Structural maintenance of chromosomes (SMC) complexes play critical roles in chromosome dynamics in virtually all organisms, but how they function remains poorly understood. In the bacterium *Bacillus subtilis*, SMC-condensin complexes are topologically loaded at centromeric sites adjacent to the replication origin. Here we provide evidence that these ring-shaped complexes tether the left and right chromosome arms together while traveling from the origin to the terminus (>2 megabases) at rates >50 kilobases per minute. Condensin movement scales linearly with time, providing evidence for an active transport mechanism. These data support a model in which SMC complexes function by processively enlarging DNA loops. Loop formation followed by processive enlargement provides a mechanism by which condensin complexes compact and resolve sister chromatids in mitosis and by which cohesin generates topologically associating domains during interphase.

In the bacterium *Bacillus subtilis*, the SMC-condensin complex is required for the resolution and segregation of newly replicated sister origins (10, 11). Condensin is recruited to the origin by the broadly conserved partitioning protein ParB bound to centromeric parS sites located adjacent to the origin (12–14). Like its eukaryotic counterparts, *B. subtilis* condensin encircles chromosomal DNA in vivo, and topological entrapment is strongly reduced in the absence of ParB, suggesting that most condensin is loaded onto the chromosome at parS sites (15). Recent Hi-C studies in *B. subtilis* revealed that recruitment of condensin to origin-proximal parS sites is required for the alignment of the left and right chromosome arms (1, 4). However, the mechanism by which condensin promotes the juxtaposition of DNA flanking its loading site remains unknown.

To investigate whether condensin-dependent DNA juxtaposition (or “zip-up”) initiates at parS and progresses down the flanking DNA, we used a strain with a single origin-proximal parS site (at −1°, where a degree is ≈11.2 kb of DNA and “−” is counterclockwise from the origin of replication) that harbors an isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible allele of *parB* as the sole source of the loader; enabling us to follow zip-up dynamics. Interarm interactions were monitored by Hi-C at 5-min intervals after ParB induction (Fig. 1A to C). Prior to the addition of IPTG, interactions between the left and right chromosome arms were undetectable (Fig. 1A), as observed previously in the absence of ParB (1, 4). Fifteen minutes after ParB induction, ~500 kb of DNA on either side of parS were juxtaposed, and 5 min later the juxtaposition had progressed ~290 kb further down the chromosome (Fig. 2A and fig. S4A). The Hi-C contact maps indicated that DNA flanking the ectopic parS sites interacted, giving characteristic zip-up patterns (Fig. 2A and fig. S4A). As observed previously (4), the zip-ups were asymmetric, containing more terminal-proximal than origin-proximal DNA (discussed below). Importantly, the ChiP-seq profiles revealed strong SMC enrichment along the DNA flanking the parS sites that correlated with the extent of juxtaposition (Fig. 2B and figs. S4B, S5, and S6A and C). Results of ChiP-seq with antibodies against the other two subunits of the condensin complex (subunits ScpA and ScpB) showed similar enrichment profiles (fig. S4D), whereas ParB enrichment was limited to small chromosomal regions (12 to 23 kb) centered on parS (12) (fig. S4C).

For unknown reasons, regardless of where the ectopic parS site was inserted along the left or right arm, the zip-up did not extend beyond a ~170-kb region surrounding the replication terminus (Fig. 2A and figs. S4A and S7). Examination of SMC enrichment in the ChiP-seq profiles indicated that condensin did not appreciably accumulate in this region (Fig. 2B and figs. S4B and S6A), suggesting that the complexes were actively dissociated. Similarly, SMC did not accumulate where enrichment ended on the origin-proximal side of parS (Fig. 2B and figs. S4B and S6A). The mechanism by which condensin is released from the DNA is currently unknown. A comparison of the Hi-C contact maps from the four strains revealed that the greater the distance between the ectopic parS site and the terminus region, the greater the extent of juxtaposition toward the origin (Fig. 2 and figs. S4, A and B, and S5, A and C). Similarly, the distance between the ectopic parS and the terminus region correlated with the extent of SMC enrichment from the parS toward the origin. These data indicate that condensin on one side of parS is influenced by condensin on the other. The simplest explanation for this “communication” between condensin complexes separated by hundreds of kilobases is that condensin functions as a tether holding the juxtaposed DNA duplexes together. In the context of this model, removal of condensin at the left and right arms. Analysis of all seven Hi-C time points indicated that the two arms zippered up at a nearly constant rate of 52 ± 5 kb/min (figs. S1 and S2). Chromatin immunoprecipitation–sequencing (ChiP-seq) using antibodies against SMC (anti-SMC) before and after ParB induction revealed modest SMC enrichment that correlated with the extent of interarm interaction observed by Hi-C (Fig. 1D and fig. S1). These results and results of previous studies (1, 4, 15, 16) suggest that condensin is loaded at parS and then progressively accumulates along the flanking DNA (Fig. 1E and figs. S3, A to C).

Condensin promotes the juxtaposition of large tracks of DNA flanking parS sites inserted at ectopic chromosomal positions (4). To determine whether SMC was specifically enriched along these juxtaposed regions, we performed Hi-C and ChiP-seq on four *B. subtilis* strains, each with an ectopic parS site at a different position along the left chromosome arm (Fig. 2 and fig. S4). The Hi-C contact maps indicated that DNA flanking the ectopic parS sites interacted, giving characteristic zip-up patterns (Fig. 2A and fig. S4A). As observed previously (4), the zip-ups were asymmetric, containing more terminal-proximal than origin-proximal DNA (discussed below). Importantly, the ChiP-seq profiles revealed strong SMC enrichment along the DNA flanking the parS sites that correlated with the extent of juxtaposition (Fig. 2B and figs. S4B, S5, and S6A and C). Results of ChiP-seq with antibodies against the other two subunits of the condensin complex (subunits ScpA and ScpB) showed similar enrichment profiles (fig. S4D), whereas ParB enrichment was limited to small chromosomal regions (12 to 23 kb) centered on parS (12) (fig. S4C).

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terminus also dissociates condensin at its juxtaposed position on the origin-proximal side of parS (S6D).

How condensin accumulates along the DNA flanking parS is not known. In one scenario, condensin rings topologically loaded at parS travel the length of the chromosome arms. Alternatively, after loading, the condensin tether might not travel far away from parS (17) but instead, by aligning the two arms, generates a template for loading new condensin complexes further down the arms (5) (fig. S8B). To distinguish between these models, we used a B. subtilis strain with a wild-type copy of smc and an IPTG-inducible copy of smc fused to the gene encoding green fluorescent protein (gfp-smc) and monitored where newly synthesized GFP-SMC accumulated along the DNA (Fig. 3). Because SMC enrichment along the chromosome arms was difficult to detect when loaded at origin-proximal parS sites (Fig. 1D and fig. S1), we used a strain harboring an ectopic parS at −94°. We performed ChIP-seq using anti-GFP on an asynchronously growing culture prior to induction and at 5-min intervals after the addition of IPTG. Ten minutes after induction, GFP-SMC was specifically enriched along a −310-kb region surrounding parS (Fig. 3B and fig. S8, A and D). Five minutes later, GFP-SMC occupied a larger expanse of DNA (−650 kb) flanking parS. Enrichment along this region was greater than at the previous time point (Fig. 3B and fig. S8, A to D), which correlated with the increase in GFP-SMC protein levels (Fig. 3A and fig. S8C). However, enrichment was virtually undetectable at sites outside of this 650-kb region. Ten minutes later, the enrichment profile largely overlapped the profile of untagged SMC prior to induction (fig. S8A). The rate of GFP-SMC accumulation from parS toward the terminus was 46 ± 5 kb/min (Fig. 3C), similar to the rate at which the left and right arms became juxtaposed (fig. S2B). Interestingly, the rate of GFP-SMC accumulation from parS toward the origin was slower (26 ± 2 kb/min), consistent with the asymmetric juxtaposition observed by Hi-C (Fig. 2A). Altogether, these results are consistent with a model in which condensin rings are loaded at parS and then travel down the flanking DNA, tethering the duplexes together.

In the context of this model, condensin movement is likely to be influenced by DNA transactions encountered along its path. We investigated the impact of convergent transcription on ring movement by analyzing the zip-up generated by a parS site at +26°. The ~300-kb region between +26° and the origin contains many highly transcribed genes that are co-oriented with replication,
including an 80-kb region between +6° and +15° that contains five ribosomal RNA operons and a ~35-kb operon that encodes abundant ribosomal proteins and translation factors (18) (fig. S9). For comparison, we analyzed the zip-up generated from a parS site at -27°. The region between -27° and the origin has few highly transcribed genes (18) (fig. S9). The zip-up generated by the +26° parS was considerably more asymmetric than the one generated by the -27° parS (Fig. 4A). Specifically, the 80-kb region between +6° and +15°

Fig. 3. Condensin complexes loaded at parS travel down the flanking DNA. Cells (B. subtilis strain BWX3690) harboring a single parS site at -94° with wild-type SMC and an IPTG-inducible gfp-smc fusion gene were analyzed before and after induction. (A) Immunoblot analysis of GFP-SMC levels using anti-SMC during the induction time course. (B) ChIP-seq enrichment performed with anti-GFP. The ratio of ChIP enrichment at the indicated time point relative to time 0 (before induction) is plotted in 1-kb bins. Dotted lines indicate the position of the parS site (black) and the extent of ChIP enrichment of untagged SMC at time 0 (blue). (C) The accumulation of GFP-SMC from the parS toward the origin (red squares) and terminus (black circles) are plotted. The rates of GFP-SMC accumulation were calculated using the first three time points. See figure S8 for unprocessed data and additional analyses.

Fig. 4. Highly transcribed genes influence DNA juxtaposition. (A) Hi-C contact maps of the strain harboring the +26° parS site (BWX3403) in the absence or presence of 25 μg/ml rifampicin (rif) for 10 and 30 min. (B) Representative images of cells from (B). 4',6-diamidino-2-phenylindole (DAPI)-stained DNA (green) and membranes (red) are shown. White bar shows scale, 4 μm. (D) Hi-C contact maps of a strain (BWX3352) harboring a single parS site at -1° and a gfp-parB fusion gene under IPTG control. Cells were induced for 20 min with IPTG and then treated with or without 25 μg/ml rifampicin for 15 min. (E) Schematic model for condensin function. (a) Condensin rings topologically loaded by ParB bound to parS travel down the flanking DNA as handcuffs, (b) resolving newly replicated sister origins by processive loop enlargement. Alternatively, a single ring composed of one or more condensin complexes could encircle the DNA on both sides of parS (not shown). (c) Condensin tethers encounter supercoiled plectonemes, DNA binding proteins, RNA polymerase, and ribosomes translating nascent transcripts. (d) Schematic model of encounters between tethered rings and either a plectoneme (gray) or a transcription complex.
interacted with >500 kb of DNA on the terminus-proximal side of the +26° parS. This asymmetry suggests that movement of condensin rings through the convergently transcribed genes is impaired, whereas movement along the DNA on the terminus-proximal side of parS is not. Condensin complexes were ultimately able to traverse this region and continue juxtaposing origin- and terminus-proximal DNA. Once condensin crossed the origin, its movement along the DNA was co-proximal DNA. Once condensin crossed the origin, its movement along the DNA was co-
imposed via transcription inhibitor rifampicin and monitored the transcriptional output, suggesting that transcription along the parS site with the tran-
scription influences... (Fig. 4B). To investigate whether condensin complexes loaded after transcription inhibition were responsible for the shift to a symmetric zip-up, we used the IPTG-inducible... observed for a parS site adjacent to the origin (52 ± 5 kb/min). The rate of DNA incorporated into the zip-up heading toward the terminus was symmetric.

To more directly test whether transcription influences DNA juxtaposition, we treated the cells containing the +26° parS site with the transcription inhibition rifampicin and monitored the impact by Hi-C (Fig. 4B). As observed previously in the bacterium Caulobacter crescentus (38), short-range interactions along the chromosome arms were rapidly lost upon inhibition of transcription, while the interactions between the DNA flanking the parS site became progressively more symmetric (Fig. 4B). To investigate whether condensin complexes in... DNA sites (Fig. 2B and fig. S6B) are consistent with a model in which two condensin rings each encircle a DNA duplex on either side of parS and tether them together by “handcuffing” (Fig. 4E and fig. S6D).

In B. subtilis, SMC complexes are required to segregate newly replicated origins (10, 11). Our data provide support for the idea that these ring-shaped complexes resolve replicated origins by processive loop enlargement (Fig. 4E). Loop formation draws contiguous DNA in on itself and away from noncontiguous DNA and facilitates the removal of entanglements by the enzyme topoisomerase IV (21). It can also explain how a limited number of condensin complexes in B. subtilis (~30 per replication origin (15)) can organize such large expanses of DNA. Our data suggest that processive loop enlargement underlies the role of condensin in resolving replicated chromosomes in all organisms and support the idea that eukaryotic cohesin complexes act similarly to generate TADs. How B. subtilis condensin rings move down the DNA is unknown. However, condensin’s progression away from the parS site scaled linearly with time (Fig. 3C and figs. S2 and S10B), consistent with an active transport mechanism. Although the prevailing view is that the adenosine triphosphatase (ATPase) cycle of SMC complexes is critical for topologically loading onto DNA, its role in providing energy for DNA translocation remains largely untested (5, 8, 16, 22, 23). Biophysical studies on B. subtilis condensin suggest that the ATPase cycle induces large structural transitions from rod- to ring-like conformations (24) and could represent a mechanochanical cycle that mediates DNA translocation (16). This structural transition could also help explain how condensin is able to thread large nucleoprotein complexes through its annulus. Finally, our data suggesting that each condensin ring encircles a single DNA duplex, and therefore each complex acts on a single helix, provide a simple model in which tethered condensin motors extrude DNA loops.

REFERENCES AND NOTES


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SUPPLEMENTARY MATERIALS

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Materials and Methods

Figs. S1 to S11

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**Bacillus subtilis** SMC complexes juxtapose chromosome arms as they travel from origin to terminus

Xindan Wang, Hugo B. Brandão, Tung B. K. Le, Michael T. Laub and David Z. Rudner (February 2, 2017)


Editor's Summary

**Tethering DNA for packing purposes**
Condensin protein complexes are critical for chromosome segregation and compaction. They form ring-shaped structures that encircle and topologically constrain DNA strands. Wang *et al.* show that *Bacillus subtilis* condensin complexes hold the two arms of the circular chromosome together (see the Perspective by Sherratt). The complexes seem to do this by encircling individual DNA duplexes and then tethering the two duplexes together by "handcuffing." The complexes actively travel along the DNA and function to enlarge DNA loops processively, leading to chromosome compaction.

*Science*, this issue p. 524; see also p. 460

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