

Template supercoiling during ATP-dependent DNA helix tracking: Studies with simian virus 40 large tumor antigen

(topoisomerase/positive supercoiling/helicase/DNA replication)

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Communicated by James C. Wang, May 25, 1989

ABSTRACT Incubation of topologically relaxed plasmid DNA with simian virus 40 (SV40) large tumor antigen (T antigen), ATP, and eubacterial DNA topoisomerase I resulted in the formation of highly positively supercoiled DNA. Eukaryotic DNA topoisomerase I could not substitute for eubacterial DNA topoisomerase I in this reaction. Furthermore, the addition of eukaryotic topoisomerase I to a preincubated reaction mixture containing both T antigen and eubacterial topoisomerase I caused rapid relaxation of the positively supercoiled DNA. These results suggest that SV40 T antigen can introduce topoisomerase-relaxable supercoils into DNA in a reaction coupled to ATP hydrolysis. We interpret the observed T antigen supercoiling reaction in terms of a recently proposed twin-supercoiled-domain model that describes the mechanics of DNA helix-tracking processes. According to this model, positive and negative supercoils are generated ahead of and behind the moving SV40 T antigen, respectively. The preferential relaxation of negative supercoils by eubacterial DNA topoisomerase I explains the accumulation of positive supercoils in the DNA template. The supercoiling assay using DNA conformation-specific eubacterial DNA topoisomerase I may be of general use for the detection of ATP-dependent DNA helix-tracking proteins.

The simian virus 40 (SV40) large tumor antigen (T antigen) is a multifunctional protein (1). It regulates viral gene expression, is responsible for most of the tumorigenic activities of SV40 virus, and is essential for viral DNA replication. In infected host cells, it also stimulates cellular DNA replication and the expression of certain cellular genes (1). Biochemical studies have demonstrated that SV40 T antigen binds with high affinity to two sites within the regulatory region of the SV40 genome. Both sites contain multiple copies of a recognition pentamer GAGGC (2). Binding of T antigen to site I, the strongest binding site, autoregulates its own synthesis by repressing early transcription. Site II is essential for initiation of SV40 viral DNA replication (3, 4). Different from site I, which has two recognition pentamers arranged in the same orientation and separated by an A/T tract, site II has four GAGGC pentamers organized into a perfect palindrome (5). In addition to its essential role in initiation of SV40 replication, T antigen also appears to be associated with the replication forks during the elongation phase of DNA synthesis (6, 7).

The recent identification of a helicase activity intrinsic to SV40 T antigen has shed light on a possible mechanism of T antigen function at least in SV40 DNA replication (6, 8, 9). Extensive unwinding of an SV40 replication origin-containing plasmid DNA by T antigen can be demonstrated in the presence of single-strand DNA binding protein and eukaryotic DNA topoisomerase I. The sequence requirement of the

T antigen unwinding reaction is similar to that of DNA replication, suggesting a direct role of the T antigen unwinding reaction in the initiation of SV40 DNA replication (10-12).

To study the possible ATP-dependent translocation of T antigen on DNA, we have employed an assay that was first developed for studies on the translocation of RNA polymerase complex along the duplex DNA (13). In the presence of eubacterial DNA topoisomerase I, which specifically removes negative supercoils, RNA transcription results in rapid accumulation of positive supercoils on the DNA template. This result has been explained in terms of a twin-supercoiled-domain model for RNA transcription (13-17). Since protein translocation along the DNA helical path should follow similar mechanics, we have studied the possible ATP-dependent translocation of T antigen in the presence of eubacterial DNA topoisomerase I. Our results suggest that, like RNA transcription, ATP-dependent movement of T antigen along the helical path of duplex DNA can also generate topoisomerase-relaxable (torsionally unconstrained) supercoils and can be sensitively detected by such an assay.

MATERIALS AND METHODS

Reagents, Enzymes, and DNAs. Phosphocreatine and creatine phosphokinase were purchased from Sigma. Dithiothreitol was from IBI. [α - 32 P]dCTP was purchased from NEN. *Escherichia coli* DNA topoisomerase I and *Micrococcus luteus* topoisomerase I were kind gifts from James C. Wang (Harvard University). Calf thymus DNA topoisomerase I and HeLa DNA topoisomerase I were purified by a modification of the published procedure (18). The immunoaffinity column of monoclonal antibody against SV40 T antigen and samples of SV40 T antigen and HeLa RP-A were kind gifts from Thomas Kelly and Mark Wold (Johns Hopkins University). Purified T antigen prepared from a baculovirus expression system was kindly provided by Bruce Stillman (Cold Spring Harbor Laboratory). pSV2.cat is a derivative of plasmid pBR322, which contains the SV40 origin of replication (19). pHu4A is a derivative of plasmid pBR322 DNA with the human histone H4 gene inserted at the *Eco*RI and *Hind*III sites (20). pUC.HSO is a derivative of pUC19, which contains the *Hind*III-*Sph* I origin-containing fragment of SV40 DNA (11). pUC.HSO.d4 is identical to pUC.HSO except for a small 4-base pair (bp) deletion in the middle of the palindrome at site II (11).

Purification of SV40 T Antigen. Human 293 cells were adapted to grow in a spinner flask in s-MEM (Eagle's minimal essential medium for suspension cultures) supplemented with

Abbreviations: SV40, simian virus 40; T antigen, large tumor antigen.

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10% fetal bovine serum. Cells were infected with recombinant adeno-SV40 virus, Ad5SVR115 (21), at a multiplicity of infection of 10 plaque-forming units per cell and incubated at 37°C for 48 hr. Infected cells were harvested, and T antigen was extracted and purified by immunoaffinity chromatography as described (22).

Supercoiling Reactions. The standard reaction mixture (20 μ l) contained 20 mM Hepes (pH 7.5), 7 mM MgCl₂, 4 mM ATP, 15 mM potassium phosphate, 0.5 mM dithiothreitol, 40 mM phosphocreatine, 100 μ g of creatine phosphokinase per ml, 50 ng of plasmid DNA, and 30 μ g of bovine serum albumin per ml. SV40 T antigen and topoisomerases were added as described in each figure legend. Reaction mixtures were incubated at 37°C for various times and terminated by adding 5 μ l of a stop solution (5% SDS/50 mM EDTA/500 μ g of proteinase K per ml). The mixtures were further incubated at 37°C for 60 min.

Enzymatic Treatment. Relaxation of plasmid DNA with calf thymus, HeLa DNA topoisomerase I, or *E. coli* DNA topoisomerase I was done under the same condition as that of the supercoiling reaction. S1 nuclease treatment was done in a reaction mixture (20 μ l) containing 30 mM sodium acetate (pH 4.6), 50 mM NaCl, 1 mM ZnCl₂, and 11 units of S1 nuclease (Pharmacia). After 30 min of incubation at 37°C, the reaction was terminated as described for the supercoiling reactions.

Electrophoresis. DNA samples were analyzed on 0.8% agarose gels in 0.5 \times TPE buffer (23). For two-dimensional gel electrophoresis, the second dimension was carried out in 0.5 \times TPE buffer containing 7 μ M chloroquine.

RESULTS

Formation of a Highly Supercoiled DNA Species in the Presence of *E. coli* DNA Topoisomerase I and SV40 T Antigen. When *E. coli* DNA topoisomerase I and SV40 T antigen were added to a reaction mixture containing pSV2.cat DNA and ATP, a DNA species that migrated faster than the relaxed pSV2.cat DNA was gradually accumulated over time (Fig. 1A, lanes H–L, see the arrow). The amount of this DNA species increased with increasing concentration of either SV40 T antigen (Fig. 1B, lanes F–J) or eubacterial DNA topoisomerase I (Fig. 2). This DNA species was later shown to be highly positively supercoiled DNA (see later sections). The slight reduction in plasmid DNA linking number in the presence of calf thymus DNA topoisomerase I and T antigen (Fig. 1A, lanes M–Q; Fig. 1B, lanes K–O) may be related to the nonspecific ATP-dependent unwinding observed previously in lower ionic strength (11).

When plasmid pHu4A, which does not contain the SV40 replication origin sequence, was used to replace pSV2.cat DNA, a less highly positively supercoiled DNA species was observed (compare lanes F–J with lanes P–T in Fig. 1B). However, more detailed studies of the origin-specificity showed that a functional origin sequence was not required; both plasmid DNA pUC.HSO, which contains the minimal origin of SV40 DNA, and pUC.HSO.d4, which is identical to pUC.HSO except for a small 4-bp deletion in the middle of the palindromic sequence of site II (11), became equally supercoiled in this assay (Fig. 1C, lanes E and F). On the other hand, the vector DNA, pUC19, which does not contain any SV40 sequence, was less active in the supercoiling assay (Fig. 1C, lane D). Studies using a number of additional mutant SV40 sequences revealed similar results in that a functional origin sequence was not required (data not shown). This positive supercoiling reaction is therefore different from the T antigen unwinding reaction or replication reaction in terms of DNA sequence specificity. As controls, T antigen unwinding reactions in the presence of a single-strand binding protein (in this case, HeLa RP-A) (24) and HeLa DNA

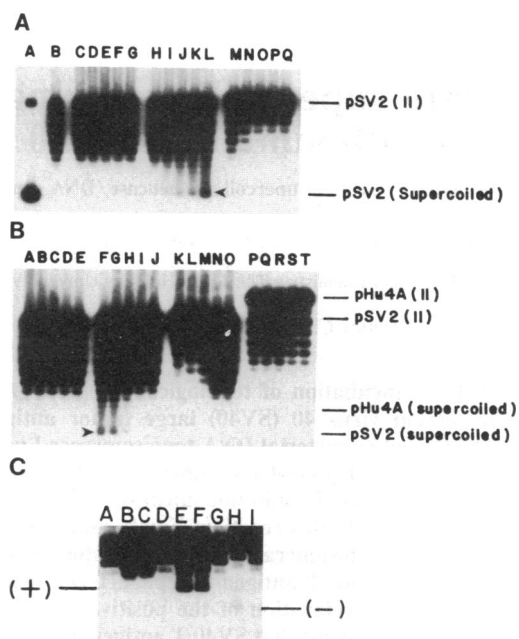


FIG. 1. Supercoiling of DNA by *E. coli* DNA topoisomerase I and SV40 T antigen. (A) Time course of supercoiling. Reaction mixtures containing 50 ng of relaxed pSV2.cat DNA and 0.6 μ g of SV40 T antigen (lanes C–Q) were incubated at 37°C for different time periods with either 3 units of *E. coli* DNA topoisomerase I (lanes H–L) or 3 units of calf thymus topoisomerase I (lanes M–Q). Lane A, form I supercoiled pSV2.cat DNA used as a mobility marker. Lane B, relaxed pSV2.cat DNA, untreated control. The same relaxed DNA was used in the rest of the reactions (lanes C–O). Lanes C, H, and M, 10 min of incubation. Lanes D, I, and N, 30 min of incubation. Lanes E, J, and O, 60 min of incubation. Lanes F, K, and P, 120 min of incubation. Lanes G, L, and Q, 180 min of incubation. (B) Effect of T antigen concentration on template supercoiling. Reaction mixtures containing either 50 ng of relaxed pSV2.cat DNA (lanes A–O) or 50 ng of relaxed pHu4A DNA (lanes P–T) were incubated at 37°C for 50 min. Lanes F–J and P–T, 3 units of *E. coli* DNA topoisomerase I. Lanes K–O, 3 units of calf thymus topoisomerase I. Lanes A, F, K, and P, 0.6 μ g of SV40 T antigen. Lanes B, G, L, and Q, 0.3 μ g of SV40 T antigen. Lanes C, H, M, and R, 0.15 μ g of T antigen. Lanes D, I, N, and S, 0.075 μ g of T antigen. Lanes E, J, O, and T, no T antigen. (C) Sequence dependence of template supercoiling. T antigen (0.6 μ g) was present in all reactions. Lanes A–C, control, no topoisomerase. Lanes D–F, each contained 3 units of *E. coli* DNA topoisomerase I. Lanes G–I, each contained 3 units of calf thymus DNA topoisomerase I and 0.2 μ g of HeLa RP-A (22). The DNA templates used in each reaction were pUC19 (lanes A, D, and G), pUC.HSO (lanes B, E, and H), and pUC.HSO.d4 (lanes C, F, and I). The highly unwound DNA is indicated by (+) and the supercoiled DNA species formed in the *E. coli* topoisomerase I reaction is indicated by (-). The reactions were terminated by adding SDS and EDTA. The samples were fractionated on a 0.8% agarose gel and analyzed by the Southern blot/hybridization method.

topoisomerase I were also performed; plasmid DNA, pUC.HSO.d4, which is defective in DNA replication *in vivo* and *in vitro* (11), was not extensively unwound by SV40 T antigen helicase activity (Fig. 1C, lane I). As expected, a small population of pUC.HSO DNA was extensively unwound by SV40 T antigen under identical conditions as evidenced by the appearance of fast-migrating, highly negatively supercoiled DNA species (Fig. 1C, lane H).

Eukaryotic DNA Topoisomerase I Cannot Substitute for Eubacterial DNA Topoisomerase I in the T Antigen-Mediated Supercoiling Reaction. The formation of the highly positively supercoiled DNA species in the T antigen reaction was dependent on the presence of eubacterial DNA topoisomerase I; either *E. coli* DNA topoisomerase I or *M. luteus* DNA topoisomerase I was active (Fig. 2, lanes g–o). However,

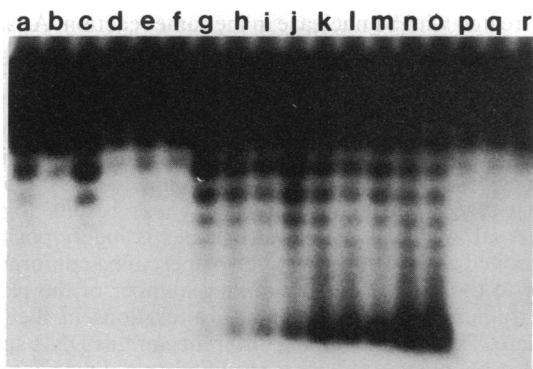


FIG. 2. Dominant effect of eukaryotic topoisomerase I over eubacterial topoisomerase I in the SV40 T antigen reaction. Each reaction mixture contained 50 ng of relaxed pSV2.cat DNA. Except for lanes b and c, each reaction mixture also contained 0.3 μ g of SV40 T antigen. Lane a, control, no topoisomerase. Lane b, 10 units of calf thymus topoisomerase I (no T antigen). Lane c, 10 units of *M. luteus* topoisomerase I (no T antigen). Lanes d–f, 10 units of calf thymus topoisomerase I. Lanes g–i, 2 units of *M. luteus* topoisomerase I. Lanes j–l, 10 units of *M. luteus* topoisomerase I. Lanes m–o, 50 units of *M. luteus* topoisomerase I. Lanes p–r, 10 units each of calf thymus topoisomerase I and *M. luteus* topoisomerase I. Reaction mixtures were incubated at 37°C for 1 hr for samples shown in lanes d, g, j, m, and p; 3 hr for samples shown in lanes e, h, k, n, and q; and 6 hr for the rest of the samples. Samples were processed as described in the legend to Fig. 1.

substitution of eubacterial DNA topoisomerase I with eukaryotic DNA topoisomerase I (calf thymus DNA topoisomerase I) failed to produce the fast migrating DNA species (Fig. 2, lanes d–f). When both eubacterial (*M. luteus*) topoisomerase I and eukaryotic (calf thymus) topoisomerase I were added together, the effect of eukaryotic topoisomerase I appeared dominant—no fast-migrating species was produced (Fig. 2, lanes p–r). We have also examined the effect of adding eukaryotic DNA topoisomerase I at different times after the formation of the fast-migrating DNA species, and in all cases, the DNA species formed in the presence of *M. luteus* topoisomerase I was immediately (within 1 min) converted to relaxed DNA by calf thymus DNA topoisomerase I (data not shown). These results suggest that the supercoils generated in this T antigen reaction are torsionally unconstrained.

The T Antigen-Mediated Supercoiling Reaction Is ATP-Dependent and Is Strongly Stimulated by Polyethylene Glycol (PEG). The addition of PEG 20,000 to the T antigen reaction greatly stimulated the formation of the highly positively supercoiled DNA species (Fig. 3A). The stimulation was dependent on the PEG concentration (Fig. 3A, lanes a–f), with 6% PEG 20,000 being optimal (Fig. 3A, lane d). Similar to the unstimulated reaction, the formation of this DNA species was time-dependent and required ATP. In the presence of 6% PEG, >80% of the relaxed DNA was converted into the supercoiled DNA species in 60 min (Fig. 3B). Though ATP was required in this reaction, the ATP regenerating system was not (Fig. 3C). Analogs of ATP (e.g., ATP[γ -S] and AMP-PCP) did not substitute for ATP either in this reaction or in the unstimulated reaction, suggesting that ATP hydrolysis was required (data not shown). The effect of PEG was probably not through topoisomerase activity, as the catalytic rate of eubacterial DNA topoisomerase I under our reaction conditions was not altered by PEG (data not shown). The sequence specificity of the T antigen reaction was much relaxed in PEG. All plasmids tested, including several SV40 replication origin mutants and plasmid vectors, were almost quantitatively converted to the supercoiled DNA species (data not shown).

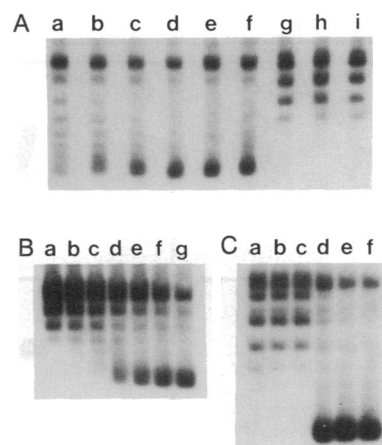


FIG. 3. The supercoiling reaction requires ATP and is stimulated by PEG. Each reaction mixture contained 50 ng of relaxed pUC.HSO DNA. (A) Effect of PEG concentration. All reaction mixtures were incubated at 37°C for 3 hr. Each reaction mixture in lanes a–f contained 50 units of *M. luteus* DNA topoisomerase I and 0.8 μ g of SV40 T antigen. Lane g, control, no topoisomerase or T antigen. Lane h, 0.8 μ g of SV40 T antigen. Lane i, 50 units of *M. luteus* topoisomerase I. PEG concentrations were 0% (lane a), 2% (lane b), 4% (lane c), 6% (lane d), 8% (lane e), and 10% (lanes f–i). (B) Time course of the reaction. Each reaction mixture contained 50 units of *M. luteus* DNA topoisomerase I, 0.8 μ g of T antigen, and 6% PEG. Reaction mixtures were incubated at 37°C for 0 min (lane a), 5 min (lane b), 10 min (lane c), 20 min (lane d), 30 min (lane e), 40 min (lane f), or 50 min (lane g). (C) ATP requirement. Each reaction mixture contained 50 units of *M. luteus* topoisomerase I, 0.8 μ g of T antigen, and 6% PEG. Lanes a–c, ATP omitted. Lanes a and d, phosphocreatin omitted. Lanes a, b, d, and e, creatine phosphokinase omitted.

The Supercoiled DNA Species Is Highly Positively Supercoiled DNA. To determine the nature of the DNA species formed in the presence of *E. coli* DNA topoisomerase I and SV40 T antigen, the reaction product was analyzed by two-dimensional gel electrophoresis. The first dimension was carried out in TPE buffer, and the second dimension was performed in TPE buffer containing 7 μ M chloroquine (Fig. 4). The electrophoretic positions of relaxed pSV2.cat DNA (Fig. 4A, streak d) and form I supercoiled pSV2.cat DNA (Fig. 4A, streak a) are shown. As expected, no mobility change was observed when relaxed pSV2.cat DNA was treated with SV40 T antigen alone (Fig. 4B, streak d). If DNA was incubated with both calf thymus topoisomerase I and T antigen (Fig. 4D, streak d), a small amount of unwinding of DNA was detected (i.e., the average linking number of the DNA decreased). However, when the DNA was treated with both T antigen and *E. coli* topoisomerase I, a fraction of the product DNA migrated in a position expected for highly positively supercoiled DNA (Fig. 4C, spot c) (15).

To confirm that this species of DNA was positively supercoiled, relaxed pUC.HSO DNA was quantitatively converted to the supercoiled DNA species in the presence of 6% PEG (Fig. 5A, spot c) and used for enzymatic treatments. The newly formed DNA species was mixed together with form I supercoiled pUC.HSO DNA (Fig. 5A, streak a) and treated with various enzymes. As expected, the newly formed DNA species and the form I negatively supercoiled pUC.HSO DNA (included as an internal control) were relaxed to completion by treatment with HeLa topoisomerase I, which is known to relax positive and negative supercoils with similar rates (Fig. 5B, streak d). In contrast, treatment with *M. luteus* DNA topoisomerase I, which preferentially relaxes negatively supercoiled DNA, caused relaxation of form I negatively supercoiled DNA but did not alter the mobility of the newly formed DNA species (Fig. 5C, streak e). To rule out the possibility that the newly formed DNA species may

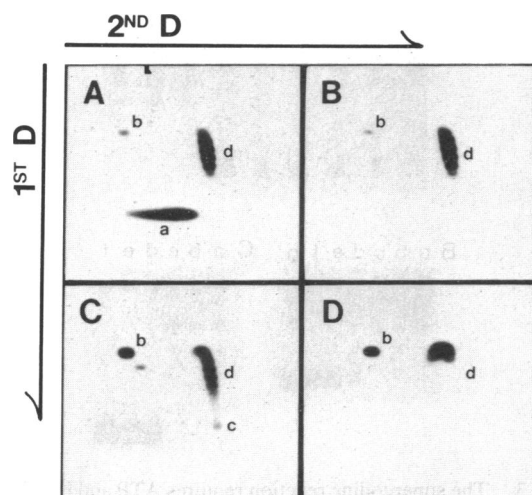


FIG. 4. Analysis of the topological structure of the supercoiled DNA species by two-dimensional gel electrophoresis. The DNA species (from the same DNA sample as shown in Fig. 1A, lane L) produced by treatment with *E. coli* DNA topoisomerase I and SV40 T antigen was analyzed by using two-dimensional gel electrophoresis. D, dimension. (A) Mixture of two DNA samples: form I supercoiled pSV2.cat DNA, shown as streak a, and relaxed pSV2.cat DNA, shown as streak d. (B) Same DNA sample as that shown in Fig. 1A, lane G (T antigen control, no topoisomerase). (C) Same DNA sample as that shown in Fig. 1A, lane L (T antigen and *E. coli* topoisomerase I). (D) Same sample as that shown in Fig. 1A, lane Q (T antigen and calf thymus topoisomerase I). Streak a, form I supercoiled pSV2.cat DNA; spot b, form II nicked pSV2.cat DNA; spot c, highly positively supercoiled DNA species; spot d, relaxed pSV2.cat DNA.

contain hairpins or other single-stranded structures, both form I supercoiled DNA and the newly formed DNA species

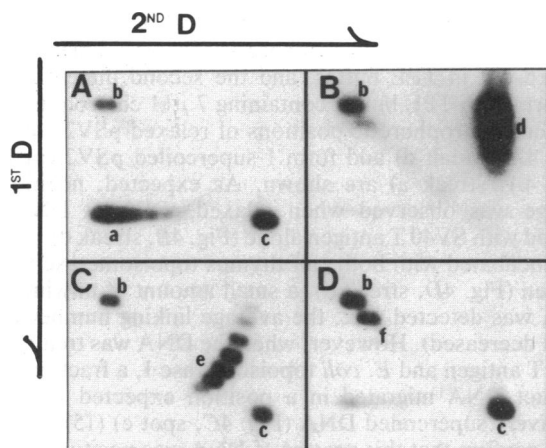


FIG. 5. Identification of the newly formed DNA species as highly positively supercoiled DNA by enzymatic treatments. Relaxed pUC.HSO DNA was used to produce the newly formed DNA species in a reaction mixture containing 6% PEG as described in the legend to Fig. 3. Nearly all pUC.HSO DNA was converted to this DNA species in such a reaction. This DNA species was then mixed with form I pUC.HSO DNA and treated with various enzymes. Each reaction mixture contained about 25 ng of the newly formed DNA species (A, spot c) and 100 ng of form I supercoiled pUC.HSO DNA (A, streak a). Two-dimensional gel electrophoresis was performed. D, dimension. (A) Control, no treatment. (B) Treatment with 90 units of HeLa topoisomerase I. (C) Treatment with 2 units of *M. luteus* topoisomerase I. (D) Treatment with 11 units of S1 nuclease. Streak a, negatively supercoiled form I pUC.HSO DNA; spot b, nicked pUC.HSO DNA; spot c, highly positively supercoiled DNA species; streak d, relaxed pUC.HSO DNA; streak e, slightly negatively supercoiled pUC.HSO DNA; spot f, linear pUC.HSO DNA.

were treated with S1 nuclease in the same reaction. As shown in Fig. 5D, most of the supercoiled form I pUC.HSO DNA was converted to the nicked form (spot b) and the linear form (spot f), but the newly formed DNA species (spot c) was resistant to S1 nuclease. Furthermore, the newly formed DNA species could be digested with a number of restriction endonucleases, and its gel mobility did not change upon brief heating to 65°C (data not shown). These results together suggest strongly that this DNA species is highly positively supercoiled and that the positively supercoiled conformation is due to the increase of the linking number of the plasmid DNA rather than other structural alterations in the DNA template. The specific linking difference of this DNA species has also been analyzed by a modified two-dimensional gel electrophoresis system (8) and shown to be greater than +0.03 (data not shown).

DISCUSSION

Our results show that highly positively supercoiled DNA can be isolated from a T antigen reaction mixture containing a eubacterial topoisomerase I, ATP, and a relaxed input plasmid DNA. The dominant effect of eukaryotic DNA topoisomerase I over eubacterial topoisomerase I also indicates that the positive supercoils are torsionally unconstrained in the T antigen reaction, unlike the production of gyrase-constrained positive supercoils described previously (25).

To explain the supercoiling activity of SV40 T antigen, we consider the recently proposed twin-supercoiled-domain model for DNA tracking processes. The tracking of a protein complex along the helical path of a double-helical DNA results in two rotations: the rotation of the tracking complex around the DNA helical axis and the rotation of the DNA around its own helical axis. The sum of the rates of these two rotations is a constant, affected only by the rate of movement of the tracking process (14, 15). In the case of RNA transcription, the longer the nascent RNA, the higher the rate of DNA rotation. Rotation of DNA around its own helical axis generates positive supercoiling waves ahead of and negative supercoiling waves behind the tracking complex. Confirming evidence for this twin-supercoiled-domain model initially comes from *in vivo* studies of RNA transcription in *E. coli* cells treated with DNA gyrase inhibitors and in yeast cells expressing eubacterial DNA topoisomerase I (15, 16). More recently, *in vitro* studies using purified RNA polymerases and eubacterial DNA topoisomerase I have also confirmed the proposed model (13). The *in vitro* studies have also demonstrated the importance of the size of the nascent RNA in positive supercoiling of the DNA template (13). To explain the observed supercoiling reaction, we propose that SV40 T antigen can track along the helical path of the DNA double helix in a reaction coupled to ATP hydrolysis. A schematic diagram of the twin-supercoiled-domain model for SV40 T antigen tracking is shown in Fig. 6. The T antigen-driven rotation of the DNA helical axis generates waves of positive supercoils ahead of and negative supercoils behind the moving T antigen helicase. Since eubacterial DNA topoisomerase I preferentially relaxes the negative supercoils, simultaneous treatment of DNA with SV40 T antigen and a eubacterial DNA topoisomerase I results in positive supercoiling of the DNA template.

Though this model can qualitatively explain our results, a quantitative explanation is difficult. As discussed previously for RNA transcription, the degree of supercoiling of the template DNA is dependent on many parameters (13–17). One of the important parameters is the size of the nascent RNA. Considering the small size of the T antigen (<400,000 daltons even assuming a tetrameric structure) and its lack of associated RNAs, it is quite surprising that such a high degree of supercoiling can be achieved in this *in vitro* reaction.

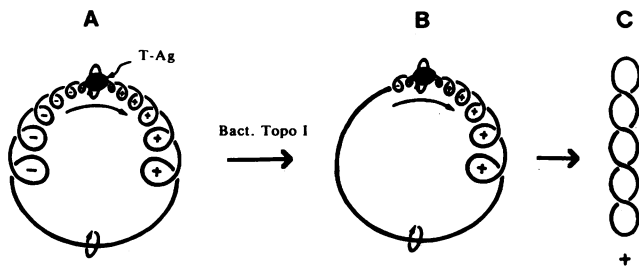


FIG. 6. Schematic diagram showing a possible mode of action of the helix-tracking activity of SV40 T antigen. (A) The movement of T antigen (T-Ag) along the helical path of the right-handed double helical DNA drives the rotation of DNA around its helix axis, resulting in the generation of a positive supercoiling wave ahead of and a negative supercoiling wave behind the moving helicase. The positive and negative supercoils can be rapidly annihilated by rotational diffusion through the intervening DNA segment. The possibility that T antigen may undergo intra- and/or intermolecular aggregation is addressed in the *Discussion*. (B) The addition of eubacterial topoisomerase I preferentially removes the negative supercoils. (C) The plasmid DNA becomes positively supercoiled upon isolation. The supercoils are arbitrarily depicted in an interwound form.

However, the reaction time is significantly longer for the T antigen reaction (1 hr) than for the RNA polymerase reaction (1 min). The longer incubation time in the presence of eubacterial topoisomerase I may explain accumulation of extensive positive supercoils in the template DNA. Alternatively, the mechanism of action of the helix-tracking activity of SV40 T antigen may involve anchorage of T antigen to another DNA-bound T antigen either on the same molecule or on a different DNA molecule. The anchorage of T antigen to another T antigen bound to the same DNA molecule effectively blocks the pathway for rotational diffusion of positive and negative supercoils. The movement of the anchored T antigen is expected to generate approximately one positive (in the shrinking domain) and one negative supercoil (in the expanding domain) for every 10- to 11-bp advancement. The anchorage of T antigen to another T antigen bound to a different DNA can also force DNA helix rotation during T antigen translocation along the DNA, although fusion of positive and negative supercoils still can occur at a rapid rate. PEG may significantly enhance intermolecular aggregation of T antigen-bound DNA and hence increase the rate of DNA helix rotation. A similar anchorage model has been proposed previously for a number of ATP-dependent enzymes such as *EcoK* restriction enzyme (26) and *RecBCD* enzyme (27).

The helix-tracking activity of SV40 T antigen may be related to the previously identified helicase activity of T antigen (6). However, the helix-tracking activity observed in our supercoiling reaction does not require a functional origin sequence. It is possible that the helix-tracking activity observed in our assay may represent a partial or uncoupled (to the replication origin) reaction of the origin-specific helicase activity of SV40 T antigen. Alternatively, the helix-tracking activity may be unrelated to the helicase activity of SV40 T antigen and represent an activity that copurifies with SV40 T antigen. However, all preparations of T antigen, including the baculovirus expressed SV40 T antigen, induce positive su-

percoiling in our assay. Further studies are necessary to determine the mechanism of T antigen entry into the duplex circle and its subsequent translocation along the DNA helix. The possible role(s) for this helix-tracking activity in various T antigen functions remains to be determined. The assay described here using DNA conformation-specific bacterial DNA topoisomerase I should be generally applicable for the detection of putative cellular helix-tracking proteins.

We are grateful to Drs. Thomas Kelly, Mark Wold, Bruce Stillman, and James C. Wang for their kind supply of materials and helpful discussions. This work was supported by National Institutes of Health Grant GM27731. L.F.L. is a recipient of an American Cancer Society Faculty Research Award.

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