A ParE−ParC Fusion Protein Is a Functional Topoisomerase[†]

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ABSTRACT: Type II topoisomerases are responsible for DNA unlinking during DNA replication and chromosome segregation. Although eukaryotic enzymes are homodimers and prokaryotic enzymes are heterotetramers, both prokaryotic and eukaryotic type II topoisomerases belong to a single protein family. The amino- and carboxyl-terminal domains of eukaryotic enzymes are homologous to the ATP-binding and catalytic subunits of prokaryotic enzymes, respectively. Topoisomerase IV, a prokaryotic type II topoisomerase, consists of the ATP-binding subunit, ParE, and the catalytic subunit, ParC. We have joined the coding regions of *parE* and *parC* in frame and constructed a fusion protein of the two subunits of topoisomerase IV. This fusion protein, ParEC, can catalyze both decatenation and relaxation reactions. The ParEC protein is also capable of decatenating replicating daughter DNA molecules during *oriC* DNA replication in vitro. Furthermore, the fusion gene, *parEC*, complements the temperature-sensitive growth of both *parC* and *parE* strains, indicating that the ParEC protein can substitute for topoisomerase IV in vivo. These results demonstrate that a fusion protein of the two subunits of topoisomerase IV is a functional topoisomerase. Thus, a heterotetrameric type II topoisomerase can be converted into a homodimeric type II topoisomerase by gene fusion.

Topoisomerases are essential enzymes that alter the linking number of DNA (*1*). There are two types of topoisomerases. Type I topoisomerases alter the linking number by breaking one strand of duplex DNA, passing the other strand through the break, and then resealing the broken strand. In contrast, type II enzymes alter the linking number by breaking both strands, passing another segment of the helix through the break, and then resealing the broken strands. Type II topoisomerases are ATP-dependent enzymes (*1*).

The identification of DNA gyrase as a target of quinolone antibacterial drugs (*2*−*4*) has led to the discovery of topoisomerases as important therapeutic targets (*5*−*7*). In eukaryotes, type II topoisomerases are the cellular targets of potent anticancer drugs (e.g., etoposide), whereas in prokaryotes both DNA gyrase and topoisomerase IV (Topo IV)[1] are the targets of the most potent broad-spectrum antibacterial agents (e.g., ciprofloxacin).

Type II topoisomerases are well conserved and form a large protein family (*1*). Eukaryotic enzymes are homodimers, whereas prokaryotic enzymes are heterotetramers of two subunits. The amino-terminal domain of eukaryotic enzymes is homologous to the ATP-binding subunit of prokaryotic enzymes, and the carboxyl-terminal domain is homologous to the catalytic subunits. DNA gyrase and Topo IV consist of GyrA and GyrB subunits and ParC and ParE subunits, respectively. GyrA and ParC subunits bind to the DNA and catalyze strand-breakage and reunion reactions, whereas GyrB and ParE subunits hydrolyze ATP (*1*).

DNA unlinking catalyzed by type II topoisomerases is critical during DNA replication and chromosome segregation (*8*). Genes encoding both DNA gyrase and Topo IV are essential. Temperature-sensitive mutations of *gyrA* (*parD*), *gyrB* (*parA*), *parC*, and *parE* have been isolated from *Escherichia coli* (*9*−*13*). Strains carrying these mutations exhibit a temperature-sensitive phenotype due to the lack of partitioning of daughter chromosomes, demonstrating that both DNA gyrase and Topo IV play critical roles during chromosome segregation. Recent studies have shown, however, that Topo IV, but not DNA gyrase, is responsible for the decatenation of daughter chromosomes (*14*−*17*).

Here, we constructed a fusion protein of the ParE and ParC subunits of *E. coli* Topo IV. The ParEC protein catalyzed decatenation and relaxation reactions in vitro. Both norfloxacin, a model quinolone drug, and Ca^{2+} stimulated ParEC-catalyzed cleavage of DNA. Site preference of cleavage was identical between Topo IV and ParEC. In addition, the ParEC protein was capable of supporting the elongation of DNA replication and the decatenation of daughter DNA molecules during *oriC* DNA replication in vitro. Furthermore, the fusion gene, *parEC*, complemented the temperature-sensitive phenotype of both *parC* and *parE* strains. These results demonstrated that the two subunits of Topo IV could function in cis.

EXPERIMENTAL PROCEDURES

Bacterial Strains, DNAs, and Proteins. E. coli strains C600*parC1215* and W3110*parE10* and their parental strains were described by Kato et al. (*14*). *E. coli* HMS174(DE3) (Novagen) was used for the overproduction of the ParEC protein.

The *Nde*I−*Bam*HI DNA fragments containing *parC* and *parE* were derived from pET3c-*parC* and pET3c-*parE* (*18*)

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[1] Abbreviations: BSA, bovine serum albumin; DTT, dithiothreitol; kDNA, kinetoplast DNA; K-Glu, potassium glutamate; Topo, topoisomerase.

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and cloned into pET-11c (Novagen) to construct pET11-*parC* and pET11-*parE*. The pET11-*parE* plasmid DNA was digested with *Bgl*II and *Bam*HI, and the DNA fragment containing both the T7 promoter and the *parE* gene was recovered after electrophoresis through a 1.2% Sea Plaque agarose (FMC) gel. The *Bgl*II/*Bam*HI fragment containing the *parE* gene was cloned into the *Bgl*II site of pET11-*parC*. This plasmid, pET11-*parE*/*parC*, contained *parE* and *parC* genes in this order and in the same direction. A fusion gene, *parEC*, was constructed using pET11-*parE*/*parC* as a starting material (see the Results section).

Purified *E. coli* replication proteins, generous gifts of Kenneth Marians (Memorial Sloan-Kettering Cancer Center), were described previously (*19*−*21*). The wild-type Topo IV was prepared according to Peng and Marians (*18*).

Overproduction and Purification of the ParEC Protein. *E. coli* HMS174(DE3)/pET11-*parEC* was grown at 37 °C in 6 L of L-broth to $OD_{600} = 0.4$. IPTG was added to 0.5 mM, and the growth continued for 2 h. Cells were harvested, resuspended in 40 mL of TES [50 mM Tris-HCl, pH 7.5 at 4 °C, 1 mM EDTA, and 10% (w/v) sucrose], frozen in liquid nitrogen, and stored at −80 °C. The cell suspension was thawed and adjusted to 50 mM Tris-HCl, pH 8.4 at 4 °C, 5 mM dithiothreitol (DTT), 20 mM EDTA, 150 mM NaCl, 0.12% (w/v) Brij 58, and 0.02% (w/v) lysozyme. The suspension was incubated for 30 min at 0 °C and then centrifuged at 100000*g* for 1 h. The supernatant (fraction 1) was decanted. Nucleic acids were precipitated by the addition of Polymin P to a final concentration of 0.075%. Protein was precipitated from the supernatant by the addition of solid $(NH_4)_2SO_4$ to a final concentration of 60%. The protein was gently resuspended in a volume equal to one-twentieth of the lysate in TBEB Buffer [50 mM Tris-HCl, pH 7.5 at 4 °C, 5 mM DTT, 1 mM EDTA, 100 mM NaCl, and 20% (v/v) glycerol] (fraction 2, 6 mL, 300 mg).

Fraction 2 was dialyzed against 1 L of buffer A [50 mM Tris-HCl, pH 7.5 at 4 °C, 5 mM DTT, 1 mM EDTA, and 20% (v/v) glycerol] plus 200 mM NaCl overnight and further diluted with buffer A to 100 mM NaCl just before being loaded onto a 15 mL Q Sepharose FF (Amersham Pharmacia Biotech) column that had been equilibrated with buffer A plus 100 mM NaCl. The column was washed with 30 mL of buffer A plus 100 mM NaCl and then developed with a linear gradient (150 mL) of 100−700 mM NaCl in buffer A. ParEC (fraction 3, 10 mL, 58 mg) eluted at 300 mM NaCl. Fraction 3 was diluted with buffer A to 100 mM NaCl and then applied to a 12 mL heparin−agarose (Sigma) column equilibrated with buffer A plus 100 mM NaCl. The column was washed with 24 mL of buffer A plus 100 mM NaCl and developed with a linear gradient (120 mL) of 100−700 mM NaCl in buffer A. ParEC (fraction 4, 12 mL, 14 mg) eluted at 500 mM NaCl. Fraction 4 was diluted with buffer A to 100 mM NaCl and loaded onto a 0.75 mL Q Sepharose FF column equilibrated with buffer A plus 100 mM NaCl. The column was washed with 1.5 mL of buffer B [25 mM Tris-HCl, pH 7.5 at 4 °C, 2.5 mM DTT, 0.5 mM EDTA, and 10% (v/v) glycerol] plus 100 mM NaCl, and ParEC (fraction 5, 0.5 mL, 7 mg) was eluted with 600 mM NaCl in buffer B. Fraction 5 was loaded onto an AKTA Sephacryl S-300 column (Amersham Pharmacia Biotech) equilibrated with buffer B plus 500 mM NaCl. The column was developed with the same buffer. The final fraction of ParEC (fraction 6, 2 mL, 3.2 mg) was collected and dialyzed against buffer C [50 mM Tris-HCl, pH 7.5 at 4 °C, 5 mM DTT, 1 mM EDTA, 200 mM NaCl, and 40% (v/v) glycerol]. This final fraction of ParEC was frozen in liquid nitrogen and stored at −80 °C.

DNA Cleavage Reaction. pBR322 DNA was linearized by digestion with *Eco*RI and was then 3′-end-labeled by incorporation of two residues of [^{32}P]dAMP with Klenow enzyme. The labeled DNA was digested with *Pst*I, and a 0.75 kb fragment, uniquely labeled at one end, was recovered after electrophoresis through a 1.4% Sea Plaque agarose (FMC) gel. This DNA fragment was used as the substrate in the DNA cleavage reaction.

Reaction mixtures (20 μL) containing 50 mM Tris-HCl (pH 7.5 at 23 °C), 10 mM $MgCl_2$ or $CaCl_2$, 100 mM K-Glu, 10 mM DTT, 50 μg/mL bovine serum albumin (BSA), 1 mM ATP, 20 fmol (as molecule) of DNA substrate, 50 fmol of either Topo IV (as tetramer) or ParEC (as dimer), and 50 μM norfloxacin when indicated were incubated at 30 °C for 10 min. SDS was added to 1%, and the reaction mixtures were incubated at 37 °C for 5 min. EDTA and proteinase K were then added to 25 mM and 100 μg/mL, respectively, and the incubation was continued for an additional 15 min. The DNA products were purified by extraction of the reaction mixtures with phenol−chloroform (1:1 v/v) and then analyzed by electrophoresis through 8% polyacrylamide (19:1 acrylamide to bisacrylamide) gels (140 × 160 × 1.2 mm) at 15 V/cm for 2 h using 50 mM Tris−borate (pH 8.3) and 1 mM EDTA as the electrophoresis buffer (TBE buffer). Gels were dried under vacuum onto DE81 paper (Whatman) and autoradiographed with Hyperfilm MP film (Amersham Pharmacia Biotech).

Decatenation of Kinetoplast DNA (kDNA). Reaction mixtures (20 μL) containing 40 mM HEPES−KOH (pH 7.6), 10 mM $MgOAc_2$, 100 mM K-Glu, 10 mM DTT, 50 μg/mL BSA, 1 mM ATP, 5 μg/mL tRNA, 0.7 μg of kDNA (Topogen), and the indicated concentrations of either Topo IV (as tetramer) or ParEC (as dimer) were incubated at 30 °C for 30 min. Reactions were terminated by addition of EDTA to 25 mM and incubation at 37 °C for 5 min. SDS and proteinase K were then added to 1% and 100 μg/mL, respectively, and the incubation was further continued at 37 °C for 15 min. The DNA products were purified by extraction of the reaction mixtures with phenol−chloroform (1:1 v/v) and then analyzed by electrophoresis through vertical 1.2% Seakem agarose (FMC) gels (14 × 10 × 0.3 cm) at 2 V/cm for 12 h in a running buffer of 50 mM Tris-HCl (pH 7.9 at 23 °C), 40 mM sodium acetate, and 1 mM EDTA (TAE). Gels were stained with ethidium bromide and photographed using an Eagle Eye II system (Stratagene).

Relaxation of Superhelical Plasmid DNA. Reaction mixtures (20 μL) containing 40 mM HEPES−KOH (pH 7.6), 10 mM $MgOAc_2$, 100 mM K-Glu, 10 mM DTT, 50 μg/mL BSA, 1 mM ATP, 5 μg/mL tRNA, 20 nM pBR322 DNA, and the indicated concentrations of either Topo IV or ParEC were incubated at 30 °C for 30 min. Reactions were terminated, and the DNA products were purified and analyzed as described in the previous section.

oriC DNA Replication Reaction. The standard *oriC* DNA replication assay was performed as described previously (*22*), except that 100 mM K-Glu was added to the reaction mixtures. Reaction mixtures (12.5 μL) containing the *oriC*

plasmid pBROTB535 type I DNA (35 fmol as molecule or 420 pmol as nucleotide), DnaA, DnaB, DnaC, DnaG, HU protein, single-stranded DNA-binding protein, the DNA polymerase III holoenzyme, and topoisomerase(s), as indicated, were incubated at 30 °C for 10 min. After termination of the reaction by addition of EDTA to 25 mM, nucleotide incorporation was measured, and the replication products were analyzed by native agarose gel electrophoresis according to Hiasa and Marians (*22*).

Complementation Assay for Topo IV Function in Vivo. The temperature-sensitive phenotype of C600*parC1215* and W3110*parE10* was used to assess the Topo IV function in vivo. These strains can grow at 30 °C but not at 42 °C due to the lack of Topo IV function required for the chromosome segregation (*12*−*14*). It has been shown that a pBR322-based plasmid carrying either the *parC* or *parE* gene, even its expression is not induced, can complement the temperature-sensitive phenotype of either C600*parC1215* or W3110*parE10*, respectively (*23*). It is likely that a low level of background expression is sufficient to produce enough amounts of the wild-type protein to substitute the inactive mutant protein in *E. coli*. Thus, these strains can be used to assess the functional activity of ParC and ParE proteins in vivo. pET11-*parC*, pET11-*parE*, pET11-*parE*/*parC*, and pET11-*parEC* were transformed into either C600*parC1215* or W3110*parE10*, and transformed cells were plated onto two Luria−Bertani agar plates containing 100 μg/mL ampicillin. One set of plates was incubated at 30 °C overnight, and the other was incubated at 42 °C overnight. The complementation efficiency was determined by comparing the colony numbers formed on plates incubated at 42 °C to those formed on plates incubated at 30 °C.

RESULTS

Gene Fusion of parE and parC and Purification of the ParEC Protein. Amino acid sequence analysis using the Blast Program (National Center for Biotechnology Information) has revealed that the last 10 amino acids of the ParE protein and the first 15 amino acids of the ParC protein show no homology to eukaryotic topoisomerase II. However, both the extreme carboxyl terminus of ParE and the extreme amino terminus of ParC are critical for tetramer formation (*18*). Thus, we suspected that these amino acids were a spacer region between the conserved, functional domains. Complementation of the temperature-sensitive growth of C600-*parC1215* and W3110*parE10* strains (*12*−*14*, *23*) was used as an in vivo assay for the functional activity of Topo IV. Deletion and Ala-scanning analyses demonstrated that amino acid changes could be made in both the last 10 amino acids of ParE and the first 10 amino acids of ParC without abolishing the functional activities of the ParC and ParE proteins (data not shown).[2]

The pET11-*parE*/*parC* plasmid DNA was used to construct a fusion gene, *parEC* (Figure 1). Restriction sites *Sal*I and *Pml*I were introduced at the end of the *parE* coding region, and *Xho*I and *Nhe*I sites were introduced at the beginning of the *parC* coding region (Figure 1B). This mutated pET11-parE/parC DNA was digested with both *Sal*I and *Xho*I enzymes and then self-ligated to generate the fusion gene *parEC* (Figure 1C). This gene fusion resulted in the loss of two amino acids and changes of four amino acids at the junction of the ParE and ParC domains of the ParEC protein.

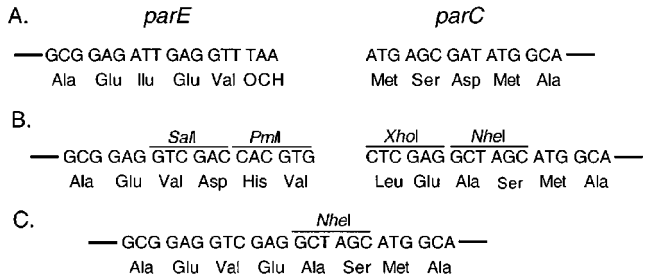

FIGURE 1: Gene fusion of *parE* and *parC*. Nucleotide and amino acid sequences at the end of the *parE* gene and the beginning of the *parC* gene (panel A), nucleotide changes and restriction sites introduced by the site-directed mutagenesis (panel B), and nucleotide and amino acid sequences of the *parEC* gene after the gene fusion (panel C) are shown. The details are in the text.

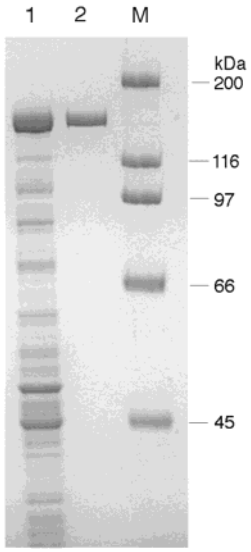

FIGURE 2: SDS−PAGE analysis of ParEC. Fractions 2 (50 μg, lane 1) and 6 (5 μg, lane 2) of ParEC were electrophoresed through a 8% polyacrylamide gel containing SDS, and then the gel was stained with Coomassie Brilliant Blue.

ParEC, a 154 kDa protein, was overexpressed in *E. coli* HMS174(DE3) and purified as described in the Experimental Procedures section. The final preparation of ParEC was greater than 96% homogeneous for a single band on SDS−polyacrylamide gel electrophoresis, and no band corresponding to either the ParC or ParE protein was detected (Figure 2).

The ParEC Protein Is a Topoisomerase. First, we assessed the topoisomerase activity of the ParEC protein using standard topoisomerase assays (Figure 3). Decatenation and relaxation assays were performed using kDNA and a negatively supercoiled plasmid DNA as a substrate, respectively (Figure 3). ParEC could decatenate kDNA (Figure 3A) and relax the negatively supercoiled plasmid DNA (Figure 3B). Under these conditions, the apparent specific activity of the ParEC protein in decatenation and relaxation reactions was 10- and 5-fold lower than that of the wild-type Topo IV, respectively. ParEC-catalyzed decatenation and relaxation reactions required ATP (data not shown). These results demonstrated that ParEC was a topoisomerase.

[2] L. S. Lavasani, K. L. Woodruff, and H. Hiasa, unpublished results.

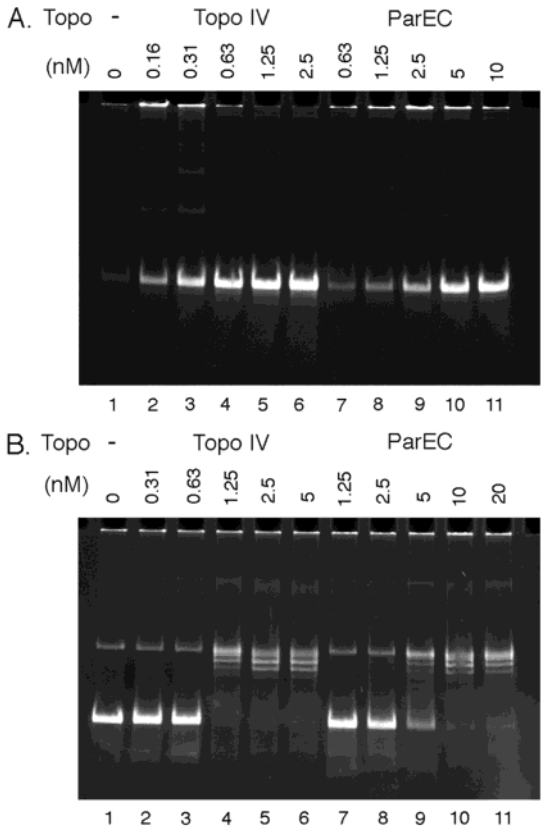

FIGURE 3: The ParEC protein catalyzes both decatenation and relaxation reactions in vitro. Decatenation (panel A) and relaxation (panel B) assays were performed using kDNA and pBR322 DNA as a substrate, respectively. Standard reaction mixtures containing the substrate DNA and the indicated concentrations of either Topo IV (as tetramer) or ParEC (as dimer) were incubated, processed, and analyzed as described in the Experimental Procedures section.

Comparison of Topo IV- and ParEC-Catalyzed Cleavage of DNA. A DNA cleavage assay was employed to assess the effect of the quinolone drug on ParEC and to compare the binding site preference of ParEC with that of Topo IV. Both norfloxacin and Ca^{2+} stimulated ParEC-catalyzed cleavage (Figure 4). ParEC and Topo IV cleaved the DNA to a similar extent and exhibited an identical site preference. These results demonstrated that the fusion of the two subunits of Topo IV did not affect its DNA binding affinity and site preference.

Functional Activity of ParEC during oriC DNA Replication in Vitro. We further examined if ParEC could decatenate replicating daughter DNA molecules during DNA replication. Standard *oriC* replication reactions were incubated in the presence of the various amounts of either ParEC or Topo IV, in addition to a constant amount of DNA gyrase, and then the DNA products were analyzed by native agarose gel electrophoresis (Figure 5A). As we have shown previously (22), in the presence of DNA gyrase alone, the majority of the replication products were late replicative intermediates and multiply linked DNA dimers (Figure 5A, lane 1). The addition of ParEC resulted in the accumulation of monomer form II DNA molecules (Figure 5A). Thus, ParEC could decatenate replicating daughter DNA molecules and produce the final monomer product. Decatenation activity of ParEC was lower than that of Topo IV, which coincided with the reduced decatenation activity of ParEC observed in the kDNA decatenation assay (Figure 3A).

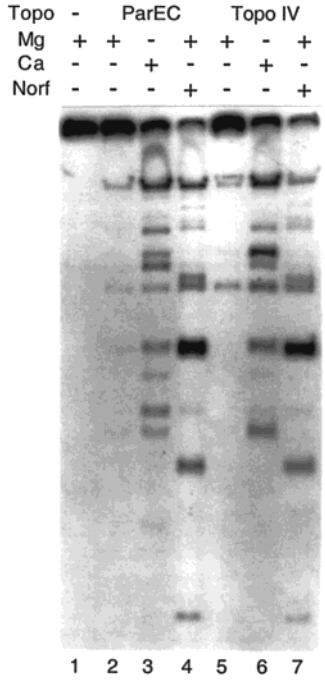

FIGURE 4: ParEC and Topo IV cleave DNA at the same sites. Fifty femtomoles of either ParEC (as dimer) or Topo IV (as tetramer) was incubated with 20 fmol (as molecule) of a 750 base pair DNA fragment labeled only at one end. DNA cleavage was induced by the presence of either 10 mM Ca^{2+} or 50 μM norfloxacin (Norf). The cleaved DNA was analyzed as described in the Experimental Proceduressection.

We also examined if ParEC alone could support *oriC* DNA replication in vitro. Standard *oriC* replication reactions were incubated in the absence of DNA gyrase and in the presence of various amounts of either ParEC or Topo IV (Figure 5B). Only early replicative intermediates accumulated when no topoisomerase was present (Figure 5B, lane 1; ref *23*). The majority of replication products were form II DNA molecules during ParEC-supported *oriC* DNA replication (Figure 5B). Thus, ParEC alone could support *oriC* DNA replication in vitro. A staged elongation assay (*24*) also confirmed that ParEC could support nascent chain elongation (data not shown).

These results demonstrated that ParEC could support the elongation of DNA replication and the decatenation of daughter DNA molecules. Thus, it was suggested that ParEC could substitute for Topo IV during DNA replication and chromosome segregation.

ParEC Can Substitute Topo IV Function in Vivo. A complementation assay was performed to examine if the ParEC protein could substitute for Topo IV in vivo. *E. coli* C600*parC1215* and W3110*parE10* grow at 30 °C but not at 42 °C due to temperature-sensitive mutations in *parC* and *parE* (*12*−*14*). The temperature-sensitive phenotype can be complemented by providing either the wild-type *parC* or *parE* gene on a plasmid (*23*). We transformed pET-11c, pET11-*parC*, pET11-*parE*, pET11-*parE*/*parC*, and pET11-*parEC* into these *E. coli* strains, and colony formation was measured after an overnight incubation at either 30 or 42 °C (Table 1). The temperature-sensitive growth of W3110-*parE10* was complemented when pET11-*parE*, pET11-*parE*/*parC*, or pET11-*parEC* was present. In contrast, pET11-*parC*, pET11-*parE*/*parC*, and pET11-*parEC* complemented the temperature-sensitive growth of C600*parC1215*. Thus,

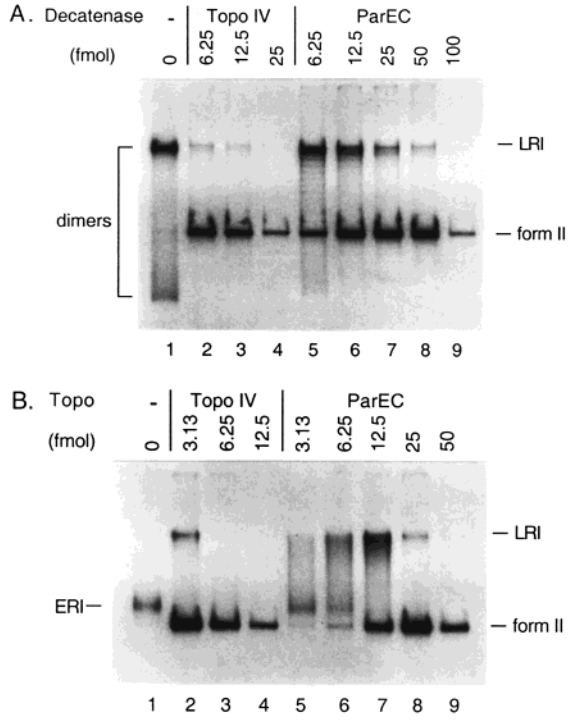

FIGURE 5: ParEC can decatenate replicating DNA molecules during *oriC* DNA replication in vitro. Standard *oriC* DNA replication reactions were incubated in the presence (panel A) or absence (panel B) of DNA gyrase (140 fmol as tetramer) and the indicated amounts of either ParEC (as dimer) or Topo IV (as tetramer), and the DNA products were analyzed by electrophoresis through 0.8% native agarose gels (*22*). The total DNA syntheses (as nucleotide) in the 10 min reaction were as follows: in panel A, lane 1, 198 pmol; lane 2, 191 pmol; lane 3, 153 pmol; lane 4, 72 pmol; lane 5, 193 pmol; lane 6, 193 pmol; lane 7, 182 pmol; lane 8, 149 pmol; lane 9, 76 pmol; and in panel B, lane 1, 53 pmol; lane 2, 129 pmol; lane 3, 111 pmol; lane 4, 62 pmol; lane 5, 84 pmol; lane 6, 127 pmol; lane 7, 118 pmol; lane 8, 106 pmol; lane 9, 58 pmol. Abbreviations: LRI, late replicative intermediates; ERI, early replicative intermediates; form II, nicked or gapped circular DNA molecules.

Table 1: The *parEC* Gene Can Complement the Temperature-Sensitive Growth of both C600*parC1215* and W3110*parE10*[a]

	strain	
plasmid	C600*parC1215*	W3110*parE10*
pET-11c	0.0% (0.0)	0.0% (0.0)
pET11-*parC*	50.8% (11.1)	0.0% (0.0)
pET11-*parE*	0.0% (0.0)	82.1% (6.4)
pET11-p*arE*/*parC*	104.3% (1.3)	78.8% (15.0)
pET11-*parEC*	69.5% (13.5)	89.1% (12.9)

[a] Plating efficiency was calculated as [(colony-forming unit at 42 °C)/(colony-forming unit at 30 °C)] × 100 (%). Each transformation was repeated at least three times, and the average values and the standard deviations are shown.

the *parEC* gene could complement the temperature-sensitive growth of both C600*parC1215* and W3110*parE10* strains. These results suggested that ParEC could substitute for Topo IV in vivo.

DISCUSSION

Type II topoisomerases are well conserved throughout evolution and form a large protein family (*1*). The active form of prokaryotic type II topoisomerases is a heterotetramer of two subunits. DNA gyrase consists of GyrA and GyrB proteins whereas Topo IV consists of ParC and ParE proteins. GyrA and ParC are the catalytic subunits and GyrB and ParE are the ATP-binding subunits. In contrast, eukaryotic type II topoisomerases are homodimers. The amino- and carboxyl-terminal domains of the eukaryotic enzymes are highly homologous to the ATP-binding and catalytic subunits of the prokaryotic enzymes, respectively (*1*).

We examined if the two subunits of Topo IV could function in cis. A fusion protein of the ParE and ParC subunits was constructed by joining two coding regions in frame (Figure 1). The ParEC protein could catalyze both decatenation and relaxation reactions in vitro (Figure 3). In addition, both norfloxacin and Ca^{2+} stimulated ParEC-catalyzed cleavage, and the site preference of Topo IV- and ParEC-catalyzed cleavages was identical (Figure 4). These results demonstrated that the fusion protein of the two subunits of Topo IV was an active topoisomerase. Thus, a heterotetrameric type II topoisomerase can be converted into a homodimeric type II topoisomerase by gene fusion.

The fusion protein provides new opportunities for exploring the function of type II topoisomerases. For instance, complementation assays can be performed using ParEC and yeast topoisomerase II to directly compare the functional activities of prokaryotic and eukaryotic type II topoisomerases in *E. coli* and yeast. Because of the fusion of the two subunits, it is not necessary to control the coexpression and colocalization of the two subunits in cells. In addition, maintenance of the subunit−subunit interaction does not limit the truncation of the ParEC protein to determine the minimal domains required for its activity. Thus, this fusion protein may be useful to determine the essential domains for the catalytic activity of type II topoisomerases. Furthermore, this fusion protein can be used as a model enzyme to investigate the structural and functional differences among prokaryotic and eukaryotic type II topoisomerases by exchange domains of these enzymes.

The specific activity of ParEC in relaxation and decatenation reactions was lower than that of the wild-type Topo IV (Figure 3). However, ParEC seemed to bind and cleave the DNA as well as Topo IV (Figure 4). It was not clear why the catalytic activity of ParEC was reduced. It is possible that amino acid changes and/or loss of two amino acids at the junction of ParE and ParC subunits (Figure 1) might affect the activity of the fusion protein. Alternatively, the physical linkage between the two subunits might alter the catalytic activity. Type II topoisomerases go through a series of drastic conformational changes to catalyze the topoisomerase reaction (*25*−*27*). Thus, a physical linkage between the two subunits could affect conformational changes and/or communication between the two subunits. Another, rather speculative but interesting, possibility is that the exchange of the ParE subunit could contribute to the efficient catalytic cycle of Topo IV. Thus, loss of subunit exchange might reduce the efficiency of ParEC-catalyzed reactions.

Both C600*parC1215* and W3110*parE10* exhibit a temperature-sensitive phenotype due to the lack of Topo IV function during chromosome segregation (*12*−*14*). The *parEC* fusion gene complemented the temperature-sensitive phenotype of both strains (Table 1). In addition, purified ParEC protein decatenated replicating daughter DNA mol-

ecules during *oriC* DNA replication in vitro (Figure 5). These results suggested that ParEC could substitute for Topo IV during DNA replication and chromosome segregation.

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