Topoisomerase I (TopA) Is Recruited to ParB Complexes and Is Required for Proper Chromosome Organization during *Streptomyces coelicolor* Sporulation

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Streptomyces species are bacteria that resemble filamentous fungi in their hyphal mode of growth and sporulation. In *Streptomyces coelicolor*, the conversion of multigenomic aerial hyphae into chains of unigenomic spores requires synchronized septation accompanied by segregation of tens of chromosomes into prespore compartments. The chromosome segregation is dependent on ParB protein, which assembles into an array of nucleoprotein complexes in the aerial hyphae. Here, we report that nucleoprotein ParB complexes are bound *in vitro* and *in vivo* by topoisomerase I, TopA, which is the only topoisomerase I homolog found in *S. coelicolor*. TopA cannot be eliminated, and its depletion inhibits growth and blocks sporulation. Surprisingly, sporulation in the TopA-depleted strain could be partially restored by deletion of *parB*. Furthermore, the formation of regularly spaced ParB complexes, which is a prerequisite for proper chromosome segregation and septation during the development of aerial hyphae, has been found to depend on TopA. We hypothesize that TopA is recruited to ParB complexes during sporulation, and its activity is required to resolve segregating chromosomes.

The bacterial chromosome recently has been shown to be very precisely organized throughout the cell cycle (1–4). It must be highly compacted to fit within the small cell volume, while remaining accessible and flexible to allow the dynamic processes of transcription, replication, and segregation. In contrast to eukaryotic cells, these processes often occur simultaneously in bacteria; for example, the spatial separation of newly replicated *oriC* (origin of replication) regions occurs soon after their duplication while the rest of chromosome is still undergoing replication (5). The segregation of bacterial chromosomes into daughter cells is a highly orchestrated process, and loci along the chromosome are transported toward specific positions in future daughter cells in a reproducible spatial pattern (1, 6, 7).

ParAB proteins mediate the spatial organization and segregation of the chromosomes in most studied bacterial species (*Escherichia coli* is an exception). The ParB proteins organize the *oriC*-proximal region by binding *parS* sites (palindromic 14- to 16-mers). Varied numbers of *parS* sites (from 3 in *Vibrio cholerae* and *Corynebacterium glutamicum* to about 20 in *Streptomyces coelicolor*) are scattered over a range of several kilobases to 100 kb near *oriC* (8). Upon binding to *parS* sites as dimers, ParB is thought to polymerize along the adjacent region to form a higher-order complex, possibly a nucleoprotein filament (9, 10). ParB binding is crucial for the positioning of the *oriC* regions within the cell and affects the positioning of other chromosomal segments (11). The positioning of ParB complexes is facilitated by their interaction with polymers of ParA protein, which exhibit dynamic properties associated with their ATPase activity (12, 13). The deletion of the genes encoding ParA and/or ParB leads to disturbed chromosome segregation and the formation of anucleate cells, with the extent dependent on the species and growth conditions (from 1% in *Bacillus subtilis* [14, 15] to 43% in *C. glutamicum* grown on minimal medium [16]).

Beyond the ParAB-mediated organization of the region prox-

imal to *oriC*, the architecture of the rest of the chromosome is crucial for its dynamics, including segregation. The proteins that influence chromosome segregation and spatial organization include condensins: proteins of the structural maintenance of chromosomes (SMC) (in *B. subtilis*, *S. coelicolor*, *Streptococcus pneumoniae*, and *Staphylococcus aureus*) or MukB (in *E. coli*) families, which generate large-scale compaction of the chromosome (17–24). Interestingly, the SMC proteins have been shown to be recruited to ParB complexes in *B. subtilis* and *S. pneumoniae*, confirming the importance of *oriC* region compaction for chromosome segregation (25, 26). In addition to SMC/MukB-induced condensation, chromosome compaction is affected by DNA supercoiling, which is maintained by topoisomerases. The main role of these enzymes is to remove the topological tension created during replication, transcription, and recombination. Chromosome segregation requires the decatenase activity of topoisomerases (mostly topoisomerase IV) to separate the interlinked circular chromosomes (27, 28). The decatenation of chromosomes may be performed in cooperation with XerCD recombinases, which are responsible for the topological maintenance.

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nance of circular chromosomes (29). The XerCD recombination system also cooperates with FtsK, a septum-associated translocase, to ensure the efficient transportation of the chromosome during the final stages of segregation (i.e., at septum closure) (30–33).

Most studies on bacterial chromosome organization have been performed on model species that possess single circular chromosomes that are segregated during replication. In contrast, *Streptomyces* spp. form filamentous multigenomic hyphae that are converted to chains of unigenomic spores during sporulation. The growth of a *Streptomyces* colony starts with the formation of branched vegetative hyphae; there, occasional septa separate adjacent multigenomic compartments in which chromosomes are replicating but not becoming spatially segregated. Sporulation is induced by starvation or stress conditions. In *S. coelicolor*, this process begins with the formation of nonbranching sporogenic aerial hyphae (34–36). Due to intensive chromosome replication, the elongated apical compartments of the aerial hyphae contain tens of nonsegregated chromosomes (37). After the aerial mycelium has stopped growing, multiple cell divisions synchronized with DNA condensation and segregation turn the apical compartments into chains of unigenomic prespores. As in other bacteria, septation is initiated by FtsZ, which forms a ladder of regularly spaced rings (34, 38, 39). The efficient segregation of chromosomes in aerial hyphae depends on the presence of ParAB proteins. Previous studies have indicated that the binding of ParB to 21 *parS* sites clustered over 200 kb of the *oriC*-proximal region results in the formation of large nucleoprotein complexes (40, 41). Before septation, the ParB complexes become uniformly aligned along the aerial hyphae via an interaction with ParA polymers (42). In *Streptomyces*, as in other bacteria, additional proteins (e.g., SMC proteins and FtsK) contribute to efficient chromosome segregation (21, 22, 43). SMC proteins condense the nucleoids in the forming prespores, and analysis of an *smc* deletion strain suggested that this chromosome compaction is required for efficient segregation (21, 22). Since *Streptomyces* chromosomes are linear, the requirement for decatenase activity during their segregation is not obvious. The ends of the *S. coelicolor* chromosome contain long terminal inverted repeats (TIRs) (44), which are associated with a telomere-binding complex composed of Tpg (telomere-protecting terminal protein), Tap (telomere-associated protein), and, interestingly, also with polymerase I (PolA) and topoisomerase I (TopA) (45). It has been suggested that the complex formed by telomere-associated proteins maintains the linear chromosome in a circularized form (46).

As a large number of chromosomes must be precisely aligned along aerial hyphae during synchronized septation, sporulation clearly imposes special requirements on the chromosome segregation machinery of *Streptomyces*. Accordingly, we examined whether ParB-mediated chromosome segregation in the aerial hyphae of *Streptomyces* requires other proteins. In our search for new proteins capable of interacting with ParB nucleoprotein complexes during the sporulation of *S. coelicolor*, we identified the putative topoisomerase I, TopA (SCO3543). Here, we report the influence of this enzyme on *Streptomyces* growth and development, focusing on the new role of TopA in reproductive cell division and its cooperation with the chromosome segregation machinery.

TABLE 1 Strains used in this study

Strain	Relevant genotype or description	Source or reference
<i>E. coli</i> strains		
DH5α	λ [−] φ80 <i>dlacZ</i> Δ <i>M15</i> Δ(<i>lacZYA-argF</i>) <i>U169</i> <i>recA1</i> <i>endA1</i> <i>hsdR17</i> (r _K [−] m _K [−]) <i>supE44</i> <i>thi-1</i> <i>gyrA</i> <i>relA1</i>	Lab stock
BW25113/pIJ790	K12 derivative; Δ <i>araBAD</i> Δ <i>rhaBAD</i> λ-Red (<i>gam</i> <i>bet</i> <i>exo</i>) <i>cat</i> <i>araC</i> <i>rep101</i> (Ts)	53
ET12567/pUZ8002	<i>dam-13::Tn9</i> <i>dcm</i> <i>cat</i> <i>tet</i> <i>hsd</i> <i>zjj-201::Tn10</i> <i>tra</i> <i>neo</i> RP4	48
BL21	F [−] <i>dcm</i> <i>ompT</i> <i>hsdS</i> _B (r _B [−] m _B [−]) <i>gal</i>	Lab stock
<i>S. coelicolor</i> strains		
M145	SCP1 [−] SCP2 [−]	44
J3305	M145 Δ <i>parB</i>	21
J3310	M145 <i>parB-egfp</i>	41
PS01	M145 Δ <i>topA::hyg</i> <i>attB</i> φC31::pIJ6902 <i>topA</i>	This study
PS02	J3310 Δ <i>topA::hyg</i> <i>attB</i> φC31::pIJ6902 <i>topA</i>	This study
PS03	J3305Δ <i>topA::hyg</i> <i>attB</i> φC31::pIJ6902 <i>topA</i>	This study
PS04	M145 Δ <i>topA::scar</i> <i>attB</i> φC31::pIJ6902 <i>topA</i>	This study
PS05	J3305 Δ <i>topA::scar</i> <i>attB</i> φC31::pIJ6902 <i>topA</i>	This study
PS06	J3305 Δ <i>topA::scar</i> <i>attB</i> φC31::pIJ6902 <i>topA</i> pIJ10257 <i>parB</i>	This study
PS07	M145/pIJ6902 <i>topA</i>	This study
PS08	J3305/pIJ6902 <i>topA</i>	This study
PS11	M145 Δ <i>topA::scar</i> <i>attB</i> φC31::pIJ6902 <i>topA</i> pWHM3Hyg	This study
MS11	M145/pWHM3Hyg	This study

MATERIALS AND METHODS

Bacterial strains, medium, and growth conditions. Basic DNA manipulations were performed according to standard protocols (47). The culture conditions, antibiotic concentrations, transformation protocols, and conjugation methods for *Streptomyces coelicolor* followed general procedures (48). All strains grew on minimal (MM) or rich (SM or R2) medium prepared according to Kieser et al. (48). The obtained mutant strains were verified by PCR and Southern blot analysis. Growth curves of the *S. coelicolor* strains were assessed in 79 medium (not supplemented with glucose) for 72 h (49). The rate of *S. coelicolor* growth was quantified by centrifugation of 25-ml culture samples and measurement of the wet weight of the cell pellet. The strains used in this study are described in Table 1. The oligonucleotides used for PCR or quantitative real-time PCR (qRT-PCR) amplification are listed in Table S1 in the supplemental material.

Pulldown experiment. For the pulldown experiments, a 250-bp fragment of chromosomal DNA from *S. coelicolor* containing two *parS* sites was amplified using the biotinylated oligonucleotide *parS2F*-biot and the nonbiotinylated H24*parSrv*. As a control, a fragment that was identical in length but lacked *parS* sites was amplified with primers *parS4440rv*-biot and *parAB4200*. Ten micrograms (60 pmol; for the pulldown from *S. coelicolor*) or 200 ng (for the pulldown with recombinant TopA) of purified DNA fragments was bound to 100 μl (for *S. coelicolor* lysate pulldown) or 10 μl (for recombinant TopA pulldown) of magnetic beads coated with streptavidin (Dynabeads) in phosphate-buffered saline (PBS) buffer for 1.5 h at room temperature. The beads were washed twice with PBS buffer, 10 to 1 μg of ParB protein was bound to the *parS*-containing DNA fragments, and unbound protein was washed off with PBS. In the pulldown from *S. coelicolor* cell lysates, samples were prepared from cultures grown for 44 h on minimal medium plates (4.5 mg of total protein in PBS buffer) and incubated with the beads in a total volume of 5 ml for 1 h at room temperature. In the pulldown experiment from the *E. coli* BL21 strain carrying pET28a-*topA*, cell extract or purified TopA protein magnetic beads were incubated with 200 μl of cell extract in PBS (total protein concentration, approximately 2.1 μg/ml) or 0.8 μg of TopA protein in PBS. To eliminate unspecific TopA binding to DNA, the pulldown experiment with purified TopA protein was performed in the presence of DNA competitor (1 μg of supercoiled pUC19 plasmid). After incubation the beads were washed three times with 1 ml of PBS buffer containing 0.3 M

NaCl and 0.5 M NaCl, and specifically bound proteins were eluted with PBS buffer containing 1 M NaCl. The eluted proteins were analyzed by SDS-PAGE. The presence of TopA protein in the specifically bound fractions was detected in Western blot analysis using anti-TopA polyclonal antibodies. In the case of the pulldown from *S. coelicolor* lysate, tryptic fragments were identified by mass spectrometry.

Construction of TopA-depleted strains. The chromosomal fragment containing the *topA* gene was first cloned as a 3,857-bp fragment digested with NcoI from cosmid H5. The recessed 3' ends were filled in with Klenow, and the 3,857-bp fragment was ligated into the integrative plasmid pIJ82 (a pSET152 derivative harboring a hygromycin resistance gene) digested with EcoRV (48). Plasmid pIJ82*topA* was used to subclone the *topA* gene into the expression vector pET-28a(+) (Novagen) (see below). Plasmid pET28*topA* was used to reclone the *topA* gene (3,372 bp, without the promoter) digested with NdeI and EcoRI into the similarly digested integrative plasmid pIJ6902 (containing the inducible *P_{tipA}* promoter). The obtained pIJ6902*topA* plasmid was introduced into *S. coelicolor* strains M145 (wild type), J3310 (expressing *parB-egfp*) (41), and J3305 (Δ *parB*), yielding strains PS07, PS09, and PS08, respectively (21). The native *topA* gene was then deleted from the obtained strains using PCR targeting (50). First, the *topA* gene within cosmid H5 was replaced with the *hyg-oriT* cassette, a hygromycin resistance cassette containing the origin of transfer region (*oriT*) flanked by FLP recombinase recognition target (FRT) sites. The cassette was amplified from pIJ10700 with primers 3543FP2 and 3543RP1 and used to transform BW25113 carrying cosmid H5 and the plasmid pIJ790 in which expression of lambda *red* genes was induced with arabinose (51). This resulted in replacement of the *topA* gene with the *hyg-oriT* cassette. The resulting cosmid, H5*topA::hyg-oriT*, was introduced by conjugation into *S. coelicolor* M145(pIJ6902*topA*), J3310(pIJ6902*topA*), and J3305(pIJ6902*topA*). Hyg^r exconjugants were screened for the loss of Kan^r, indicating the presence of a double-cross-over allelic exchange in the native *topA* locus, yielding strains PS01, PS02, and PS03, respectively. The unmarked *topA* deletion in strains PS01 and PS03 was created by using a FLP-mediated excision of the *hyg-oriT* cassette from the H5*topA::hyg-oriT* cosmid, leaving only an in-frame "scar" sequence. In order to facilitate conjugative transfer of the cosmid, the H5*topA::scar* cosmid was then used to replace the Amp^r gene in the SuperCos portion of cosmid H5 with the apramycin resistance cassette containing the *oriT* region [*acc3(IV)-oriT*] (amplified from pIJ773 with primers blap1apraRV and blap2apraFW), to yield H5*topA::scar bla::acc3(IV)-oriT*. This construct was conjugated into strains PS01 and PS03. Kan^r exconjugants were screened for the loss of both Kan^r and Hyg^r, indicating a double-cross-over allelic exchange of the *topA* locus of PS01 and PS03, to yield strains PS04 and PS05, respectively. The PS05 strain was transformed with plasmid pIJ10257*parB*, in which expression of the *parB* gene was under the control of the constitutive *P_{erm}* promoter. The *parB* gene was amplified using ParBEcoRI and ScB_NdeF oligonucleotides, digested with NdeI and EcoRI (the EcoRI site was subsequently blunted), and ligated to pIJ10257 (52) digested with NdeI and PacI (the PacI site was previously blunted).

RNA isolation, quantitative RT-PCR, and Western blotting. Total RNA was isolated from bacterial cultures cultivated in 79 medium for 48 h, according to the modified Kirby's protocol described by Kieser et al. (48). The purity and quality of total RNA samples were analyzed by standard 1% agarose gel electrophoresis, and cDNA was prepared from 2 μ g of total RNA using a High Capacity RNA-to-cDNA Kit (Applied Biosystems). The cellular levels of *topA* transcripts in strains M145 (wild type) and PS01 were analyzed using qRT-PCR (comparative threshold cycle method, $\Delta\Delta C_T$) with the *hrdB* gene used as a reference gene.

Cloning and purification of TopA protein. For purification of TopA protein, the *topA* gene was first cloned into the pET-28a(+) vector as a 3,857-bp fragment digested with BamHI and EcoRI from pIJ82*topA*, yielding pET28*topA*3857. To put *topA* gene expression under the control of the pET28 *P_{lac}* promoter, a 576-bp fragment of the N-terminal region of *topA* was amplified with primers 3543Ndefw and 3543Fserv and ex-

changed with the fragment encompassing the upstream region and beginning of the *topA* gene in pET28*topA*3857. The obtained PCR product was digested with NdeI and FseI and cloned into NdeI- and FseI-digested pET*topA*3857 to yield pET28*topA*, which was used to overexpress TopA in *E. coli* strain BL21. For protein expression, cells were grown at 37°C to an optical density at 600 nm (OD₆₀₀) of ~0.4; isopropyl- β -D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.3 mM, and the culture was continued for 4 h at 37°C. The cells were collected by centrifugation, resuspended in buffer A (50 mM NaH₂PO₄, pH 8.1, 300 mM NaCl, and 20 mM imidazole), and sonicated. The resulting cell lysate was purified in a fast protein liquid chromatography (FPLC) system using HisTrap HP columns (GE Healthcare), followed by gel filtration using a HiLoad 26/60 Superdex 200 column (GE Healthcare) equilibrated with buffer B (50 mM NaH₂PO₄, 300 mM NaCl, and 10% glycerol). Nuclease contamination was tested by incubation of 250 ng of purified TopA protein with 200 ng of supercoiled pUC19 plasmid in 0.5 $\times$ buffer B supplemented with 10 mM MgSO₄ and 1 mg/ml bovine serum albumin (BSA) for 24 h in 37°C. Degradation of DNA was analyzed by standard 1% agarose gel electrophoresis.

Preparation of antisera. To generate polyclonal antibodies against the TopA protein, 500 ng of purified recombinant protein was mixed with TiterMax Gold adjuvant (Sigma-Aldrich) according to the manufacturer's suggestions and used to immunize rabbits. A second booster injection was performed 2 weeks after the first injection, and serum samples were obtained 2 weeks later. The protocol to produce rabbit polyclonal antibodies raised against the TopA protein was approved by the 1st Local Ethics Committee for Experiments with the use of Laboratory Animals, Wrocław, Poland (permit number 3/2007, 17 January 2007), Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland. All efforts were made to minimize suffering. The serum was diluted in Tris-buffered saline plus Tween (TBST) buffer (1:10,000), and Western blot analysis showed that it could specifically detect TopA in M145 cell lysates. For chromatin immunoprecipitation with high-throughput sequencing (ChIP-Seq) analysis, anti-TopA antibodies were purified by ammonium sulfate precipitation (to 40% saturation), followed by affinity chromatography. The affinity column was prepared by immobilizing 2 mg of purified recombinant TopA protein (previously dialyzed against carbonate buffer [0.1 M NaHCO₃, pH 8.3, and 0.5 M NaCl]) on CNBr-activated Sepharose 4B (Thermo Scientific), according to the supplier's instructions. The anti-TopA antibodies were eluted with 0.1 M glycine, pH 2.5, and immediately neutralized by the addition of 1 M Tris-HCl buffer, pH 9.5.

Immunoprecipitation assay. The protein-DNA complexes were cross-linked by incubation of 5 ml of *S. coelicolor* culture (grown in 79 medium for 48 h) with 1% paraformaldehyde for 5 min. As a negative control, we used non-cross-linked cultures of each analyzed strain. Cells were collected by centrifugation and washed twice with cold PBS buffer, and then 50 to 60 mg of cells (wet weight) was resuspended in 300 μ l of cold PBS buffer and subjected to sonication for cell disruption and DNA fragmentation. The average length of the DNA fragments was ~300 to 1,000 bp, as quantified by standard agarose gel electrophoresis. Each cell extract (50 μ l) was diluted with 500 μ l of IMP buffer (50 mM Tris-HCl, pH 7.5, 300 mM NaCl, 2% Nonidet P-40, 1 mM EDTA, 1 mg/ml BSA, and 0.02% Na₂S₂O₃) and incubated with 4 μ g of purified anti-TopA antibodies for 20 h at 4°C. Then, each sample was mixed with 20 μ l of 50% protein A-Sepharacyl resin (Thermo Scientific) equilibrated with IMP buffer and incubated for 4 h at 4°C. The anti-TopA IgG-TopA-DNA complex-bound resin was collected by centrifugation and washed six times with 300 μ l of IMP-wash buffer (50 mM Tris-HCl, pH 7.5, 300 mM NaCl, 0.1% SDS, 0.5% Nonidet P-40, and 0.02% Na₂S₂O₃). After samples were washed, the resin was resuspended in 80 μ l of Tris-EDTA (TE) buffer, 5 μ l of 10% SDS, 5 μ l of 2 mg/ml proteinase K, and 10 μ l of 50 mM CaCl₂. The samples were incubated overnight at 65°C with vigorous shaking. Thereafter, the DNA was extracted with phenol-chloroform-isoamyl alcohol (25:24:1), precipitated with isopropanol in the presence of sodium ace-

tate, and washed with 70% ethyl alcohol. The obtained DNA was amplified by PCR (for sequences of the oligonucleotides used, see Table S1 in the supplemental material) and subjected to next-generation sequencing on an Illumina HiSeq2000 apparatus at The Genome Analysis Centre (TGAC; Norwich Research Park, Norwich, United Kingdom). The sequencing yielded about 10 to 20 million reads with average lengths of 50 bp aligned to the *S. coelicolor* chromosome, and the coverage frequencies of specific DNA fragments were plotted against their positions on the chromosome.

Fluorescence microscopy. For microscopic observations, spore dilutions were inoculated at the acute-angled junctions of coverslips inserted at 45° into minimal medium agar plates supplemented with 1% mannitol (48) and grown for 48 or 72 h. Cross-linking and cell preparation for fluorescence microscopy were performed as described by Jakimowicz et al. (42). 4',6'-Diamidino-2-phenylindole (DAPI) (or propidium iodide [PI]) and wheat germ agglutinin (WGA)-Texas red (or WGA-Alexa Fluor 350) were used for visualization of DNA and cell walls, respectively. All fluorescent dyes were obtained from Invitrogen, and images were analyzed with the AxioVision Rel, version 4.8, software (Zeiss).

Assay for plasmid supercoiling *in vivo*. To assess the level of DNA supercoiling *in vivo*, we used pWHM3Hyg, a derivative of plasmid pWHM3 (48) in which the fragment containing the *tsr*, *bla*, and *lacZ* genes was replaced with a hygromycin resistance cassette and *oriT* region using the redirect procedure (53). Here, pWHM3Hyg was introduced via conjugation (48) into wild-type M145 and PS04, yielding MS11 and PS11, respectively. pWHM3Hyg was isolated from 3-ml bacterial cultures grown in yeast extract-malt extract (YEME) medium supplemented with hygromycin B (50 µg/ml) and MgCl₂ (5 mM) for 24, 48, or 72 h at 30°C and 180 rpm. Cells were collected by centrifugation, washed with cold PBS buffer, resuspended in 500 µl of lysozyme solution (25 mM Tris-HCl, pH 8.0, 0.6 M sucrose, 2 mg/ml lysozyme, and 50 µg/ml RNase), and incubated for 30 min in 37°C. For lysis, cells were incubated with 250 µl of 0.3 M NaOH supplemented with 2% SDS for 15 min in 70°C. Lysates were vortexed in the presence of acidic phenol-chloroform (1:1), and plasmids were precipitated with isopropanol. Each pellet was washed with 70% ethanol and dissolved in TE buffer. DNA (2 to 6 µg) was resolved by 0.8% agarose gel electrophoresis in Tris-acetate-EDTA (TAE) buffer for 14 h at low voltage (2 V/cm). Nucleic acids were stained with ethidium bromide in TAE buffer for 30 min at room temperature, and the results were analyzed using a Typhoon 8600 Imager (Molecular Dynamics).

RESULTS

ParB complexes are bound by topoisomerase I *in vitro*. To examine whether proteins other than ParA may interact with ParB complexes in *Streptomyces* hyphae, we performed pulldown experiments using ParB-DNA nucleoprotein complexes as the bait. We used a 250-bp DNA fragment containing two *parS* sites separated by 50 bp, located ~14 kb downstream of the *parAB* operon; this region, designated the H24-19 region, was previously shown to be efficiently bound by ParB and the included sites that were considered “strong *parS* sites” (40). Purified ParB was bound to biotinylated DNA fragments that were immobilized on magnetic beads (Dynabeads). A crude lysate prepared from sporulating *S. coelicolor* grown for 44 h on minimal medium was used to fish out the proteins that interacted specifically with the ParB-DNA complexes. Proteins were eluted from the beads with a buffer containing increasing salt concentrations and were analyzed by SDS-PAGE. We obtained a protein with an apparent size of ~100 kDa from beads containing ParB-bound DNA fragments but not control beads harboring unbound DNA fragments. Mass spectrometry identified the bound protein as SCO3543, a putative topoisomerase I (TopA). To confirm the identified interaction, we performed a similar pulldown assay using TopA overproduced in

E. coli BL21. TopA from BL21 cell extract bound efficiently to the ParB-DNA complex-bound beads and only weakly to control beads bound only with DNA, while purified TopA required the presence of competitor DNA to bind selectively to ParB complexes and not to DNA (Fig. 1A). To examine whether TopA interacts directly with ParB, we used a bacterial two-hybrid system, a pulldown experiment in which TopA protein was immobilized on chromatography resin (BD-Talon; Clontech), and far-Western blotting in which TopA was bound to a nitrocellulose membrane. We did not observe any direct interaction with ParB in these experiments, suggesting that TopA interacts with the nucleoprotein complex of ParB, presumably with DNA that is constrained by ParB binding (data not shown).

TopA is recruited to ParB complexes *in vivo*. To confirm the interaction of TopA with ParB-DNA complexes *in vivo*, we performed a ChIP assay in which DNA bound to TopA was immunoprecipitated using purified rabbit polyclonal anti-TopA antibodies from wild-type *S. coelicolor* (M145) and from a *parB* deletion strain (J3305). Using the PCR we tested whether TopA interacts with *parS*-containing regions in a ParB-dependent manner. The relevant PCR-amplified DNA fragment containing the two *parS* sites (H24-19, using primers H24parSfw and H24parSrv) was seen only with the immunoprecipitated DNA released from cross-linked TopA complexes isolated from the wild-type strain (Fig. 1B, lane 2) and was absent from similarly prepared samples from the *parB* deletion strain (Fig. 1B, lane 6), from control samples isolated from the topoisomerase-depleted strain, PS01 (see below), and from non-cross-linked cultures (Fig. 1B, lanes 3, 4, and 7). Additionally, a fragment carrying the two strong *parS* sites of the *parAB* promoter region (H24-5) was present in the DNA immunoprecipitated from the wild-type strain (Fig. 1B, lane 2) but not in that from the *parB* deletion strain J3305 (as identified using primers parASmaRV and 2parABamHI) or in the negative control (Fig. 1B, lanes 3, 4, and 7). Furthermore, a fragment of the *hrdB* gene (encoding a constitutively expressed sigma factor) that is located about 2 Mb away from the *oriC* region and does not contain *parS* sites was not present in the immunoprecipitated DNA (as assessed by PCR using oligonucleotides hrdBfw and hrdBrv) (Fig. 1B), while a fragment encompassing *topA* (detected using primers RT3543FWD and RT3543RV) served as the positive control in the experiment (possibly due to coupled transcription and translation, which explains why the *topA* gene which is transcribed at a low level in the TopA-depleted strain was detected also in negative control) (Fig. 1B, lane 3). Thus, the binding of TopA to ParB complexes that we observed *in vitro* was confirmed by our *in vivo* immunoprecipitation assays.

To map the interactions of TopA with ParB complexes, we used high-throughput sequencing and analyzed total DNA fragments obtained from ChIP experiments performed using cross-linked and non-cross-linked (control) M145 *S. coelicolor* cultures. We confirmed the presence of regions H24-19 and H24-5 but found that the other *parS*-containing regions were not abundant in the immunoprecipitated DNA (Fig. 1C). Region H24-19, which contained the two *parS* sites used for the pulldown experiment, exhibited the highest coverage, suggesting that it had the highest affinity for TopA binding (similarly to the *oriC* region). Interestingly, the highest enrichment of DNA in the TopA-immunoprecipitated fractions was not observed directly within the *parS* sites but, instead, encompassed the 150-bp regions adjacent to the *parS*

FIG 1 Binding of TopA to ParB complexes. (A) Binding of TopA to ParB-DNA complexes in pull-down experiments. The proteins bound to the beads were eluted with 1 M NaCl and subjected to SDS-PAGE analysis. Cell extracts from BL21 cells overproducing TopA were incubated with ParB-DNA complex or DNA alone bound to beads (top left panel, as indicated) or negative-control beads without DNA bound (top right panel). Purified TopA was incubated with ParB-DNA complex or DNA alone bound to beads in presence or absence of competitor DNA (Comp.) (bottom panel, as indicated). (B) PCR-based detection of the H24-16 and H24-5 DNA regions in TopA-immunoprecipitated samples. The *hrdB* region served as a negative control while *topA* served as the positive control. Lane 1, total input DNA of wild-type strain (M145); lane 2, cross-linked immunoprecipitated DNA of wild-type strain (M145) (imp-DNA); lane 3, cross-linked immunoprecipitated DNA of TopA-depleted strain (PS01; negative control); lane 4, non-cross-linked immunoprecipitated DNA of wild-type strain (M145; negative control); lane 5, total input DNA of $\Delta parB$ strain (J3305); lane 6, cross-linked immunoprecipitated DNA of $\Delta parB$ strain (J3305); and lane 7, non-cross-linked immunoprecipitated DNA of $\Delta parB$ strain (J3305). (C) ChIP-Seq analysis of TopA-binding sites within the *oriC*-proximal region. The numbers of reads are plotted against the fragments of the chromosome containing the 21 *parS* sites. The chromosomal localizations of the identified *parS* sites are marked with black (weak *parS* site) and blue (strong *parS* site) rectangles, while the *oriC* region is marked with a pink rectangle. The localizations of TopA-binding sites in the vicinity of the H24-5 region, the H24-19 region, and the *oriC* region are enlarged in insets a, b, and c, respectively. Within each inset, the maximal number of detected DNA fragments covering the region is given in pink.

sites in H24-19 and H24-5 (Fig. 1C, insets a and b). These results suggest that TopA binds in close proximity to the ParB complexes.

Alteration of chromosome supercoiling inhibits growth. A previous study showed that deletion of the *topA* gene is lethal for *S. coelicolor* (45), and our attempts to delete *topA* (data not shown) confirmed the prior report. This may be because the *S. coelicolor* genome encodes only one type I topoisomerase (44). Thus, to analyze the phenotypic effect of TopA depletion, we constructed strain PS01, in which the chromosomal *topA* gene was deleted and plasmid pIJ6902, encoding *topA* expressed under the control of the thiostrepton-inducible *P_{tipA}* promoter, was integrated into the chromosome. Although the growth of strain PS01 was severely retarded, it was still viable even in the absence of the inducer, suggesting that a residual level of *P_{tipA}* promoter activity allowed the production of a low level of TopA. We used qRT-PCR and Western blotting with an anti-TopA antibody to measure the cellular levels of *topA* transcripts and TopA proteins, respectively, in the presence of different concentrations of the inducer (0 to 10 μ g/ml). When the TopA-depleted strain, PS01, was cultivated in liquid 79 medium in the absence of thiostrepton, the TopA protein level was about 20-fold lower than that in the wild-type strain (Fig. 2A and B). The concentration of thiostrepton that restored the wild-type cellular level of TopA was estimated to be approximately 2 μ g/ml. In the presence of 2 μ g/ml thiostrepton, PS01 growth was fully restored, confirming that the observed growth retardation was due to a decreased TopA level (Fig. 3A). Interestingly, cultivation of PS01 in R2 liquid medium or on R2 agar plates (without the inducer) resulted in the overproduction of acti-

norhodin, which is a blue secondary metabolite of *S. coelicolor* with antibacterial activity. This effect decreased as the inducer concentration increased and was not observed in the presence of 2 μ g/ml thiostrepton (the concentration restoring physiological TopA level) (Fig. 3B).

To test whether the slow growth and actinorhodin overproduction of the TopA-depleted strain were due to alterations in the supercoiling level of the chromosome, we used a nonintegrating plasmid, pWHM3Hyg, as a marker for the topological state of the *S. coelicolor* chromosome. The analysis of the topology of pWHM3Hyg showed that it varied from a more condensed form during the exponential phase to a more relaxed form during the early stationary phase (48 h of culture) (data not shown), presumably reflecting changes in global DNA supercoiling. Our analysis of pWHM3Hyg isolated from the TopA-depleted strain (PS11) grown with different concentrations of the inducer revealed different ratios of topoisomers: the plasmid was mostly in the supercoiled form in the presence of a low concentration of the inducer, whereas a higher concentration of the inducer was associated with a higher fraction of relaxed topoisomers (Fig. 2C). This confirms that the TopA depletion phenotype may be correlated with the increased DNA supercoiling.

TopA depletion inhibits sporulation, but *parB* deletion partially restores the wild-type phenotype. Since TopA depletion inhibited growth, we examined whether alterations in the level of TopA (and thus DNA supercoiling) could also affect differentiation. We grew the TopA-depleted strain (PS01) on solid medium for these experiments as *S. coelicolor* is able to sporulate only in

FIG 2 The influence of TopA levels on DNA supercoiling *in vivo*. (A) The level of the *topA* transcripts in noninduced PS01 compared with that in the wild-type (M145), as quantified by qRT-PCR. (B) The level of TopA proteins in the PS01 strain subjected to thiostrepton induction, as quantified by Western blotting using anti-TopA polyclonal antibodies. (C) Agarose gel electrophoresis showing the topoisomer distribution of pWHM3Hyg plasmids isolated from PS01 (liquid culture; 48 h in 79 medium) induced with different concentrations of thiostrepton (0 to 10 $\mu\text{g/ml}$). WT, wild type; RQ, relative quantification; conc, concentration.

FIG 3 The effect of TopA depletion on growth and antibiotic production of *S. coelicolor*. (A) Growth of TopA-depleted PS01 without or with 2 $\mu\text{g/ml}$ thiostrepton versus that of wild-type M145 (all in 79 medium). (B) Depletion of the TopA protein affects the secondary metabolism of *S. coelicolor*, leading to overproduction of actinorhodin (blue) in PS01 grown in R2 liquid medium for 48 h. (C) Sporulation is inhibited by TopA depletion in PS01 (white aerial mycelium), but this effect is partially restored by *parB* deletion (strain PS03, TopA depletion in the ΔparB background; gray aerial mycelium). (D) Cultures were grown on SM medium supplemented with 0.1, 2.0, and 10.0 $\mu\text{g/ml}$ thiostrepton. Sporulation blockage returns in PS06 when the *parB* gene is provided in *trans* in a strain with TopA depletion in the *parB* deletion background (PS05). Cultures were grown on SM supplemented with 2.0 and 10.0 $\mu\text{g/ml}$ thiostrepton.

solid cultures. PS01 grown on SM agar with no inducer developed white aerial hyphae that did not turn into gray spores even after a prolonged (more than 5 days) incubation (sporulation was not affected in the control strains containing an additional copy of *topA* in the pIJ6902 integrating vector or empty pIJ6902 vector) (Fig. 2C). The formation of gray spores by PS01 was partially restored in cultures supplemented with 2.0 $\mu\text{g/ml}$ thiostrepton and completely restored in the presence of 10 $\mu\text{g/ml}$ thiostrepton. The higher level of TopA induction required to reestablish sporulation may be due to either lower activity of *tipA* promoter in aerial hyphae or higher demand for TopA. Since TopA interacts with ParB nucleoprotein complexes, we next questioned how TopA depletion affects sporulation in the absence of ParB. Surprisingly, the growth analysis of the strain in which TopA was depleted in the ΔparB background (PS03) revealed that lower concentrations of inducer were sufficient to restore sporulation; spores were observed in the presence of 0.5 $\mu\text{g/ml}$ thiostrepton, and 2.0 $\mu\text{g/ml}$ thiostrepton completely restored sporulation (Fig. 3C). However, in strain PS06 the expression of the *parB* gene delivered in *trans* (expressed from strong constitutive promoter, P_{ermE} , in pIJ10257 in the PS05 background [$\Delta\text{parB topA}::\text{scar pIJ6902topA}$]) resulted in a return to sporulation blockage (Fig. 3D). This strongly suggests that TopA depletion inhibits sporulation, but this effect is dependent on the ParB.

Together, these results suggest that the changes in chromosome supercoiling induced by TopA depletion inhibit growth and sporulation, but the elimination of ParB partially restores this sporulation defect. Moreover, the overproduction of actinorhodin suggests that chromosome supercoiling may have an effect on secondary metabolism though it is possible that this is an indirect effect of reduced growth rate.

TopA depletion affects septation and chromosome condensation. In *Streptomyces*, chromosome segregation occurs only during sporulation, when ParAB proteins actively distribute tens of chromosomes along the sporogenic hyphae. To examine how TopA depletion affects chromosome segregation in *S. coelicolor*, we stained the aerial hyphae of TopA-depleted strains PS01 (wild-type background) and PS03 (ΔparB background) with PI and

FIG 4 Defects in septation and chromosome segregation are seen in TopA-depleted PS01. Fluorescence microscopy was used to compare the noninduced PS01 strain (two sets of example images, as indicated) to the wild-type control (M145, as indicated). Images of aerial hyphae show cell walls stained with WGA-Alexa Fluor 350 (blue) or DNA stained with propidium iodide (PI; red) and merged fluorescence, as indicated. Scale bar, 5 μ m.

WGA-Alexa Fluor 350 conjugate (for visualization of DNA and septa, respectively). Microscopy observations confirmed that when TopA was not induced in PS01 and PS03 strains, spores failed to form even if the culture time exceeded 96 h. In contrast to wild-type sporulating hyphae, in which septa are visible as regularly spaced ladders, in the aerial hyphae of the TopA-depleted strain, no septa were detected; instead, there were accumulations of peptidoglycan at the cell membrane (Fig. 4). DAPI staining showed a lack of chromosome segregation in the depleted strains, but the irregular intensity of the fluorescence suggested local accumulations of chromosomal DNA (Fig. 4).

When PS01 and PS03 strains were grown with a suboptimal concentration of the inducer (0.5 μ g/ml, which was not sufficient to provide the wild-type level of TopA protein), spore chains were visible in both strains after 48 h. The number of microscopically visible spore chains after 72 h of growth was noticeably higher in strain PS03 than in strain PS01, confirming our analysis of plate growth (Fig. 3C). In both strains grown at the suboptimal inducer concentration, we observed irregular but intensive DNA staining that suggested high, but nonuniform, chromosome compaction and/or defects in chromosome distribution (Fig. 5A). Although TopA depletion in the wild-type background (PS01) did not yield a typical missegregation phenotype, as we did not detect any

anucleate prespores, the analysis of the CFU count suggested that spore viability is about one-third that of the wild-type spores (see Fig. S2 in the supplemental material). Interestingly, TopA depletion in the Δ *parB* background (PS03) led to a decrease in chromosome segregation abnormalities (8.9% anucleate prespores) compared to that in the *parB* deletion strain (J3305, with 19.5% anucleate prespores) (21). This may indicate that increased DNA compaction may partially rescue the chromosome segregation defect observed in the *parB* deletion strain. Additionally, septal staining revealed that the distances between septa were reduced in PS01 and PS03 cultured in the presence of 0.5 μ g/ml of thiostrepton (Fig. 5C, right histogram); the most frequent length of the prespore compartments was 0.9 to 1.1 μ m in PS01 and PS03, compared with 1.3 to 1.4 μ m in the wild-type control (M145). The distributions of the distances between septa in PS01 and PS03 were very similar, suggesting that the TopA depletion-induced irregularities in septation are ParB independent. In the presence of a higher concentration of inducer ($\sim$ 2 μ g/ml), the distributions of the between-septa distances in the two TopA-depleted strains were closer to the distribution observed in the wild-type strain, at 1.1 to 1.3 μ m in PS01 (Fig. 5C, left histogram) and 1.0 to 1.2 μ m in PS03 (data not shown). Although the typical prespore size was larger in the presence of the higher concentration of inducer, the chromosome segregation defects in PS03 were still lower (7.3% anucleate prespores versus 8.9% at the lowest inducer concentration) than in the *parB* deletion strain, perhaps suggesting that the wild-type level of TopA was not completely restored in aerial hyphae.

In summary, our microscopy analyses revealed that TopA depletion affects chromosome condensation and septation in aerial hyphae. Moreover, a suboptimal level of TopA reduced the chromosome segregation defects arising from ParB deletion, suggesting that increased chromosome supercoiling may promote chromosome segregation.

TopA depletion does not inhibit the formation of ParB complexes but does impair their regular distribution. The formation of regularly spaced ParB complexes precedes chromosome condensation and segregation in *S. coelicolor* aerial hyphae. The observed inhibition of chromosome segregation in the TopA-depletion strain prompted us to analyze the influence of TopA levels on the distribution of ParB complexes. Strain J3310, which produces a ParB-enhanced green fluorescent protein (EGFP) fusion protein (41), was modified to obtain a TopA-depleted strain, PS02, which showed the same white phenotype as PS01 (TopA depletion in the wild-type background) (data not shown). Fluorescence microscopy of PS02 aerial hyphae grown without the inducer revealed the presence of ParB-EGFP foci with signal intensities similar to those observed in the control strain, J3310 (Fig. 6A). Interestingly, in contrast to the regular distribution of ParB-EGFP nucleoprotein complexes along the aerial hyphae of the control strain, J3310, in PS02 ParB-EGFP showed a disrupted pattern with irregularly distributed foci that were often visible as doublets (Fig. 6A, red arrows). Measurements of the distances between the foci (for about 360 ParB complexes) confirmed that in the control strain the typical distance between ParB foci was 1.3 to 1.4 μ m (41), and 74% of the measurements fell in the range of 0.9 to 1.7 μ m. In contrast, for PS02, although the average distance between two ParB foci was also 1.36 μ m, only 34% of the distances were within the range of 0.9 to 1.7 μ m. Moreover, in PS02 the distance between ParB-EGFP foci was shorter than 0.9 μ m in 34%

FIG 5 Defects in prespore length under TopA-depleted conditions in *parB*⁺ (PS01) and *parB* deletion (PS03) backgrounds. (A and B) PS01 cells were induced with 0.5 µg/ml (A) and 2.0 µg/ml (B) thiostrepton. Images of hyphae show cell walls stained with WGA-Alexa Fluor 350 (blue), DNA stained with propidium iodide (PI; red), and merged fluorescence, as indicated. Scale bar, 5 µm. (C) Distribution of prespore sizes is presented for PS01 induced with different concentrations of thiostrepton (thio) (left histogram) and for PS01 and PS03 induced with a low concentration of thiostrepton (right histogram). The dashed lines indicate the values 0.9, 1.2, and 1.5 (left histogram) or the values 0.9 and 1.2 (right histogram).

of the analyzed complexes, compared to only 14% for J3310 (Fig. 6B).

These results indicate that a proper level of TopA and accurate supercoiling are required for the regular distribution of ParB nucleoprotein complexes and suggest that the inhibition of their segregation may explain the blockage of sporulation.

DISCUSSION

During chromosome segregation, ParB proteins are responsible for organizing the *oriC*-proximal region of the chromosome. ParB-DNA complexes due to the interaction with polymers of ParA are distributed to specific positions in the future daughter cells; in *Streptomyces* they are uniformly arranged along the aerial hyphae. Our present studies revealed that the segregation complexes formed by ParB in *S. coelicolor* recruit topoisomerase I, TopA, which is required for their efficient distribution along the aerial hyphae.

Topoisomerases of classes I and II (gyrases) are responsible for maintaining chromosome topology, which is crucial for many important cellular processes, such as replication, transcription, and recombination, so a minimal set of these enzymes is essential in all species. Class I topoisomerases counteract gyrases removing negative supercoils from DNA by cutting a single strand of the DNA molecule. Previous reports from other groups (45) and our studies have shown that *S. coelicolor* TopA exhibits topoisomerase activity with features typical of the class I topoisomerases (54–56) in

that it relaxes negatively supercoiled DNA in the presence of divalent cations without requiring ATP (see Fig. S1 in the supplemental material). Most bacteria possess more than one gene encoding class I topoisomerases; for example, three *topA* paralogs are present in the *Helicobacter pylori* J99 genome. The *S. coelicolor* genome, however, appears to encode only a single TopA, which differs from the other class I topoisomerases in that it has a long C-terminal domain and lacks zinc fingers. The chromosomes of some *Streptomyces* species include genes encoding IB topoisomerases (a short poxvirus-origin topoisomerase of class I), but their expression and functions have not yet been studied. The presence of only one topoisomerase of class I in *S. coelicolor* is somewhat surprising but possibly could be connected with linearity of the *Streptomyces* chromosome and/or its growth as multigenomic hyphae in which the chromosomes do not appear to be condensed. Interestingly, the *Actinomycetes*, which exhibit a complex life cycle, possess a chromosome that seems to have become linearized during evolution (57). The advantage of linearization is not yet known, but it may be connected with hyphal growth and/or sporulation. Nevertheless, the telomere-associated proteins (TPs; mostly Tpg), which stabilize the ends of a chromosome, engage in intramolecular interactions that may cause the chromosome to assume a circular conformation with negative superhelicity. Interestingly, TopA was suggested to be associated with TP though in our studies we could not detect binding of TopA to the ends of a chromosome. Earlier studies indicated that the *topA* gene is essential for

FIG 6 The presence of clustered ParB-EGFP complexes in a TopA-depleted strain. (A) Fluorescence microscopy of noninduced PS02 (TopA depletion in the *parB-egfp* background). Images show DNA staining (red) and ParB-EGFP complexes (green) in aerial hyphae of PS02 and the control strain, J3310, as indicated; clustered ParB complexes are marked with red arrows. Scale bar, 5 μm. Note that ParB complexes are relatively regularly spaced in the control compared to the TopA-depleted strain. (B) Histogram showing the distribution of distances between ParB-EGFP foci in J3310 (the reference) and noninduced PS02.

the viability of *S. coelicolor* (45). Here, we demonstrate that the changes in chromosome supercoiling induced by TopA depletion inhibit growth and affect secondary metabolism and sporulation. Since TopA is the single class I topoisomerase in *S. coelicolor* and is the enzyme maintaining chromosome topology during transcription and replication, its indispensability is not unexpected. Interestingly, *topA* has been deleted from some other bacterial species. For example, transposon mutagenesis introduced mutations into the *topA* genes of *E. coli*, *Salmonella enterica* serovar Typhimurium, and *Shigella flexneri*, suggesting that the enzyme is not essential in these species (58–60). However, the viability of the *E. coli topA* mutants is the subject of some debate as Stockum et al. (61) showed that the growth of $\Delta topA$ cells failed unless compensatory mutations were present. In many pathogenic bacteria, such as *Mycobacterium tuberculosis* and *H. pylori*, attempts to delete *topA* have proven unsuccessful, suggesting that TopA is essential in these organisms (62, 63).

In addition to inhibiting growth, TopA depletion completely inhibited sporulation at the stage of septation and chromosome condensation in the aerial hyphae. In *Streptomyces* vegetative hyphae, chromosome replication is not followed by segregation, and the arrangement of the multiple chromosome copies does not seem to be precisely controlled; but during sporulation, the organization of the chromosomes faces a particularly challenging task. Shortly following their extensive replication, tens of chromosomes must be uniformly arranged along the hyphae and condensed to fit within the prespore space. Several proteins have been reported to affect chromosome compaction in *Streptomyces*, in-

cluding HU, Dps, and SMC proteins (21, 22, 64, 65). Different paralogs of HU and Dps are encoded in the *S. coelicolor* genome, and at least one of these is closely associated with sporulation and is responsible for ensuring the proper compaction of chromosomes in the spores. Similarly, analysis of *smc* mutants revealed the greatest phenotypic effect at the time of sporulation. Consistent with our observation that TopA depletion led to a higher degree of chromosome supercoiling, we found that a slightly suboptimal level of TopA was associated with high compaction of chromosomes in prespores and shortened distances between septa. This suggests that chromosome arrangement influences septation in *S. coelicolor*, as previously indicated by studies showing that disturbed chromosome segregation in ParA and ParB mutants was linked with irregular septation (i.e., the formation of minicompartments) (42, 66). Interestingly, the lowest level of TopA was associated with complete blockage of sporulation at the stage of septation and chromosome segregation in aerial hyphae. In line with the hypothesis that chromosome arrangement and compaction affect septal positioning, this inhibition of septation could be due to the presence of nonsegregated chromosomes. However, since TopA depletion resulted in a higher level of chromosome compaction, inhibition of chromosome segregation was somewhat surprising at first. One explanation for this phenomenon could be a lack of activation of supercoiling-sensitive genes required for the progress of sporulation. Such supercoiling-sensitive genes have been described in a few bacterial species, including *E. coli* (67), but have not yet been studied in *Streptomyces*. Alternatively, or additionally, the inhibition of sporulation may be connected with the interaction of TopA with ParB complexes, as suggested by our observation that a lower level of TopA is sufficient to overcome sporulation blockage in the absence of ParB, while providing ParB in *trans* brings back sporulation inhibition. It is therefore tempting to suggest that TopA is required to eliminate topological tensions created by the formation of ParB complexes and is thus necessary for their separation.

In *S. coelicolor*, ParB interacts with 21 *parS* sites (out of 24 in total) distributed within 400 kb around the *oriC* region of the chromosome. Some of these *parS* sites are paired in close proximity, and our earlier immunoprecipitation experiments suggested that these sites are the most efficiently bound by ParB (i.e., “strong” *parS* sites) (40). The results presented here indicate that TopA interacts with ParB complexes assembled on these strong *parS* sites. We did not find any evidence of direct binding between TopA and ParB, indicating that TopA interacts with the DNA that is engaged in complex formation. Analysis of TopA-DNA coimmunoprecipitation profiles revealed that the most efficient binding appeared to occur in the regions adjacent to *parS* sites. A previous study has shown that ParB binding affects the DNase I sensitivity of neighboring sequences, indicating that ParB may modify the DNA structure (40). Consistent with this observation, it has been demonstrated that ParB (Spo0I) in *Bacillus subtilis* spreads from the *parS* sites to adjacent regions of the chromosome (68). Structural studies of the *Caulobacter crescentus* chromosome showed that binding of *parS* sites can induce DNA compaction across more than 100 kb (11). Similarly, ParB of *Mycobacterium tuberculosis* was suggested to undergo conformational changes and induce the formation of large complexes (69). On the basis of these results, we hypothesize that in *S. coelicolor*, ParB binding imposes some topological tension on DNA and that this is detected and neutralized by TopA.

The formation of ParB complexes in the aerial hyphae of *S. coelicolor* is dependent on the developmental induction of the *parAB* promoter. Thus, ParB complexes were not observed in the nonsporulating (white) *Streptomyces* mutants (70). Earlier studies showed that in *S. coelicolor*, as in other bacteria, the ParA protein is required for efficient formation and regularly spaced placement of ParB complexes (42). Furthermore, the ParB-EGFP complexes were less intensive in the *S. coelicolor* Δsmc strain than in the wild-type control (21), possibly due to inefficient DNA compaction in the absence of SMC proteins. However, the distribution of ParB complexes seemed to be unaffected in the *S. coelicolor* Δsmc strain. In other bacteria, ParB complexes have been shown to recruit SMC proteins (25, 26). An *S. coelicolor* TopA-depleted strain showed inhibited sporulation and the “white” phenotype but, interestingly, no disturbance in the efficient formation of ParB complexes (i.e., their intensity was similar to levels observed in the wild-type background [data not shown]). On the other hand, TopA depletion inhibited the efficient separation of ParB complexes, which often remained paired. This may indicate that the inhibition of sporulation in the TopA-depleted strain arises solely from altered separation of ParB complexes rather than from inhibition of other important sporulation pathways. This notion is supported by the phenotype of the TopA depletion strain lacking ParB, in which complexes do not form and which is able to sporulate at lower levels of TopA. The partial restoration of sporulation in the TopA-depleted strain when *parB* was deleted confirms that sporulation inhibition, at least to some extent, results from lack of an interaction of TopA with ParB complexes that, presumably, is required for their resolution.

In summary, our present results prompt us to hypothesize that the formation of ParB complexes creates topological tension on adjacent DNA and that these are the sites of TopA recruitment. Furthermore, we suggest that TopA activity is required for the separation of ParB complexes, and inhibition of this process blocks septation. According to our hypothesis, the TopA-dependent resolution of ParB-bound chromosomes should occur before their compaction into prespores.

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