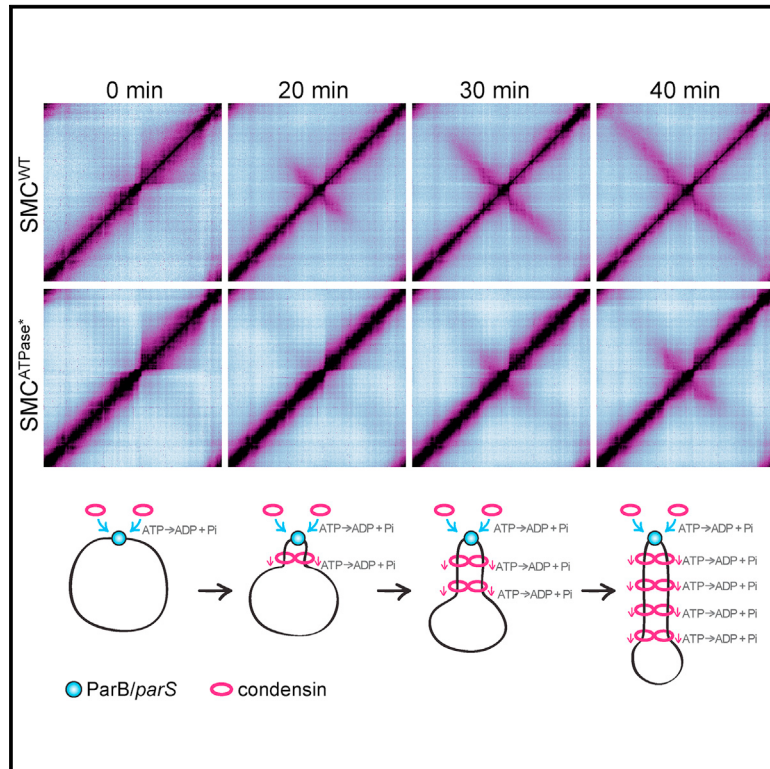


Molecular Cell

***In Vivo* Evidence for ATPase-Dependent DNA Translocation by the *Bacillus subtilis* SMC Condensin Complex**

Graphical Abstract



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In Brief

Using time-resolved chromosome conformation capture and SMC point mutants with reduced ATPase activity, Wang et al. provide evidence that *Bacillus subtilis* SMC complexes utilize cycles of ATP hydrolysis to extrude DNA loops.

Highlights

- SMC point mutants with reduced ATPase activity exhibit delays in DNA juxtaposition
- SMC ATPase mutants translocate down the chromosome arms more slowly than wild-type
- *In vivo* evidence that SMC complexes function as loop extruders

In Vivo Evidence for ATPase-Dependent DNA Translocation by the *Bacillus subtilis* SMC Condensin Complex

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SUMMARY

Structural maintenance of chromosomes (SMC) complexes shape the genomes of virtually all organisms, but how they function remains incompletely understood. Recent studies in bacteria and eukaryotes have led to a unifying model in which these ring-shaped ATPases act along contiguous DNA segments, processively enlarging DNA loops. In support of this model, single-molecule imaging experiments indicate that *Saccharomyces cerevisiae* condensin complexes can extrude DNA loops in an ATP-hydrolysis-dependent manner *in vitro*. Here, using time-resolved high-throughput chromosome conformation capture (Hi-C), we investigate the interplay between ATPase activity of the *Bacillus subtilis* SMC complex and loop formation *in vivo*. We show that point mutants in the SMC nucleotide-binding domain that impair but do not eliminate ATPase activity not only exhibit delays in *de novo* loop formation but also have reduced rates of processive loop enlargement. These data provide *in vivo* evidence that SMC complexes function as loop extruders.

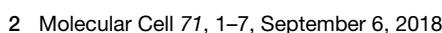
INTRODUCTION

Structural maintenance of chromosomes (SMC) complexes are present in most eubacteria and all eukaryotes, where they play central roles in a wide array of DNA transactions, including the compaction and resolution of sister chromatids in mitosis and the formation of topologically associating domains (TADs) in interphase (Hirano, 2016; Uhlmann, 2016). These broadly conserved complexes are composed of two SMC subunits, a kleisin family member, and accessory factors (Haering and Gruber, 2016). SMC proteins contain a long (40–50 nm) antipar-

allel coiled coil. At one end of the coiled coil, the N- and C-terminal domains form an ATP-binding “head” domain that resembles those found in ATP-binding cassette (ABC) transporters. The hinge domain at the other end interacts with the hinge of a second SMC protein, forming a V-shaped dimer. The kleisin subunit bridges the two nucleotide-binding domains, generating a large tripartite ring (Figure 1A).

Studies in a number of organisms suggest that these ring-shaped assemblies are topologically loaded onto DNA and then redistribute along the chromosome arms (Cuylen and Haering, 2011; Ercan et al., 2009; Gligoris et al., 2014; Hu et al., 2011; Minnen et al., 2016; Schmidt et al., 2009; Wilhelm et al., 2015). ATP-dependent head engagement is critical for association of the SMC complex with its DNA loading site (Hu et al., 2011; Minnen et al., 2016), while hydrolysis is required for topological entrapment and release from this site. It has been suggested that the rings then passively slide along the DNA buffeted by RNA polymerase complexes engaged in transcription elongation (Davidson et al., 2016; Ocampo-Hafalla and Uhlmann, 2011). Alternatively, it has been proposed that cycles of ATP hydrolysis by SMC’s ATPase domains provide the motive force for DNA transport (Alipour and Marko, 2012; Minnen et al., 2016). This translocation activity has been invoked to explain how SMC complexes structure genomes. In this model, tethered rings (or a single ring embracing a small loop) load onto DNA and translocate away from this site processively enlarging a DNA loop (Alipour and Marko, 2012; Dekker and Mirny, 2016; Fudenberg et al., 2016; Goloborodko et al., 2016; Kimura et al., 1999; Nasmyth, 2001; Naumova et al., 2013; Nichols and Corces, 2015; Sanborn et al., 2015). Loop extrusion by SMC complexes loaded along chromosome arms provides a simple solution for how condensin complexes linearly compact and resolve sister chromatids in mitosis and how cohesin complexes, aided by the insulator protein CTCF, generate TADs during interphase.

Recent single-molecule experiments using purified condensin complexes from *Saccharomyces cerevisiae* provide biochemical support for the loop-extrusion model (Eeftens et al., 2017; Ganji et al., 2018; Keenholz et al., 2017; Terakawa et al., 2017). These



to the fact that *B. subtilis* SMC forms a homodimer, and therefore, both subunits in the complex contain these amino acid substitutions. Four of the SMC mutants (including Q143A) had intermediate phenotypes. These grew slower than wild-type in the presence of ParB at 37°C and failed to grow in its absence (Figures S1C and S1E). The remaining 11 mutants formed colonies in the presence of ParB that were indistinguishable from wild-type. In the absence of ParB, four of these (K12R, D42A, R57A, and R57K) were unable to form colonies, one (F66Y) had a small-colony phenotype, and five (including D42E) resembled wild-type (Figures 1B, S1C, and S1E). When grown on defined rich (casein hydrolysate [CH]) medium at 37°C, SMC(K12R), SMC(R57A), and SMC(F66Y) mutants lacking ParB formed smaller colonies than the matched wild-type control (Figure 1B).

We purified 9 mutants that encompassed the full range of phenotypes described above, including a canonical Walker A motif mutant (K37I) that does not bind ATP (Hirano et al., 2001), and wild-type *B. subtilis* SMC from *E. coli* as SMC-His6 fusions (Figure S2). As anticipated, the mutants that phenocopied the *smc* null (K37I in the Walker A motif, G1092S in the signature motif, D1124E in the D-loop, and N33A) (Figures S1C and S1D) had virtually undetectable ATPase activity *in vitro* (Figures S2B and S2C). One of the mutants that grew slowly in the presence of ParB (Q143A) (Figures S1C and S1E) had <1% of the ATPase activity of wild-type. Finally, three of the hypomorphic mutants (K12R, R57A, and F66Y) with relatively mild growth defects in the absence of ParB had reduced ATPase activity ranging from 6%–20% of wild-type (Figures 1C, 1D, and S2). A previous study found a similar reduction in ATPase activity for the R57A mutant (Hirano and Hirano, 2006). Similar reductions were observed in the presence of single- and double-stranded DNA (Figure S2E) (Hirano and Hirano, 2006).

To investigate whether a decrease in condensin's ATPase activity impacts DNA translocation and loop formation, we analyzed the rate of DNA juxtaposition by time-resolved Hi-C. In this assay, strains harbor an isopropyl β-D-1-thiogalactopyranoside (IPTG)-inducible allele of the condensin loader ParB and a single origin-proximal *parS* site. Upon addition of IPTG, the extent of juxtaposition of the left and right arm is monitored in a Hi-C time course. Importantly, our previous ChIP-seq analysis indicates that all three condensin subunits are specifically enriched along the juxtaposed region. Furthermore, time-resolved ChIP-seq experiments using an ectopic *parS* site inserted at –94° indicate that SMC travels along the chromosome arms at rates comparable to DNA juxtaposition (Wang et al., 2017). Here, we use rates of juxtaposition as a proxy for condensin movement, because when condensin is loaded at the origin, ChIP-seq does not provide sufficient resolution of SMC enrichment for rate determination (Wang et al., 2017). Wild-type SMC and the three mild hypomorphic mutant strains were grown in defined rich (CH) medium in the absence of inducer at 22°C to ensure similar growth rates (Figure 1B). Cultures were then treated with IPTG and shifted to 37°C, and samples were collected for Hi-C at 5- or 10-min intervals. Importantly, upon addition of IPTG, all four strains grew at indistinguishable rates ($\tau = 34$ min) and had similar levels of SMC, ScpA, ScpB, and ParB (Figure 2D). As observed previously (Wang et al., 2017),

in cells with wild-type SMC, 20 min after ParB induction, ~685 kb of DNA on either side of the origin-proximal *parS* site was juxtaposed (Figure 2A). 5 min later, ~930 kb was aligned. Analysis of the complete Hi-C time course revealed that the left and right chromosome arms zipped up at a rate of 50 ± 2 kb/min (Figures 2C and S3).

Consistent with the idea ATP hydrolysis is required for topological loading of the condensin ring (Wilhelm et al., 2015), all three SMC mutants displayed a lag prior to the appearance of juxtaposed DNA flanking the origin (Figures 2B, 2C, and S3). The mild SMC(F66Y) mutant had a 4-min delay compared to wild-type, while the stronger mutants (K12R and R57A) were delayed by ~10 min. Importantly, after the lag, the left and right arms processively zipped up in all three mutants. Analysis of the rates of DNA juxtaposition revealed that the mild F66Y mutant zipped up the arms at 41 ± 2 kb/min, while K12R and R57A had rates of 30 ± 3 and 33 ± 1 kb/min, respectively. These rates of DNA juxtaposition are 18%–40% slower than wild-type and were reproducible in an independent Hi-C time course (Figure S4). The reduced rates of condensin-mediated juxtaposition did not directly correlate with the decrease in ATPase activity of the SMC mutants, and we suspect that this is due to the limitations of the *in vitro* assays available for the *B. subtilis* SMC complexes (Hirano and Hirano, 2004). However, we note that the lag times in the initiation of juxtaposition, which likely reflect the requirement for ATP hydrolysis for topological loading *in vivo*, correlated with the slower rates of movement down the arms.

Importantly, the SMC mutant (Q143A) that had a more severe growth defect (Figures S1C and S1E) and <1% of the ATPase activity of wild-type (Figures S2B and S2C) failed to juxtapose the chromosome arms even after 40 min of ParB induction (Figure S5C). By contrast, one of the mutants (D42E) with no discernable growth phenotype (Figures S1C and S1E) and 82% of the ATPase activity of wild-type (Figures S2B and S2C) zipped up the two arms in a manner similar to wild-type (Figure S5C). Finally, a strain in which SMC itself was under IPTG-inducible control juxtaposed the left and right arms without a lag and at rates similar to wild-type upon addition of inducer (Figures S5A and S5B). That the juxtaposition was similar to wild-type in this strain indicates that any DNA entanglements that might have arisen during growth prior to SMC induction in this strain and prior to ParB induction in SMC mutants studied here (Gruber et al., 2014; Wang et al., 2014) did not impair condensin movement after their production.

DISCUSSION

These data extend the analysis of mammalian cohesin in lymphoid cells (Vian et al., 2018) and complement the single-molecule studies of the *S. cerevisiae* condensin complex (Eeftens et al., 2017; Ganji et al., 2018; Keenholtz et al., 2017; Terakawa et al., 2017), providing *in vivo* support for the model that SMC complexes translocate along chromosomes powered by ATP hydrolysis and could therefore structure genomes by loop extrusion. Intriguingly, the *in vitro* rate of DNA extrusion determined for *S. cerevisiae* condensin (up to 1.5 kb/s) (Ganji et al., 2018) is similar to the rate of loop formation we observe *in vivo* (1.67 kb/s, 0.83 kb/s per chromosome arm). Furthermore,

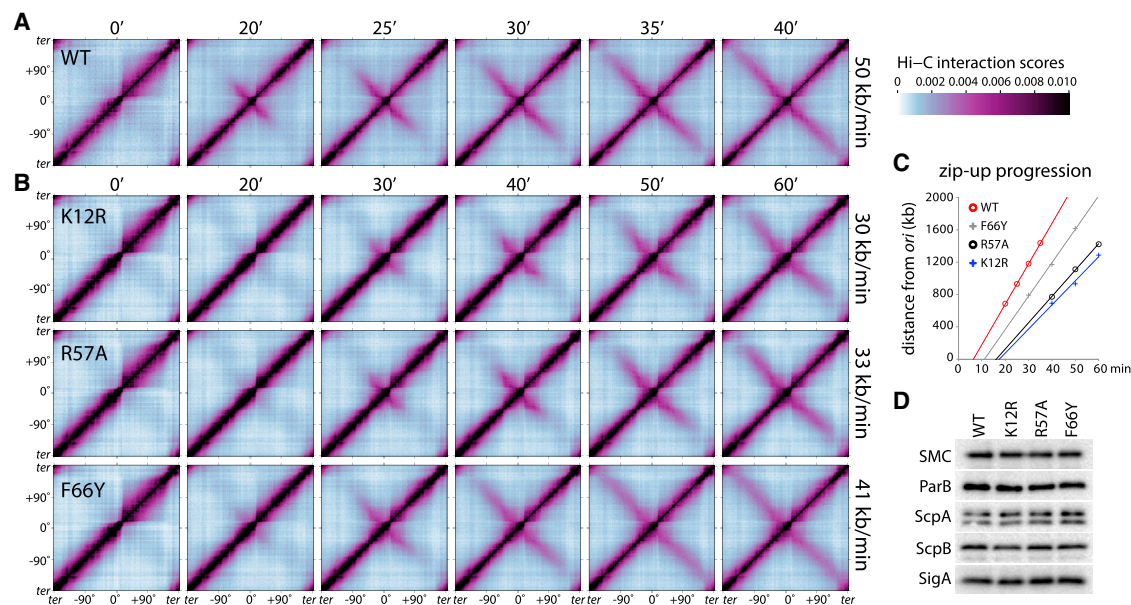


Figure 2. SMC ATPase Mutants Have Reduced Rates of DNA Juxtaposition

(A) Normalized Hi-C interaction maps displaying contact frequencies for pairs of 10-kb bins. The strain (BW4077) contains wild-type *smc*, a single *parS* site at -1° , and an IPTG-inducible *parB* allele. Maps show interaction frequencies before and after addition of IPTG. Time after induction is indicated above the maps. Axes present genome positions in degrees and are oriented with the replication origin at the center. The rate of DNA juxtaposition is indicated on the right. See Figure S3 for analysis. The scale bar depicts Hi-C interaction scores for all contact maps presented in this study.

(B) Hi-C time course of strains containing the *smc* point mutations K12R (BW4149), R57A (BW4078), and F66Y (BW4152).

(C) Extent of juxtaposition over time averaged for the left and right arms in the indicated strains. Extrapolation to the abscissa shows the relative lag before juxtaposition begins. See Figure S3 for details.

(D) Immunoblot analysis of the same strains presented in (A) and (B) in the presence of IPTG. The levels of SMC variants ScpA, ScpB, and ParB are similar in all four strains. For the ScpA blot, the bottom band is ScpA and the top band is nonspecific. SigA controls for loading.

See also Figures S3–S5 and Table S1.

the ensemble rates of ATP hydrolysis for *B. subtilis* SMC and the *S. cerevisiae* condensin complex are within a factor of two of each other. Thus, these rates may represent a biological optimum for loop extrusion efficiency.

While we attribute the reduced translocation rates of the SMC hypomorphic mutants to their decreased ATPase activity, other possibilities could account for our findings. For example, it is possible that the mutations affected some other aspect of the complex that hindered its movement along the chromosome arms. We consider this unlikely, because we obtained similar results with three distinct mutations in the nucleotide-binding domain. It is also possible that the mutants affected the on or off rates of the complex on DNA. While formally possible, we note that previous studies (Tran et al., 2017; Wang et al., 2017) indicate that condensin-dependent DNA juxtaposition initiates at *parS*, suggesting that productive loading at other positions along the chromosome is negligible. An alternative model is that the number of SMC complexes loaded onto the DNA influences the rate of DNA translocation (Terakawa et al., 2017), and mutations that reduce the rate of loading and therefore the number of SMC complexes indirectly affect the rate of DNA translocation. This model predicts that as more and more condensin complexes are loaded onto the chromosome, the rate of DNA transport will increase. However, we observed that the rates of DNA juxtaposition were constant as a function of time

in all strains tested despite continuous loading of condensins at the *parS* site and their increasing numbers on the DNA (Wang et al., 2017). These considerations lead us to conclude that our data are most consistent with a model in which cycles of ATP hydrolysis by the SMC head domains are required for DNA transport (Badrinarayanan et al., 2012).

Structural and biophysical studies on the *B. subtilis* condensin indicate that the complex undergoes a large structural transition from a ring- to rod-like conformation during its ATPase cycle (Soh et al., 2015). More recent studies have led to the proposal that DNA translocation is mediated by stepwise loop formation involving a peristalsis-like mechanism in which two regions of DNA along the duplex separately interact with the head and hinge of the (ATP-bound) ring-shaped complex (Burmam et al., 2017; Diebold-Durand et al., 2017). The ring-to-rod transition triggered by ATP hydrolysis and nucleotide release drives the DNA bound at the hinge toward the head. The two regions of DNA that constitute the base of a loop are then captured in a meta-chamber created by the kleisin subunit and the SMC head domains (Kschonsak et al., 2017). The re-binding of ATP leads to ring opening and capture of a new chromosomal loop, likely facilitated by conformational fluctuations of the DNA (Lawrimore et al., 2017; Marko et al., 2018). ATP hydrolysis drives this second captured loop into the meta-chamber, where it merges with one held in this compartment (Marko et al., 2018). In this

way, cycles of ATP hydrolysis expand the loop in a stepwise manner. Consistent with this model, the *in vitro* single-molecule studies on *S. cerevisiae* condensin have shown that the complex can extrude a loop by anchoring DNA and reeling the duplex in from one side (Ganji et al., 2018). Studies in *B. subtilis* and *C. crescentus* (Tran et al., 2017; Wang et al., 2017) indicate that bacterial condensin complexes loaded at the origin generate a symmetric loop as they translocate down the chromosome arms. These data raise the possibility that paired SMC complexes might be the functional unit for loop extrusion (Diebold-Durand et al., 2017). Future studies *in vitro* and *in vivo* will be required to establish how these novel DNA translocases travel along the chromosome while accommodating all of the DNA transactions encountered along their paths.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information includes five figures and three tables and can be found with this article online at <https://doi.org/10.1016/j.molcel.2018.07.006>.

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AUTHOR CONTRIBUTIONS

X.W. and D.Z.R. designed the study. X.W. constructed strains and performed Hi-C and immunoblot experiments. H.B.B. analyzed Hi-C data. A.C.H. and X.W. purified proteins. A.C.H., B.W., and J.C.C. performed ATPase assays. A.C.H., C.L., and M.G.O. carried out FPLC. A.C.K. performed homology

modeling and guided SMC mutagenesis. X.W. and D.Z.R. wrote the manuscript. All authors read the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-SMC polyclonal rabbit antibody	(Lindow et al., 2002)	N/A
Anti-ParB polyclonal rabbit antibody	(Lin et al., 1997)	N/A
Anti-ScpA polyclonal rabbit antibody, affinity purified	(Wang et al., 2017)	N/A
Anti-ScpB polyclonal rabbit antibody, affinity purified	(Wang et al., 2017)	N/A
Anti-SigA polyclonal rabbit antibody	(Fujita, 2000)	N/A
Chemicals, Peptides, and Recombinant Proteins		
Benzonase	EMD	Cat # 7046
Ni-NTA agarose	QIAGEN	Cat # 30230
HiTrap Q HP	GE Healthcare	Cat # 17115301
Bradford Assay Kit	ThermoFisher	Cat # 23236
ATP	Dot Scientific	Cat # DSA30030-5
NADH	Acros Organic	Cat # 271100010
Phosphoenolpyruvate	Tokyo Chemical Industry	Cat # P0256
Rabbit Pyruvate Kinase	Roche Diagnostics	Cat # 10109045001
Lactate Dehydrogenase	Sigma, Darmstadt, Germany	Cat # L2500-25KU
Formaldehyde 37%	Sigma	Cat # F8775
Ready-Lyse Lysozyme	Epicentre	Cat # R1802M
HindIII	NEB	Cat # R0104M
Klenow	NEB	Cat # M0210L
Biotin-14-dATP	ThermoFisher	Cat # 19524016
T4 DNA ligase	NEB	Cat # M0202M
Proteinase K	NEB	Cat # P8107S
T4 DNA Polymerase	NEB	Cat # M0203L
Critical Commercial Assays		
ATPase/GTPase Activity Assay Kit	Sigma	Cat # MAK113
NEBNext Ultra II DNA Library Prep Kit	NEB	Cat # E7645S
Deposited Data		
Raw and analyzed Hi-C data	This paper	GEO: GSE95137
Original Data	Mendeley Data	https://doi.org/10.17632/tygwp234gr.1
Experimental Models: Organisms/Strains		
<i>Bacillus subtilis</i> strains, see Table S1		
Oligonucleotides		
See Table S3		
Recombinant DNA		
See Table S2		
Software and Algorithms		
MATLAB 8.5 (R2015)	(Wang et al., 2017)	https://www.mathworks.com/
hiclib	(Imakaev et al., 2012)	https://bitbucket.org/mirnylab/hiclib

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for reagents may be directed to, and will be fulfilled by the Lead Contact, David Rudner (rudner@hms.harvard.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Bacillus subtilis Strains and Growth

Bacillus subtilis strains were derived from the prototrophic strain PY79 (Youngman et al., 1983). Strains, plasmids and oligonucleotides are listed in Tables S1–S3. For the Hi-C time courses, cells were grown overnight (for 12–14 h) in defined rich (Casein Hydrolysate, CH) medium (Harwood and Cutting, 1990) in the absence of IPTG at 22°C. The following morning, logarithmically growing cultures were diluted into fresh CH medium to an OD₆₀₀ of 0.075 and incubated with aeration at 22°C for ~2 h until an OD₆₀₀ reached 0.15. IPTG was added to a final concentration of 1 mM and the flasks were placed at 37°C. Samples were taken before or after induction at specified time points.

METHOD DETAILS

Homology Modeling

A structure of *B. subtilis* SMC has been reported (PDB ID 3ZGX) (Bürmann et al., 2013), but this structure lacks a bound nucleotide and is poorly ordered in the ATPase domain, offering limited guidance for the design of mutants. However, high resolution structural data are available for *Pyrococcus furiosus* SMC in complex with ATP (PDB ID 1XEX) (Lammens et al., 2004). To enable rational design of mutants in the *B. subtilis* protein, we employed a multiple template homology modeling method using the two structures. Briefly, the two template structures were aligned to each other by secondary structure matching in Coot (Emsley and Cowtan, 2004). A sequence alignment was constructed on the basis of the aligned structures and was used for multi-template modeling in MODELER (Webb and Sali, 2016). Residues 199–1195 are located far from the ATPase domain, and were omitted from the modeling. A bound ATP and Mg²⁺ ion were included during modeling as rigid bodies, positioned based on the *P. furiosus* SMC structure. A total of 20 models were created and the top scoring model was used as the basis for the design of mutagenesis experiments and the preparation of Figures 1A and S1B.

Protein Purification

SMC-His₆ wild-type and mutants were expressed in *E. coli* BL21 DE3 pLysS. Cells were grown in LB at 37°C to an OD₆₀₀ of 0.4 and induced with 0.5 mM IPTG and harvested after 2 hours. All subsequent manipulations were carried out at 4°C. 250 mL of cells were harvested by centrifugation and resuspended in 20 mL lysis buffer (50 mM HEPES Na⁺ pH 7.6, 75 mM NaCl). A crude extract was prepared by freeze-thawing the cells followed by the addition of 5 mM β-Mercaptoethanol, 1 mM PMSF and 1 mM MgCl₂, 28 U/ml Benzonase (EMD 7046) and Lysozyme (0.5 mg/ml), and then sonication. The lysates were clarified at 100,000 g for 1 hour. To the soluble fraction, NaCl and Imidazole were added to achieve a final concentration of 300 mM and 10 mM respectively. The soluble fraction was then loaded onto a 3 mL Ni²⁺-NTA agarose (QIAGEN) column equilibrated with Buffer I (50 mM HEPES Na⁺ pH 7.6, 300 mM NaCl, 5 mM β-Mercaptoethanol, 10 mM Imidazole). Bound proteins were washed with Buffer II (20 mM HEPES Na⁺ pH 7.6, 300 mM NaCl, 5 mM β-Mercaptoethanol, 50 mM Imidazole, 10% glycerol) followed by elution in Buffer II containing 500 mM Imidazole. Peak fractions were pooled and dialyzed with three changes into Buffer A (20 mM HEPES K⁺ pH 7.6, 50 mM KCl, 1 mM EDTA, 10% glycerol, 5 mM β-Mercaptoethanol). The dialysate was loaded onto 2 tandem 1 mL HiTrap Q column on a FPLC (AKTA, GE Healthcare Life Sciences), washed with Buffer A and eluted with a linear KCl gradient. Peak fractions were pooled, dialyzed into Buffer A, aliquoted and flash frozen in liquid nitrogen. Protein concentrations were determined using the Bradford reagent from Thermo Scientific.

ATPase Activity Assays

Steady-state ATPase activity was monitored using the NADH-coupled assay (De La Cruz et al., 2000; Hass et al., 1961; Imamura et al., 1966; Trentham et al., 1972). Purified SMC wild-type and mutant proteins were diluted to 400 nM in reaction buffer (20 mM HEPES K⁺ pH 7.6, 50 mM KCl, 1 mM EDTA, 10% glycerol, 5 mM β-Mercaptoethanol). In a separate tube, a 2x NADH cocktail was prepared, which contained reaction buffer, 2 mM ATP, 4 mM MgCl₂, 0.8 mM NADH (Acros Organic), 1 mM phosphoenolpyruvate (Tokyo Chemical Industry), 10 U/mL rabbit pyruvate kinase (Roche Diagnostics), 16 U/mL lactate dehydrogenase (Sigma, Darmstadt, Germany). 40 μL of the diluted proteins were mixed with 40 μL of 2x NADH cocktail. After mixing, 30 μL of each reaction was transferred into a 384-well plate in duplicate. The plate was pulse centrifuged at 1000 g and placed in a microplate spectrophotometer (BioTek, Winooski, VT) where NADH oxidation was monitored at 340 nm over time. The amount of NADH oxidized (equivalent to the amount of ADP produced) was calculated via a NADH standard curve. ADP product formation was plotted over time and fitted to a line. The obtained rates were divided by the final SMC concentration. The experiment was performed four times using aliquots of proteins from the same protein preparation.

For comparison, a malachite green assay was used for a subset of the mutants. SMC (WT), SMC (K37I), SMC (K12R), SMC (R57A) and SMC (F66Y), were separately purified and subjected to a malachite green assay using Sigma-Aldrich ATPase/GTPase Activity Assay Kit (MAK113), in a buffer containing 20 mM HEPES K⁺ pH 7.6, 50 mM KCl, 2 mM MgCl₂ and 1 mM ATP. The assays were performed at 37°C for 30 min in triplicate. To ensure the reactions were in the linear range of the assay, 50 nM wild-type SMC, 200 nM SMC (R57A) and SMC (F66Y), and 400 nM SMC (K12R) and SMC (K37I) were used. The ATPase rates were divided by the protein concentration. For the 5 mutants tested, the ATPase rates measured using NADH-coupled assay were ~25% lower than using the malachite green assay (Figures S2C and S2D). However and importantly, the relative rates of the mutants compared to the wild-type were very similar between the two assays.

Hi-C

The detailed Hi-C procedure was previously described in Wang et al., 2015 (Wang et al., 2015). Briefly, cells were crosslinked with 3% formaldehyde at room temperature for 30 min, quenched with 125 mM glycine for 5 min. 5×10^7 cells were used for each Hi-C reaction. Cells were lysed using Ready-Lyse Lysozyme (Epicentre, R1802M) followed by 0.5% SDS treatment. Solubilized chromatin was digested with HindIII in the presence of 1% Triton for 2 h at 37°C. The cleaved ends were filled in with Klenow and Biotin-14-dATP, dGTP, dCTP, dTTP. The products were then ligated with T4 DNA ligase overnight at 16°C. Crosslinking was reversed at 65°C overnight in the presence proteinase K. The DNA was then extracted twice with phenol/chloroform/isoamylalcohol (25:24:1) (PCI), precipitated with ethanol, and resuspended in 20 μ L of QIAGEN EB buffer. Biotin from non-ligated ends was removed using T4 polymerase (4 h at 20°C) followed by extraction with PCI. The DNA was then sheared by sonication in 10 s on - 10 s off cycles for 12 min with 60% amplitude using a Qsonica Q800 water bath sonicator. The sheared DNA was used for library preparation with the NEBNext Ultra II DNA Library Prep kit (E7645S) according to the manufacturer's instructions for end repair, adaptor ligation, and size selection. Biotinylated DNA fragments were purified using 10 μ L streptavidin beads. 5 μ L DNA-bound beads were used for PCR in a 50 μ L reaction for 14 cycles. PCR products were purified using Ampure beads and sequenced using Illumina NextSeq 500.

Generation of Hi-C contact maps

Paired-end sequencing reads were mapped to the genome of *B. subtilis* PY79 (NCBI Reference Sequence NC_022898.1) using the same pipeline described previously (Wang et al., 2015, 2017). The *B. subtilis* PY79 genome was first divided into 404 10-kb bins. The interaction matrices were generated, and normalized using an iterative normalization procedure, implemented using the hiclib library for Python (<https://bitbucket.org/mirnylab/hiclib>) (Imakaev et al., 2012; Le et al., 2013; Wang et al., 2015). Subsequent analysis and visualization were done using R scripts or MATLAB 8.5 (R2015) (MathWorks, Natick, MA). For presentation, the circular genome was linearized at the replication terminus to generate origin-centered contact maps.

Mapping endpoints of DNA juxtaposition on Hi-C maps

The endpoints of DNA juxtaposition on Hi-C maps (Figures 2A and 2B) were determined as in Wang et al., 2017 (Wang et al., 2017). Essentially, a three-step process was used. First, the mean and standard deviation (σ) of contact probabilities for each Hi-C map were computed. To minimize biases to the estimates of the mean and standard deviation (σ) from contributions arising from chromosome features like condensin-dependent interactions, we used robust statistics: as an estimate of the mean, we calculated the median; and as an estimate of the standard deviation, we calculated the median absolute deviation and multiplied it by 1.4826 (Rousseeuw and Croux, 1993). The contact probabilities equal to 0.25x, 0.50x, 0.75x, 1.00x, or 1.25x standard deviations (σ) above the mean were set as the threshold values. Next, interaction probabilities above or below the threshold were assigned a value of 1 or 0 respectively, generating an enrichment map with a binary profile showing points with a Hi-C score above the specified threshold (Figure S3, lower panels points in light green and yellow). Lastly, a point-connecting algorithm was employed to identify the largest contiguous region of enrichment (Figure S3, lower panels, highlighted in yellow) after applying a mask of 30-35 pixels (300-350 kb) to remove contributions arising from the primary diagonal that represents short-range interactions along the chromosome arms.

Lastly, to avoid missing entire sections of connected segments, especially at higher threshold values, we chose to connect neighbors separated by as many as 15 unconnected bins. This was performed by the "imclose()" function in MATLAB 8.5 (R2015) using a diamond structuring element. From this contiguous region, the coordinates of the maximum excursion away from the *parS* site were obtained (white dotted lines), and labeled on the Hi-C contact maps (Figure S3, upper panels, blue dotted lines). For consistency throughout the study, the measurements using a threshold of $0.50x\sigma$ (0.50 standard deviation above the mean) were reported in the main text.

Calculating rates of DNA juxtaposition

The rates of DNA juxtaposition were calculated from a linear fit to the position-versus-time plots, where position was the endpoint of DNA juxtaposition determined as described above. The reported rates and their errors were calculated from the slope of the line-of-best-fit and the standard error of the regression, respectively. To more accurately determine the rates, the early time points when the juxtaposition endpoints were close to the primary diagonal and late time points when the two arms appear fully juxtaposed were omitted from the analysis (Figure S3).

Immunoblot analysis

Immunoblot analysis was performed as described previously (Wang et al., 2015). Cells were collected and resuspended in lysis buffer (20 mM Tris pH 7.0, 1 mM EDTA, 10 mM MgCl₂, 1 mg/ml lysozyme, 10 μg/ml DNase I, 100 μg/ml RNase A, with protease inhibitors: 1 mM PMSF, 1 μg/ml leupeptin, 1 μg/ml pepstatin) to a final OD₆₀₀ of 10 for equivalent loading. The cells were incubated at 37°C for 10 min followed by the addition of an equal volume of sodium dodecyl sulfate (SDS) sample buffer (0.25 M Tris pH 6.8, 4% SDS, 20% glycerol, 10 mM EDTA) containing 10% β-Mercaptoethanol. Samples were heated for 5 min at 80°C prior to loading. Proteins were separated by SDS-PAGE on 10% (SMC and SigA) or 15% (ParB, ScpA, and ScpB) polyacrylamide gels, electroblotted onto Immobilon-P membranes (Millipore) and blocked in 5% nonfat milk in phosphate-buffered saline (PBS) with 0.5% Tween 20. The blocked membranes were probed with anti-ParB (1:5,000) (Lin et al., 1997), anti-SMC (1:5,000) (Lindow et al., 2002), anti-SigA (1:10,000) (Fujita, 2000), anti-ScpA (1:10,000) (Wang et al., 2017), or anti-ScpB (1:10,000) (Wang et al., 2017) diluted into 3% BSA in 1x PBS with 0.05% Tween 20. Primary antibodies were detected using horseradish peroxidase-conjugated goat anti-rabbit IgG (BioRad) and Western Lightening Plus chemiluminescence reagent as described by the manufacturer (Perkin Elmer). The signal was captured using Bio-Rad ChemiDoc XRS.

Plasmid construction

pWX722 [*yvbJ::Pspank (optRBS) spo0J (ΔparS) (cat)*] was constructed by inserting *spo0J (ΔparS)* containing an optimized ribosomal binding site (*optRBS*) (amplified from pWX599 (Wang et al., 2015) using oWX1668 and oWX999 and digested with XmaI and NheI) into pER134 between XmaI and NheI. pER134 [*yvbJ::Pspank (cat)*] is an ectopic integration vector with an IPTG-inducible promoter (D.Z.R., unpublished data).

pWX740 [*smc(K12R)*-(his)6 (kan)], **pWX741** [*smc(R57A)*-(his)6 (kan)], **pWX742** [*smc(F66Y)*-(his)6 (kan)], **pWX743** [*smc(K37I)*-(his)6 (kan)], **pWX758** [*smc(N33A)*-(his)6 (kan)], **pWX759** [*smc(D42E)*-(his)6 (kan)], **pWX760** [*smc(Q143A)*-(his)6 (kan)], **pWX762** [*smc(G1092S)*-(his)6 (kan)], **pWX763** [*smc(D1124E)*-(his)6 (kan)] were generated using site-directed mutagenesis from template pKM309 [*smc*-(his)6 (kan)] (Sullivan et al., 2009), with oligos oWX1732 and 1733 (for pWX740), oWX1736 and 1737 (for pWX741), oWX1738 and 1739 (for pWX742), oWX1744 and 1745 (for pWX743), oWX1625 and 1626 (for pWX758), oWX1666 and 1667 (for pWX759), oWX1634 and 1635 (for pWX760), oWX1792 and 1793 (for pWX762), oWX1794 and 1795 (for pWX763). The *smc* gene was sequenced using oWX848, oWX1194, oWX1195, oWX1196, oWX1746 and oWX1747.

Strain construction

BWX3976: *smc* (WT) *loxP*-kan-*loxP*.

In the wild-type cells, a *loxP*-flanked *kan* cassette was inserted upstream of the *smc* gene using isothermal assembly. The isothermal assembly reaction contained 3 PCR products: 1) a region upstream of *smc* (amplified from wild-type genomic DNA using oWX1620 and oWX1621); 2) *loxP*-kan-*loxP* cassette (amplified from pWX470 using universal primers oWX438 and oWX439) and 3) a region containing the 5' end of *smc* (amplified from wild-type genomic DNA using primers oWX1622 and oWX822). The insertion was verified using oWX1623 and oWX1624. The *smc* gene was sequenced using oWX523, oWX848, oWX1194, oWX1195 and oWX1196 and oWX1624. The genomic DNA of this strain (BWX3976) was transformed into BWX4070, which harbors a single *parS* at −1° and an IPTG-inducible *parB*, to build BWX4077.

BWX3990: *smc* (R57A) *loxP*-kan-*loxP*.

The *smc*(R57A) mutation was constructed by transforming the product of an isothermal assembly reaction into wild-type cells. The isothermal assembly reaction contained 2 PCR products: 1) a region upstream of *smc* and *loxP*-kan-*loxP* cassette (amplified from BWX3976 genomic DNA using oWX1620 and oWX1631) and 2) a downstream region (amplified from BWX3976 using oWX1633 and oWX822). The *smc* gene was sequenced using oWX523, oWX848, oWX1194, oWX1195 and oWX1196 and oWX1624. The genomic DNA of this strain (BWX3990) was transformed into BWX4070, which harbors a single *parS* at −1° and an IPTG-inducible *parB*, to build BWX4078. The *smc* (R57A) mutation in the final strain was sequence-confirmed using oWX1624.

BWX4129: *smc* (K12R) *loxP*-kan-*loxP*.

The *smc*(K12R) mutation was constructed and verified using the same method, except that the 2 PCR products used for isothermal assembly were: 1) the region upstream of *smc* and *loxP*-kan-*loxP* cassette (amplified from BWX3976 genomic DNA using oWX1620 and oWX1696) and 2) the downstream region (amplified from BWX3976 using oWX1661 and oWX822).

BWX4137: *smc* (F66Y) *loxP*-kan-*loxP*.

The *smc*(F66Y) mutation was constructed and verified using the same method, except that the 2 PCR products used for isothermal assembly were: 1) the region upstream of *smc* and *loxP*-kan-*loxP* cassette (amplified from BWX3976 genomic DNA using oWX1620 and oWX1702) and 2) the downstream region (amplified from BWX3976 using oWX1703 and oWX822).

QUANTIFICATION AND STATISTICAL ANALYSIS

For the NADH-coupled ATPase assay, absorbance at 340 nm was measured every 10 s for 60 min. The extent of NADH oxidized (equivalent to the amount of ADP produced) was calculated using an NADH standard curve. ADP product formation was plotted over time and fitted to a line. The obtained rates were divided by the input SMC concentration. The experiment was performed four times using aliquots of proteins from the same purification. The data were averaged and the standard deviation was calculated.

For the malachite green ATPase assay, absorbance at 620 nm was measured 30 min after the start of the reaction. The experiment was performed three times using aliquots of proteins from the same purification. The data were averaged and the standard deviation was calculated.

DATA AND SOFTWARE AVAILABILITY

Protein gels and immunoblot analyses are available at Mendeley data <https://doi.org/10.17632/tygwp234gr.1>. The accession number for the Hi-C data (raw and analyzed) reported in this paper is GEO: GSE95137. The scripts for the Hi-C analyses are available upon request.