Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 1212–1216, February 1991
Biochemistry

ATP-dependent partitioning of the DNA template into supercoiled domains by *Escherichia coli* UvrAB

(DNA supercoiling/helix tracking/UV damage)

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Communicated by Christian B. Anfinsen, November 26, 1990

ABSTRACT **The helicase action of the *Escherichia coli* UvrAB complex on a covalently closed circular DNA template was monitored using bacterial DNA topoisomerase I, which specifically removes negative supercoils. In the presence of *E. coli* DNA topoisomerase I and ATP, the UvrAB complex gradually introduced positive supercoils into the input relaxed plasmid DNA template. Positive supercoils were not produced when *E. coli* DNA topoisomerase I was replaced by eukaryotic DNA topoisomerase I or when both *E. coli* and eukaryotic DNA topoisomerases I were added simultaneously. These results suggest that like other DNA helix-tracking processes, the ATP-dependent action of the UvrAB complex on duplex DNA simultaneously generates both positive and negative supercoils, which are not constrained by protein binding but are torsionally strained. The supercoiling activity of UvrAB on UV-damaged DNA was also studied using UV-damaged plasmid DNA and a mutant UvrA protein that lacks the 40 C-terminal amino acids and is defective in preferential binding to UV-damaged DNA. UvrAB was found to preferentially supercoil the UV-damaged DNA template, whereas the mutant protein supercoiled UV-damaged and undamaged DNA with equal efficiency. Our results therefore suggest that the DNA helix-tracking activity of UvrAB may be involved in searching and/or prepriming the damaged DNA for UvrC incision. A possible role of supercoiled domains in the incision process is discussed.**

UvrABCD enzymes of *Escherichia coli* are involved in the repair of damaged DNA (1–3). Studies using individually purified Uvr proteins have started to reveal details of the mechanism of action of UvrABCD enzymes. UvrA recognizes thymine dimers introduced by UV irradiation and other bulky adducts to DNA (4–7). UvrA dimerizes and forms a complex with UvrB in the presence of ATP (8–10). Following UvrAB complex formation on the damaged site, UvrC produces two nicks encompassing the damaged site on the same DNA strand (11, 12). The 11- to 12-base-pair incised oligonucleotide is removed by UvrD, which is a DNA helicase, and the gap is filled by DNA polymerase I, followed by ligation at the nicks (13, 14).

Oh and Grossman (15, 16) have demonstrated that the UvrAB complex is a DNA helicase that can displace a short (20- to 50-mer) but not a long (>300-mer) oligonucleotide in a 5′ to 3′ direction. This discovery has led to the speculation that this limited helicase activity of UvrAB complex may be involved in searching for the damaged sites on DNA (17). Alternatively, the limited helicase activity of UvrAB may be involved in prepriming the damaged site on DNA for UvrC incision (9, 18). However, the lack of an assay that can monitor the movement of a helicase on duplex DNA without ends has hampered further analysis of the helicase action of UvrAB complex.

Bacterial DNA topoisomerase I (ω protein) has been used as a DNA conformation-specific probe for studies of protein translocation along duplex DNA (19, 20). This assay is based on the theoretical consideration that vectorial movement of a macromolecular complex with sufficient mass along the helical path of duplex DNA necessitates rotation of the DNA helical axis and hence the generation of strained positive and negative supercoils in the vicinity of the translocating complex (21). Specific removal of negative supercoils by bacterial DNA topoisomerase I results in accumulation of positive supercoils in the DNA template. With such an assay, RNA polymerases (19), simian virus 40 (SV40) large tumor (T) antigen (20), and a DNA helix-tracking protein from *Xenopus laevis* (22) have been shown to be capable of entering duplex DNA and effecting an ATP-dependent movement along the helical path of DNA. The possibility that UvrAB may track along duplex DNA to search for and/or to prime the damage sites (9, 17, 18) prompted us to use bacterial DNA topoisomerase I to monitor UvrAB movement on duplex DNA. We report that the ATP-dependent action of UvrAB on duplex DNA produces both positive and negative supercoiled domains.

MATERIALS AND METHODS

Enzymes, Chemicals, and DNAs. *E. coli* DNA topoisomerase I was kindly provided by James C. Wang (Harvard University). *E. coli* DnaB was obtained from Roger McMacken (The Johns Hopkins University) and UvrD from Floyd Ransom Bryant (The Johns Hopkins University). UvrA and UvrB were purified from *E. coli* cells overexpressing these proteins (4). Mutant UvrA(ΔC40) and UvrB(K45A) proteins were prepared as described (23, 24). P1 nuclease, S1 nuclease, ATP, adenosine 5′-[β,γ-imido]triphosphate (ATP-[β,γ-NH]), and adenosine 5′-[β,γ-methylene]triphosphate (ATP[β,γ-CH_2]) were purchased from Pharmacia. Plasmid pUC.HSO is a derivative of pUC19 containing the *Hin*dIII–*Sph* I fragment of SV40 DNA (25). pAO DNA was constructed by deleting the *Eco*RI–*Ava* I fragment from pAT153 (26). pAOSLO was constructed by inserting an essential *lac* repressor binding sequence (27) to the *Aat* II site of pAO (28). UV-damaged DNA was prepared by irradiating DNA at a dose of 720 J/m^2 with a germicidal lamp (254 nm).

Positive Supercoiling Reaction. Relaxed plasmid DNAs were prepared by treating negatively supercoiled plasmid DNAs with eukaryotic topoisomerase I. The relaxed plasmid DNAs (40 ng) were incubated with various amounts of UvrA, UvrB, and *E. coli* topoisomerase I at 37°C in a buffer (20 μl) containing 20 mM Hepes (pH 7.5), 50 mM KCl (except for

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Abbreviations: ATP[β,γ-NH], adenosine 5′-[β,γ-imido]triphosphate; ATP[β,γ-CH_2], adenosine 5′-[β,γ-methylene]triphosphate; SV40, simian virus 40; T antigen, large tumor antigen.

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experiments shown in Fig. 2A, where 20 mM KCl was used), 8 mM $MgCl_2$, 4 mM ATP, 1 mM dithiothreitol, and bovine serum albumin at 30 μg/ml. The reaction proceeded for 1 hr and was stopped by adding EDTA (25 mM) and SDS (0.5%). Proteinase K (300 μg/ml) was then added and the digestion continued at 37°C for 2 hr.

Enzymatic Reactions. The UvrAB reaction product of input pUC.HSO DNA was mixed with an equivalent amount of negatively supercoiled pUC.HSO DNA. The DNA mixture (total, 40 ng) was treated with HeLa topoisomerase I (20 units) or *E. coli* topoisomerase I (80 ng) in the positive supercoiling reaction buffer (20 μl) at 37°C for 1 hr. S1 nuclease (10 units) digestion was carried out in a buffer (20 μl) containing 30 mM sodium acetate (pH 4.6), 50 mM NaCl, 1 mM zinc acetate, and 5% (vol/vol) glycerol at 37°C for 30 min. The reactions were terminated as above, followed by proteinase K digestion.

Gel Electrophoresis and Indirect Labeling of DNA. Electrophoresis was carried out using 1% agarose gels in 0.5× TPE buffer (29). For two-dimensional gel electrophoresis, the second dimension was run in the presence of 8 μM chloroquine diphosphate. Agarose gels were dried and hybridized *in situ* (30) using pUC.HSO DNA nick-translated with [α-^{32}P]-dCTP.

RESULTS

UvrAB Produces Highly Supercoiled DNA Products in the Presence of Prokaryotic Topoisomerase I and ATP. Translocation of the UvrAB complex along the helical path of duplex DNA is expected to generate local positive and negative supercoils if the UvrAB complex can enter closed circular duplex DNA (see Fig. 1A, model I, where the arrow indicates the direction of movement of DNA relative to the protein). Selective relaxation of negative supercoils by *E. coli* DNA topoisomerase I should result in accumulation of positive supercoils in the DNA template. Indeed, when purified UvrA and UvrB proteins were incubated with a relaxed plasmid DNA (pUC.HSO DNA) in the presence of ATP and *E. coli* topoisomerase I (ω protein), the plasmid DNA became gradually supercoiled (Fig. 1B). This supercoiling reaction required UvrA, UvrB, ATP, and *E. coli* topoisomerase I (Fig. 2A, lanes a–n). The omission of any one of these components abolished the supercoiling reaction. The requirement for ATP was further studied using nonhydrolyzable ATP analogues. ATP[β,γ-NH] and ATP[β,γ-CH_2] could not substitute for ATP in the supercoiling reaction, suggesting that ATP hydrolysis was necessary for the supercoiling reaction (data not shown). The supercoiling assay was also performed using a mutant, UvrB(K45A), which has the lysine residue at the ATPase site replaced by alanine and has been shown to be defective in helicase activity and damage-specific nucleoprotein complex formation (17, 24). UvrB(K45A) could not supercoil DNA in the presence of UvrA, *E. coli* topoisomerase I, and ATP (data not shown).

The requirement for bacterial topoisomerase I is almost certainly due to its DNA conformation specificity. HeLa topoisomerase I, which relaxes both positive and negative supercoils at about equal rates, could not substitute for *E. coli* topoisomerase I. In addition, simultaneous addition of both *E. coli* topoisomerase I and HeLa topoisomerase I abolished the supercoiling reaction (Fig. 2B). These results suggest that both positive and negative supercoils are generated during the ATP-dependent action of UvrAB on duplex DNA and that the supercoils are torsionally stressed and not constrained by protein binding.

The supercoiling activity of UvrAB appeared to be nonspecific. A number of plasmid DNAs with different DNA sequences were equally efficient in the supercoiling assay (data not shown). This supercoiling activity of UvrAB was not shared by other DNA helicases, including *E. coli* Rep protein, UvrD, and DnaB (data not shown).

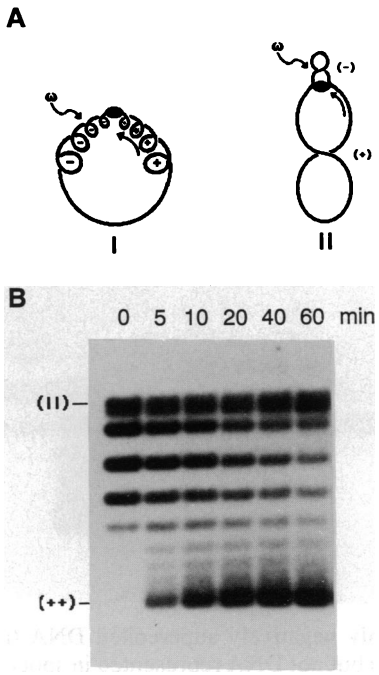

FIG. 1. A DNA supercoiling assay for the DNA helix-tracking activity of *E. coli* UvrAB helicase. (A) Two possible models for the supercoiling activity of a DNA helix-tracking protein (solid ellipse). In model I, the helix-tracking protein generates positive (+) and negative (−) supercoils on the opposite sides of the protein during its movement along the DNA helix. *E. coli* DNA topoisomerase I (ω protein) relaxes the transient negative supercoils before the negative supercoils fuse with positive supercoils through rotation of the DNA segments between the oppositely supercoiled domains. The relatively small size of the UvrAB complex has prompted us to consider another possibility, model II, where the helix-tracking protein contacts two separate sites on a DNA molecule. During the helix-tracking process, DNA moves through one contact site, while the other site is anchored. Arrows within the circular duplex DNAs indicate the direction of DNA translocation relative to the helix-tracking protein. (B) Time course of the supercoiling reaction. Relaxed pUC.HSO DNA (40 ng) was incubated with UvrA (100 ng), UvrB (100 ng), and *E. coli* DNA topoisomerase I (40 ng) in reaction buffer containing 4 mM ATP. At left, positions of nicked (II) and highly positively supercoiled (++) DNA are indicated.

Identification of the Supercoiled DNA Products of the UvrAB Reaction as Highly Positively Supercoiled DNA. The DNA conformation of the UvrAB reaction product was determined by two-dimensional gel electrophoresis with the second dimension in 8 μM chloroquine (30). The reaction product of UvrAB migrated in the two-dimensional gel as a streak of spots (Fig. 3A, regions marked c and d). DNA topoisomers migrating in spot c were shown previously to represent highly positively supercoiled plasmid DNA (30). To prove that the higher mobility of DNA topoisomers in spot c is due to their positively supercoiled conformation, a number of tests were performed. First, the two-dimensional gel pattern did not change upon heating of the reaction product to 65°C for 5 min prior to electrophoresis (data not shown). Second, the reaction product (Fig. 3A, spot c and streak d) was mixed with negatively supercoiled pUC.HSO DNA (Fig. 3A, streak a), which served as an internal control, and then treated with various DNA conformation-specific enzymes. Treatment of the mixed DNA with HeLa topoisomerase I resulted in complete conversion of all the DNA into streak d, which represented relaxed DNA topoisomers (Fig. 3B). Treatment of the mixed DNAs with *E. coli* topoisomerase I, however,

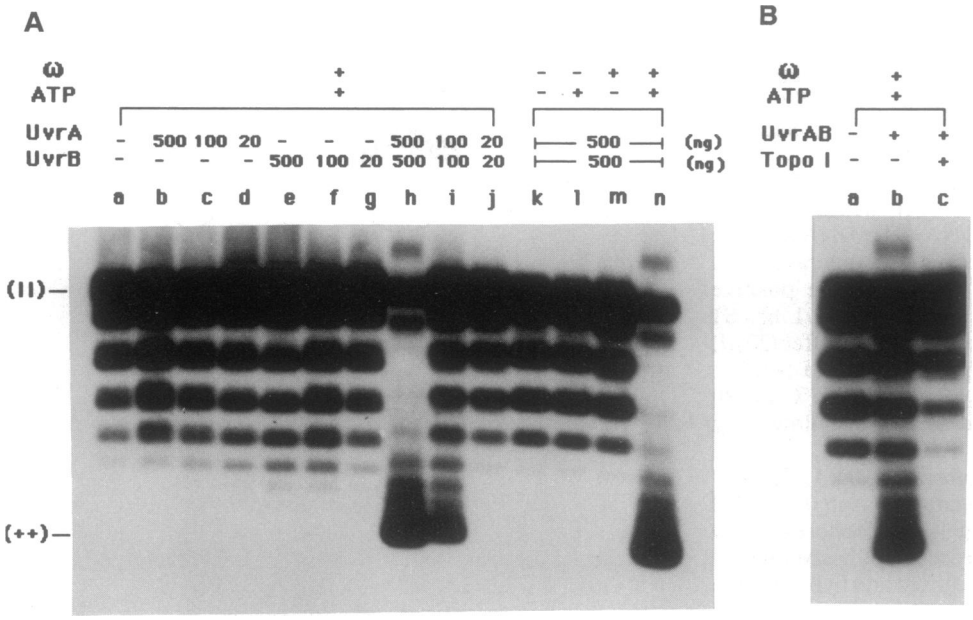

FIG. 2. Characterization of the supercoiling reaction catalyzed by UvrA and UvrB. (*A*) Relaxed pUC.HSO DNA was incubated with the indicated amounts of UvrA and UvrB in the presence (+) or absence (−) of 80 ng of *E. coli* topoisomerase I (ω) and 4 mM ATP. (*B*) Relaxed pUC.HSO DNA was incubated with UvrA (100 ng) and UvrB (100 ng) in the presence of *E. coli* topoisomerase I (ω, 40 ng) and HeLa topoisomerase I (Topo I, 10 units).

converted only negatively supercoiled DNA (internal control, streak a) but not DNA represented in spot c into relaxed topoisomers (streak d) (Fig. 3*C*). These results strongly suggested that the UvrAB reaction product represented in spot c was highly positively supercoiled DNA with a linking number significantly higher than the input relaxed DNA. To further confirm our assignment, the mixed DNAs were treated with S1 nuclease, which specifically nicks and linearizes negatively supercoiled DNA. As predicted, S1 nuclease treatment of the mixed DNAs resulted in almost complete conversion of the negatively supercoiled DNA (streak a) into the linear form (Fig. 3*D*, spot e) but insignificant conversion of the UvrAB reaction product represented in spot c and streak d (Fig. 3*D*).

Preferential Supercoiling of UV-Damaged DNA by UvrAB. The preferential binding of UvrA to UV-damaged DNA (4, 5, 31) prompted us to study the effect of UV-damaged DNA on the supercoiling activity of UvrAB. Monomeric pAOSLO DNA was UV-irradiated to produce about eight thymine dimers per plasmid DNA, mixed with undamaged dimer species, and then treated with UvrAB in the presence of *E. coli* DNA topoisomerase I and ATP. The undamaged dimeric pAOSLO DNA was used in the same reaction as an internal control. The formation of positively supercoiled plasmid DNA was >10 times as efficient for UV-damaged pAOSLO monomer DNA as for its undamaged counterpart (Fig. 4, compare lanes c and d). Unlike UvrAB, SV40 T antigen did not preferentially supercoil the UV-damaged DNA in the positive supercoiling reaction (Fig. 4, lanes g and h). The preferential positive supercoiling of the UV-damaged DNA by UvrAB may be due to the higher binding affinity of UvrAB for UV-damaged DNA. This conjecture was supported by an experiment using a UvrA mutant, UvrAΔC40, which has the 40 C-terminal amino acids deleted and has been shown to have lost the preferential affinity for UV-damaged DNA (36). In the reactions with UvrAΔC40 and UvrB, positive supercoiling of UV-damaged pAOSLO DNA proceeded at a slower rate than that of undamaged pAOSLO DNA (Fig. 4, compare lanes e and f). The decreased rate of supercoiling on UV-damaged DNA could be partly due to inhibition of *E. coli* topoisomerase I by thymine dimers (32). The preferential action of UvrAB on the UV-damaged DNA template therefore suggests that the binding of UvrA to UV-damaged DNA may be rate-limiting in the supercoiling reaction.

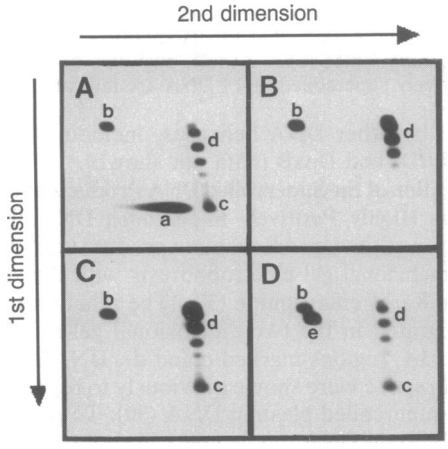

FIG. 3. Identification of the UvrAB reaction product as highly positively supercoiled DNA by two-dimensional gel electrophoresis. Letters a, b, c, and d indicate negatively supercoiled, nicked, positively supercoiled, and relaxed forms of plasmid DNA, respectively. (*A*) A mixture of negatively supercoiled pUC.HSO DNA (streak a) and the UvrAB reaction product of pUC.HSO DNA (spot c and streak d) (see lane i of Fig. 2*A*). The UvrAB reaction product was purified by phenol extraction and ethanol precipitation and then mixed with the same amount of negatively supercoiled pUC.HSO DNA. (*B*) The DNA mixture in *A* was treated with HeLa topoisomerase I. (*C*) The same mixture as in *A* was treated with *E. coli* topoisomerase I. (*D*) The same mixture as in *A* was treated with S1 nuclease.

Increased P1 Nuclease Sensitivity in the Presence of UvrAB and ATP. Positive supercoiling by UvrAB in the presence of *E. coli* topoisomerase I suggests that ATP-dependent translocation of UvrAB on duplex DNA may generate both positive and negative supercoils. To detect the existence of negative supercoils in the DNA template during UvrAB action, P1 nuclease, which is specific for single-stranded DNA, was used in the supercoiling reaction (Fig. 5). P1 nuclease acts preferentially on negatively supercoiled DNA because of the propensity of the DNA to form single-stranded regions. To increase the sensitivity of the assay, linearized pAO DNA was used instead of relaxed DNA. For undamaged linear pAO DNA (Fig. 5, lanes a, c, e, g, i, and k), P1 nuclease sensitivity was markedly increased only when all the three components (UvrA, UvrB, and ATP) were present (lane e). UV-damaged linear pAO DNA has higher sensitivity to P1 nuclease than intact DNA (compare lanes c and d). This is probably due to DNA structural deformations around thy-

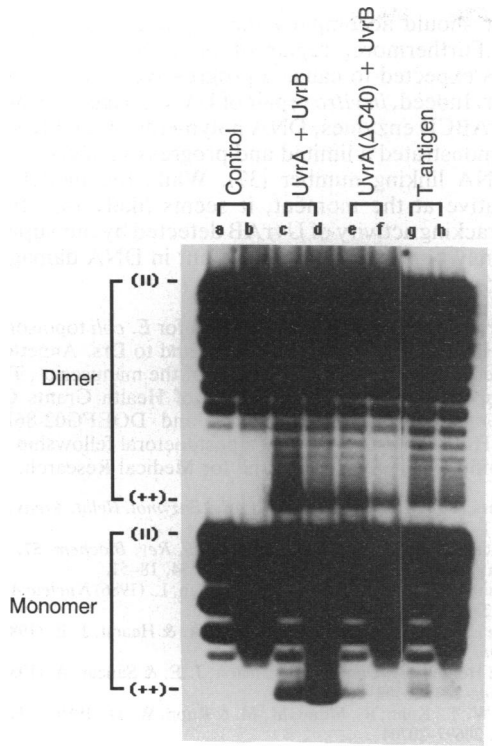

FIG. 4. Preferential supercoiling of UV-damaged DNA by UvrAB. Dimeric pAOSLO DNA (20 ng each) was mixed with equal amounts of intact monomeric pAOSLO DNA (lanes a, c, e, and g) or UV-damaged monomeric pAOSLO DNA (lanes b, d, f, and h), and the supercoiling reaction was performed. Lanes a and b: no DNA helix-tracking proteins. Lanes c and d: UvrA (20 ng) and UvrB (500 ng); the relative molar ratio of UvrA and plasmid DNA in the reaction was 9:1. Lanes e and f: UvrAΔC40 (125 ng) and UvrB (500 ng). Lanes g and h: SV40 T antigen (200 ng).

mine dimers (33, 34). Again, P1 sensitivity of the UV-damaged DNA was increased when UvrA, UvrB, and ATP were all present in the reaction (lane f). The nonhydrolyzable ATP analogues ATP[β,γ-NH] and ATP[β,γ-CH_2] could not

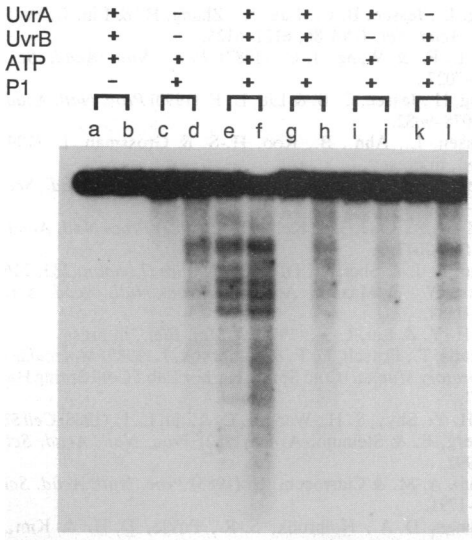

FIG. 5. P1 nuclease sensitivity of the DNA template in the presence of UvrAB and ATP. Linear pAO DNA was prepared by digesting the plasmid DNA with *Pst* I. The linear pAO DNA (40 ng) was incubated with UvrA (250 ng), UvrB (500 ng), and P1 nuclease (1 unit) in the presence of 4 mM ATP in the supercoiling reaction buffer. After 30 min at 37°C, the reaction was terminated as described for the positive supercoiling reaction.

substitute for ATP (data not shown), suggesting that ATP hydrolysis is required for P1 sensitivity. The increased P1 sensitivity supports the existence of negative supercoils during ATP-dependent UvrAB action on duplex DNA.

DISCUSSION

Our results are most consistent with a model in which positive and negative supercoils are simultaneously generated as a result of ATP-dependent translocation of UvrAB complex along the helical path of duplex DNA. Studies using both prokaryotic and eukaryotic DNA topoisomerases I also suggest that both positive and negative supercoils generated by UvrAB are torsionally strained and not constrained by protein binding. Because the DNA template is covalently closed circular DNA, UvrAB must be able to effect its DNA helix-tracking process on intact duplex DNA without ends.

How UvrAB supercoils the DNA template during its ATP-dependent translocation is not clear. We consider two possibilities, which are schematically shown in Fig. 1*A*. One possibility (I) is that the large mass of the translocating complex is sufficient to cause rotation of the DNA helical axis and hence the generation of both positive and negative supercoils in the vicinity of the traversing complex. If this is the case, the negative supercoils are most likely localized in a small region behind the traversing complex. The localization of negative supercoils in a small region ensures a high degree of local superhelical tension and hence the binding of *E. coli* DNA topoisomerase I (35). The other possibility (II) is that UvrAB may anchor to another site on DNA while translocating along the DNA helix. An anchored translocation results in the formation of positive and negative supercoiled domains in DNA. However, it seems unlikely that such an anchored mode of protein translocation, if it exists, can occur processively over a long distance, since we failed to demonstrate extensive template unwinding using eukaryotic DNA topoisomerase I and *E. coli* single-stranded-DNA-binding protein (unpublished results).

The supercoiling activity of UvrAB as defined by our supercoiling assay may be related to the helicase (strand-displacement) activity of UvrAB. Both activities require ATP hydrolysis and most likely involve protein translocation along the DNA helix. However, unlike the supercoiling activity, the strand-displacement activity of UvrAB is inhibited rather than stimulated on UV-damaged DNA (15, 16). It is possible that the two assays may monitor different aspects of the same reaction. Two possible functions have been proposed for the helicase activity (assayed by strand displacement) of UvrAB. One possible function of UvrAB helicase is to search for damaged sites along the DNA (17). The other proposed function is to preprime UV-damaged sites for UvrC incision (9, 18). Significant supercoiling activity of UvrAB on undamaged DNA suggests that the DNA helix-tracking activity of UvrAB may be involved in searching for the damaged sites on duplex DNA. However, preferential supercoiling of the UV-damaged DNA template by UvrAB is more consistent with the latter possibility of prepriming. If the DNA helix-tracking activity is involved in searching for the damaged sites and is inhibited upon reaching the damaged site, the supercoiling activity of UvrAB should be inhibited rather than stimulated on the UV-damaged DNA template. It is possible that the DNA helix-tracking activity may be involved in both searching for the damaged sites on DNA and prepriming the damaged sites for UvrC incision. It remains to be answered why UV damage has opposite effects in the strand-displacement reaction and our supercoiling assay. Note, however, that the effect of UV damage in the strand-displacement reaction may be primarily due to UV damage on the single-stranded region of the DNA template, rather than on the duplex region (15, 16).

The possibility that the DNA helix-tracking activity of UvrAB may be involved in searching and prepriming damage sites led us to consider the following model (Fig. 6). Upon binding to undamaged DNA, UvrAB may scan the duplex DNA, using its DNA helix-tracking activity to reach the damaged site. Once bound to the damaged site, UvrAB can preprime (see below) the damage site by using the same DNA helix-tracking activity. Prepriming of the damaged site by UvrAB involves ATP-dependent translocation of UvrAB at the damaged site. UvrAB is presumed to have a stoichiometry of 2:1 (8–10). One UvrA subunit binds to the damaged site (marked by x), while the other binds a DNA segment nearby to serve as an anchor during ATP-dependent translocation of UvrAB. Anchored translocation of UvrAB at the damage site may displace the damaged site into the small negatively supercoiled domain (Fig. 6*D*). On undamaged DNA, UvrAB may scan the DNA by the same mechanism of anchored translocation. The higher supercoiling activity of UvrAB on damaged DNA may be related to the more stable translocation intermediate at the damaged site. The small highly negatively supercoiled domain of the translocation intermediate at the damage site may be the substrate for UvrC incision. In the presence of *E. coli* topoisomerase I (ω protein), the negative supercoils in the small domain are removed and the template becomes positively supercoiled. This speculative model has two interesting features. (*i*) UvrC incision occurs in a small domain that is topologically separated from the rest of the DNA. Any swivels (e.g., a nick or gap) generated during incision and/or later excision will not significantly affect the superhelical state of the rest of DNA. (*ii*) The damage site is under high negative superhelical tension in a small domain. The high negative superhelical tension may facilitate UvrC incision. A strong prediction of this model is that only a very limited increase of linking number should accompany the repair of a damage site on DNA. Furthermore, repair of multiply damaged plasmid DNA is expected to cause a progressive increase in linking number. Indeed, *in vitro* repair of UV-damaged plasmid DNA by UvrABCD enzymes, DNA polymerase I, and DNA ligase has demonstrated a limited and progressive increase in plasmid DNA linking number (37). While the model remains speculative at the moment, it seems likely that the DNA helix-tracking activity of UvrAB detected by our supercoiling assay may be functionally important in DNA damage repair by Uvr proteins.

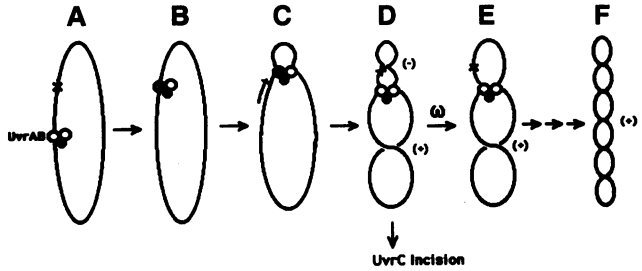

FIG. 6. A speculative model on the possible role(s) of the DNA helix-tracking activity of UvrAB. The cross (x) on the plasmid DNAs indicates the site of DNA damage (e.g., a thymine dimer). UvrAB is drawn as a complex of two molecules of UvrA and one molecule of UvrB. UvrAB first binds to an undamaged site on DNA (*A*) and subsequently moves to the damaged site (*B*). This searching mode of UvrAB may involve the DNA helix-tracking activity. It is assumed that one UvrA molecule is used for binding to the damaged site, based on the studies by Claassen and Grossman (36). The other UvrA molecule in the UvrAB enzyme binds to DNA near the damaged site. The DNA segment between the two UvrA molecules may be very small (20 base pairs) but is exaggerated in the drawing. ATP-dependent protein translocation occurs at the damage site, using the second UvrA molecule as an anchor (*C*). Arrow indicates the direction of DNA movement relative to the UvrAB enzyme. Anchored translocation at the damaged site results in formation of two oppositely supercoiled domains and displacement of the damage site into the small negatively supercoiled domain (*D*). Note that the small size of the negatively supercoiled domain may translate into high local superhelical tension. The relative size of the negatively supercoiled domain may be highly exaggerated in the drawing. Relaxation of negative supercoils by bacterial DNA topoisomerase I (ω protein) results in accumulation of positive supercoils in the template DNA (*E*). Repeated action of UvrAB in the presence of bacterial DNA topoisomerase I produces highly positively supercoiled plasmid DNAs (*F*).

We are grateful to Dr. James C. Wang for *E. coli* topoisomerase I, to Dr. Hai-Young Wu for discussions, and to Drs. Annette Bodley and Peter D'Arpa for critical readings of the manuscript. This work was supported by National Institutes of Health Grants GM27731 (L.F.L.) and GM22846, GM31110, and DOEFG02-86ER60396 (L.G.). H.-S.K. is supported by a postdoctoral fellowship from the Jane Coffin Childs Memorial Fund for Medical Research.

1. Weiss, B. & Grossman, L. (1987) *Adv. Enzymol. Relat. Areas Mol. Biol.* **60**, 1–34.
2. Sancar, A. & Sancar, G. B. (1988) *Annu. Rev. Biochem.* **57**, 29–67.
3. Van Houten, B. (1990) *Microbiol. Rev.* **54**, 18–51.
4. Yeung, A. T., Mattes, W. B. & Grossman, L. (1986) *Nucleic Acids. Res.* **14**, 2567–2582.
5. Van Houten, B., Gamper, H., Sancar, A. & Hearst, J. E. (1987) *J. Biol. Chem.* **262**, 13180–13187.
6. Van Houten, B., Gamper, H., Hearst, J. E. & Sancar, A. (1988) *J. Biol. Chem.* **263**, 16553–16560.
7. Pu, W. T., Kahn, R., Munn, M. M. & Rupp, W. D. (1989) *J. Biol. Chem.* **264**, 20697–20704.
8. Orren, D. K. & Sancar, A. (1988) *UCLA Symp. Mol. Cell. Biol. New Ser.* **83**, 87–94.
9. Orren, D. K. & Sancar, A. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5237–5241.
10. Oh, E. Y., Claassen, L., Thiagalingam, S., Mazur, S. & Grossman, L. (1989) *Nucleic Acids Res.* **17**, 4145–4159.
11. Sancar, A. & Rupp, R. D. (1983) *Cell* **33**, 249–260.
12. Yeung, A. T., Mattes, W. B., Oh, E. Y. & Grossman, L. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6157–6161.
13. Caron, P. R., Kushner, S. R. & Grossman, L. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4925–4929.
14. Husain, I., Van Houten, B., Thomas, D. C., Abdel-Monem, M. & Sancar, A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6774–6778.
15. Oh, E. Y. & Grossman, L. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 3638–3642.
16. Oh, E. Y. & Grossman, L. (1989) *J. Biol. Chem.* **264**, 1336–1343.
17. Seeley, T. & Grossman, L. (1990) *J. Biol. Chem.* **265**, 7158–7165.
18. Caron, P. R. & Grossman, L. (1988) *Nucleic Acids Res.* **16**, 7855–7865.
19. Tsao, Y. P., Wu, H.-Y. & Liu, L. F. (1989) *Cell* **56**, 111–118.
20. Yang, L., Jessee, B. C., Lau, K., Zhang, H. & Liu, L. F. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 6121–6125.
21. Liu, L. F. & Wang, J. C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 7024–7027.
22. Zhang, H., Jessee, C. B. & Liu, L. F. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 9078–9082.
23. Claassen, L., Ahn., B., Koo, H.-S. & Grossman, L. (1991) *J. Biol. Chem.*, in press.
24. Seeley, T. & Grossman, L. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 6577–6581.
25. Wold, M. S., Li, J. J. & Kelly, T. J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 3643–3647.
26. Twigg, A. J. & Sherratt, D. (1980) *Nature (London)* **283**, 216–218.
27. Gilbert, W. & Maxam, A. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 3581–3584.
28. Wu, H.-Y. & Liu, L. F. (1991) *J. Mol. Biol.*, in press.
29. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), p. 6.7.
30. Wu, H. Y., Shyy, S. H., Wang, J. C. & Liu, L. F. (1988) *Cell* **53**, 433–440.
31. Seeberg, E. & Steinum, A. L. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 988–992.
32. Pedrini, A. M. & Ciarrocchi, G. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1787–1791.
33. Pearlman, D. A., Holbrook, S. R., Pirkle, D. H. & Kim, S. (1985) *Science* **227**, 1304–1308.
34. Husain, I., Griffith, J. & Sancar, A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2558–2562.
35. Wang, J. C. & Liu, L. F. (1979) in *Molecular Genetics*, ed. Tailor, J. H. (Academic, New York), Part III, pp. 65–88.
36. Claassen, L. & Grossman, L. (1991) *J. Biol. Chem.*, in press.
37. Backendorf, C., Olsthoorn, R. & van de Putte, P. (1989) *Nucleic Acids Res.* **17**, 10337–10351.