REPORT

CHROMOSOMES

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

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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 assemblies 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.

Recent chromosome conformation capture (Hi-C) studies (*1*–*4*) and polymer simulations (*2*, *5*–*7*) have reinvigorated a model proposed over a decade ago (*8*) in which structural maintenance of chromosomes (SMC) complexes generate DNA loops through processive loop enlargement (also referred to as loop extrusion) (*5*, *6*). In this model, these ring-shaped complexes encircle the DNA flanking their loading site, tethering the DNA duplexes together. As these tethers move away from their loading site, they generate loops. Moreover, if SMC rings are continuously loaded at the same site, then the DNA duplexes within the loop segment become juxtaposed (Fig. 1E). De novo loop generation along chromosome arms provides a simple solution to explain how condensin complexes could compact and resolve replicated chromosomes into rod-shaped structures during mitosis and provides a mechanism for the formation of topologically associating domains (TADs) by the SMC-cohesin complex during interphase (*3*, *6*, *9*). Although compelling in its simplicity, this model remains largely untested.

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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 left and right arms. Analysis of all seven Hi-C time points indicated that the two arms zipped-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 fig. 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 terminus-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 S6, A 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 from *parS* 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

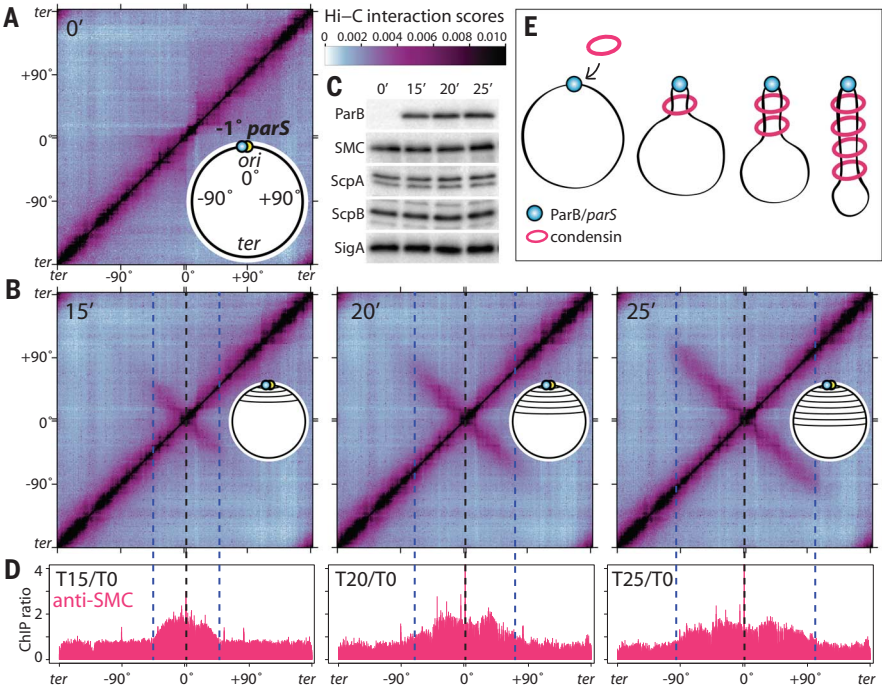

Fig. 1. DNA juxtaposition propagates from condensin's loading site. (**A** and **B**) Normalized Hi-C interaction maps displaying contact frequencies for pairs of 10-kb bins (or genomic regions). The *B. subtilis* strain BWX3352 contains a single *parS* site at –1° and an IPTG-inducible *gfp-parB* fusion gene. Maps show interaction frequencies before (A) and after (B) induction with IPTG. Axes show genome positions in degrees and are oriented with the replication origin at the center. Schematics of the juxtaposed regions are shown. The scale bar depicts Hi-C interaction scores for all contact maps presented in this study. Dotted lines indicate the –1° *parS* site (black) and the leading edge of the juxtaposed DNA (blue). (**C**) Immunoblot analysis of the same samples from (A) and (B) showing GFP-ParB accumulation, amounts of condensin complex components (SMC, ScpA, and ScpB), and SigA, a loading control. (**D**) Anti-SMC ChIP-seq performed under the same conditions as in (A) and (B). Sequencing reads from ChIP and input samples were normalized to the total number of reads. The ratio of ChIP enrichment (ChIP/input) at each time point relative to time 0 is shown in 1-kb bins. (**E**) Schematic interpretation of the Hi-C and ChIP-seq data. Progressive juxtaposition enlarges a DNA loop centered on *parS*.

terminus also dissociates condensin at its juxtaposed position on the origin-proximal side of *parS* (fig. 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,

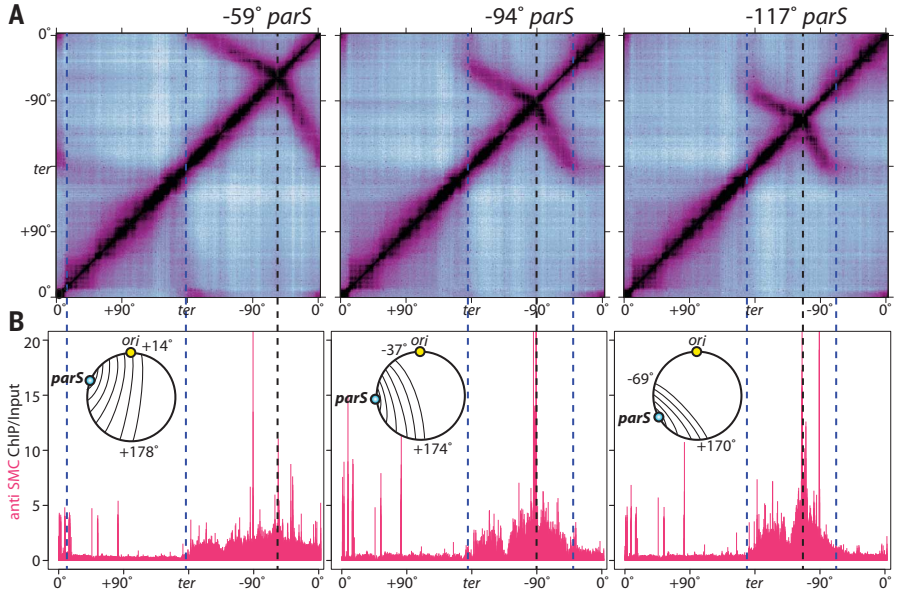

Fig. 2. Condensin is specifically enriched along juxtaposed DNA. (**A**) Hi-C contact maps of *B. subtilis* strains harboring single *parS* sites at –59° (BWX3377), –94° (BWX3270), and –117° (BWX3381). To visualize interactions in the terminus region, the genome was oriented with the terminus (*ter*) at the center of the maps. Dotted lines indicate the position of the *parS* sites (black) and the extent of DNA juxtaposition (blue). (**B**) Anti-SMC ChIP-seq was performed on the same samples as in (A). ChIP enrichment (ChIP/input) was plotted in 1-kb bins. Schematics of the juxtaposed regions are shown. SMC enrichment at the highly transcribed genes outside the juxtaposed regions is probably nonspecific (see fig. S3).

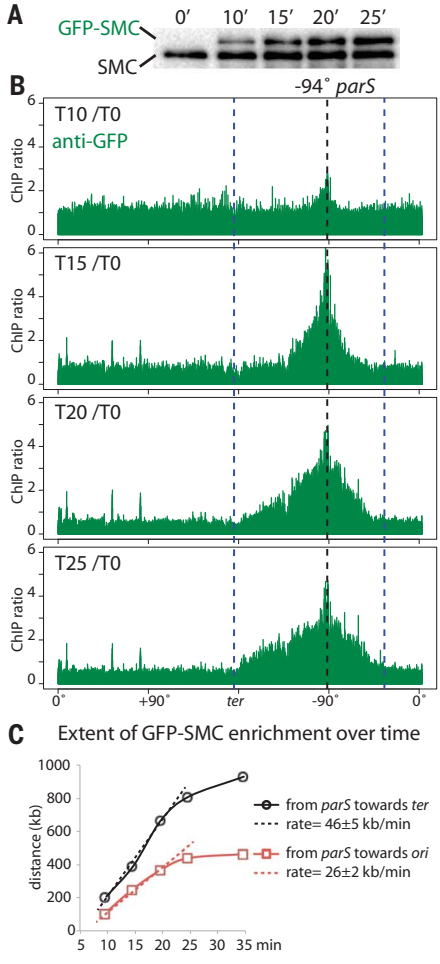

Fig. 3. Condensin complexes loaded at *parS* travel down the flanking DNA. Cells (*B. subtilis* strain BWX3690) harboring a single *parS* site at −94° with wild-type SMC and an IPTG-inducible *gfp-smc* fusion gene were analyzed before and after induction. (**A**) Immunoblot analysis of GFP-SMC levels (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.

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°

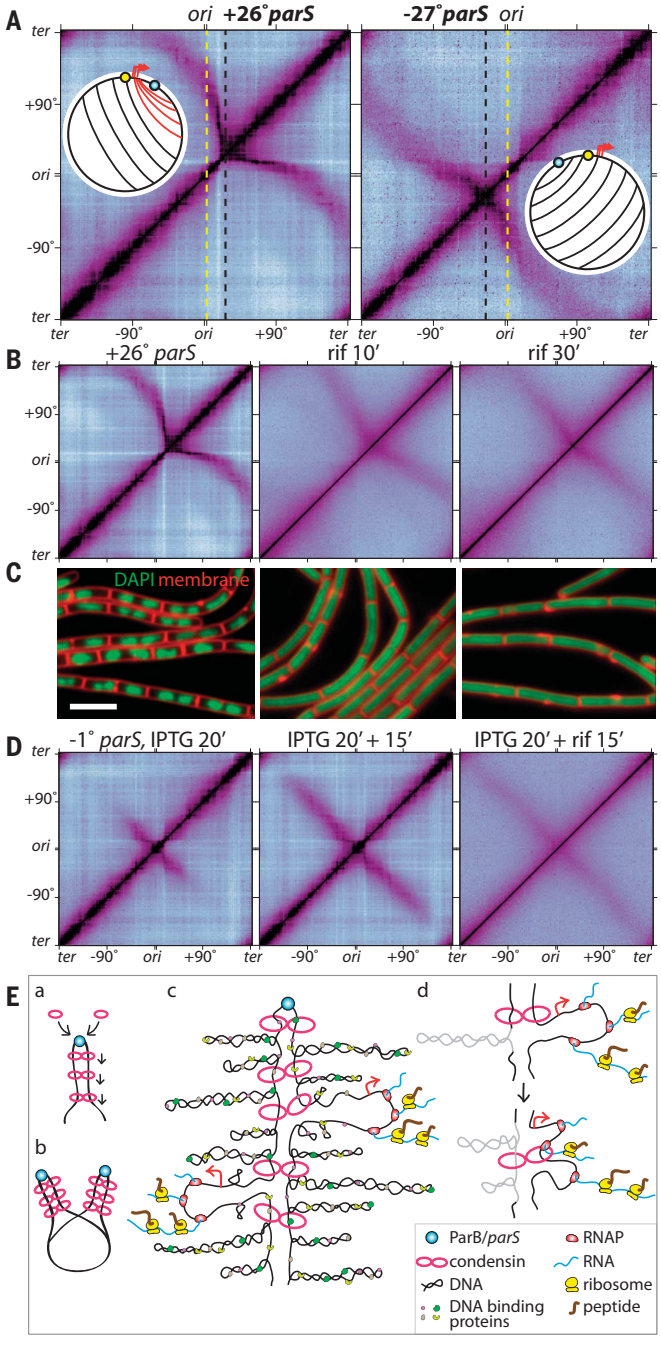

Fig. 4. Highly transcribed genes influence DNA juxtaposition. (**A**) Hi-C contact maps of *B. subtilis* strains harboring a single *parS* site at +26° (strain BWX3403) or −27° (strain BWX3268). The maps are oriented with the origin at the center of the axes. Dotted lines indicate the positions of the origin (yellow) and the *parS* sites (black). Schematics show DNA juxtaposition. Red lines highlight the asymmetric juxtaposition adjacent to the highly transcribed genes (red arrows). (**B**) 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. (**C**) 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-oriented with the transcription of most genes (*18*), and the resulting zip-up was symmetric.

To more directly test whether transcription influences DNA juxtaposition, we treated the cells containing the +26° *parS* site with the transcription inhibitor rifampicin and monitored the impact by Hi-C (Fig. 4B). As observed previously in the bacterium *Caulobacter crescentus* (*19*), 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 loaded after transcription inhibition were responsible for the shift to a symmetric zip-up, we used the IPTG-inducible *parB* strain described in Fig. 1 and added rifampicin 20 min after induction of ParB. DNA juxtaposition continued unabated after transcription was inhibited (Fig. 4D). The rate of zip-up appeared to be slightly faster in the presence of rifampicin than in its absence, suggesting that transcription along the arms also affects the rate of condensin movement from the origin. As reported previously in studies of the bacterium *Escherichia coli* (*20*), inhibition of transcription caused a rapid and pronounced decompaction of *B. subtilis* chromosomes (Fig. 4C). That the DNA flanking the +26° *parS* site remained juxtaposed and the zip-up continued unabated after decompaction provide additional evidence that condensin physically tethers the DNA duplexes. Altogether, these results indicate that transcription influences condensin-mediated DNA juxtaposition and provide additional support for the idea that condensin rings traverse the flanking DNA as they tether the duplexes together.

Finally, to assess the rate of juxtaposition away from the +26° *parS* as a proxy for condensin movement, we followed zip-up progression by Hi-C after induction of *parB*, as described in Fig. 1 (figs. S10 and S11). The rate of DNA incorporated into the zip-up from *parS* heading toward the terminus was 50 ± 5 kb/min (figs. S10B and S11), similar to the rate 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 origin appeared biphasic (figs. S10B and S11). The zip-up from *parS* through the highly transcribed genes was slow (~8 kb/min), but once the juxtaposition traversed this region, the rate (50 ± 4 kb/min) was similar to the zip-up of the terminus-proximal DNA. These data suggest that convergent transcription on one DNA duplex impairs condensin movement along this track of DNA but does not influence the rate of condensin movement on the partner DNA duplex. A similar explanation can account for the differential rates of GFP-SMC movement on either side of the –94° *parS* (Fig. 3C). These results and the asymmetric enrichment of SMC on either side of the ectopic *parS* 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 mechanochemical 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.

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SUPPLEMENTARY MATERIALS

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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.

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