A Physical and Functional Interaction between Escherichia coli FtsK and Topoisomerase IV*

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FtsK and topoisomerase (Topo) IV are both involved in chromosome segregation in Escherichia coli. The former protein resides at the septal ring and is required for resolution of chromosome dimers. The latter protein is the chromosomal decatenase. We have demonstrated recently that Topo IV activity is concentrated at the septal proximal regions of the nucleoids late in the cell cycle. Here we demonstrate that FtsK and Topo IV physically and functionally interact. Topo IV was recovered in immunoprecipitates of FtsK. Two-hybrid analysis and immunoblotting showed that this interaction was mediated by the ParC subunit of Topo IV. In addition, we show that the C-terminal motor domain of FtsK stimulates the decatenation activity of Topo IV but not that of DNA gyrase, the other type II topoisomerase in the cell. Topo IV and FtsK appear to cooperate in the cell as well. Rescue of a parE temperature-sensitive mutation by overproduction of DnaX, which leads to stabilization of the temperature-sensitive Topo IV, required both the C-terminal domain of FtsK and parE, whereas rescue by overproduction of Topo III, which bypasses Topo IV function, did not. The interaction between FtsK and Topo IV may provide a means for concentrating the latter enzyme at the cell center.

In bacteria, the division plane at mid-cell is the crucial locus where most of the events leading to cytokinesis take place. The DNA polymerases assemble (1, 2) and replicate DNA at mid-cell (3, 4). This replication factory remains stationary, suggesting that the DNA is drawn through the factory during replication. These observations have led to the development of the “extrusion-capture” model of chromosome partitioning (5), whereby replication itself drives DNA segregation by pushing the newly replicated DNA outward from the anchored factory toward opposite cell poles. Transcription may also participate in chromosome dynamics, with relatively stationary RNA polymerases playing the role of motor proteins that also act to drive the sister chromosomess away from the cell center (6), because transcription units tend to be oriented away from oriC.

Chromosome segregation and cytokinesis are functionally linked. Delays in chromosome segregation that lead to prolonged residence of the nucleoid in the cell center will prevent cell division (7). The major structure for cytokinesis is the septal ring. Formation starts early after the initiation of replication with the assembly of the FtsZ ring at mid-cell. FtsZ is a tubulin-like protein (8), and contraction of the ring results in cell division.

The septal ring is also linked to chromosome segregation via FtsK. FtsK is a bifunctional protein composed of three domains (9). The N-terminal domain consists of four transmembrane segments. This domain is required for cell viability and is presumed to be involved in closure of the septal ring (10). The C-terminal part of FtsK is cytoplasmic and is required for XerCD-catalyzed resolution of chromosomal dimers at dif (11). This portion of FtsK is composed of two domains: a proline- and glutamine-rich domain of unknown function and a 500-amino acid C-terminal domain (domain 3) that is necessary for normal chromosome segregation (9), in part because of its effect on XerCD recombination at dif (12). Domain 3 is homologous to the C-terminal domain of SpoIIIE, a protein involved in DNA transfer from the mother cell to the prespore in Bacillus subtilis (13). The purified FtsKparE fragment of the protein, which includes FtsK amino acids 179–230 linked to the N terminus of the ParC subunit of Topo IV, can alter the topology of DNA and affect the direction of XerCD-catalyzed resolution in vitro (14).

Topoisomerase (Topo) IV was discovered by Kato et al. (15) as a new topoisomerase activity that appeared when the products of parE and parC were co-overexpressed in Escherichia coli. Mutations in these genes produce partition-defective, conditionally lethal phenotypes (15, 16). The purified enzyme consists of a heterotetramer of a dimer of ParE, which contains the ATPase domain, and a dimer of ParC, which contains the DNA binding and DNA cleavage and religation domains (17). Topo IV can decatenate replicating daughter chromosomes in vitro (18) and in vivo (19), demonstrating that it, and not DNA gyrase, the other type II topoisomerase present in bacteria, was responsible for topological unlinking of the daughter chromosomes during segregation and partition.

We have shown that Topo IV activity is temporally regulated in the cell (2). This regulation manifests itself because the two subunits of the enzyme reside in different locations in the cell for a significant fraction of the cell cycle. ParC is associated with the replication factory, whereas ParE appears to be distributed in the DNA-free spaces in the cell. As a result, Topo IV activity becomes concentrated in the cell center toward the end of the cell cycle after the replication factory disassembles. Interestingly, a strong Topo IV cleavage site has been found very close to dif, whereas they are scarce for 35–54 kbp on either side of it (20). These observations prompted us to ask whether Topo IV and FtsK were associated in the cell. We show here that this is indeed the case, that this association is mediated by the ParC subunit of Topo IV, and that the C-terminal domain 3 of FtsK stimulates the chromosome decatenation activity of Topo IV.

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† The abbreviations used are: Topo IV, topoisomerase IV; PBS, phosphate-buffered saline; DTT, dithiothreitol; BSA, bovine serum albumin.
Topo IV in *vitro* and is required for the Topo IV-mediated chromosome decatenation pathway *in vivo* when Topo IV function is compromised.

**EXPERIMENTAL PROCEDURES**

**FtsK** Immunoprecipitation—W3110(pFX90) (a gift of D. Sherratt, Oxford University) was grown in LB medium at 37 °C until reaching an A600 of 0.2. Either arabinose (0.2%) or glucose (0.2%) was then added to either induce or repress, respectively, expression of FtsKc, and the cultures (40 ml) were incubated at 37 °C until they reached an A600 of 0.5. Protein-protein cross-links were induced by addition of formaldehyde to 2% followed by incubation for 20 min at 37 °C. Cross-linking was reversed by the addition of glycine to 200 mM followed by incubation for 5 min at 37 °C. The cells were harvested, and FtsKc was isolated as described below except that lysis was in 5 ml. The lysis buffer included 20 μg/ml DNase I, 200 μl of FLAG antibody beads were used per culture, and elution of FtsKc was in batches with 1 ml of FLAG peptide (100 μg/ml) for 1 h. Western blot analysis was performed as described by Levine and Marinas (21) except that the samples (25 μl of eluted material) were heated at 100 °C for 10 min to reverse the cross-links. Antiserum used were rabbit polyclonal antibodies to Topo IV, FtsZ, and GyrA and mouse monoclonal antibodies to GyrB and the FLAG epitope.

**Immunoblotting**—200 μl of 1× PBS and then 200 μl of the indicated protein in 1× PBS was spotted onto a nitrocellulose membrane (Hybond ECL) by using a Schleicher & Schuell MiniMold II dot blotting apparatus, and the membrane was then air-dried for 10 min. The membrane was then blocked by incubation in 1× PBS, 5% non-fat dried milk for 1 h at room temperature. FtsKc (2 μg/ml) was then incubated with the membrane for 15 min in a solution containing 40 mm HEPES-KOH (pH 8.0), 12 μM MgOAc, 5 μM DTT, 0.1 mg/ml BSA, and 4% sucrose. Glutaraldehyde was then added to a final concentration of 0.1%, and the incubation was continued for 45 min. The membrane was then washed with 1× TBS (137 mM NaCl, 2.7 mM KCl, 25 mM Tris-HCl (pH 8.0), 2% milk, and 200 mM glycine and then washed four times (10 min each wash) with 1× PBS, 2% milk. Monoclonal anti-FLAG antibody (1:2500 dilution) in 1× PBS was then incubated with the blot overnight at 4 °C. The blot was washed three times quickly, once for 15 min, and three times for 5 min each with ≥50 ml of 1× PBS, 2% milk, and then incubated with goat anti-rabbit IgG coupled to horseradish peroxidase at a 1:5000 dilution in 1× PBS, 2% milk, for 1 h at room temperature. The blot was washed again, as after application of the primary antibody, and FtsKc was detected using ECL-Western blotting detection reagents as described by the manufacturer (Amersham Biosciences).

**Two-hybrid Analysis**—DNA-binding fusions were made to the first 202 amino acids of LexA in the pCA-1 plasmid (a gift of S. Keene, Sloan-Kettering Institute). In this plasmid, expression of a nuclear localization signal-modified N-terminal LexA fragment is driven by an actin promoter. The expression cassette was followed by the Gal11 transcription terminator. The FtsKc open reading frame was amplified by PCR using primers containing SaI and BglII restriction endonuclease sites, and the DNA fragment was digested and cloned into the SaI and BglII sites of the plasmid to give the cplx-ftsKc fusion gene. Transcriptional activator fusions were made to the activator domain of Gal4 (amino acids 768–881) in the pACT2 plasmid (Clontech). In this plasmid, expression of a nuclear localization signal-modified Gal4 activation domain is driven by an alcohol dehydrogenase promoter followed by the alcohol dehydrogenase transcriptional terminator. The ParE and ParC open reading frames were amplified by PCR using primers containing NcoI and BglII restriction endonuclease sites and cloned into the NcoI and BglII sites of the plasmid to give the g4l-parE and gal4-parC fusion genes.

To assay for interaction, *Saccharomyces cerevisiae* strain L40 (In-vitro) carrying the two plasmids of interest was grown at 30 °C in minimal media lacking Leu and Trp but containing the other 18 amino acids at 1 μg/ml each. 0.5–1.0 μg of this fusion plasmid was then assayed as described by Bartel and Fields (22) using 0.5 ml of cell culture and 600 of 0.2. Either arabinose (0.2%) or glucose (0.2%) was then added to either induce or repress, respectively, expression of the FLAG-tagged FtsKc, respectively, and growth was continued until the cultures reached an OD of 0.5. Formaldehyde was then added to induce protein-protein cross-links, and cross-linking was then blocked by the subsequent addition of glycine. Soluble lysates were prepared, and the FLAG-tagged FtsKc was isolated via the use of antibodies against the FLAG epitope linked to agarose beads. To ensure specificity, proteins were eluted from the beads by competition with FLAG peptide and analyzed by SDS-PAGE and Western blotting (Fig. 1).

As expected, FLAG-FtsKc was only present in the lysate when arabinose was added to the culture. Essentially all of the FLAG-FtsKc in the lysate was captured by the antibody. Both subunits of Topo IV, ParE and ParC, were detected in the peptide eluate from the immunoprecipitate when the cultures had been exposed to arabinose and FtsKc was produced, whereas no Topo IV was evident when the cultures had been exposed to glucose. The third band detected with the Topo IV antibodies is a specific degradation product of ParE that we observe consistently in cell extracts. These observations indicated that the presence of Topo IV in the immunoprecipitate was dependent on the presence of FtsKc. Note that because DNase I was included during preparation of the extracts, the presence of Topo IV in the FtsKc immunoprecipitate was not the result of co-residence of the two enzymes on the same DNA fragment. The specificity of the interaction between Topo IV and FtsKc was reinforced by the finding that neither of the
subunits of DNA gyrase could be found in the peptide eluate of the immunoprecipitate under any condition.

We have described a temporal regulation of Topo IV activity whereby ParC associates with the replication factory during most of the cell cycle, associating with ParE when the factory disassembles (2). These events cause Topo IV activity to be concentrated at the end of the cell cycle in the central region of the cell, either at or in the vicinity of the invaginating septum. FtsKc, lacks the N-terminal membrane domain that localizes the intact FtsK to the septal ring. We have found, not surprisingly for a domain that self-associates, that FtsKc localizes to the septal ring via interaction with the C-terminal domain of the intact protein (data not shown). This observation likely accounts for our ability to co-immunoprecipitate FtsKc with both subunits of Topo IV. Because of the residence of FtsKc at the septal ring, it was also reasonable to expect that FtsZ would be found in the immunoprecipitate. This proved to be the case. Like Topo IV, FtsZ could be found in the peptide eluate from immunoprecipitates from cultures treated with arabinose.

The findings described by the experiment shown in Fig. 1 indicated that Topo IV interacted with FtsK in the cell. However, this experiment could not differentiate between a direct or indirect interaction between the two proteins. To address this issue, we performed two-hybrid analysis in yeast (Table I). FtsKc was fused to the DNA-binding domain of LexA in plasmid pCA-1, whereas ParE and ParC were fused to the transcriptional activator domain of Gal4 in plasmid pACT2. The indicated pairs of plasmids were transformed into S. cerevisiae strain L40; cultures were grown to late log phase in the presence of arabinose as described by Aussel et al. (14). Purified FtsKc, (Fig. 3A) exhibited a powerful ATPase activity that was absolutely dependent on the presence of DNA (Fig. 3B). Aussel et al. (14) reported that this fragment of FtsKc was a very poor ATPase that, unlike the FtsK50c fragment, was not DNA-dependent. They attributed the difference in activities to the probable requirement of the region from amino acid residues 179-230 for oligomerization.

However, they noted that FtsKc could activate Xer recombination in vitro, indicating that the fragment was active and that other factors in the cell, most likely increased concentration, could lead to its oligomerization. The ATPase activity of FtsKc displays a strong sigmoidal response to protein concentration (Fig. 3C); thus we suspect that oligomerization, which is presumably required for activity, was aided by both the presence of DNA and the high FtsKc concentration that we were able to achieve in the reaction mixtures. We also note that FtsKc ATPase activity is strongly inhibited if Mg2+ is in excess of ATP (activity at a Mg:ATP ratio of 2:1 is one-tenth that at a ratio of...
ably greater values of Lki than those in the 1st lane because the topoisomers present in the preparation, and its Lki multiplied is given by the sum of the products of the fraction of any decatenated (fmol)/min. Note that the total amount of linkages deriving a rate of decatenation, which is measured in linkages per mole (mM) at 0 time and after the 5-min incubation and then phosphorimaging the amount of total linkages present (in femtomoles) at 0 time and after the 5-min incubation and then derivating a rate of decatenation, which is measured in linkages decatenated (fmol)/min. Note that the total amount of linkages is given by the sum of the products of the fraction of any particular topoisomer in the preparation, and its Lk, multiplied by one-half the total amount of monomer DNA circles present. Thus, for example, although there is more DNA present in each of the topoisomers in the 3rd lane of Fig. 4 than in the 1st lane, because the topoisomers present in the 1st lane have considerably greater values of Lk than those in the 3rd lane, the total number of linkages present in the 1st lane is much greater than in the 3rd lane. Note also that unlike the common decatenation assay that measures unlinking of the mini- and maxi-circles of kinetoplast DNA (29), because one strand passage event leads to the reduction of the Lk of the multiply linked DNA dimers by a value of 1, the assay used here measures the actual rate of strand passage events. Because DNA circles in the kinetoplast DNA are linked only once, that assay measures only the rate of daughter chromosome decatenation. This reaction can be modeled in vitro with the use of multiply linked DNA dimers as a substrate (24). This DNA form arises in and can be purified from DNA replication reactions in vitro that utilize small plasmid DNAs as templates. They consist of the daughter DNA molecules linked together as toroidal catenanes and as such are precisely the same substrate that is seen by Topo IV in the cell. The number of linkages between the daughter chromosomes is described by Lk, the intermolecular topological linking number. Typically, these molecules are purified from DNA replication reactions where DNA ligase has been omitted, and thus the product DNA is form II. Therefore, when analyzed by agarose gel electrophoresis, the form II:form II DNA dimers appear as a ladder of bands with a certain distribution, with each step in the ladder representing one intermolecular linkage between the two daughter DNAs (Fig. 4, 1st lane). When treated with either Topo IV (Fig. 4, 2nd and 3rd lanes) or DNA gyrase (Fig. 4, 4th and 5th lanes), decatenation can be observed as a shift in the distribution of linkages to a lower average value of Lk. If the reactions shown in Fig. 4 had been allowed to proceed to completion, the final product would have been the monomer form II DNA. The reactions are quantitated by determining by PhosphorImaging the amount of total linkages present (in femtomoles) at 0 time and after the 5-min incubation and then derivating a rate of decatenation, which is measured in linkages decatenated (fmol)/min. Note that the total amount of linkages is given by the sum of the products of the fraction of any particular topoisomer in the preparation, and its Lk, multiplied by one-half the total amount of monomer DNA circles present. Thus, for example, although there is more DNA present in each of the topoisomers in the 3rd lane of Fig. 4 than in the 1st lane, because the topoisomers present in the 1st lane have considerably greater values of Lk than those in the 3rd lane, the total number of linkages present in the 1st lane is much greater than in the 3rd lane. Note also that unlike the common decatenation assay that measures unlinking of the mini- and maxi-circles of kinetoplast DNA (29), because one strand passage event leads to the reduction of the Lk of the multiply linked DNA dimers by a value of 1, the assay used here measures the actual rate of strand passage events. Because DNA circles in the kinetoplast DNA are linked only once, that assay measures only the rate of product accumulation.

Befitting their different roles in the cell, Topo IV is the superior decatenating enzyme to DNA gyrase (30, 31). Thus, only 0.2 nM Topo IV was required to establish a decatenation rate of 0.85 (Fig. 4, 2nd lane), whereas 25 nM DNA gyrase gave a rate about twice that (1.83) (Fig. 4, 4th lane). The presence of 1.2 μM FtsKc stimulated Topo IV-catalyzed decatenation 2.5-fold (Fig. 4, compare 2nd and 3rd lanes), whereas it inhibited DNA gyrase-catalyzed decatenation by 25% (Fig. 4, compare 4th and 5th lanes). Although the range of FtsKc concentration that elicited a stimulation of Topo IV activity was high (0.6–1.2 μM as monomer) relative to the concentration of either the substrate or the topoisomerase used, it mirrored the response of the ATPase activity to the FtsKc concentration. Therefore, it is likely that the high concentration of FtsKc required for stimulation reflects the requirement for oligomerization for activity of the AAA domain. Although the observed stimulation of Topo IV activity could be indirect, the lack of a similar stimulation of gyrase activity argues strongly that there is a functional interaction between Topo IV and FtsKc. To probe this issue further, we reasoned that such a functional interaction might become obvious in vivo under conditions where Topo IV activity was compromised.

We have shown that the temperature-sensitive and partition phenotypes of the parE10 and parC1215 alleles can be suppressed by the overproduction of a number of genes. Suppression occurs via two pathways: overexpression of dnaX, encoding the γ and θ subunits of the DNA polymerase III holoenzyme, leads to the stabilization of the temperature-sensitive Topo IV at the non-permissive temperature (2), whereas...
overexpression of Topo III bypasses Topo IV function at the non-permissive temperature (26). Thus, we expected that if there were a functional interaction between Topo IV and FtsK, it would be observed under conditions of suppression by overexpression of dnaX and not topB.

Three isogenic strains were constructed: W3110parE10, W3110parE10topB, and W3110parE10topB::cam. These strains were transformed with pBAD-topB and pLex-dnaX(y) (i.e. a dnaX gene engineered to produce only y, not r), as well as their respective parental plasmids. The strains were grown to mid-log at 30 °C and then plated for viable cells at both 30 and 42 °C (Table II). As expected, overexpression of both dnaX and topB allowed W3110parE10 to grow at 42 °C. However, when either the C-terminal domain of FtsK was removed or dif was inactivated, overexpression of dnaX no longer was able to rescue the parE phenotype at the non-permissive temperature. On the other hand, neither the removal of the C-terminal domain of FtsK nor the inactivation of dif had any effect on the rescue of the parE phenotype by overexpression of topB at the non-permissive temperature. These results indicate that the functional interaction observed between Topo IV and FtsK, in vivo is likely to be reflective of in vitro as well.

**DISCUSSION**

The past few years has seen a profound change in our understanding of chromosome dynamics in bacteria. DNA replication in most bacteria appears to take place in centrally located, stationary replication factories (1, 2). Parental DNA is therefore drawn through the factory (4). Thus, as would be expected under these circumstances, movement of specific regions of DNA in the cell can be detected cytologically. In E. coli, a recent definitive study demonstrated that the newly duplicated origin regions segregate to opposite sides of the cell soon after initiation of replication, whereas segregation of the terminus region occurs very late in the cell cycle, as the daughter cells separate (32). Newly replicated DNA appears to transit through an aggregate of SeqA (33), an event that contributes to segregation of oriC to prevent reinitiation, and also probably helps direct the newly replicated DNA away from the cell center. Upon exiting this SeqA tunnel, the DNA probably associates with the SMC-like protein MukB (34), which is likely to act to condense the DNA.

In order for the sister chromosomes to be segregated, the topological linkage between the two parental strands must be reduced to zero. This task is accomplished by Topo IV. Remarkably, we have found that Topo IV activity manifests itself on the DNA only late in the cell cycle, apparently when most replication has been completed (2). By using terminal dUTP nick-end labeling to label quinolone-induced DNA breaks made by Topo IV, we also found that the activity of this enzyme was concentrated at the septal-proximal edges of the nucleoids. We have demonstrated a physical interaction between Topo IV and FtsK that is likely to be between ParC and domain 3 of FtsK, based on two-hybrid analysis, co-immunoprecipitation, and immunoblotting. We have proposed previously that the temporal regulation of Topo IV activity reflects the fact that for much of the cell cycle the subunits of the enzyme are found in different locations in the cell: ParC at the replication factory and ParE in the DNA-free spaces. Active Topo IV is constituted when the factory disassembles when replication is complete. This Topo IV may now be captured by the C-terminal domain of FtsK. Because FtsK resides at the septal ring, such capture would effectively concentrate Topo IV at the cell center.

The potential for an association between FtsK and Topo IV had been suggested in previous studies. Hojgaard et al. (20) demonstrated the presence of a preferred Topo IV cleavage site at dif. Interestingly, Topo IV cleavage at dif was dependent on the presence of the XerCD recombinase but not on the presence of FtsK domain 3. Given the association between FtsK and Topo IV reported here and the suggestion of Barre et al. (28) that at the termination of replication an arrangement holds whereby dif is effectively connected to the septal ring either directly via an interaction with FtsK or indirectly because of an interaction between XerCD and FtsK, we would have expected a greater influence of FtsK domain 3 on Topo IV cleavage at dif. Perhaps the explanation for this discrepancy lies in the fact that because Hojgaard et al. (20) used norfloxacin to trap covalent Topo IV-DNA complexes, their results do not accurately reflect the relative steady-state occupancy of the site at dif in the presence and absence of the C-terminal domain of FtsK.

We have also demonstrated a functional interaction between FtsK and Topo IV, showing that FtsK, stimulates in vitro the very activity of Topo IV that underlies its essentiality in vivo, decatenation of multiply linked daughter chromosomes. In addition, both the C-terminal domains of FtsK and dif were required in vivo when a parE temperature-sensitive mutation was rescued at the non-permissive temperature by overproduction of the γ subunit of the DNA polymerase III holoenzyme, conditions where the temperature-sensitive Topo IV was stabilized, but neither was required when rescue was effected by overproduction of Topo III, conditions that bypass the need for Topo IV. This observation indicates that FtsK and Topo IV cooperate in vivo as well. This cooperation in vivo may reflect the stimulation of Topo IV by FtsK observed in vitro. Under these circumstances, the requirement for dif could indicate that this region is the site of interaction between the two enzymes. The specificity of the interactions between Topo IV and FtsK is underscored by the lack of any physical or functional interaction between FtsK and DNA gyrase, the other type II topoisomerase in the cell and a close homolog of Topo IV.

The mechanism by which FtsK stimulates Topo IV-catalyzed decatenation is unclear. Assel et al. (14) demonstrated that FtsKtop5c was a motor protein that could track along a circular double-stranded DNA molecule partitioning it into positively and negatively supercoiled domains. These authors proposed that the protein utilized this activity in a local manner to directly alter the conformation of the Xer synaptic complex from one suitable for XerC strand exchanges to one suitable for XerD strand exchanges. Supercoiling stimulates decatenation of multiply linked DNA dimers (24). However, the monomer DNA rings of the multiply linked dimers used in the experiments reported here were not covalently closed. Thus, supercoiling of the substrate is unlikely to be the sole basis of the observed stimulation. In addition, supercoiling would have been expected to stimulate both gyrase and Topo IV, which was not observed. It seems more likely that the FtsK motor activity generates usable substrate at a locally higher concentration in the vicinity of Topo IV. This could be accomplished by the protein-protein interaction between the two proteins serving as one anchor of a topological domain and the FtsK motors domain serving as the other. Tracking of the motor could then result in the pumping of supercoiled DNA into the topological domain where it would be readily available for Topo IV.

**Table II**

| Plasmid          | Strain                  |
|------------------|-------------------------|
| pBAD18           | parE10                  |
| pBAD-topB        | <0.01                   |
| pLex5BA          | 0.91                    |
| pLex-dnaX(y)     | <0.01                   |

Plating efficiency was at 42/30 °C.
We have argued that the purpose of concentrating Topo IV in the center of the cell is to ensure the decatenation of the correct pair, of multiple pairs, of chromosomes that must clear the division plane in order for cytokinesis to occur in a rapidly growing cell. An interaction between Topo IV with FtsK that stimulates the activity of the latter enzyme is consistent with this argument. Concentration of Topo IV at the cell center late in the cell cycle may provide additional advantages as well. Sherratt et al. (35) have suggested that localization of Topo IV might be required to ensure efficient removal of any knots or catenanes that become trapped as a result of XerCD resolution of the sister chromosomes happens just as the cells divide. Thus, it is possible that a complex of proteins may exist in the cell center late in the cell cycle whose purpose is to ensure final resolution of the sister chromosomes.

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