Type I topoisomerase activity is required for proper chromosomal segregation in *Escherichia coli*

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Type I DNA topoisomerases are ubiquitous enzymes involved in many aspects of DNA metabolism. *Escherichia coli* possesses two type I topoisomerase activities, DNA topoisomerase I (Topo I) and III (Topo III). The gene encoding Topo III (*topB*) can be deleted without affecting cell viability. Cells possessing a deletion of the gene encoding Topo I (*topA*) are only viable in the presence of an additional compensatory mutation. In the presence of compensatory mutations, Topo I deletion strains grow normally; however, if Topo III activity is repressed in these cells, they filament extensively and possess an abnormal nucleoid structure. These defects can be suppressed by the deletion of the *recA* gene, suggesting that these enzymes may be involved in RecA-mediated recombination and may specifically resolve recombination intermediates before partitioning.

The requirement for topoisomerases during replication has been observed in all organisms. DNA topoisomerases fall into two classes: type I and type II. In *Escherichia coli*, type II topoisomerases [DNA gyrase (1) and topoisomerase IV (Topo IV) (2)] are involved in many DNA metabolic processes, among which are decatenation (Topo IV) and the maintenance of superhelical density within the cell (DNA gyrase) (for a review see ref. 3).

There are also two type I topoisomerases in *E. coli*, topoisomerase I (Topo I), encoded by *topA* (4, 5), and topoisomerase III (Topo III), encoded by *topB* (6–9). Genetic studies of *topA* deletion strains of *E. coli* showed that these strains are viable only because they acquire a secondary compensatory mutation. In the *topA* deletion strains DM750 and DM800, these mutations have been mapped to the *gyrA* and *gyrB* genes, respectively (10, 11). These mutant alleles compensate for the loss of Topo I by encoding DNA gyrase subunits with diminished supercoiling activity (12, 13). This adaptation appears to be in response to the chromosome accumulating excess negative supercoils in the absence of Topo I activity. One consequence of the accumulation of the excess supercoils appears to be R-loop formation, which can inhibit cell growth (14, 15). This evidence suggests that the major function of Topo I *in vivo* may be in the regulation of superhelical density. Cells lacking Topo I activity, which have acquired compensatory mutations, grow normally, and no gross chromosomal segregation defects have been identified.

Topo III is extremely efficient in the decatenation of gapped, multiply interlinked DNA dimers and DNA replication intermediates *in vitro*. Based on this observation, it has been proposed that Topo III may play a role in chromosomal segregation (8). In addition, Topo III, in conjunction with the RecQ helicase, is capable of decatenating completely double-stranded interlinked DNA molecules (16), presumably via two sequential strand passage reactions. Previously, the decatenation of completely double-stranded interlinked circular DNA molecules was ascribed solely to type II topoisomerases. This observation has led to further speculation that type I topoisomerases may be involved in the segregation of nascent chromosomes.

Strains lacking *topB* exhibit a RecA-independent hyperrecombination phenotype between small direct repeated sequences (17). Interestingly, *topA* deletion strains also exhibit a similar phenotype (18) although it is unclear whether the two enzymes are involved in overlapping or distinct pathways. This suggests that type I topoisomerases also could be involved in the suppression of RecA-independent illegitimate recombination.

Although *topB* mutants in *E. coli* exhibit a hyperrecombination phenotype (17), they are viable and do not appear to acquire compensatory mutations (9). A possible explanation for this observation is that Topo I and Topo III may have partially overlapping activities. Consistent with this hypothesis, studies have indicated that *E. coli topA* deletion strains DM750 and DM800 resist chromosomal disruption of the *topB* gene (19). Although it is clear that Topo I plays a role in the maintenance of the superhelical density of chromosomal DNA in the cell, it is still unclear what role, if any, Topo III or type I topoisomerases in general play in chromosomal decatenation or recombination. In an effort to further define the role of *E. coli* type I topoisomerases in DNA metabolism, we have characterized the effect of repressing both type I topoisomerase activities in *E. coli*. Cells lacking both topoisomerase activities filament extensively and do not appear to segregate chromosomal DNA. The phenotype of these cells is not suppressed by the expression of the potent decatenating enzyme Topo IV; however, deletion of the *recA* gene suppresses this phenotype, suggesting that Topo I and Topo III may have a significant role in RecA-mediated DNA recombination rather than decatenation.

Materials and Methods

Bacterial Strains. The *E. coli* (K-12) strains used in this work are listed in Table 1 and Table 2, which is published as supplemental material on the PNAS web site, www.pnas.org.

Microbiological Techniques. Expression of the *topB* gene was studied by using minimal M9 medium supplemented with 0.25% casamino acids, 0.2% glycerol, and 0.005% arabinose or glucose. The cultures were grown at 37°C overnight in M9 medium containing arabinose. The overnight culture was diluted to $OD_{600} = 0.01$ into arabinose or glucose containing medium. The growth rates of cultures were determined by measuring the cell density at various times. Viable cell count was determined by plating on M9 agar medium supplemented with 0.25% casamino acids and 0.005% arabinose or glucose and incubated overnight at 37°C.

The bacterial strain DH5-α, which was used to prepare all plasmid DNAs, was prepared by $CaCl_2$ treatment (20) and used for transformation. Antibiotics, when required, were at the following concentrations: ampicillin, 100 μg/ml; chloramphenicol, 30 μg/ml, kanamycin, 30 μg/ml.

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Abbreviations: Topo I, topoisomerase I; Topo III, topoisomerase III; Topo IV, topoisomerase IV; DAPI, 4′,6-diamidino-2-phenylindole; IPTG, isopropyl β-D-thiogalactoside.

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Table 1. Bacterial strains

Strains	Genotype	Source or reference
MG1655	F^-, λ^-	Coli Genetic Stock Center
TP606	*sulA6209* (cotransducible with tet^r)	33
DM4100	*cysB am242*	10, 11
DM750	Δ(*cysB topA*)*218 acrA12 gyrA224*	10, 11
DM800	Δ(*cysB topA*)*204 acrA13 gyrB225*	10, 11
GD504	MG1655 *lexA3*::*malTn10*	M. Drolet
MA972	RFM443 Δ*recA306 srlR301*::*Tn10*	M. Drolet
QZ101	DM4100/pDE1	This work
QZ102	DM4100 Δ*topB*:: *aphA*/pDE1	This work
QZ103	MG1655 Δ*topB*:: *aphA*	P1:QZ102 × MG1655, kan^r
QZ104	DM750/pTBE302	This work
QZ105	DM750/pTBE33	This work
QZ106	DM750 Δ*topB*:: *aphA*/pTBE302	P1:QZ103 × QZ104, kan^r
QZ107	DM750 Δ*topB*:: *aphA*/pTBE302/pLex5BA-parEC	This work
QZ108	DM750/pTBE302/pMAYrecA	This work
QZ109	DM750 Δ*recA306 srlR301*::*Tn10*/pTBE302/pMAYrecA	P1:MA972 × QZ107, tet^r
QZ110	DM750 Δ*recA306 srlR301*::*Tn10* Δ*topB*:: *aphA*/pTBE302/pMAYrecA	P1:QZ103 × QZ108, kan^r tet^r
QZ111	DM750 Δ*recA306 srlR301*::*Tn10* Δ*topB*::*aphA*/pTBE302	This work
QZ112	DM750 Δ*topB*::*aphA lexA3*::*malTn10*/pTBE302	P1:GD504 × QZ106, tet^r
QZ113	DM750 Δ*topB*::*aphA sulA6209*/pTBE302	P1:TP606 × QZ106, kan^r tet^r

The genotypes and source of the *E. coli* strains used in this study are indicated. In the case of P1 transductions, relevant antibiotic selection markers also are provided.

GENETICS

P1 Transduction, Strain, and Plasmid Construction. Phage P1*vir* was grown on host strains carrying different antibiotic-resistant markers and used to transduce mutations into strains by using the method described by Miller (21). A complete *topB* deletion was constructed by amplifying 1,000 bp upstream and downstream of *topB* by PCR. A kanamycin-resistance cassette (*aphA*) then was inserted between the two regions, generating a complete deletion of the *topB* gene within a pMAK705 vector (pBK1). The procedure of Hamilton *et al.* (22) was then used to create the gene deletion in the *E. coli* strain DM4100, containing *topB* expression plasmid pDE1 (QZ101) (9). The expression plasmid was used in case deletion of the *topB* gene was detrimental to cell growth. Strain DM4100 Δ*topB* harboring plasmid pDE1(QZ102) was then used to generate a P1 transducing phage stock and to transduce a wild-type strain MG1655 to Δ*topB* (QZ103).

Construction of the *recA* deletions was accomplished by using pMAYrecA (provided by T. Hill, University of North Dakota). This plasmid contains the *recA* gene on a pMAK705 plasmid (22). Cells harboring pMAYrecA were transduced to Δ*recA* at 30°C and then the cells were cured of the plasmid by incubating the cells at 42°C (pMAK705 contains a temperature-sensitive replicon).

To achieve physiologically appropriate levels of Topo III expression, highly regulated transient expression plasmids were used to create expression plasmids pTBE302 and pTBE33. These pBAD plasmids contain the P_{BAD} promoter of the *araBAD* (arabinose) operon, the P_{BAD} regulatory gene araC, and a relatively low copy number plasmid pACYC origin (23). Plasmid pBAD33 contains the chloromphenicol-resistant (cat^r) gene, whereas pBAD30 contains the ampicillin-resistance (amp^r) marker. The *topB* gene sequence was excised from the pET *topB* expression plasmid pL-1 at *Nde*I and *Eco*RI sites and was filled in by using Klenow fragment (plasmid pL-1 contains an *Nde*I restriction site within the initiation codon of *topB* and an *Eco*RI restriction site immediately downstream of the translational stop codon). The fragment was then ligated into pBAD30 and pBAD33 that were digested and filled in at the *Xba*I site to create pTBE302 and pTBE33, respectively. This manipulation places the *topB* gene under the control of a poor Shine–Delgarno sequence within the vectors.

DNA gyrase compensatory mutations were transduced into strains by taking advantage of the fact that the gyrase compensatory mutations are associated with an unusual phenotype, Bgl^+ (10). These strains, unlike wild-type strains, are able to constitutively use β-glucosides as a carbon source. Wild-type *E. coli* K12 strains are Bgl^- and are unable to use β-glucosides because the genes of the *bgl* operon, which are required for catabolism of β-glucosides, are uninducible. Because the Bgl^+ phenotype is inseparable from the DNA gyrase compensatory mutations, it has been used to map the compensatory mutations. Screening for Bgl^+ was carried out on plates containing 5-bromo-4-chloro-3-indolyl β-D-glucoside (Sigma) at 40 μg/ml. The sugar fermentors (Bgl^+) are blue and nonfermentors (Bgl^-) are white colonies.

Western Blotting. Proteins were separated by SDS/PAGE (24) and transferred to a poly(vinylidene difluoride) membrane (Micron Separations, Westboro, MA). Immunodetection was performed with polyclonal antibodies against ParC and ParE as the primary and goat anti-rabbit Ig G conjugated to horseradish peroxidase (New England Biolabs) as the secondary antibody (25). ECL substrate (Amersham Pharmacia) was used to detect the secondary antibody, and the ParC and ParE proteins were visualized by using Kodak X-Omat film.

Fluorescence Microscopy. A 97-μl aliquot of the cell culture was mixed with 3 μl toluene, incubated at 37°C for 15 min. The mixture was then incubated with 11 μl of 5 μg/ml 4′,6-diamidino-2-phenylindole (DAPI) (Sigma) at room temperature for 5 min. Stained cells (2–3 μl) were spread on slides pretreated with poly-L-lysine (Sigma) and sealed with a cover glass. Fluorescence micrographs were recorded on a Nikon E600 equipped with a ×100 oil immersion objective, a 100-W mercury lamp, and standard DAPI filter sets. Images were captured with a SONY DKC 5000 catseye digital still camera system and processed with Adobe PHOTOSHOP. Exposures were between 1/50 and 1/15 s for phase-contrast and DAPI images.

Results

The Effect of *topB* Suppression in *topA* Deletion Strains. Although Topo III is not essential for growth and viability, previous studies

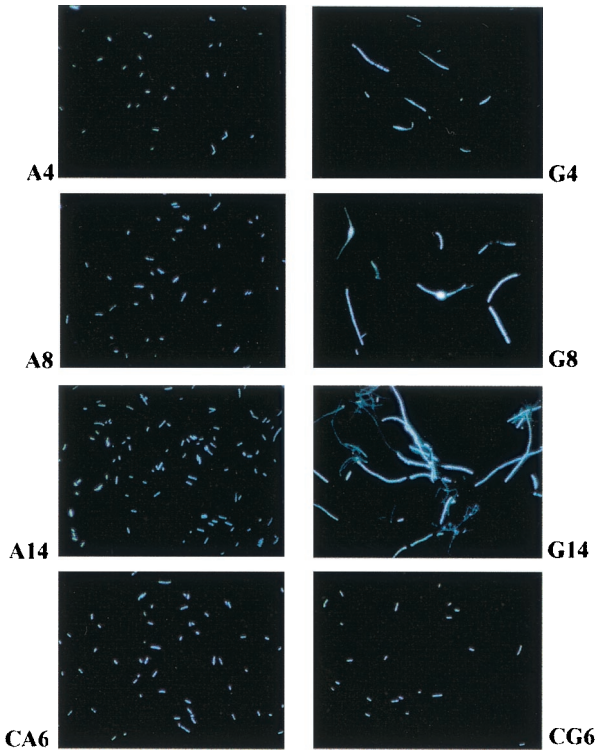

Fig. 1. The effect of the suppression of Topo III activity in DM750 cells. *E. coli* DM750, containing plasmid pTBE302, was transduced to Δ*topB*::*aphA* (QZ106) in the presence of arabinose. The cells were prepared as described in *Materials and Methods*. Their morphology was visualized by using fluorescence microscopy and DAPI staining. Cells grown in the presence of arabinose (A) or glucose (G) were observed after 4 (A4, G4), 8 (A8, G8), and 14 (A14, G14) h. The isogenic parent strain (DM750), containing pTBE302, was grown in glucose (CG) or arabinose (CA) containing medium and observed after 6 h.

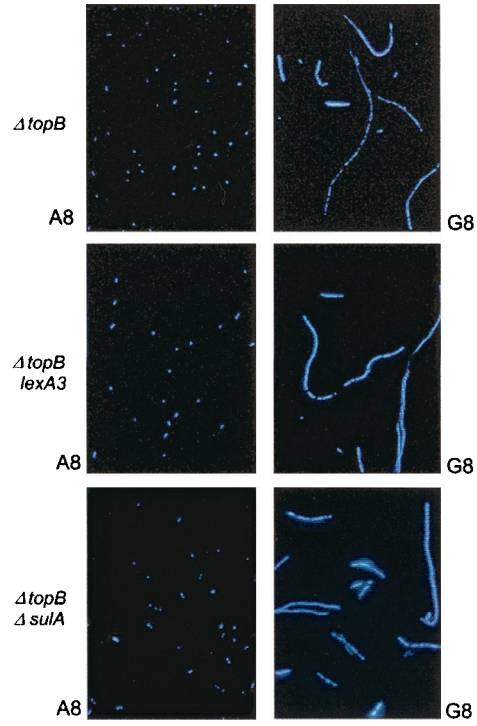

Fig. 2. *lexA3* and Δ*sulA* mutations have no effect on the chromosomal defects exhibited by DM750 Δ*topB* cells. DM750 Δ*topB* (QZ106), DM750 Δ*topB lexA*3 (QZ112), and DM750 Δ*topB* Δ*sulA* (QZ113) strains containing plasmid pTBE302 were grown in the presence of arabinose or glucose for 8 h. Similar to the isogenic parent strain (QZ106), the *lexA3* and Δ*sulA* derivatives exhibited a chromosomal segregation defect in the presence of glucose (G8), but grew normally in the presence of arabinose (A8).

have shown that it is extremely difficult to disrupt the *topB* gene in *topA* deletion strain DM750 unless the strain harbors a plasmid that is capable of expressing *topB* (19). This finding suggested that at least one type I topoisomerase activity may be required for viability in *E. coli*. Because Topo III was nonessential for cell growth (9), it seemed possible that Topo I could substitute for Topo III function *in vivo*. To analyze the effect of the inactivation of type I topoisomerases on DNA metabolism in "real time," a highly regulated pBAD transient *topB* expression plasmid (pTBE302) was constructed (23) and used to study the effect of repressing Topo III activity in *topA* deletion strains. Using this system, Topo III activity can be expressed in the presence of arabinose or repressed in the presence of glucose.

Strain DM750, harboring plasmid pTBE302 (QZ104), was grown in the presence of arabinose, and transduced to Δ*topB*::*aphA* (QZ106). This strain was then grown overnight in the presence of arabinose and diluted into and grown in M9 medium containing either arabinose or glucose. The cells grown in arabinose-containing medium exhibited a doubling time of about 1 h as assessed by viable cell count (Table 3, which is published as supplemental material). These cells and those of the isogenic parent strain, DM750, grew at a similar rate and possessed similar morphology (Fig. 1, compare CA6 and CG6 with A4, A8, and A14). Cells grown on glucose, however, had a viable cell count that was 6 orders of magnitude lower than the cells grown in arabinose. In addition, these cells became highly filamented over time (Fig. 1 G4, G8, and G14) and were characterized by aberrant nucleoid structures. These data indicated that, at least in this genetic background, it is essential to have at least one type I topoisomerase activity present in the cells and that these enzymes may play a role in chromosome segregation and cell division. Similar results were obtained when *E. coli* strain DM800 was used in the same experiments (data not shown).

The *topA* deletion strains DM750 and DM800 contain compensatory mutations in the genes encoding gyrase subunits (*gyrA224* in DM750 and *gyrB225* in DM800) that allow growth in the absence of Topo I activity (10, 11). It was unclear, therefore, whether the observed phenotype was caused by the repression of both type I topoisomerase activities or the repression of Topo III activity in the presence of gyrase compensatory mutations. To address this issue, the viability of *gyrA224* Δ*topB*::*aphA* and *gyrB225* Δ*topB*::*aphA* mutant strains was assessed (see Table 4, which is published as supplemental material). The presence of either compensatory allele had no effect on the transduction frequency relative to the wild-type strain, indicating that the *topB* deletion strains were viable in the presence of the gyrase compensatory mutations (Table 4). In addition, the morphology of the cells and nucleoids were identical to the wild-type strain (data not shown). This finding strongly suggests that the observed phenotype was caused by the repression of type I topoisomerase activity in the cells.

A *lexA3* or *sulA* Mutation Cannot Suppress the Growth Defect Phenotype of Δ*topA* Δ*topB* Cells. During normal cell growth, the LexA repressor of *E. coli* represses a set of genes called the SOS regulon (26). When DNA is damaged or replication is inhibited, RecA promotes the cleavage of the LexA repressor by inducing a specific proteolytic cleavage near the center of the repressor, resulting in induction of genes involved in the SOS response. This response helps organisms survive the lethal effect of DNA damage by

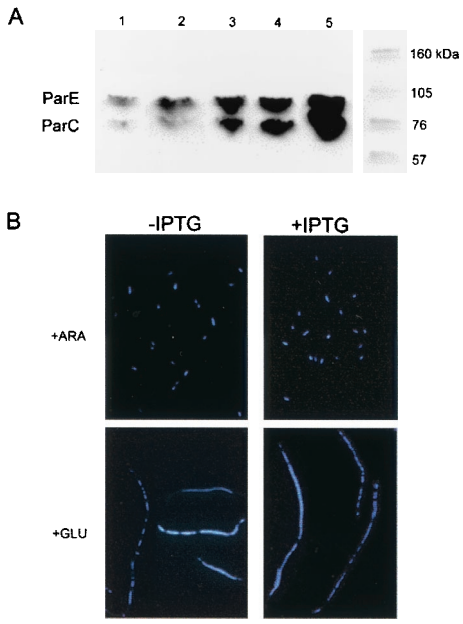

Fig. 3. Overexpression of Topo IV does not suppress the chromosomal defects of cells lacking type 1 topoisomerase activity. (*A*) DM750 cells carrying plasmid plex5BA-parEC were grown in culture for 8 h at 37°C. ECL-Western analysis was performed as described in *Materials and Methods* with anti-ParC and anti-ParE antibodies. Lane 1, DM750 cells; lanes 2–5, DM750 cells containing plasmid plex5BA-parEC in the presence of 0 μM (lane 2), 5 μM (lane 3), 25 μM (lane 4), and 100 μM (lane 5) IPTG. (*B*) DM750 Δ*topB* cells containing Topo III expression plasmid pTBE33 and Topo IV expression plasmid plex5BA-parEC were transduced to Δ*topB*::*aphA* (QZ107) in the presence of arabinose. An overnight culture was harvested and washed with medium containing no arabinose, and the cells were diluted into M9 medium containing arabinose or glucose (to repress Topo III activity) in the absence or presence of 5 μM IPTG (to induce Topo IV activity). Cells were removed after 8 h of incubation at 37°C, and their morphology was visualized by DAPI staining by using fluorescence microscopy.

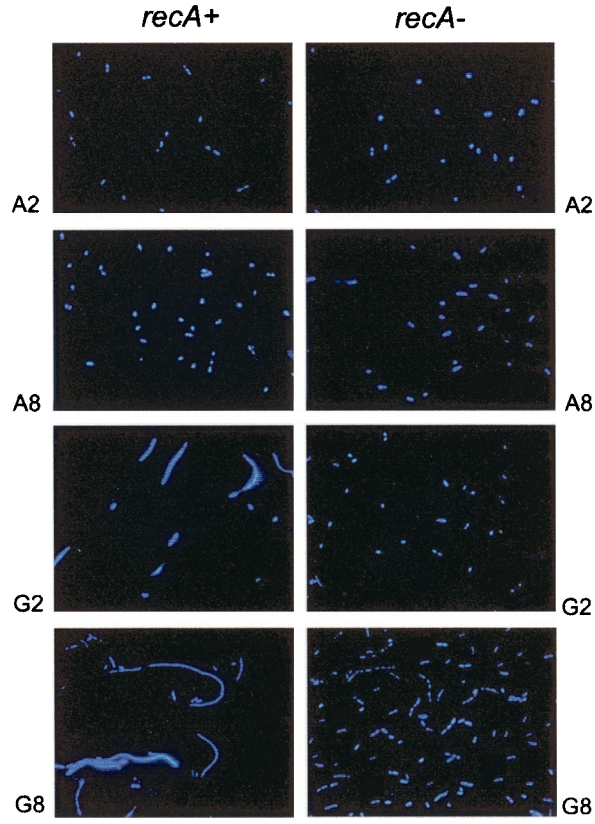

Fig. 4. A *recA* deletion suppresses the chromosomal defects of DM750Δ*topB* cells. DM750 Δ*topB* (QZ106) and DM750 Δ*topB* Δ*recA* (QZ111) cells, containing plasmid pTBE302, were grown overnight in the presence of arabinose. The cells were washed in medium without arabinose and then diluted into medium containing either glucose (G) or arabinose (A). The state of chromosomal DNA, observed after 2 and 8 h of incubation, was visualized by DAPI staining by using fluorescence microscopy. In the *recA* deletion mutants, the chromosomes began to segregate after 2–4 h of incubation regardless of the presence of arabinose or glucose.

combining increased expression of genes involved in excision, recombination, and mutagenic repair mechanisms with control of cell division exerted through *sulA* (*sfiA*), which delays cell division while repair is affected and causes cell filamentation (26).

Cells are highly filamented in the absence of both type I topoisomerase activities, suggesting that the SOS response may be responsible for the filamentation observed in the Δ*topA* Δ*topB* mutant strains. To assess this hypothesis, we transduced the *lexA3* mutation into DM750 cells. The *lexA3* allele encodes a noncleavable repressor protein so that the SOS response cannot be induced. DM750 Δ*topB* cells, carrying plasmid pTBE302, were transduced to *lexA3* (QZ112). The morphology of these cells and the state of the chromosomes, grown in the presence of glucose and arabinose, were then observed by using fluorescence microscopy (Fig. 2). In comparison to its SOS-inducible parent strain, neither the morphology nor the state of intracellular chromosomes of these *lexA3* mutant cells were found to have changed (i.e., they were still highly filamented and exhibited a segregation defect). In addition, DM750 Δ*topB*, harboring plasmid pTBE302, was transduced to Δ*sulA* (QZ113). These cells also filamented in the absence of type I topoisomerase activity (Fig. 2), suggesting that filamentation phenotype of DM750 Δ*topB* cells was not caused by SOS induction.

The Chromosomal Segregation Defect of Δ*topA* Δ*topB* Cells Is Not Caused by a Defect in Decatenation but by a Defect in Recombination. Evidence, *in vitro*, has shown that Topo III is a potent decatenase (8). Therefore, it was possible that the abnormal nucleoid morphology in cells lacking type I topoisomerase activity may have been caused by the presence of catenated bacterial chromosomes. Topo IV plays a primary role in decatenating newly replicated daughter chromosomes in *E. coli* (27). To investigate whether the nature of the aberrant nucleoid morphology in cells lacking type I topoisomerase activity was caused by the accumulation of catenated chromosomes, we examined whether the overexpression of Topo IV could suppress the phenotype of these mutants. An isopropyl β-D-thiogalactoside (IPTG)-inducible Topo IV expression plasmid, plex5BA-parEC (25), was used to increase Topo IV activity within the cells. Plasmid plex5BA-parEC was introduced into DM750 Δ*topB* containing plasmid pTBE33 (QZ107), a *topB* expression plasmid compatible with plex5BA-parEC. Cells were grown in the presence of arabinose, washed, and then diluted into medium containing glucose and IPTG. Overexpression of Topo IV activity was unable to rescue the growth deficient phenotype (Fig. 3), indicating that decatenation activity of Topo IV could not suppress the mutant phenotype (this finding also was confirmed in DM800, data not shown). The same result also was observed if Topo IV activity was induced before repression of Topo III activity (data not shown).

It has been observed that *topB* deletion strains exhibit an increased RecA-independent recombination frequency between small direct repeats; therefore, Topo III is responsible, either directly or indirectly, for the suppression of illegitimate recom-

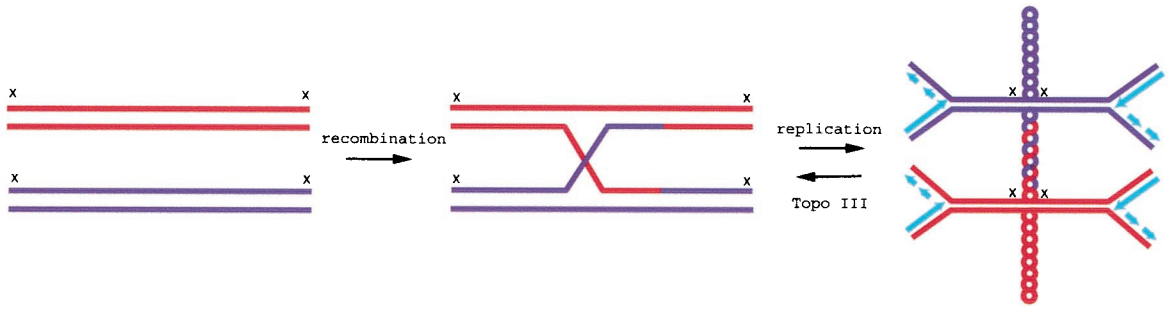

Fig. 5. Model for the role of type 1 topoisomerase activity in recombination. Two DNA helices (red and purple) initiate a strand exchange. As replication forks (shown in blue) approach the crossover junction, the twists in the helix of the exchanging strands (for simplicity, the twists in the exchanging strands are not shown) are converted into interlinks within the recombination junction. Topo III may unwind these interlinks to generate a structure that is recognized by the Holliday junction resolvase. X represents two homologous sequences located at a distance from one another on the same DNA segment. These sequences are brought together by this process and a deletion can be generated by another crossover event or by replication across the overwound junction.

bination in these strains. If this is the case, Topo III may be involved in the resolution of recombination intermediates. Failure to resolve such intermediates would lead to an increase in the recombination frequency in the cell. Because the *recA* gene is essential to all of the homologous recombination pathways in the bacterial cell, we tested this hypothesis by introducing a *recA* gene deletion into the chromosome of DM750 cells containing plasmid pTBE302. DM750 Δ*recA* cells, harboring pTBE302, then were transduced to Δ*topB*::*aphA* (in the presence of arabinose), and the strain (QZ111) was then analyzed for viability and morphology in the presence of arabinose or glucose. As previously described, the DM750 Δ*topB* cells (harboring plasmid pTBE302 (QZ106)) form no colonies in the presence of glucose at 37°C, but DM750 Δ*topB* Δ*recA* (QZ111) forms colonies in the presence of either glucose or arabinose. These cells, observed by DAPI staining, exhibited regularly spaced nucleoids similar to those observed in the parent strain in the presence of glucose or arabinose (Fig. 4). Interestingly, although the chromosomal segregation defect was completely suppressed in these cells, filamentation was only partially suppressed. Although cells exhibited normal morphology during log-phase growth, the cells became slightly filamented after entry into stationary phase. The same phenotype was observed by using DM800 Δ*topB* Δ*recA* cells in a similar experiment (data not shown).

Discussion

A highly regulated *topB* expression, plasmid pTBE302, was introduced into *topA* deletion strains to study the effect of the repression of type I topoisomerase activity in real time. The repression of Topo III activity in these strains was deleterious to cell growth and caused the cell to filament. Filamentation was also accompanied by abnormal nucleoid structures in which the DNA was strewn throughout the filament, suggesting that type I topoisomerases may be involved in some aspect of chromosomal segregation. Mutations that suppress SOS-induced filamentation, *lexA3* and Δ*sulA*, do not affect the nucleoid abnormalities or filamentation exhibited by Δ*topA* Δ*topB* cells. This finding does not rule out the possibility that SOS is induced in these cells; however, the defects observed in these cells are clearly not a result of SOS induction.

The phenotype of Δ*topA* Δ*topB* cells is not associated with the presence of compensatory mutations that are present in *topA* deletion strains (10, 11). A *topB* deletion was easily transduced into strains that contained only the gyrase compensatory mutations. The cells grew normally and possessed normal morphology, suggesting that the chromosomal segregation defects observed upon the repression of type I topoisomerase activity are caused solely by the absence of these enzymatic activities and are not related to the presence of any other mutations.

The defect in chromosomal segregation observed in Δ*topA* Δ*topB* mutants was not caused by a defect in chromosomal decatenation because overexpression of Topo IV, a potent decatenase, did not suppress the phenotype of the Δ*topA* Δ*topB* mutants. The segregation defect of Δ*topA* Δ*topB* cells was, however, suppressed by the deletion of the *recA* gene. The Δ*topA* Δ*topB* Δ*recA* cells exhibit distinct nucleoids regardless of the expression of Topo III, suggesting that type I topoisomerases are primarily involved in recombination as opposed to being directly involved in chromosomal decatenation. Interestingly, although Δ*topA* Δ*topB* Δ*recA* cells have isolated and distinct nucleoids, they still filament slightly during stationary phase. This phenomenon is also independent of the SOS response because Δ*recA* cells are incapable of SOS induction.

An essential question is what step in recombination could be affected by type I topoisomerases? Whether or not recombination is legitimate or illegitimate, a Holliday junction is formed between the two participating molecules. During this process, two independent DNA molecules are covalently linked via the junction. If DNA replication occurs in molecules undergoing recombination, torsional stress could be transferred to the recombination junction, resulting in the accumulation of interlinks between the homologous strands engaged in the exchange (Fig. 5). The presence of a topoisomerase with a preference for binding single-strand DNA could play a role in the unlinking of this intermediate and generate an "open" structure that could be recognized by the Holliday junction resolvase. This model predicts that mutations that affect events before strand incision (*recA* and *recBCD* mutations) should suppress the chromosomal segregation defect observed in cells lacking type I topoisomerase activity. Mutations that affect events after Holliday junction formation (*recG*, *ruvABC*), on the other hand, should not suppress the segregation defect.

It recently has been shown that yeast Topo III interacts with the Sgs1 helicase (28, 29). The Sgs1 helicase is a member of the RecQ family of helicases. In addition, *E. coli* Topo III and RecQ protein also appear to act synergistically with one another, creating a very efficient catenation activity (16). It is tempting to hypothesize that *E. coli* type I topoisomerases and helicases, similar to what has been proposed for eukaryotes (30- 32), are capable of interacting to form a "toposome" that is capable of acting as an unwinding machine. In support of this hypothesis, have we recently found that Δ*topA* Δ*recQ* mutants exhibit a phenotype identical to Δ*topA* Δ*topB* mutants (Q.Z. and R.J.D., unpublished work). Therefore, a toposome could be essential to resolving recombination intermediates before chromosome partitioning in both prokaryotes and eukaryotes.

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