Topoisomerase III Can Serve as the Cellular Decatenase in Escherichia coli*

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Escherichia coli has four DNA topoisomerases, two type IIA DNA gyrase and topoisomerase (Topo)\(^I\) IV, and two type IA Topo I and Topo III. The roles in the cell played by three of these proteins is reasonably well defined (1–4). DNA gyrase is the major facilitator of DNA replication, acting to convert the positive supercoils generated directly to negative ones. Mutations in either gyrA or gyrB, encoding the subunits of gyrase, are conditionally lethal, and incubation of such mutated strains at the non-permissive temperature causes a rapid cessation of DNA relaxation. Topo IV is the cellular decatenase, responsible for unlinking the daughter chromosomes so that chromosome segregation is accomplished in a timely manner. Mutations in either parE or parC, encoding the subunits of Topo IV, are also conditionally lethal. Incubation of either parE or parC temperature-sensitive strains at the non-permissive temperature results in the elaboration of a par phenotype, where a large DNA mass accumulates in the center of an elongated cell. This DNA mass results from repeated initiation of replication on chromosomes that have not segregated. Topo IV appears also to be able to support replication fork progression at about one-half the rate supported by gyrase (5). Topo I acts as a balancing force to gyrase, such that the net negative superhelicity of the chromosome is maintained within an optimal range. Inactivation of Topo I is tolerated only in the presence of compensatory mutations in gyrase that act to reduce its activity. In the case of Topo III, there is more known about its biochemical activities than its role in the cell.

Topo III was detected originally as a superhelical DNA relaxing activity present in extracts prepared from cells deficient in Topo I (6) and as an activity that bound to novobiocin affinity columns (7). We purified Topo III as an activity capable of decatenating replicating daughter chromosomes (8) after we determined that gyrase was inefficient at this task (9). Whereas Topo III was inefficient at superhelical DNA relaxation, requiring high temperature for optimal activity, it was very efficient at decatenating multiply linked daughter chromosomes as long as one of the DNA circles contained a small gap. These observations suggested that the productive binding site for the enzyme was single-stranded DNA (10), which has been borne out by all subsequent studies.

Cloning of the gene for Topo III (topB (10)) indicated that the protein had considerable identity to Topo I, although it lacked the zinc finger region of the latter enzyme. Rothstein and colleagues (11) reported contemporaneously that EDRI, a gene in yeast that, when mutated, caused enhanced deletion of SUP4-o when it was flanked by \(\delta\) sequences (the long terminal repeats of the Ty retrotransposon) (12), encoded a protein that had significant similarity to \(E.\ coli\) Topo I and therefore renamed the gene TOP3. TOP3 was actually more homologous to \(E.\ coli\) Topo III than to \(E.\ coli\) Topo I and proved to be the first eukaryotic member of the Topo III family identified.

Disruption of topB was noted originally not to cause any obvious phenotype (10). Subsequently, Schofield et al. (13) showed that mutR, a gene that, when mutated, caused a 5-fold increase in RecA-independent recombination between short directly repeated sequences, was allelic with topB. To date, this observation describes the only known phenotype of \(topB\) deficiencies over an otherwise wild-type background. Interestingly, the situation in eukaryotes is very different, where the phenotypes of deficiencies in Top3 clearly indicate that the enzyme plays a significant role in the maintenance of genomic integrity. Deletion of \(TOP3\) in Saccharomyces cerevisiae causes a slow growth phenotype, the hyper-recombination phenotype mentioned above (11), and sensitivity to several DNA-damaging agents as a result of disruption of the intra-S phase checkpoint (14). In Schizosaccharomyces pombe, \(TOP3\) is an essential gene. Cells deleted for \(TOP3\) undergo only a few rounds of abnormal nuclear division before death (15, 16). In this organism, dysregulation of Top3 function has been linked to low Cdc2-cyclin B activity, causing a defect in the late stages of homologous recombination (17). In vertebrates, there are two Top3 isozymes, \(\alpha\) (18) and \(\beta\) (19). Deletion of the former causes embryonic lethality in mice (20). Many of these effects are thought to be manifested via interaction, or the lack thereof, of Top3 with members of the RecQ-like family of DNA helicases (21).

Here we show that \(E.\ coli\) Topo III can act as the principal
cellular decatenase, capable of unlinking replicating daughter chromosomes in vivo, and provide evidence that this decatenation proceeds via the removal of precatenanes.

EXPERIMENTAL PROCEDURES

Enzymes, Reagents, Proteins, and Antibodies—Restriction enzymes and bacteriophage T4 DNA ligase were from New England Biolabs. Pfu polymerase was from Stratagene. Hybrid ECL nitrocellulose membrane and ECL-Western blotting detection reagents were from Amer sham Biosciences. DNA polymerase I, RNase H, and E. coli DNA ligase were from Roche Molecular Biochemicals. Topo III was prepared as described by Peng and Marians (23). DNA gyrase was prepared as described under “Materials and Methods.” DNA polymerase I, RNase H, and polymerase were from Stratagene. Hybond ECL nitrocellulose membrane and ECL-Western blotting detection reagents were from Amersham Biosciences. DNA polymerase I and Bsm HI sites. ParEC316Y expressed from this plasmid was 600 of 0.5. Equal volumes of cell culture and a solution of 4% paraformaldehyde and 1 μl/ml 4,6-diamidino-2-phenylindole in 1× phosphate-buffered saline were mixed, and the cells were fixed for 30 min at 4 °C on a rotator. Aliquots (40 μl) of the fixed cells were spread on polysine-coated glass slides and allowed to air-dry. Samples were observed with an Olympus AX-70 microscope. Fluorescence and differential interference contrast images were acquired with a Hamamatsu Orca-ER CCD camera using MetaMorph software (Universal Imaging). Figures were prepared for publication using Adobe Photoshop 7.0 software.

Plasmid Constructions—The construction of the plasmid kan-inc3 suppression library has been described (26). The construction of plasmid kan-inc3 suppressor library carrying the genomic insert described under “Results.” plasmid suppressor-6 is a plasmid from the plasmid suppressor-6 plasmid DNAs, was from Invitrogen. Cultures were grown in LB with appropriate antibiotics to an A600 of 0.5. Equal volumes of cell culture and a solution of 4% paraformaldehyde and 1 μg/ml 4,6-diamidino-2-phenylindole in 1× phosphate-buffered saline were mixed, and the cells were fixed for 30 min at 4 °C on a rotator. Aliquots (40 μl) of the fixed cells were spread on polysine-coated glass slides and allowed to air-dry. Samples were observed with an Olympus AX-70 microscope. Fluorescence and differential interference contrast images were acquired with a Hamamatsu Orca-ER CCD camera using MetaMorph software (Universal Imaging). Figures were prepared for publication using Adobe Photoshop 7.0 software.

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Experimental Microbiological Techniques—W3110 (28) was used. A DNA fragment was constructed by overlap PCR such that 33 random, coding bp were inserted in-frame between the ATG and TAA codons of parE flanked by about 700 bp of upstream and downstream genomic sequence. The extreme outside primers also contained a BamHI site. The DNA was digested with BamHI and introduced into the BamHI site of pK30, which carries cat and sacB, and is replicated via a temperature-sensitive pSC101 origin, to give pK30parEKob. This plasmid was transformed into W3110, and the transformants were plated at 42 °C. A chloramphenicol-resistant colony was used to grow cells for transformation with pLEXparE (27) at 42 °C. A single chloramphenicol-resistant, ampicillin-resistant (because of pLEXparE) colony was diluted in media and plated at 30 °C on plates containing 100 μg/ml IPTG, 5% sucrose, and ampicillin. These colonies were then replica-plated in the presence and absence of IPTG and also challenged with a mixture containing chloramphenicol and chloramphenicol-resistant IPTG-dependent colony was then checked by PCR for the presence of the genomic parE deletion that has the sequence Met-His-Ala-Ile-Thr-

RESULTS

Identification of topB as a High Copy Suppressor of parC1215—We previously conducted a screen for high copy suppressors of the conditional lethality of W3110 (26), which revealed an interaction between Topo IV and the σ70 and γ subunits of the DNA polymerase III holoenzyme. Further investigation of this observation led to the discovery that the Topo IV activity was temporally regulated during the cell cycle (33). We conducted a screen for high copy suppressors of the conditional lethality of C600parC1215 (24). A plasmid library (26) was generated by inserting size-selected E. coli genomic DNA into a pBR322 vector (pBR322-kan-inc3) that carried a mutation giving it a slightly higher copy number than normal. All E. coli genes present in this library were expressed from their natural promoters. Library DNA was transformed into C600parC1215, and about 80,000 transformants were plated at 42 °C. Of the 13 colonies that grew, 6 regrew at both the permissive and nonpermissive temperatures. Plasmid DNA was isolated from these six clones and retransformed into C600parC1215. Plasmid DNA from all six potential suppressor clones conferred to C600parC1215 the ability to grow at 42 °C (Table I). This screen yielded four different genes that functioned as high copy suppressors of the temperature sensitivity of parC1215: parC (two clones), as expected; topB (two clones), as discussed in this report; dnaN (one clone), encoding the β subunit of the DNA polymerase III holoenzyme; and spaC (suppressor of parC A) (one clone), an integral membrane protein that links chromosome segregation to cellular architecture. The interaction of the proteins encoded by these latter two genes with Topo IV will be the subject of a separate report. The ends of the genomic DNA insert in pBR-suppressor-6 were determined using primers flanking the BamHI insertion...
**Characterization of the Rescue of W3110parE10 by Overexpression of Topo III**—Stationary overnight cultures grown at 42 °C of W3110(pBADTopB) and W3110parE10(pBADTopB) were diluted to an A₆₀₀ of 0.02 in fresh media, and the growth of the cultures were followed at 42 °C (Fig. 1A). The wild-type culture commenced growth immediately; on the other hand, the mutant culture exhibited a pronounced lag but then grew at a rate that was roughly the same as the wild-type culture. It should be noted that under the conditions described, W3110parE10 itself will not grow at all, cells having reached a terminal phenotype during the original overnight incubation at the non-permissive temperature. Furthermore, examination of the cell and nucleoid morphology under conditions of topB rescue demonstrated that Topo III was acting to decatenate the daughter chromosomes (Fig. 1, B and C).

Wild-type and mutant parE10 cells grown at 30 °C in the presence of pBADTopB and arabinose gave essentially indistinguishable morphologies with about 95% of the population appearing as small cells with either two or four nucleoids. The other 5% of the population was made up of an assortment of abnormally appearing cells (Fig. 1, B and C). When these two strains were grown at the non-permissive temperature as described above, the fraction of wild-type appearing cells in the W3110(pBADtopB) culture decreased to 83%, and there was a significant fraction of small cells with condensed, non-separated nucleoids (type 2, Fig. 1C). In addition, filaments could be observed that contained nucleoids that either were condensed (type 6, Fig. 1C) or appeared normal (type 5, Fig. 1C); anucleates were observed as well (type 3, Fig. 1C). Interestingly, the largest population of abnormally appearing cells were small cells clearly containing two nucleoids that were linked together by DNA (type 4, Fig. 1C), suggesting that either chromosome decatenation or segregation was delayed at 42 °C compared with 30 °C. On the other hand, whereas the culture of W3110parE10(pBADTopB) grown at the non-permissive temperature contained a significant fraction of wild-type appearing cells (43%, Fig. 1C), the proportion of type 2 and type 3 cells was elevated, suggesting that the rescue by overexpression of Topo III was not completely penetrant. However, when compared with the cellular morphologies present in a culture of W3110parE10 grown at the non-permissive temperature, it was clear that overexpression of Topo III resulted in rescue of the Topo IV defect and affected decatenation of the daughter chromosomes. W3110parE10(pBR-kan-inc3) cells grown overnight at 30 °C, diluted by 100-fold, and then shifted to 42 °C for 2 h exhibited a classic par phenotype (Fig. 1B). Half the cells in the population were long filaments with either a central very large, dense nucleoid or two symmetrically placed, somewhat smaller dense nucleoids. The other half of the population consisted of anucleate cells (Fig. 1C). No wild-type cells were observed at all.

pBR-suppressor-6 and pBADtopB exhibited different efficiencies of rescue of C6000parC1215, suggesting that there was a certain minimal level of Topo III needed to effect chromosome decatenation. We therefore examined the levels of Topo III produced from these plasmids in W3110parE10 growing at 42 °C (Fig. 2). We used the parE10 strain, rather than C6000parC1215, because in the former strain both plasmids rescued to the same extent, suggesting that we would obtain an unbiased value for the extent of overproduction. The pBRsuppressor-6 plasmid produced about 60-fold more Topo III at 42 °C than was present in W3110parC1215, whereas the pBADtopB overproduced Topo III by about 180-fold. The level of Topo III in wild-type cells is very low, having been estimated at about 10 copies per cell. Our current estimate for Topo IV is about 1000 copies per cell (33); thus, Topo III works effectively as the cellular decatenase when it is present at roughly the same concentration as Topo IV.

**Topo III Is Substituting for the Decatenation Activity of Topo IV**—If the observed rescue by Topo III was a result of it substituting completely for the activity of Topo IV, then overexpression of Topo III should also rescue E. coli in the complete absence of Topo IV. To assess this issue, we constructed a conditional parE null strain. W3110ΔparE(pLEXparE) carries a precise deletion of the chromosomal parE covered by an expression plasmid (pLEX5BA (35)) where the parE ORF is under the tightly regulated control of a modified lac operator. Consequently, growth of this strain at any temperature is dependent on the presence of IPTG to induce ParE expression. pBADtopB could not rescue growth of this strain at 37 °C in the presence of arabinose when IPTG was omitted from the culture medium. In the presence and absence of arabinose to induce Topo III expression, the plating efficiency of W3110ΔparE(pLEXparE, pBADtopB) was 0.96 (with arabinose/without arabinose) when IPTG was also present. In the absence of IPTG, no growth was observed at all in either the presence or absence of arabinose. We considered three explanations for the lack of rescue of the parE deletion strain. The first relates to
the possibility that the observed rescue by overexpression of Topo III in the temperature-sensitive parE and parC strains was dependent on a partial level of Topo IV decatenation activity, and therefore, because the ParE deletion strain had no Topo IV, higher levels of Topo III were required to effect rescue.

To address this possibility, we constructed a modified topB expression vector that could produce higher levels of Topo III than pBADtopB; however, we found that even modest increases in the levels of Topo III expression over those produced by pBADtopB were toxic to wild-type cells. Thus, we probed the nature of the Topo IV activity present in W3110 parE10 by purifying the mutant protein (the amino acid substitution is C316Y) and assaying its activity in vitro (Fig. 3). Both the superhelical DNA relaxation and the decatenation activity of

**Fig. 1. Characterization of rescue of W3110 parE10 by overexpression of topB.** A, growth curves of W3110(pBADtopB) and W3110 parE10(pBADtopB) at 42 °C. Overnight cultures grown in rich medium were diluted to an A600 of 0.02, and their growth in rich medium was followed at 42 °C. B, cell and nucleoid morphologies of W3110(pBADtopB), W3110 parE10(pBADtopB), and W3110 parE10(pBR-kan-inc3) growing at 30 and 42 °C. Samples were taken from cultures of the former two strains at an A600 of 0.7. The latter strain was grown as an overnight culture at 30 °C; fresh media were inoculated to an A600 of 0.02, and the culture was then grown for 2 h at 42 °C. Preparation of cells and fluorescence microscopy were as described under “Experimental Procedures.” C, comparison of the types of cells present in the three different cultures at 42 °C. Roughly 300–400 cells from each culture were examined at random and assigned, based on cell and nucleoid morphology, to the six cell types illustrated in the figure. Type 1 cells were wild type in appearance. Type 2 cells were small but contained condensed nucleoids. Type 3 cells were anucleates. Type 4 cells were normal-sized but had nucleoids that appeared connected by DNA. Type 5 cells were filaments with non-condensed nucleoids, whereas type 6 cells were filaments with condensed nucleoids.
III to levels roughly comparable with those of Topo IV in a null strain. We therefore conclude that overexpression of Topo III in some decatenation activity of the latter enzyme. Overexpression of Topo III that W3110parE10 can rescue E. coli strain W3110 (parE−/H9004) growing at 37°C, yet still could not rescue the W3110parE10/H9004 strain, where, presumably, because the parE deletion strain reflects a physical requirement for ParE at some stage of the cell cycle and that this requirement is distinct from the requirement for ParE to form Topo IV that is active for decatenation. ParEC316Y mutation is likely to cause folding defects, at the nonpermissive temperature the same situation holds, i.e. there is a surfeit of free ParC in the cell. On the other hand, our experience with various parC expression constructs is consistent with high level, overexpression of ParC being toxic to wild-type cells.

A third possibility is that the failure of Topo III overexpression to rescue the parE deletion strain reflects a physical requirement for ParE at some stage of the cell cycle and that this requirement is distinct from the requirement for ParE to form Topo IV that is active for decatenation.

Topo III Is Very Efficient at Removing Precatenanes—Under most circumstances, Topo III will be a very poor superhelical DNA relaxing enzyme. This is because of its strong dependence on single-stranded DNA for activity and an apparently poor ability to bind and partially denature double-stranded DNA to create its substrate. Thus, maximal relaxation activity in vitro requires elevated temperature to increase the single-stranded character of negatively supercoiled substrates (8). Conse-
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The precatenane unlinking activity of Topo III does not require gaps between Okazaki fragments in the nascent daughter chromosomes. Treatment of the precatenated DNA with RNase H, DNA ligase, and DNA polymerase I (RLP) seals all the Okazaki fragments. The denaturing alkaline-agarose gel shows the nascent DNA present in the precatenated DNA before (lane 1) and after (lane 2) treatment with RLP. Because the plasmid replicates bidirectionally, when sealed, all the Okazaki fragments synthesized by the clockwise-moving fork will be joined to the leading strand synthesized by the counterclockwise-moving fork and vice versa, resulting in a continuous linear nascent DNA product that spans the distance between the two Ter sites (5 kb).

Plasmid prevention. We showed previously, using neutral agarose gel electrophoresis and electron microscopy, that the partially replicated daughter duplexes in these molecules are intertwined in precatenanes. The precatenanes take the form of a ladder of bands during neutral agarose gel electrophoresis, the mobility and spacing of which almost precisely matches that of complete catenanes. The steps in the ladder differ by one precatenane.

A replication system reconstituted with purified proteins was used to replicate a plasmid DNA template carrying oriC sites 2 kb clockwise and 3 kb counterclockwise from the origin. Both Ter sites were oriented to stop replication fork progression. Thus, when the replication reaction is performed in the presence of Tus, the forks formed at oriC will be terminated and complete replication of the plasmid prevented. We showed previously, using neutral agarose gel electrophoresis and electron microscopy, that the partially replicated daughter duplexes in these molecules are intertwined in precatenanes. The precatenanes take the form of a ladder of bands during neutral agarose gel electrophoresis, the mobility and spacing of which almost precisely matches that of complete catenanes. The steps in the ladder differ by one precatenane. Such a ladder can be observed in the partially replicated DNA that we prepared (Fig. 4A, lane 1). The gaps on the lagging strand persist at the replication fork.

As noted originally by Champoux and Been, the positive linkages between replicating daughter chromosomes that arise as a result of unwinding of the parental duplex can distribute either ahead of the replication fork as positive supercoils or behind the replication fork as precatenanes, torus-like topological linkages that, if replication were completed, would become catenanes. This equilibrium is governed by the extent of DNA that is replicated: with positive supercoils predominating when the majority of the template is replicated and precatenanes predominating when the majority of the template is unreplicated (38). The differential action of Topo IV and DNA gyrase in the cell can be explained, at least in part, by their preference for positive supercoils or catenanes as substrates.

The precatenated DNA substrate was treated with either DNA gyrase, Topo III, or Topo IV (Fig. 4A). Both Topo III and Topo IV could clearly remove the precatenanes, as evinced by the disappearance of the DNA ladder and the accumulation of LRI DNA. On the other hand, gyrase was very inefficient at precatenane removal, even at concentrations 25-fold greater than those at which Topo III and Topo IV were effective. These data are consistent with previous observations that address the substrate specificity of Topo IV and gyrase. Topo III and Topo IV were powerful precatenane-removing enzymes, with activity detectable at sub-nanomolar concentrations (Fig. 4B and C). The specific activities of the two enzymes in this reaction were comparable (Fig. 4C). The DNA replication reaction used to prepare the precatenated substrate lacked DNA ligase; thus there are nicks present between the Okazaki fragments that could represent the sites of Topo III action. To assess if this was the case, the precatenated substrate was treated with a combination of DNA polymerase I, DNA ligase, and RNase H to remove the RNA primers and seal the Okazaki fragments, resulting in the conversion of all the nascent DNA to a long linear strand equal in length to the distance between the two Ter sites (Fig. 5A). Interestingly, this treatment had no effect on the ability of Topo III to remove the precatenanes (Fig. 5B). However, this process does not elimi-
nate all the single-stranded gaps on the lagging strand. We showed previously that when a replication fork collides with the Tus-Ter complex, the site of leading-strand arrest is within a nucleotide or two of the edge of Ter site, whereas the site of the last primer on the lagging strand is about 60 nucleotides upstream, leaving a gap at the fork in the partially replicated molecule (41). This arrangement represents a snapshot of the disposition of the nascent strands at the growing point of a replication fork. This gap cannot be sealed by the treatment described above and thus represents a probable position for the site of Topo III action.

These studies demonstrate directly that Topo III can remove precatenanes. It is thus likely that the ability demonstrated here of Topo III to substitute for the decatenation activity of Topo IV in vivo is a manifestation of this capacity.

**DISCUSSION**

Although the biochemical properties of Topo III are well established, the manner in which these activities are expressed in the cell are far from clear. The purified protein is capable of relaxing supercoiled DNA and decatenating linked DNA circles (8). Its activity is strongly dependent on single-stranded DNA to provide a productive binding site on the substrate. Thus, negatively supercoiled DNA serves as a substrate because of its underwound character, but positively supercoiled DNA, with its overwound character, is not a substrate. Similarly, Topo III-catalyzed decatenation requires that the DNA rings contain discontinuities in the strands and is stimulated when actual gaps are provided (8). We have shown that Topo III can support both nascent strand progression (40) and daughter chromosome decatenation (22) in oriC replication systems in vitro. However, deletion of topB is of little consequence to the cell (8). Here we report a genetic system where survival of E. coli is dependent on overexpression of Topo III. Increasing the level of Topo III in the cell by about 70-fold was sufficient to allow cells with temperature-sensitive mutations in either parC or parE, the genes encoding the subunits of Topo IV, to grow at the nonpermissive temperature. Although, as indicated by the cellular and nucleoid morphologies, rescue was not complete, it was clear that Topo III was decatenating the replicating daughter chromosomes, allowing them to be partitioned and thus permitting successful cytokinesis.  

There are two possible modes of strand unlinking during θ-type DNA replication (36): removal of positive supercoils ahead of the replication fork and removal of positive precatenanes behind the fork. Execution of the latter mode is ultimately required for complete removal of all topological linkages between daughter chromosomes. The biochemical properties of Topo III suggested that it likely acts behind the fork, removing precatenanes. In support of this, we demonstrated that the rate of Topo III-supported fork progression from early replication intermediates where replication forks had stalled because of accumulation of positive supercoils actually increased as the extent of the template replicated increased. In contrast, the rate of gyrase-supported fork progression decreased as the extent of the template replicated increased. We interpreted these observations to mean that at the beginning of the reaction, when only about 20% of the DNA was replicated, most of the positive windings existed as positive supercoils, substrates for DNA gyrase, not Topo III. As replication proceeded, precatenanes, substrates for Topo III and not gyrase, accumulated.

Where is Topo III acting in vivo? In support of the previous observations, we have demonstrated directly in this report that Topo III and Topo IV, but not gyrase, can efficiently remove precatenanes. We therefore think it likely that the observed ability of Topo III to support daughter chromosome decatenation arises as a result of it working behind the fork to unlink precatenanes. However, the actual sites on the chromosome that can be occupied successfully by Topo III to accomplish this task is limited because of its requirement for single-stranded DNA. Topo III could be acting in one of two general locations: at gaps produced on the lagging strand during the process of sealing of Okazaki fragments or at the replication fork itself, in the gap on the lagging strand between the 3'-end of the nascent leading strand and the 5'-end of the last primer for Okazaki fragment synthesis. We favor the latter as the likely preferred location of Topo III because chromosome segregation requires that the topological linkage between the daughters be reduced to zero. Thus, in order for Topo III to support daughter chromosome unlinking, it must be able to act after the last parental duplex turn is unwound and before all gaps in the daughters are sealed. The gap on the lagging strand at the forks will persist after complete unwinding of the parental duplex until the leading strand from the clockwise-moving fork meets up with the 5'-end of the last Okazaki fragment from the counter-clockwise-moving fork, for example (Fig. 6). The gaps between Okazaki fragments may be too transient to provide a site at which Topo III can complete its task. This competition between sealing of gaps in the daughter chromosomes and productive Topo III binding provides a possible explanation for the incomplete penetrance of the rescue by Topo III, because once all the gaps are sealed, Topo III will not be able to work.

Topo III could not, when expression of the ParE was shut down, rescue growth of a strain that had been deleted for parE and was dependent on expression of ParE from an IPTG-inducible promoter. We do not think that this observation reflects the fact that some residual Topo IV activity was required for the rescue of the temperature-sensitive parE and parC strains by Topo III. At least in the case of the parE strain, we could demonstrate that the endogenous Topo IV was clearly temperature-sensitive for both its superhelical DNA relaxation and decatenation activities, and the expression of a 3-fold greater level of Topo III than was sufficient to rescue the temperature-sensitive parE strain was still insufficient to rescue the ΔparE strain in the absence of IPTG. The simplest explanation for the lack of rescue of the null is that free ParC is toxic to the cell. ParC must be complexed with ParE for its DNA cleavage and religation activity to be manifested; however, ParC alone is a DNA-binding protein, and its indiscriminate binding to the chromosome could interfere with processes such as replication and transcription. Although this is the most likely possibility, it is interesting to note that both the ΔparE strain in the absence of IPTG and the temperature-sensitive parE strain at the nonpermissive temperature presumably both have free ParC, yet Topo III overexpression rescued the latter and not the former. This observation suggests another, admittedly speculative, possibility that ParE, and possibly Topo IV, has two distinct roles in the cell, only one of which (decatenating the daughter chromosomes) depends on the formation of catalytically active Topo IV. What this second task could be is obscure. Additional studies are required to resolve this issue.

In eukaryotic cells, Topo III and the RecQ-like DNA helicases appear to cooperate to preserve genomic integrity, perhaps by processing some types of recombination intermediates that can form at stalled replication forks (21). Little evidence of such cooperation exists in E. coli, although our observation that Topo III can remove precatenanes in vivo supports its potential involvement in such reactions. Harmon et al. (42) have reported that the combination of E. coli RecQ and Topo III can catenate covalently closed, superhelical DNA in vitro. These authors proposed that a functional interaction between these two proteins allowed Topo III to execute the required two sequential strand passage events by exploiting the ability of
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RecQ to unwind the superhelical DNA. The existence of such a mechanism in E. coli would obviate the issue discussed above with respect to the requirement for a single-stranded gap for Topo III action in decatenating the replicating daughter chromosomes. Accordingly, we asked whether disruption of recQ affected the ability of Topo III to rescue the parE temperature-sensitive strain. Interestingly, it did not (data not shown). Of course, this experiment may not be an accurate test of the hypothesis given the requirement for overproduction of Topo III to effect rescue of the parE strain.

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