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The RuvAB Branch Migration Complex Can Displace Topoisomerase IV·Quinolone·DNA Ternary Complexes*

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Molly E. Shea and Hiroshi Hiasa‡

From the Department of Pharmacology, University of Minnesota Medical School, Minneapolis, Minnesota 55455

Quinolone antimicrobial drugs target both DNA gyrase and topoisomerase IV (Topo IV) and convert these essential enzymes into cellular poisons. Topoisomerase poisoning results in the inhibition of DNA replication and the generation of double-strand breaks. Double-strand breaks are repaired by homologous recombination. Here, we have investigated the interaction between the RuvAB branch migration complex and the Topo IV·quinolone·DNA ternary complex. A strand-displacement assay is employed to assess the helicase activity of the RuvAB complex *in vitro*. RuvAB-catalyzed strand displacement requires both RuvA and RuvB proteins, and it is stimulated by a 3′-non-hybridized tail. Interestingly, Topo IV·quinolone·DNA ternary complexes do not inhibit the translocation of the RuvAB complex. In fact, Topo IV·quinolone·DNA ternary complexes are reversed and displaced from the DNA upon their collisions with the RuvAB complex. These results suggest that the RuvAB branch migration complex can actively remove quinolone-induced covalent topoisomerase·DNA complexes from DNA and complete the homologous recombination process *in vivo*.

The generation of genetic diversity is a result of homologous recombination but it is not the main function of recombination (1–3). Recent studies, especially in *Escherichia coli*, have revealed the close link between DNA replication and recombination (4–8). Recombinational repair of stalled or collapsed replication forks takes place frequently, even under the normal growth conditions. A similar link between replication and recombination is also observed in eukaryotes (3, 9). Thus, the primary function of homologous recombination seems to be the repair of prematurely terminated replication forks. In addition, double-strand breaks (DSBs)[1] are repaired only by homologous recombination in prokaryotes, which may explain why the mutations in recombination genes cause the hypersensitivity to DNA damaging agents (10).

There are three steps in the major pathway for conjugational recombination in *E. coli*: the initiation of recombination by RecBCD, RecA-mediated homologous pairing and strand exchange, and the branch migration and the resolution of the Holliday junction by RuvABC (1, 11–14). During the late stage of homologous recombination, RuvA forms a tetramer, binds specifically to the Holliday junction, and then loads RuvB, a hexameric helicase. Two RuvB hexamers sandwich two RuvA tetramers at the Holliday junction to form the RuvAB branch migration complex. The RuvB helicase functions as a molecular motor to promote the branch migration process. RuvC dimer interacts with the RuvAB complex and cleaves the Holliday junction in a sequence-specific manner (14).

Topoisomerases are responsible for DNA unlinking (15, 16). These enzymes break and rejoin the DNA strand by forming a covalent linkage between the enzyme and the DNA at the site of scission. This covalent topoisomerase·DNA complex is normally a fleeting catalytic intermediate, and the steady-state level of the covalent topoisomerase·DNA complex depends on the cleavage-religation equilibrium. Some topoisomerase inhibitors affect the cleavage-religation equilibrium of a topoisomerase and trap a covalent topoisomerase·DNA complex as a topoisomerase·drug·DNA ternary complex. These drugs, with proven clinical antimicrobial and anticancer activities, are often referred to as "topoisomerase poisons" (17–19). Formation of drug-induced covalent topoisomerase·DNA complexes is necessary, but not sufficient, for the cytotoxicity of the drug. In fact, topoisomerase·drug·DNA ternary complexes are completely reversible. An active DNA transaction, such as the passage of a replication fork, must take place to disrupt a ternary complex and generate a permanent DNA damage. Topoisomerase poisoning results in the inhibition of DNA replication and the generation of DSBs (18, 19).

Quinolone antimicrobial drugs, such as norfloxacin and ciprofloxacin, target both DNA gyrase and topoisomerase IV (Topo IV) and convert these essential enzymes into cellular poisons (17–20). Recent studies (20–25) have demonstrated that collisions between advancing replication forks and topoisomerase·quinolone·DNA ternary complexes formed with type II topoisomerases result in the inhibition of DNA replication, but not the generation of DSBs. However, the downstream events of replication fork arrest, including the mechanisms of DSB generation, have not been completely understood. Mutations in genes for homologous recombination, such as *recA* and *recB*, increase the susceptibility to quinolone drugs (23). Thus, homologous recombination plays a critical role in the repair of quinolone-induced DNA damage.

DNA helicases function as molecular motors and play essential roles in many aspects of DNA transaction including DNA replication, DNA repair, and recombination (26, 27). The unwinding of duplex DNA catalyzed by DNA helicases is critical for the continuous progression of such processes. We have shown that Topo IV·norfloxacin·DNA ternary complexes inhibit the unwinding activities of DnaB, T7 Gene 4, and SV40 T-antigen helicases (28). All of these replicative helicases are hexameric helicases (27). Topo IV·norfloxacin·DNA ternary

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‡ To whom correspondence should be addressed: 6-120 Jackson Hall, 321 Church St. SE, Minneapolis, MN 55455. Tel.: 612-626-3101; Fax: 612-625-8408; E-mail: hiasa001@tc.umn.edu.

[1] The abbreviations used are: DSB, double-strand break; Topo IV, topoisomerase IV; nt, nucleotide(s); oligo, oligonucleotide.

complexes also inhibit the action of UvrD, a repair helicase (28). Thus, it is concluded that the formation of Topo IV·norfloxacin·DNA ternary complexes causes a general inhibition of DNA helicases.

Here, we investigated the effect of Topo IV·norfloxacin·DNA ternary complexes on the RuvAB branch migration complex. The homologous recombination process, including the RuvAB-catalyzed branch migration, must proceed in the presence of topoisomerase·quinolone·DNA ternary complexes to repair DSBs generated by the quinolone treatment and/or restart the replication fork stalled by a topoisomerase·quinolone·DNA ternary complex. The question was: Does the Topo IV·norfloxacin·DNA ternary complex inhibit the translocation of the RuvAB complex? We employed a strand-displacement assay to address this question. RuvAB was capable of reversing and displacing Topo IV·norfloxacin·DNA ternary complexes to continue its translocation *in vitro*. These results indicated that the RuvAB branch migration complex could remove topoisomerase·quinolone·DNA ternary complexes to complete the recombinational repair of DSBs and/or the replication restart *in vivo*.

EXPERIMENTAL PROCEDURES

DNAs and Proteins—The construction of M13-T440 (28), a recombinant M13 containing a defined Topo IV binding site (29), and the preparation of the single-stranded circular DNA of M13-T440 were described previously (28). A pair of partial duplex DNAs were prepared according to Shea and Hiasa (28). Briefly, a 62-nucleotide (nt) oligonucleotide (oligo), T4–62, 5′-GCTCGTATCTAGACTCCTAAAAATCCGGGGTATACCCCGGATTTTTAGGAGTGTGTCGCGAT-3′ and an 87-nt oligo T4–62-tail, 5′-GCTCGTATCTAGACTCCTAAAAATCCGGGGTATACCCCGGATTTTTAGGAGTGTGTCGCGATAGTGGAGGCCTACGGCTGCAACTGC-3′ (a 25-nt non-hybridized tail is underlined), were 5′-end-labeled using T4 polynucleotide kinase (Invitrogen) and [γ-^{32}P]ATP (Amersham Biosciences) and then hybridized to the M13-T440 single-stranded DNA to prepare the 5′-end-labeled partial duplex DNAs. These substrates were referred to as T440 and T440T, respectively (see Fig. 1).

Overproduction and purification of *E. coli* ParC and ParE proteins and the reconstitution of Topo IV were described previously (22, 30). *E. coli ruvA* and *ruvB* genes were generated by PCR using *E. coli* C600 genomic DNA as a template and cloned into pET vectors (Novagen). RuvA and RuvB proteins were overexpressed and purified according to protocols described previously (31, 32). The final preparations of RuvA and RuvB were greater than 97% homogeneous for a single band on SDS-polyacrylamide gel electrophoresis (data not shown). The concentrations of purified proteins were determined by the Bradford assay (Bio-Rad) using bovine serum albumin as a standard. Purified RuvA and RuvB proteins, generous gifts of Kenneth Marians (Memorial Sloan-Kettering Cancer Center), were also used.

Strand-displacement Assay for the RuvAB Complex—Standard reaction mixtures (12.5 μl) contained 50 mM Tris-HCl (pH 8.0 at 23 °C), 10 mM magnesium chloride, 10 mM dithiothreitol, 50 μg/ml bovine serum albumin, 2 mM ATP, and 10 fmol (as molecule) DNA substrate, either T440 or T440T. Three hundred fmol (as tetramer) of Topo IV, either in the absence or presence of 400 μM norfloxacin, were bound to the DNA substrate in the first stage incubation of 5 min at 37 °C. The indicated amounts of RuvA (as tetramer) and RuvB (as hexamer) proteins were then added, and the reaction mixtures were incubated during the second stage for 15 min at 37 °C.

Reactions were terminated by adding EDTA to 25 mM, followed by the addition of a quarter volume of a dye mixture containing 15% glycerol, 2% sarkosyl, 0.05% xylene cyanol, 0.05% bromphenol blue, and 50 mM EDTA. Aliquots were analyzed by electrophoresis through vertical 1% agarose (Seakem ME, BioWhittaker Molecular Applications) gels (140 × 120 × 3 mm) at 5 V/cm for 2 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 buffer). Portions of reaction mixtures were also analyzed by electrophoresis through 10% polyacrylamide (19:1, acrylamide to bisacrylamide) gels (140 × 160 × 1.2 mm) at 25 V/cm for 1 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 papers (Whatman) and autoradiographed with Hyperfilm MP films (Amersham Biosciences). Strand displacement was quantitated by scanning images by a STORM 840 PhosphoImager (Amersham Biosciences).

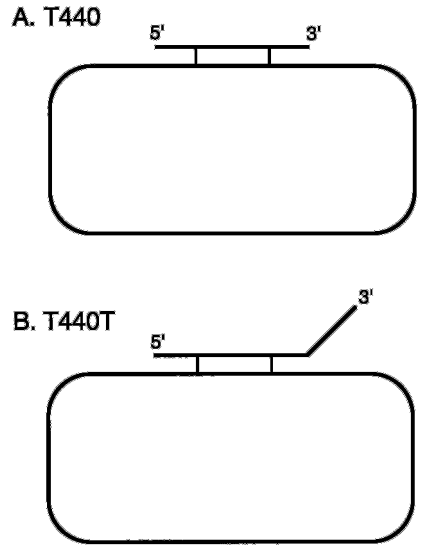

FIG. 1. **Schematic presentations of partial duplex DNAs.** Annealed oligo was 5′-end-labeled by T4 polynucleotide kinase. The 40-bp defined Topo IV binding site (29) located in the middle of the duplex region is shown as an *open square*. Details were described under "Experimental Procedures."

Topo IV-catalyzed Cleavage Assay—Standard reaction mixtures (12.5 μl) containing 50 mM Tris-HCl (pH 8.0 at 23 °C), 10 mM magnesium chloride, 10 mM dithiothreitol, 50 μg/ml bovine serum albumin, 1 mM ATP, 10 fmol (as molecule) DNA substrate, either T440 or T440T, 300 fmol (as tetramer) of Topo IV, and the indicated concentrations of norfloxacin were incubated at 37 °C for 10 min (or the indicated times). 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), heat-denatured by heating at 100 °C for 5 min, and then analyzed by electrophoresis through 10% polyacrylamide (19:1, acrylamide to bisacrylamide) gels (140 × 160 × 1.2 mm) at 25 V/cm for 1 h using TBE buffer. Gels were dried under vacuum onto DE81 papers (Whatman) and autoradiographed with Hyperfilm MP films (Amersham Biosciences). The extent of cleavage was quantitated by scanning images by a STORM 840 PhosphoImager (Amersham Biosciences).

Displacement Assay for Topo IV·Quinolone·DNA Ternary Complexes—The reaction mixtures (200 μl) contained 50 mM Tris-HCl (pH 8.0 at 23 °C), 10 mM magnesium chloride, 10 mM dithiothreitol, 50 μg/ml bovine serum albumin, 2 mM ATP, and 80 fmol (as molecule) T440T DNA. First, 2.4 pmol (as tetramer) of Topo IV, in the presence of 400 μM norfloxacin, were bound to the DNA substrate in the first stage incubation of 5 min at 37 °C. Four pmol each of RuvA (as tetramer) and RuvB (as hexamer) were then added, and the reaction mixtures were incubated during the second stage at 37 °C. Portions (25 μl each) were taken at indicated times. One half of the sample was used for the strand-displacement assay to assess the extent of strand displacement, and the other half was used for the cleavage assay to determine the extent of ternary complexes remained on the DNA. For the strand-displacement assay, reactions were terminated by adding EDTA to 25 mM. DNA products were analyzed by agarose gel electrophoresis as described in the previous section. For the cleavage assay, reactions were terminated by adding SDS to 1%. DNA products were processed, purified, and analyzed by polyacrylamide gel electrophoresis as described in the previous section.

RESULTS

Partial Duplex DNAs—A pair of partial duplex DNAs, T440 and T440T (Fig. 1), were prepared by annealing 5′-end-labeled oligos, T4–62 and T4–62-tail, to the M13-T440 single-stranded DNA, respectively. These oligos are complementary to the cloned Topo IV binding site and the 40-bp-long Topo IV binding site was located in the middle of the 62-bp duplex region on both partial duplex DNAs (28). Thus, these two partial duplex DNAs differed only by the presence of a 25-nt non-hybridized

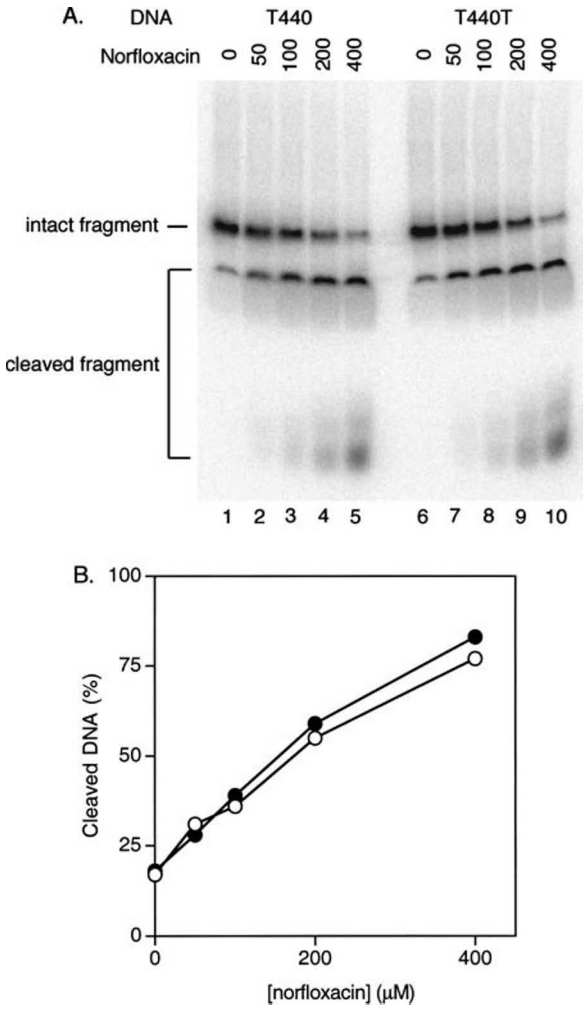

FIG. 2. **Norfloxacin-induced, Topo IV-catalyzed cleavage of the partial duplex DNAs.** *A*, the DNA cleavage assay using Topo IV, the partial duplex DNA, either T440 or T440T, and the indicated concentrations of norfloxacin were performed and analyzed as described under "Experimental Procedures." *B*, the extent of cleavage as a function of the norfloxacin concentration was quantitated using a PhosphorImager. Either T440 (*open circle*) or T440T (*closed circle*) was used as a substrate.

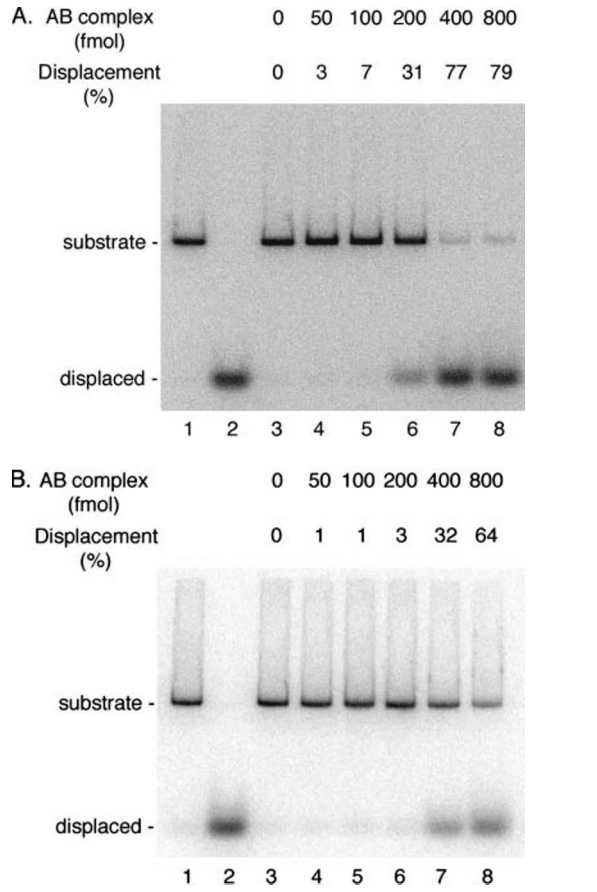

FIG. 3. **A 3′-non-hybridized tail stimulates RuvAB-catalyzed strand displacement activity.** The strand-displacement assay was performed using either T440T (*A*) or T440 (*B*) as a substrate. Reaction mixtures containing 10 fmol of the partial duplex DNA and indicated amounts (as RuvA tetramer·RuvB hexamer complex) were incubated and DNA products were analyzed as described under "Experimental Procedures." *Lanes 1* and *2* in both *panels* show intact and heat-denatured substrate, respectively. *AB complex*, the RuvAB complex.

tail at the 3′-end of the duplex region in T440T DNA (Fig. 1).

First, we examined the stimulation of Topo IV-catalyzed cleavage by norfloxacin using either T440 or T440T as a substrate. As we have reported previously (28), we observed cleavage of the annealed oligo as a result of a norfloxacin-stimulated, Topo IV-catalyzed cleavage (Fig. 2*A*). Both T440 and T440T were cleaved to a similar extent (Fig. 2*B*). We have previously demonstrated that the norfloxacin-stimulated, Topo IV-catalyzed DNA cleavage and the nofloxacin-stimulated Topo IV binding to the DNA coincided with each other (28, 29). Thus, we assumed that the Topo IV-catalyzed cleavage in the presence of various concentrations of norfloxacin shown in Fig. 2 correlated with the extent of Topo IV·norfloxacin·DNA ternary complexes formed on the partial duplex DNA. In addition, because of the length of the duplex region, it was likely that only one molecule of Topo IV could bind to each partial duplex DNA. Note that Topo IV does not bind to single-stranded DNA (30). This system allowed us to monitor the events during the collision between a RuvAB complex and a Topo IV·norfloxacin·DNA ternary complex.

A 3′-Non-hybridized Tail Stimulates RuvAB-catalyzed Helicase Activity in Vitro—The strand-displacement assay is a useful and the most commonly used system to measure the unwinding activity of DNA helicases (26). A partial duplex DNA, an oligo annealed to the single-stranded M13 DNA, is often used as a substrate. In the initial characterization of RuvAB helicase activity, a strand-displacement assay was used to demonstrate its helicase activity (33, 34).

We used a similar strand-displacement assay to assess the helicase activity of the RuvAB branch migration complex (Fig. 3). Either T440 or T440T was used as a substrate. RuvAB could displace an oligo either in the presence (Fig. 3*A*) or absence (Fig. 3*B*) of a 3′-non-hybridized tail. However, the 3′-tail stimulated RuvAB-catalyzed strand displacement activity. Both RuvA and RuvB proteins were required for the strand displacement activity and the maximum activity was observed when the molar ratio of RuvA tetramer to RuvB hexamer was 1:1 (data not shown). These results coincided well with the initial studies of the helicase activity of the RuvAB complex (33, 34).

Topo IV·Norfloxacin·DNA Ternary Complexes Do Not Inhibit the Translocation of RuvAB—The RuvAB branch migration complex plays an essential role during the late stage of homologous recombination to complete the process (14). DSBs generated by the quinolone treatment are repaired by the recombinational repair pathway (9). In addition, homologous recombination could be involved in the restart of a replication fork stalled by a topoisomerase·quinolone·DNA ternary complex (4–8). Thus, the RuvAB complex is likely to function on the DNA, where topoisomerase·quinolone·DNA ternary complexes are formed. The question was: What happens when a

RuvAB complex collides with a topoisomerase·quinolone·DNA ternary complex during the branch migration? It is possible that the ternary complex could inhibit the translocation of RuvAB. A strand break could be generated as a result of the collision between a RuvAB complex and a topoisomerase·quinolone·DNA ternary complex. Another possibility is that RuvAB might somehow translocate through ternary complexes. To directly address this question, we assessed the effect of the Topo IV·norfloxacin·DNA ternary complex on RuvAB (Fig. 4).

RuvAB could displace about 80% of the annealed oligo from T440T DNA under the conditions used (Fig. 4*A*, *lane 3*). Either norfloxacin alone or Topo IV alone did not affect RuvAB-catalyzed strand displacement activity (Fig. 4*A*, *lanes 4* and *5*). In contrast to the replicative helicases (28), formation of a Topo IV·norfloxacin·DNA ternary complex did not inhibit the helicase activity of the RuvAB complex (Fig. 4*A*, *lane 6*). Displaced DNA fragments in the presence of ternary complexes were the intact 87-nt oligo (Fig. 4*B*), demonstrating that collisions between RuvAB and Topo IV·norfloxacin·DNA ternary complexes did not generate any strand breaks at ternary complexes. These results showed that Topo IV·norfloxacin·DNA ternary complexes did not block the passage of the RuvAB branch migration complex. Similar results were obtained when T440 DNA was used as a substrate (data not shown). These results suggested that RuvAB could promote the branch migration reaction in the presence of topoisomerase·quinolone·DNA ternary complexes.

RuvAB Reverses Topo IV·Norfloxacin·DNA Ternary Complexes—To displace the intact oligo from the partial duplex DNA, RuvAB must reverse a Topo IV·norfloxacin·DNA ternary complex and displace it from the DNA. If this were the case, the extent of norfloxacin-induced, Topo IV-catalyzed cleavage of T440T DNA would decrease, as RuvAB would unwind the duplex DNA and displace the annealed oligo. To directly examine this possibility, time courses of RuvAB-catalyzed strand displacement (Fig. 5) and norfloxacin-induced, Topo IV-catalyzed cleavage (Fig. 6) were measured simultaneously.

RuvAB-catalyzed strand displacement in the absence of ternary complexes (Fig. 5) and the stability of Topo IV·norfloxacin·T440T DNA ternary complexes in the absence of RuvAB (Fig. 6) were also measured as controls. RuvAB complex could displace about 85 and 80% of annealed oligo in 30 min in the absence and presence of Topo IV·norfloxacin·DNA ternary complexes, respectively (Fig. 5*B*). These results suggest that the RuvAB complex could reverse the most, if not all, of the ternary complexes it encountered. No displacement of the annealed oligo was observed in the absence of the RuvAB complex (data not shown). Topo IV cleaved about 75% of T440T DNA and, in the absence of RuvAB, the extent of cleavage did not change during the time period of these experiments (Fig. 6*B*). In contrast, the extent of norfloxacin-induced, Topo IV-catalyzed cleavage, that is, the amounts of ternary complexes remained on the DNA, reduced with time when the RuvAB complex was present (Fig. 6*B*). In fact, the increase of RuvAB-catalyzed strand displacement (Fig. 5*B*) correlated well with the decrease of norfloxacin-induced, Topo IV-catalyzed cleavage (Fig. 6*B*). These results demonstrated that the RuvAB complex could force the reversal of Topo IV·norfloxacin·DNA complexes and displace them from the DNA as it unwinds the duplex DNA.

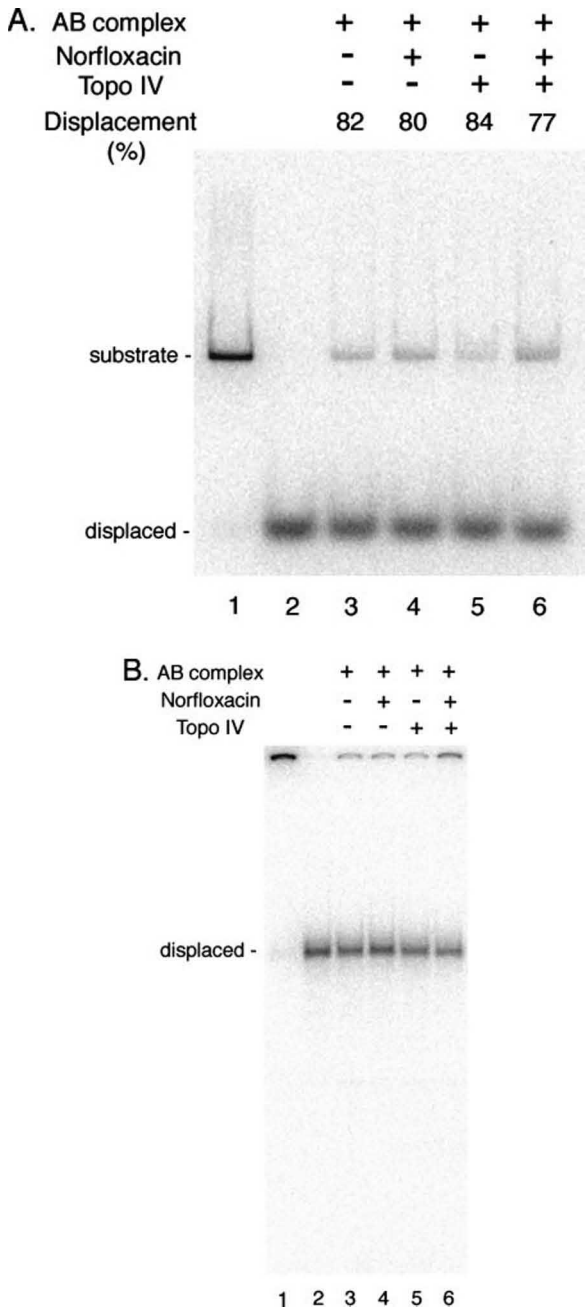

FIG. 4. **Topo IV·norfloxacin·DNA ternary complex does not block RuvAB translocation *in vitro*.** The strand-displacement assay for RuvAB in the absence and presence of Topo IV and norfloxacin, as indicated, was performed as described under "Experimental Procedures." Ten fmol of T440T was used as a substrate. RuvAB (as RuvA tetramer·RuvB hexamer complex) and Topo IV (as tetramer) were 50- and 30-fold molar excess over substrate, respectively, and norfloxacin was at 400 μM when present. DNA products were analyzed by either agarose (*A*) or polyacrylamide (*B*) gel electrophoresis. *Lanes 1* and *2* in both *panels* show intact and heat-denatured substrate, respectively. *AB complex*, the RuvAB complex.

DISCUSSION

E. coli DNA gyrase was the first topoisomerase to be identified as a cellular target of quinolone antibacterial drugs (35, 36). Quinolone drugs block DNA replication not by depriving the cell of gyrase but by converting gyrase into a poison of DNA replication (20). Recent studies have demonstrated that quinolones also target and poison Topo IV, another bacterial type II topoisomerase (21, 23, 30, 37). Some anticancer drugs that target human topoisomerases convert their targets into poisons in a similar manner (18, 19, 38). The poisoning of topoisomerases is mediated by the formation of a topoisomerase·drug·DNA ternary complex. It has been demonstrated that collisions between replication forks and topoisomerase·drug·DNA ternary complexes result in the inhibition of DNA replication (22, 24, 25). We have shown that Topo IV·quinolone·DNA ternary com-

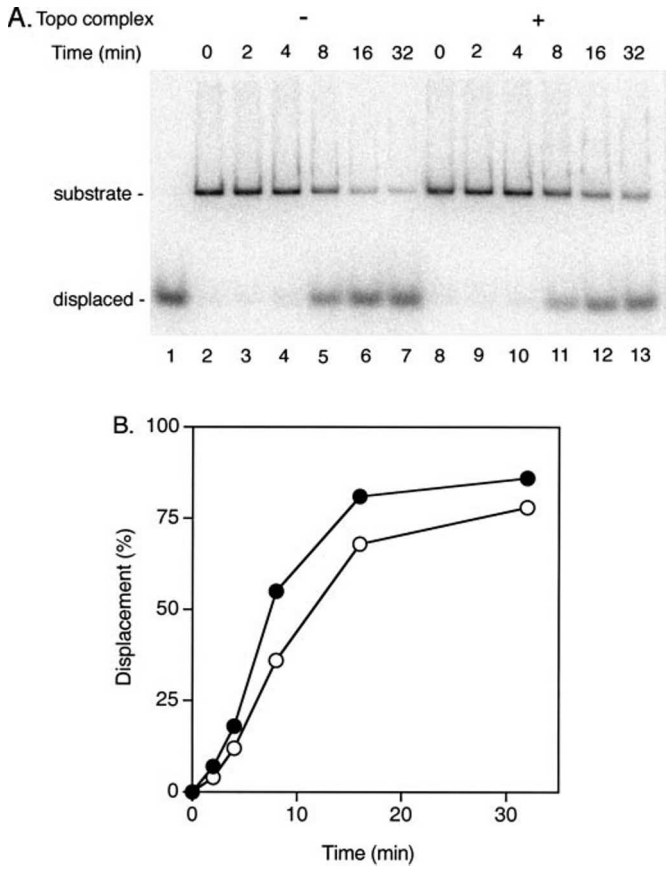

FIG. 5. **Time course of RuvAB-catalyzed helicase activity.** *A*, a time course of RuvAB-catalyzed helicase activity was measured in the absence or presence of Topo IV·norfloxacin·DNA ternary complexes as described under "Experimental Procedures." T440T was used as a substrate. RuvAB (as RuvA tetramer-RuvB hexamer complex) was 50-fold molar excess over substrate. Topo IV (as tetramer) and norfloxacin were 30-fold molar excess over substrate and at 400 μM, respectively, when present. *B*, the amounts of the displaced oligo, in the absence (*closed circle*) or presence (*open circle*) of Topo IV·norfloxacin·DNA ternary complexes, at indicated times were quantitated using a PhosphorImager.

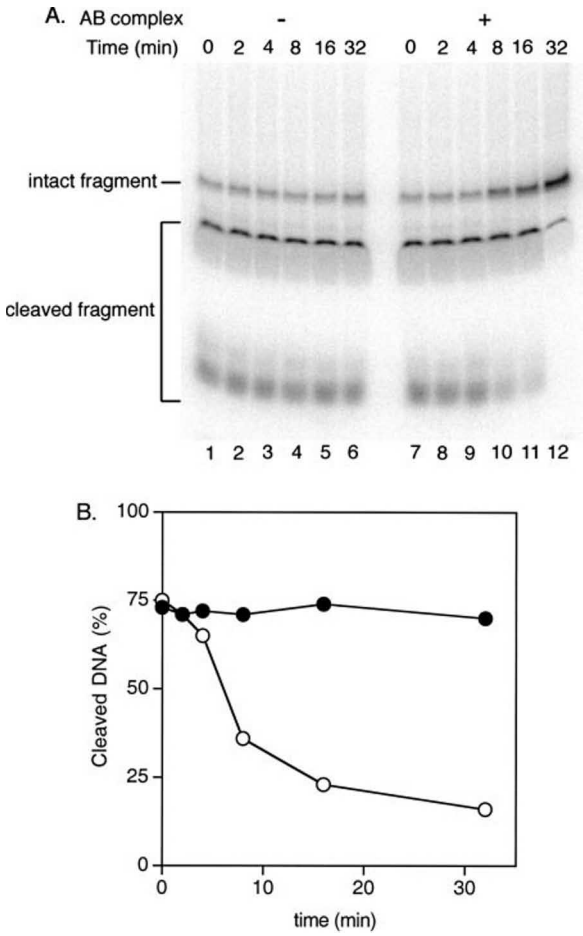

FIG. 6. **RuvAB can displace Topo IV·norfloxacin·DNA ternary complexes.** *A*, a time course of Topo IV-catalyzed cleavage of the T440T DNA was measured in the absence or presence of RuvAB as described under "Experimental Procedures." Thirty-fold molar excess over substrate of Topo IV (as tetramer) and 400 μM norfloxacin were present. RuvAB (as RuvA tetramer-RuvB hexamer complex) was 50-fold molar excess over substrate when present. *B*, the extent of the cleavage, in the absence (*closed circle*) or presence (*open circle*) of RuvAB, at indicated times was quantitated using a PhosphorImager.

plexes block the translocation of replicative helicases (28). Replication fork arrest by the topoisomerase·quinolone·DNA ternary complex may be cause by the inhibition of DnaB translocation. Topo IV·quinolone·DNA ternary complexes also inhibited UvrD repair helicase (28, 39). Thus, topoisomerase·quinolone·DNA ternary complex seems to function as a general inhibitor of DNA helicases.

DSBs are potentially lethal forms of DNA damage that could cause chromosome rearrangement and lead to cell death. Homologous recombination plays a critical role in DSB repair both in prokaryotes and eukaryotes (40, 41). Mutations in recombination genes confer hypersensitivity to DNA damaging agents, such as ionizing radiation and UV radiation (10). *E. coli* strains carrying these mutations also increase the susceptibility to quinolone drugs (23). Thus, homologous recombination is mainly responsible for the repair of DNA damage caused by quinolone drugs. After the quinolone treatment, the homologous recombination machinery must operate in the presence of topoisomerase·quinolone·DNA ternary complexes to repair the damage. This includes the branch migration process catalyzed by the RuvAB complex (14). We asked if a Topo IV·norfloxacin·DNA ternary complex would inhibit the translocation of the RuvAB branch migration complex. A strand-displacement assay revealed that the RuvAB complex could reverse Topo IV·norfloxacin·DNA ternary complexes and continue its translocation (Figs. 5 and 6). These results suggest that the RuvAB complex can complete the branch migration process in the presence of topoisomerase·quinolone·DNA ternary complexes *in vivo*.

We have shown that a Topo IV·norfloxacin·DNA ternary complex could arrest the translocation of hexameric replicative helicases, such as *E. coli* DnaB, T7 Gene 4, and SV40 T-antigen (28). Here, we showed that RuvB, another hexameric helicase, could translocate through a Topo IV·norfloxacin·DNA ternary complex. What are the differences between RuvB and DnaB helicases? One obvious and drastic difference is the fact that the RuvB helicase function as the RuvAB complex. Two RuvB hexamers sandwich two RuvA tetramers at the Holliday junction to form the RuvAB branch migration complex (14). RuvAB is thought to drive branch migration by passing the DNA strands through the two oppositely oriented RuvB hexamers. The DNA strands move through first the RuvA tetramer and then the RuvB hexamer. Thus, it is likely that a Topo IV·norfloxacin·DNA ternary complex would encounter the RuvA tetramer, not the RuvB hexamer, during the collision between a Topo IV-norfloxacin-DNA ternary complex and a RuvAB complex. Either the changes of the local structure of DNA caused by the RuvA tetramer (14) or the interaction between RuvA and Topo IV could cause the reversal of the

ternary complex. Alternatively, although both DnaB and RuvB are hexameric helicases, DnaB and RuvB could influence Topo IV·norfloxacin·DNA ternary complexes in distinct manners. These helicases might alter the local structures of DNA in front of the hexameric ring differently and distinct structural changes caused by approaching helicases could have different effects on the ternary complex. Or, the interactions between these helicases and Topo IV in the ternary complex could affect Topo IV differently. As a result, RuvB, but not DnaB, causes the reversal of Topo IV·norfloxacin·DNA ternary complexes.

Recent studies using *in vitro* replication systems have revealed that not every collision between a topoisomerase·drug·DNA ternary complex and an advancing replication fork results in replication fork arrest and subsequent cytotoxic events (42–44). It is not clear why some drug-induced covalent topoisomerase·DNA complexes, but not others, can arrest replication fork progression. Studies using drug-induced human topoisomerase I·DNA complexes have demonstrated that effective collisions leading to the cytotoxic events, including replication fork arrest and DSB generation, require a covalent topoisomerase I·DNA complex to be formed on the template for leading-strand synthesis, but not on the template for lagging-strand synthesis (42). These results suggest that the protection of the downstream region of the scission site by the topoisomerase may influence the fate of a replication fork. Ternary complexes formed with gyrase can not arrest replication fork progression when gyrase in the ternary complex does not wrap the DNA strand (43, 44). These observations indicate that the binding mode of the topoisomerase in a ternary complex is critical for replication fork arrest by the ternary complex. Further studies are necessary to distinguish ternary complexes that arrest replication fork progression from those that do not.

In addition to the repair of DSBs caused by the quinolone treatment, RuvAB and other recombination proteins might be involved in the restart of a replication fork arrested by a topoisomerase·quinolone·DNA ternary complex. Recent studies in *E. coli* have revealed the pathways for replication restart when replication fork progression is halted prematurely (4–8). Reassembly of replication forks involves the homologous recombination process and it is likely that different recombination pathways would be utilized depending on the cause of replication fork arrest, the extent of replication fork disassembly, and the local structure of DNA at the site of fork arrest. When a replication fork is arrested, fork regression may take place to form a Holliday junction (5–8) and the Holliday junction is recognized and processed by RuvA, RuvB, and RuvC proteins. It seems reasonable to argue that replication forks stalled by topoisomerase·quinolone·DNA ternary complexes are recognized in a manner similar to those stalled by DNA lesions. In this scenario, it is interesting to speculate that the RuvAB complex could bind to the Holliday junction formed at the fork arrest and displace the topoisomerase·quinolone·DNA ternary complex from the DNA. As a result, replication restart could take place without triggering the cytotoxic events. Recent studies demonstrated that *E. coli* strains carrying mutations in *ruv* genes exhibited modest hypersensitivity to quinolone drugs.[2] These results also support the possibility that RuvABC is involved in the repair of quinolone-induced DNA damage.

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