Structural Maintenance of Chromosomes Protein of *Bacillus subtilis* Affects Supercoiling In Vivo

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Structural maintenance of chromosomes (SMC) proteins are found in nearly all organisms. Members of this protein family are involved in chromosome condensation and sister chromatid cohesion. *Bacillus subtilis* SMC protein (BsSMC) plays a role in chromosome organization and partitioning. To better understand the function of BsSMC, we studied the effects of an *smc* null mutation on DNA supercoiling in vivo. We found that an *smc* null mutant was hypersensitive to the DNA gyrase inhibitors coumermycin A1 and norfloxacin. Furthermore, depleting cells of topoisomerase I substantially suppressed the partitioning defect of an *smc* null mutant. Plasmid DNA isolated from an *smc* null mutant was more negatively supercoiled than that from wild-type cells. In vivo cross-linking experiments indicated that BsSMC was bound to the plasmid. Our results indicate that BsSMC affects supercoiling in vivo, most likely by constraining positive supercoils, an activity which contributes to chromosome compaction and organization.

The mechanisms by which bacterial chromosomes are organized and compacted into small volumes and faithfully partitioned to opposite halves of a dividing cell are poorly understood. Supercoiling, negative or positive, is a means to compact and organize DNA. Several bacterial proteins are known to play important roles in supercoiling: DNA gyrase, which introduces negative supercoils into DNA; topoisomerase I, which relaxes negative supercoils; and topoisomerase IV, which decatenates chromosomes and removes supercoils (25, 44). In addition to topoisomerases, there are other proteins that affect DNA topology, although they do not act as topoisomerases. One such protein is the structural maintenance of chromosomes (SMC) protein.

SMC proteins play key roles in chromosome dynamics in prokaryotes and eukaryotes. Members of the SMC protein family are involved in chromosome condensation, sister chromatid cohesion, chromosome partitioning, dosage compensation, DNA repair, and recombination (reviewed in references 5, 8, 15, 17, 30, and 41). SMC proteins have globular N- and C-terminal domains separated by long coiled-coil regions with a central flexible hinge (13, 21, 28, 33, 39). Biochemical analysis of *Bacillus subtilis* SMC (BsSMC) has revealed that the protein forms a homodimer capable of hydrolyzing ATP, binding DNA, and aggregating single-stranded DNA in an ATP-dependent manner (1, 13–16, 18, 27, 28).

In *B. subtilis*, *smc* null mutations cause a variety of complex phenotypes, consistent with a primary role for BsSMC in chromosome organization and compaction. *B. subtilis* *smc* null mutants have defects in chromosome partitioning, producing a significant fraction of cells with abnormal nucleoids, including cells that lack a chromosome as well as those with decondensed and guillotined chromosomes (4, 29). The positioning of the origin and terminus of replication within a cell is often aberrant in an *smc* null mutant (4, 7, 29). The defects in partitioning and positioning are likely secondary effects of improper chromosome compaction (4, 29).

In addition to the effects on chromosome partitioning, *B. subtilis* *smc* mutations also cause defects in growth and development. Null mutations are temperature sensitively lethal in rich growth medium and cause a growth defect under permissive growth conditions (4, 29). *smc* mutations are also synthetically lethal with mutations in other genes (*spo0J*, *spoIIIE*, and *recU*) involved in chromosome partitioning (3, 36). Finally, *smc* null mutants are defective in sporulation (4, 29).

Mutations in *smc* or *smc*-like genes in other bacteria cause phenotypes similar to those in *B. subtilis*. *smc* null mutations in *Caulobacter crescentus* cause defects in growth and chromosome partitioning (20). *mukB* of *Escherichia coli* encodes a functional and structural analog of bacterial SMC proteins. *mukB* null mutations cause phenotypes remarkably similar to those of *B. subtilis* *smc* mutants, including partitioning defects and temperature-sensitive growth (12, 32). Together, the properties of bacterial *smc* and *mukB* mutants indicate that the proteins function similarly in a wide range of organisms.

BsSMC could affect chromosome compaction by affecting the level of supercoiling in cells. The SMC-containing 13S condensin complex of eukaryotes, but not the purified SMC protein heterodimer alone, is able to constrain positive supercoils in vitro (9, 22–24). In addition, *E. coli* MukB appears to affect the supercoiling of plasmid and chromosomal DNAs in vivo (45, 46). *mukB* null mutants are also hypersensitive to inhibitors of DNA gyrase and are partially suppressed by mutations that decrease the activity of topoisomerase I (35, 38, 46), which functions to relax negatively supercoiled DNA.

Based on the properties of eukaryotic SMC-containing complexes and *E. coli* MukB, we wanted to determine if BsSMC affects DNA topology in vivo. We found that: (i) a *B. subtilis* *smc* null mutant was hypersensitive to the DNA gyrase inhibitors coumermycin A1 and norfloxacin, (ii) depletion of topoisomerase I substantially suppressed the chromosome-partitioning defect of the *smc* null mutant, and (iii) BsSMC affected
the supercoiling of plasmid DNA in vivo. These results indicate that BsSMC helps to compact chromosomes by globally affecting the level of supercoiling.

**Materials and Methods**

**Bacterial strains and plasmids.** _B. subtilis_ strains (Table 1) were derived from JH642 (tpcC pheA1) and were constructed by standard methods (10). pJCL56 contains a 541-bp fragment of _tpcC_ (from 30 to 511 with respect to the start codon) cloned into integration vector pDH88 (49), which contains the LacI-repressible, isopropyl-β-D-galactopyranosidase (IPTG)-inducible promoter. _Pspac, pJCL56_ was integrated into the chromosome by single crossover to generate a fusion of _Pspac_ to the entire _tpcC_ (topoisomerase I) open reading frame (Pspac-topA).

Plasmids used to measure the effects of _SMC_ on supercoiling in vivo included pDL125 (cat) (26), which undergoes bidirectional theta replication from the _Bacillus subtilis_ plasmid pLS32 origin, oriC (11, 42), and pH13 (cat mhs), which undergoes rolling-circle replication from the origin of _plasmid pTA1060_ (10).

**Media and growth conditions.** Strains were grown in _Luria-Bertani_ (LB) medium or defined S7 minimal medium supplemented with 1% glucose, 0.1% glutamate, and required amino acids as described previously (19, 43). All _Δsmc_ strains and plasmid-containing strains were grown at 25°C (LB medium) or 30°C (defined minimal medium).

**Cross-linking and immunoprecipitation assays.** Cross-linking and immunoprecipitation assays were performed essentially as described previously (2, 26, 37). Briefly, cells were grown to mid-exponential phase in LB medium, and 10-ml samples were fixed with 1% formaldehyde for 20 min. Samples were lysed and incubated overnight at 4°C with polyclonal antiserum (rabbit) to _BsSMC_. Immunoprecipitates were isolated, cross-links were reversed, and DNA in the immunoprecipitates was analyzed by PCR. In all cases, dilutions of the templates were used such that the PCR products appeared to be in the linear range. The templates were total DNA (prior to immunoprecipitation) and the immunoprecipitates were analyzed by PCR. In all cases, dilutions of the templates were used such that the PCR products appeared to be in the linear range. All samples were corrected for the dilution, in the immunoprecipitated sample as a fraction of the corresponding sample in the gel. Supercoiled DNA runs faster than nicked DNA molecules in an agarose gel. As the amount of DNA intercalator is increased, the plasmid becomes less negatively supercoiled until the plasmid is relaxed, reducing the mobility of the plasmid in the gel. At even higher DNA intercalator concentrations, the plasmid becomes increasingly more positively supercoiled and displays increased mobility in the gel.

To test whether the DNA was positively supercoiled at 50 µg/ml, we also ran the plasmid samples on gels containing 3 µg of chloroquine/ml (DNA should be negatively supercoiled). At this concentration of chloroquine, the plasmid from the _smc_ null mutant had a higher mobility than the plasmid from the wild type. The reverse results were true at 50 µg of chloroquine/ml: the plasmid from wild-type cells had a higher mobility than the plasmid from _smc_ null mutant cells. Thus, plasmid DNA is negatively supercoiled at 5 µg of chloroquine/ml and positively supercoiled at 50 µg/ml.

**Inhibition of DNA gyrase in vivo.** Coumermycin A1 and norfloxacin were used to inhibit DNA gyrase (and perhaps topoisomerase IV). _JCL236_ (wild type) and RB35 (_Δsmc_) were grown at 25°C in LB medium. Serial dilutions (10 µl) were spotted onto LB agar plates containing 0, 0.25, 0.5, or 1 µg of coumermycin A1/ml and scored for viability after 2 to 4 days at 24°C. To ensure that the inhibition seen in the _smc_ null mutant was not due to a polar effect on the downstream _srb_ gene, we also tested the effect of coumermycin A1 on a strain in which the _srb_ gene is separated from the _promoter_ (4). Coumermycin A1 did not inhibit the growth of the _srb_ mutant, indicating that the growth inhibition seen in the _smc_ null mutant was due to the absence of BsSMC and not Srb (data not shown).

The MICs of norfloxacin for the wild type and the _smc_ null mutant were determined by adding 100 µl of cells growing exponentially at room temperature to various concentrations of norfloxacin in the wells of a 96-well microtiter plate. Norfloxacin concentrations were 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 µg/ml, and samples were assayed in triplicate. Wells were examined after 3 days (for the wild type) or 5 days (for the _smc_ mutant) for growth by using a Molecular Devices plate reader and Softmax Pro software.

**TopA depletion assays.** Strains RB123 (Pspac-topA Δsmc::kan) and JCL245 (Pspac-topA) were grown in minimal glucose or LB medium containing 5 µg of chloramphenicol/ml. Cultures were inoculated from single colonies grown on LB agar plates (containing 5 µg of chloramphenicol/ml and 1 mM IPTG) at 24°C. For depletion of topoisomerase I, cells were grown for a minimum of nine generations in the absence of IPTG to allow for dilution of the _topA_ gene product during growth and division and to allow the cells to reach some approximation of steady-state growth.

**Temperature sensitivity assays.** RB123 (Pspac-topA Δsmc::kan) and JCL245 (Pspac-topA), JCL346 (Δsmc::kan spoI4A-lacZ), and JCL27 (spoI4A-lacZ) were grown in liquid medium in the presence of IPTG to allow the expression of _topA_. Samples were then streaked on rich medium (LB) plates containing chloramphenicol (to select for the integrated Pspac plasmid) in the presence or absence of IPTG. LB plates were incubated at 22, 24, 30, 33, 35, 37, 42, 45, and 53°C and scored for growth after 2 days. RB123 (Pspac-topA Δsmc::kan) and JCL245 (Pspac-topA) were streaked on minimal glucose plates; incubated at 24, 30, 33, 36, 38, 42, 44, and 52°C; and scored for growth after 3 to 4 days. JCL346 and JCL27 contained a gene conferring chloramphenicol resistance linked to an _spoI4A-lacZ_ fusion to allow growth in chloramphenicol.

**Sporulation assays.** RB35 (_Δsmc::kan_), RB123 (Pspac-topA Δsmc::kan), and JCL245 (Pspac-topA) were grown in liquid_ _Difco_ sporulation medium (for RB35) or _Difco_ sporulation medium containing 5 µg of chloramphenicol/ml (for RB123 and JCL245) with or without 1 mM IPTG at room temperature. Serial dilutions were plated on rich medium (LB or LB containing 5 µg of chloramphenicol/ml and 1 mM IPTG) before and after dilutions were heated to 80°C for 20 min. The percent sporulation was calculated by dividing the number of spores per milliliter (CFU following heat treatment) by the total number of cells per milliliter (CFU prior to heat treatment).

**Results**

_smc_ null mutants are hypersensitive to inhibitors of DNA gyrase. To test whether _smc_ null mutants are sensitive to the level of supercoiling of the chromosome, we measured the viability of wild-type and _smc_ null mutant cells in the presence of coumermycin A1, an antibiotic that reduces the level of supercoiling by functioning as an inhibitor of DNA gyrase (and perhaps topoisomerase IV). The viability of wild-type cells was unaffected by low concentrations of coumermycin A1. 

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**Table 1. B. subtilis strains used**

| Strain | Genotype |
|--------|----------|
| JH642  | _tpcC pheA1_ |
| RB35   | _Δsmc::kan_ (4) |
| RB123  | _Δsmc::kan topA::Pspac-topA cat_ |
| JCL7   | _pPH13_ |
| JCL27  | _spoI4A-lacZ_ (cat) |
| JCL245 | _topA::Pspac-topA cat_ |
| JCL28  | _Δsmc::kan_ pDL125 |
| JCL289 | _Δsmc::kan_ pH13 |
| JCL346 | _Δsmc::kan spoI4A-lacZ_ |

*a* All strains were derived from JH642 and contained the _tp_ and _phe_ alleles.

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were grown from single colonies with 1 mM IPTG (anucleate cells). Three independent experiments were performed. The cultures grew in rich medium (LB). Second, the depletion of topoisomerase I did not suppress the growth defects caused by the smc null mutation. The smc null mutant was unable to grow at or above 30°C on rich medium or at 52°C on minimal medium even when the cells were depleted of topoisomerase I. Third, the sporulation defect of the smc null mutant was unchanged when cells were depleted of topoisomerase I. In the absence of IPTG, the Pspac promoter is somewhat leaky, and there is probably some low level of expression of topoisomerase I. We have not determined whether a further reduction of topoisomerase I levels or activity would completely suppress the phenotypes of the smc mutant, and our attempts to construct a topA null mutant have not been successful. However, we are reluctant to conclude definitively that topA is essential.

**BsSMC affects the level of supercoiling of plasmid DNA.** The results described above indicate that BsSMC may affect supercoiling of the chromosome. To further test the effects of BsSMC on supercoiling in vivo, we measured the topoisomer distribution of plasmid DNA isolated from smc null mutant and wild-type cells. PDL125 (26) is a low-copy-number plasmid that replicates bidirectionally from an origin, oriN, isolated from a plasmid from B. natto (11, 42). Wild-type and smc mutant cells containing PDL125 were grown in parallel, and plasmid DNA was isolated and run on gels containing chloroquine, a DNA intercalator, to resolve topoisomers (see Materials and Methods). The topoisomer distribution of DNA from each strain is shown in Fig. 1. The peak of the distribution was shifted approximately three to six topoisomers in the smc null mutant relative to the wild type (Fig. 1A and B). On gels containing 50 μg of chloroquine/ml, the plasmid isolated from the smc null mutant migrated more slowly than the plasmid isolated from the wild type (Fig. 1A and B). At 3 μg of chloroquine/ml, we observed the reverse results; the plasmid from the smc null mutant migrated faster than the plasmid from the wild type (data not shown). Together, these results indicate that, in the absence of bound protein, plasmid DNA from the smc null mutant is more negatively supercoiled than plasmid DNA from the wild type and that BsSMC likely constrains positive supercoils in vivo.

We also tested whether BsSMC affects the topoisomer distribution of a plasmid, pHPI3, which undergoes rolling-circle replication rather than bidirectional replication. The topoisomer distribution of pHPI3 in the wild type differed from that in the smc null mutant, ranging from no shift (Fig. 1C and D) to a shift of two to five topoisomers (Fig. 1E and F). We do not understand the nature of this variability. However, when shifted, the plasmid isolated from smc null mutant cells was more negatively supercoiled than the plasmid isolated from wild-type cells.

To test whether BsSMC binds to both plasmids in vivo, we used formaldehyde-mediated cross-linking followed by immunoprecipitation of BsSMC and BsSMC-bound DNA. We then detected plasmid sequences by PCR. BsSMC bound to both plasmids in vivo (Fig. 2), and we were not able to detect reproducible differences in BsSMC binding to the plasmids. BsSMC bound to PDL125 at a level ~50-fold over the background (the signal in an immunoprecipitation assay with an smc null mutant) and to pHPI3 at a level ~80-fold over the background.

### Table 2. smc null mutants are hypersensitive to an inhibitor of DNA gyrase

| Coumermycin A1 concn (μg/ml) | % Viabilitya of: | Wild type | Δsmc mutant |
|------------------------------|------------------|-----------|-------------|
| 0                            | 100              | 100       |
| 0.25                         | 100              | 3.7       |
| 0.5                          | 100              | 2.5       |
| 1                            | 2.1              | 0.02      |

*a On LB agar plates with the indicated concentration of coumermycin A1 relative to plates without coumermycin A1. Each value is the average of at least three experiments.

### Table 3. Depletion of topoisomerase I suppresses the anucleate phenotype of smc null mutants

| Relevant genotype (strain) | IPTG | % Anucleate cells (total no. of cells scored) |
|---------------------------|------|---------------------------------------------|
| Δsmc (RB35)               | −    | 13 (455)                                   |
| Pspac-topA Δsmc (RB123)   | +    | 13 (807)                                   |
| Pspac-topA Δsmc (RB123)   | −    | 0.1 (748)                                  |

*a Cells were grown in defined minimal glucose medium at 30°C and scored for anucleate cells. Three independent experiments were performed. The cultures were grown from single colonies with 1 mM IPTG (+) or no IPTG (−).
DISCUSSION

Our results indicate that BsSMC has global effects on DNA topology that likely affect the compaction of the nucleoid. In *B. subtilis*, smc null mutants are unable to properly organize and partition their chromosomes, resulting in anucleate cells and many cells with nucleoids that appear abnormal (4, 29). We suspect that the partitioning defect of smc null mutants is a secondary manifestation of a global defect in chromosome compaction.

BsSMC affects the level of supercoiling of plasmid DNA and probably affects the supercoiling of the chromosome, too. BsSMC probably does not act as a topoisomerase, as no SMC protein-containing complexes have been shown to have topoisomerase activity in vitro (22–24). Rather, we suspect that BsSMC stabilizes positive supercoils.

We found that plasmid DNA from an smc null mutant was more negatively supercoiled than that from wild-type cells, indicating that the normal role of SMC proteins is probably to constrain positive supercoils, at least on plasmids. BsSMC binds DNA both in vivo (this work) and in vitro (13, 14). We suspect that in vivo, this binding constrains a positive supercoil without breaking a DNA strand. A compensatory negative supercoil is then likely to form elsewhere on the DNA. If the compensatory negative supercoil is not constrained, then topoisomerase I (or topoisomerase IV) can remove it. When the proteins are removed from the DNA (upon extraction from the cells), a positive supercoil remains because the topoisomerase covalently changed the supercoiling of the plasmid. This interpretation can explain why plasmids isolated from the wild type are less negatively supercoiled than plasmids isolated from the smc null mutant.

FIG. 1. SMC affects the level of supercoiling of plasmid DNA in vivo. (A, C, and E) Agarose gels containing 50 μg of chloroquine/ml show topoisomers and linear DNA (arrows). (B, D, and F) Topoisomer distributions are indicated in black for plasmids isolated from wild-type cells and in gray for plasmids isolated from smc null mutant cells. Arrowheads in all panels denote the peaks of the topoisomer distributions (black for plasmids from the wild type and gray for those from the smc mutant). The peak of the distribution was identified by using area integration of each topoisomer and identifying the highest peak as the one bordered by topoisomers of decreasing areas on both sides. In many cases, the peak coincided with that of linear DNA (black arrow in A), as determined with a linearized plasmid standard (leftmost lanes in panels A, C, and E). Data presented are from representative experiments, and similar results were obtained in at least three independent experiments. Plasmids from smc null mutant strains were shifted three to six topoisomers for pDL125 and zero to five topoisomers for pH13 relative to plasmids from wild-type strains. Topoisomer distributions are as follows: A and B, pDL125 (bidirectional replication); C and D, pH13 (rolling-circle replication) with no shift; E and F, pH13 with a shift.

FIG. 2. BsSMC binds both pDL125 and pH13. Formaldehyde was added to cells to cross-link protein and DNA, and DNA was prepared as described in Materials and Methods. Samples were collected prior to immunoprecipitation (total DNA) and after immunoprecipitation with antibodies to BsSMC (IP DNA). Dilutions of total DNA and IP samples were used as templates for quantitative PCRs. Data are from a representative experiment, and similar results were obtained in at least three independent experiments. In all cases, the PCR signal from the immunoprecipitation was at least 10-fold above the background, as determined in parallel experiments with an smc null mutant. Note that different dilutions of template DNAs were used for the PCRs with the two plasmids (see below). (A and B) BsSMC binds pDL125. PCRs were done with DNAs isolated from wild-type cells (JCL282) (A) and an smc null mutant (JCL287) (B). In panel A, dilutions of total DNA were 1:8,000, 1:16,000, and 1:32,000 (left to right, respectively), and dilutions of IP DNA were 1:5, 1:25, and 1:125. In panel B, dilutions of total DNA were 1:16,000, 1:32,000, and 1:64,000, and dilutions of IP DNA were 0, 1:5, and 1:25. (C and D) BsSMC binds pH13. PCRs were done with DNAs isolated from wild-type cells (JCL7) (C) and an smc null mutant (JCL289) (D). Dilutions of total DNA were 1:16,000, 1:32,000, and 1:64,000, and dilutions of IP DNA were 1:10, 1:50, and 1:250.
It is possible (although we think unlikely) that BsSMC constrains negative supercoils on the chromosome, in contrast to its effect on plasmids. This is a simple interpretation of the hypersensitivity of smc null mutants to gyrase inhibitors and suppression by depletion of topoisomerase I and assumes that these interactions are due to an effect on the direction of supercoiling. Inhibition of DNA gyrase will reduce the level of negative supercoiling in the cell, and it is easy to imagine that the hypersensitivity of smc null mutants occurs because these mutants already have a reduced level of negative supercoiling and reducing it further is deleterious. Conversely, depletion of topoisomerase I should increase the level of negative supercoiling. It is easy to imagine that smc null mutants have a decreased level of negative supercoiling and that this is partly restored by the depletion of topoisomerase I, resulting in suppression of the anucleate phenotype of the mutants.

We favor an alternative interpretation in which the effects of smc on overall compaction cause the phenotypic interactions between smc and DNA gyrase and topoisomerase I. In this view, BsSMC constrains positive supercoils on plasmids and on the chromosome. Increased supercoiling, regardless of direction (positive or negative), increases DNA compaction. If BsSMC acts to compact the chromosome by constraining positive supercoils, then the chromosome would be less compacted in smc null mutants and hypersensitive to treatments that further decrease compaction. Inhibiting DNA gyrase decreases negative supercoiling and should decrease compaction of the chromosome, exacerbating the phenotype caused by the loss of smc. Conversely, depleting cells of topoisomerase I should increase the amount of negative supercoiling and thus increase compaction of the chromosome, partly compensating for the loss of smc. We believe that the simplest model that is consistent with all of our data is that BsSMC constrains positive supercoils, thereby affecting DNA compaction.

BsSMC could constrain supercoils by binding across a supercoil node to constrain a positive supercoil. Since BsSMC appears to be a symmetric homodimer, directionality (i.e., constraining positive supercoils rather than negative supercoils) may come from interactions with other proteins, either on the DNA or in a complex. Purified BsSMC alone was not able to constrain supercoils in vitro (14), consistent with the notion that other proteins may be involved. Two likely candidates are ScpA and ScpB (40). Null mutations in scpA or scpB result in phenotypes similar to those of smc null mutants, and yeast two-hybrid experiments have indicated that BsSMC, ScpA, and ScpB interact (40).

Despite the many similarities between BsSMC and E. coli MukB, the constraining effect of BsSMC on supercoils appears to be opposite that of MukB in E. coli (38, 45). That is, BsSMC appears to constrain positive supercoils, and MukB appears to constrain negative supercoils. BsSMC and MukB are similar in organization and structure (14, 28, 31–33). MukB is known to interact with at least two other proteins, MukE and MukF, neither of which is found in B. subtilis or other SMC protein-containing bacteria (34, 48). These interacting proteins could be responsible for conferring directionality to MukB function and making that directionality different from that of BsSMC.

The phenotypes of the B. subtilis smc null mutant and the E. coli mukB null mutant are complex. The mutants produce a large number of anucleate cells, have many guillotined chromosomes, have altered chromosome organization, and are temperature sensitive for growth in rich media (4, 29, 32, 45–47). Extragenic suppressors of the mukB phenotypes have been isolated in E. coli. Some are able to suppress the partitioning phenotype but not the temperature-sensitive growth, and others suppress the growth phenotype but not the partitioning defect (31, 35, 47). These findings indicate that these two phenotypes are separable and not causal. In E. coli, mutations in topA can suppress both the growth and the partitioning phenotypes of mukB null mutants (38), whereas in B. subtilis, depletion of topoisomerase I suppressed only the partitioning defect and not the temperature-sensitive growth phenotype. This difference may be due to the nature of the alleles used. More interesting is the possibility that the differences reflect the different modes of action of the two proteins, BsSMC constraining positive supercoils and E. coli MukB constraining negative supercoils. In any case, we suspect that the primary role of both BsSMC and E. coli MukB is to constrain supercoils, contributing to compaction of the chromosome, which aids significantly in chromosome partitioning. The loss of BsSMC (or MukB) causes defects in these processes and probably also has effects on growth and gene expression. It will be important to determine which phenotypes of the mutants are primary and which are secondary.

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