blue) and indicated chromosomal loci (green and red) in the *smc^{ts}* mutant (BWX2378, BWX2116, BWX2110) grown in CH medium for 1.5 hr after shifting to 42°C. Schematic representations of the loci analyzed and their subcellular locations are shown above the micrographs; 72%–76% ($n > 1320$ per strain) had nucleoids and foci similar to those shown in (A).

(B) Origin segregation is impaired in ParB mutants when SMC levels are reduced. Representative micrographs of DAPI-stained nucleoids (false-colored red) and origins (green) in cells (BWX1497, left panel) harboring an *smc-ssrA* fusion that results in a 2.5-fold reduction in SMC levels. The cells in the middle panel (BWX2551) harbor an in-frame deletion of *parA*, and those in the right panel (BWX1569) lack *parB*. A single bright origin focus was found in 2% ($n = 1165$) of *smc-ssrA* cells, 8% ($n = 1038$) of $\Delta parA$, *smc-ssrA* cells, and 34% ($n = 1214$) of $\Delta parB$, *smc-ssrA* cells. An unsegregated

nucleoid was found in 5%, 13%, and 80% of cells, respectively. Representative images of ParA and ParB mutants that have wild-type levels of SMC and quantitative analysis of nucleoid size in all strains can be found in Figure S2D. Scale bars represent 4 μ m.

(C) Immunoblot analysis of SMC, ScpB, and a loading control (σ^A) in wild-type and the *smc-ssrA* mutant under conditions in which the adaptor protein is not induced.

See also Figure S2.

SMC-Mediated Origin Individualization Is Not Dependent on Topoisomerase IV

The failure to resolve the origins upon inactivation of SMC in *B. subtilis* was reminiscent of the segregation defects in temperature-sensitive mutations in *parE* and *parC* (encoding the subunits of Topoisomerase IV [Topo IV]) in *E. coli* [17]. Intriguingly, the *E. coli* SMC analog (called MukB) has been shown to stimulate some of Topo IV's activities [18–20]. Accordingly, we wondered whether the failure to individualize the origins upon SMC inactivation was due to impaired Topo IV activity. To explore this possibility, we generated a temperature-sensitive

mutant of ParC (Figure 1B) and monitored segregation of chromosomal loci at the nonpermissive temperature. As expected, upon inactivation of Topo IV the nucleoid mass increased in size (Figure 3). Consistent with the idea that the replicated arms remain intertwined in the absence of the decatenase, chromosomal loci at $\pm 87^\circ$ formed clusters of foci in opposite cell halves, and chromosomal loci at -87° and -120° formed adjacent clusters (Figure 3A). However (and surprisingly), the origins were able to individualize and segregate in this mutant background (Figure 3B), indicating that condensin does not act through Topo IV to mediate origin resolution.

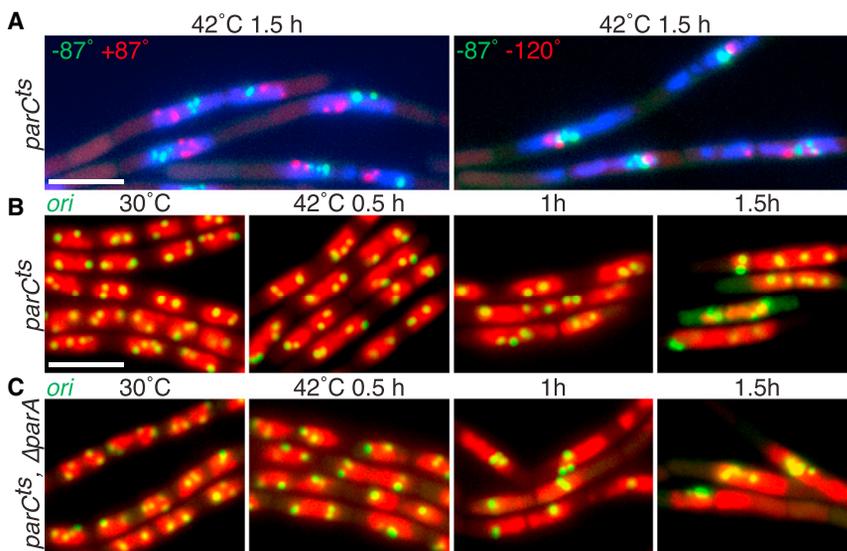


Figure 3. Topoisomerase IV Is Essential for Bulk Chromosome Segregation but Is Not Necessary to Resolve Replicated Origins

(A) Representative images of DAPI-stained nucleoids (blue) and indicated chromosomal loci (green and red) in a *parC^{ts}* mutant (BWX2112 and BWX2106) grown in CH medium for 1.5 hr after shifting to 42°C. Clusters of green and red foci reflect a failure to segregate the replicated chromosomes. Nucleoids and foci similar to those shown in (A) were found in 70%–75% of the cells ($n > 1190$ per strain).

(B) Representative images of nucleoids (red) and origin loci (green) in a *parC^{ts}* mutant (BWX2082) grown at 30°C and after shifting to 42°C.

(C) The same time course as in (B) with a *parC^{ts}* mutant that also lacks ParA (BWX2574). Origin foci are more often clustered in the absence of ParA, although this is not as pronounced as upon SMC inactivation (Figure 1). At 1.5 hr, 70% ($n = 1143$) of *parC^{ts}* and 19% ($n = 1238$) of $\Delta parA$, *parC^{ts}* had nucleoids with three or more well-resolved foci or clusters of foci. Scale bars represent 4 μ m.

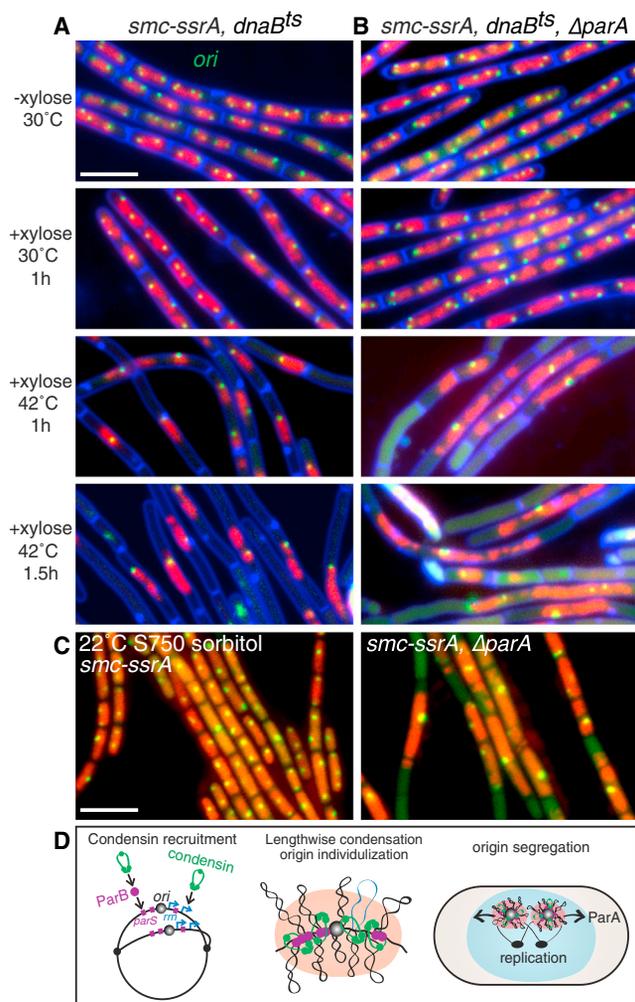


Figure 4. The *parABS* Partitioning System and the SMC Complex Contribute to Origin Segregation

(A and B) Chromosome resolution and segregation occur in the absence of SMC if new rounds of replication are blocked. Representative micrographs of DAPI-stained nucleoids (false-colored red) and origin foci (green) in a strain (BW1527) harboring the *smc-ssrA* degradable allele and a temperature-sensitive replication initiation mutant (*dnaB^{ts}*). After induction of SMC-SsrA degradation for 1 hr, most cells had unsegregated nucleoids with unresolved origin foci. Inhibition of replication leads to resolution and segregation of the chromosomes if the *parABS* system is intact (61%; $n = 1284$) (A), but not if ParA is absent (strain BWX2558) (29%; $n = 1262$) (B). (C) Cells lacking SMC and ParA grown under permissive conditions have severe defects in origin segregation. Cells with intact *parABS* (BW1497) and lacking *parA* (BWX2551) were induced to degraded SMC-SsrA under permissive growth conditions (minimal S750 medium supplemented with sorbitol at 22°C). Representative images of nucleoids (false-colored red) and origin foci (green) are shown. Unsegregated nucleoids were found in 11% ($n = 1595$) of *smc-ssrA* cells and 51% ($n = 1287$) of $\Delta parA$, *smc-ssrA* cells; 2% and 30%, respectively, had a single bright origin focus. Scale bars represent 4 μm .

(D) Schematic model depicting origin segregation in *B. subtilis*. The SMC condensin complex (green) is recruited to the origin by ParB (purple) bound to origin-proximal *parS* sites and is enriched at highly transcribed genes, including ribosomal RNA genes (*rrn*) (blue), that reside adjacent to the origin [10, 11]. Compaction of contiguous DNA segments leads to individualization of the sister origins that are resolved and segregated, in part, through the action of ParA acting on ParB/*parS* complexes.

See also Figure S3.

Inhibition of Replication Suppresses the Chromosome Segregation Defect in SMC-Depleted Cells

During slow growth, SMC null mutants segregate their chromosomes, albeit inefficiently [3, 4] (Figures 1A and S1A). We therefore wondered whether the unsegregated chromosomes that accumulate upon SMC inactivation could be segregated if new rounds of DNA synthesis were halted. To address this, we analyzed chromosome segregation in a strain harboring a degradable allele of SMC and a temperature-sensitive replication-initiation mutant (*dnaB^{ts}*) [21]. Cells were grown at 30°C to allow normal replication and growth. Degradation of SMC was then induced for 1 hr to generate unsegregated nucleoids. Finally, replication initiation was blocked by shifting the cells to 42°C. Strikingly, over the next 1.5 hr the DNA mass resolved into segregated chromosomes containing a single replication origin (Figure 4A). Similar results were obtained using the ScpB degradable allele (Figure S3A). Moreover, replicated and unsegregated loci on the left and right arms also resolved into single foci after replication was inhibited (Figure S3B). These results support the idea that condensin complexes become increasingly important for chromosome segregation as the rate of DNA synthesis increases.

The *parABS* Partitioning System and the SMC Complex Contribute to Origin Segregation

Although our data indicate that the condensin complex plays a central role in promoting origin segregation, previous work suggests that the *parABS* partitioning system also functions in this capacity [11, 12]. In support of this idea, we found that origin segregation in the absence of Topo IV was reduced in a ParA mutant (Figure 3C). To further explore the role of the *parABS* module in origin segregation, we investigated whether it was required for the successful resolution of the unsegregated chromosomes in the cells in which SMC was degraded and DNA synthesis was blocked (Figure 4A). We generated a strain lacking ParA that harbors a degradable allele of SMC and the *dnaB^{ts}* mutant. When we induced SMC degradation and then blocked new rounds of replication by shifting to 42°C, most of the origins and the unsegregated chromosomes failed to resolve as they had in the ParA+ strain (Figure 4B). Thus, a critical role for the *parABS* partitioning system is specifically revealed under conditions in which SMC is absent and replication is blocked.

Grossman and coworkers previously reported synthetic interactions between mutations in *parA* (or *parB*) and the genes encoding components of the condensin complex [3, 12]. The double mutants were found to have a significant increase in the production of anucleate cells. These data were interpreted in the context of the SMC null phenotype and its proposed role in condensation of the chromosome as a whole. Our findings raise the possibility that these synthetic phenotypes are a direct consequence of a failure to segregate replicated origins. To test this, we constructed a strain lacking ParA and SMC. However, the double mutant was so infirmed that it could not stably maintain fluorescent reporters and/or operator arrays. Accordingly, we used a ParA mutant that harbors the *smc-ssrA* degradable allele. SMC was degraded from ParA+ and $\Delta parA$ strains grown under permissive growth conditions (minimal medium at 22°C), and the origins were analyzed by fluorescence microscopy. ParA+ cells resembled the *smc* null mutant, with aberrant and heterogeneous nucleoids and some bright origin foci (Figure 4C). By contrast, most cells lacking ParA had elongated nucleoids with a single bright origin focus (Figure 4C). Furthermore, as reported

previously, many cells also had septa that bisected unsegregated nucleoids, resulting in anucleated cells [3]. These results indicate that the *parABS* system becomes critical for origin segregation in the absence of SMC and highlights the complementary roles played by the condensin complex and the ParAB/*parS* module in segregating replicated origins.

Conclusions

Our results support a model in which origin-localized SMC complexes constrain contiguous DNA segments, drawing the origin region in on itself and away from its sister origin (Figure 4D) [22]. This so-called lengthwise condensation [23, 24] supplemented by the *parABS* partitioning system could overcome factors that maintain origin cohesion [12] and promote efficient origin individualization and segregation. Once the origins have been segregated, further lengthwise compaction mediated by SMC and/or other factors like small nucleoid-associated proteins or negative supercoiling likely drives segregation of the bulk DNA [22]. Finally, we have shown that in the absence of SMC, the *parABS* system, which normally plays a supporting role in segregating origins, becomes critical. Thus, ParB bound to *parS* serves as a central hub in origin segregation. This nucleoprotein complex recruits the SMC condensin complex to the origin and is acted upon by the ParA ATPase [25, 26]. Together, the activities of these two molecular machines ensure efficient resolution and segregation of replicated origins.

Experimental Procedures

General Methods

B. subtilis strains were derived from the prototrophic strain PY79 [27]. *E. coli* SspB was induced with 0.5% xylose or 0.5 mM isopropyl β -D-1-thiogalactopyranoside (IPTG) as specified for degradation of SsrA-tagged proteins [13]. At least two biological replicates were performed for all experiments, and >500 cells per strain were scored for each experiment. The generation and identification of temperature-sensitive mutants are described in Supplemental Experimental Procedures. Strains, plasmids, and oligonucleotides used in this study are listed in Tables S1–S3.

Fluorescence Microscopy

Fluorescence microscopy was performed with a Nikon Ti microscope equipped with Plan Apo 100 \times /1.4NA phase-contrast oil objective and a CoolSnapHQ² charge-coupled device camera (Photometrics). Cells were immobilized on 2% agarose pads containing growth media. Membranes were stained with TMA-DPH (0.01 mM) or FM4-64 (3 μ g/ml). DNA was visualized with DAPI (2 μ g/ml) (Molecular Probes) or an HBsu-GFP fusion (a merodiploid) that had no impact on growth or nucleoid morphology. Images were cropped and adjusted using MetaMorph software (Molecular Devices). Final figure preparation was performed in Adobe Illustrator.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, three figures, and three tables and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2013.11.050>.

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