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A protein factor from *Xenopus* oocytes with simian virus 40 large tumor antigen-like DNA supercoiling activity

(helicase/topoisomerase/ATPase)

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ABSTRACT **The ATP-dependent movement of simian virus 40 large tumor antigen on DNA can cause positive supercoiling of template DNA in the presence of bacterial DNA topoisomerase I, which specifically relaxes negative supercoils. With such a supercoiling assay, an activity capable of ATP-dependent, positive supercoiling of DNA in the presence of bacterial DNA topoisomerase I was found in 7S particles prepared from *Xenopus laevis* oocytes but not in purified transcription factor IIIA. The positive supercoils accumulated during the reaction are strained, as evidenced by their sensitivity to eukaryotic DNA topoisomerase I. Purification of this activity led to the identification of a DNA-dependent ATPase. Molar excess of transcription factor IIIA or spermidine was found to strongly stimulate the supercoiling reaction of the purified ATPase. These results suggest that this DNA-dependent ATPase can interact with and translocate along closed-circular duplex DNA, producing local alterations in the supercoiled state of the DNA template in a manner analogous to that of simian virus 40 large tumor antigen.**

Vectorial movement of a macromolecular complex along the helical path of DNA has been postulated to produce positive supercoils ahead of and negative supercoils behind the traversing complex, and studies of the process of RNA transcription elongation support such a theory (1–7). More recently, simian virus 40 (SV40) large tumor antigen has also been shown to produce similar effects (8), suggesting that the state of supercoiling of intracellular DNA may be affected by multiple cellular functions that involve tracking of macromolecules along the DNA helix.

In *Xenopus laevis* oocytes, plasmid DNAs microinjected into the germinal vesicles are immediately relaxed and then gradually become negatively supercoiled (9). Studies using *X. laevis* oocyte extracts (S-150) have shown that, in addition to ATP-independent chromatin assembly, supercoils could also be introduced into a relaxed DNA template in a reaction requiring ATP and the 5S RNA gene transcription factor TFIIIA (10). Results of the latter experiments, however, have been controversial and irreproducible (11).

In view of the effect of DNA helix-tracking processes on the supercoiling state of DNA, we have investigated the possibility that an ATP-dependent DNA helix-tracking process might exist in *X. laevis* oocyte extracts. By using the recently developed assay specific for DNA helix-tracking processes (8), we have identified an ATP-dependent DNA helix-tracking activity in 7S-particle preparations from *Xenopus* oocytes. This DNA helix-tracking activity, which is stimulated greatly by molar excess of TFIIIA or spermidine, is shown to be due to a DNA-dependent ATPase. This protein, like SV40 large tumor antigen, probably translocates along DNA and thereby alters DNA supercoiling in a manner analogous to that of SV40 large tumor antigen.

MATERIALS AND METHODS

Purification of 7S Particles and TFIIIA from *X. laevis* Oocytes. Oocytes from three female *X. laevis* were used to prepare the S-150 cytoplasmic extract according to the published method (10), except that KCl was at 25 mM. 7S particles were then purified according to published procedures (12, 13) with slight modifications. TFIIIA was purified from the 7S-particle DE-52 fraction. The 7S-particle fraction was treated with RNase A at 150 μg/ml on ice for 30 min, mixed with an equal volume of 10 M urea in buffer A (12), and then chromatographed on a BioRex 70 column. TFIIIA was distributed between the 0.5 M and 1 M KCl fractions.

Supercoiling Reactions. The positive supercoiling reaction (15 μl) occurred in 30 mM Tris·HCl, pH 7.9/1 mM dithiothreitol/4 mM ATP/8 mM $MgCl_2$/bovine serum albumin at 50 μg/ml. To the reaction buffer, 2 units of *Escherichia coli* DNA topoisomerase I, 25 ng of relaxed pXbs201 DNA, 330 ng of 7S particles (from 0.32 M KCl fraction of the DE-52 chromatographic step), and RNase A at 150 μg/ml were added. The complementation reaction was done without RNase A by using 250 ng of TFIIIA and 330 ng of a 7S-particle fraction (or 15 ng of purified TFIIIA-complementing protein, see below). After 2-hr incubation at 25°C, each reaction was stopped by adding 5 μl of 80 mM EDTA/2.0% SDS/proteinase K at 600 μg/ml. The proteinase K treatment was continued at 37°C for 1 hr. The negative supercoiling reaction was done as described above, except that *E. coli* topoisomerase I was replaced with HeLa topoisomerase I.

Purification of a TFIIIA-Complementing Protein from 7S-Particle Preparations. The TFIIIA-complementing protein was purified from the ovaries of 27 adult female *X. laevis*. Briefly, ovaries were excised and washed several times in OR-2 medium (14). The ovaries were homogenized in a Dounce homogenizer with a loosely fitting pestle at 4°C in buffer A (12). The homogenate was centrifuged in a Beckman JA20 rotor at 18,000 rpm and 4°C for 30 min. The supernatant was loaded onto a 100-ml BioRex 70 column preequilibrated with buffer B (same as buffer A except that KCl was increased to 0.1 M and no protease inhibitor was present). The flow-through fraction from the BioRex 70 column was loaded directly onto a 200-ml DE-52 column preequilibrated with buffer C (20 mM Hepes, pH 7.4/15% (vol/vol) glycerol/0.5 mM dithiothreitol/0.2 mM EDTA/100 mM KCl), and the column was eluted with a linear 0.1–1 M KCl gradient in buffer C. Buffer C was used for all subsequent steps. The TFIIIA-complementing activity was eluted with 0.3 M KCl. The activity-containing fractions were pooled, loaded onto a

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Abbreviation: SV40, simian virus 40.

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50-ml hydroxylapatite column, and eluted with a linear gradient of 0.1–0.6 M potassium phosphate (KP_i), pH 7.0. The activity, which was in the 0.35 M KP_i fraction, was pooled and dialyzed against buffer C and then chromatographed on a 5-ml BioRex 70 column. The activity (0.4 M KCl eluate) from the BioRex 70 column was loaded onto a 0.5-ml ATP-agarose column. The activity could not be eluted with 20 mM ATP but was eluted with 0.4 M KP_i, pH 7.0. The activity was pooled and dialyzed against 50% glycerol in buffer C and stored at −70°C. Part of the activity was loaded onto a 15–35% glycerol gradient in buffer C and centrifuged at 40,000 rpm for 36 hr at 4°C with a Beckman SW41 rotor. The gradient was fractionated into 60 fractions by using an ISCO density-gradient fractionator. Gel filtration was performed by loading the 0.4 M KP_i eluate from the ATP-agarose column onto a fast protein liquid chromatography (Pharmacia) Superose 12 column preequilibrated with buffer C.

RESULTS

An ATP-Dependent Supercoiling Activity in 7S-Particle Preparations: Probing with DNA Conformation-Specific Bacterial DNA Topoisomerase I. One possible explanation for the observed ATP- and TFIIIA-dependent supercoiling activity in S-150 extracts (10) is that TFIIIA itself might be an ATP-dependent, DNA helix-tracking protein. To test this possibility, an assay using DNA conformation-specific bacterial DNA topoisomerase I was employed (see Fig. 1*A* for schematic diagram of the assay) (7, 8). If the ATP-dependent DNA helix-tracking protein translocates along the DNA, producing both positive and negative supercoils, preferential relaxation of negative supercoils by bacterial DNA topoisomerase I would cause the accumulation of positive supercoils in template DNA. Fig. 1*C* shows that with bacterial DNA topoisomerase I, ATP, 7S particles prepared according to the steps described in Fig. 1*B*, and RNase A (presumably to release TFIIIA from 7S particles), a significant population of the input-relaxed pXbs201 DNA, which contains a copy of the *Xenopus borealis* 5S RNA gene (12), became supercoiled.

The supercoiled pXbs201 DNA product was shown to consist of highly positively supercoiled topoisomers by two-dimensional gel electrophoresis (Fig. 2*A*, spot a) (4). These positively supercoiled topoisomers were further identified by their sensitivity to eukaryotic DNA topoisomerase I (Fig. 2*C*), which relaxes both positive and negative supercoils, and by their resistance to treatment with DNA conformation-specific bacterial DNA topoisomerase I (Fig. 2*D*), which relaxes only negative supercoils.

Formation of positive supercoils specifically required bacterial DNA topoisomerase I; eukaryotic DNA topoisomerase I could not substitute for the function of bacterial DNA topoisomerase I. Furthermore, addition of eukaryotic DNA topoisomerase I to the preincubated reaction mixture containing bacterial DNA topoisomerase I rapidly relaxed the positive supercoils (data not shown), indicating that the positive supercoils formed in the supercoiling reaction were strained and were not constrained by proteins. These results suggested that an ATP-dependent DNA helix-tracking protein might be present in the 7S-particle preparation.

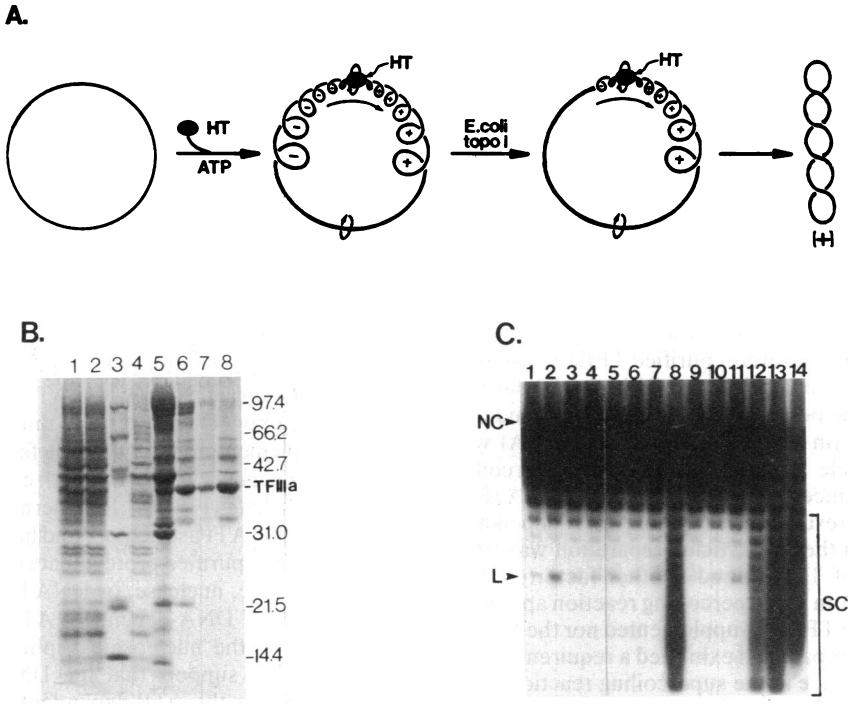

FIG. 1. 7S-particle preparations from *X. laevis* oocytes exhibit an ATP-dependent DNA supercoiling activity. (*A*) Schematic diagram showing the supercoiling assay used for detecting ATP-dependent DNA helix-tracking processes. Binding and subsequent ATP-dependent translocation of a DNA helix-tracking protein (HT) along the right-handed double-stranded DNA helix may drive DNA rotation around its helical axis, generating positive and negative supercoils near the moving helix-tracking protein. Preferential removal of negative supercoils by *E. coli* DNA topoisomerase I during the ATP-dependent DNA helix-tracking process would result in an accumulation of positive supercoils in the DNA template. (*B*) Preparation of 7S particles from *X. laevis* oocytes. Various fractions of the 7S-particle preparation were analyzed by electrophoresis in SDS/12.5% PAGE. Lanes: 1, S-150 extract; 2, flow-through fraction from BioRex 70 column; 3, molecular weight markers (2 μg each); 4–8, DE-52 column fractions; 4, flow-through fraction; 5, 0.16 M KCl step eluate; 6 and 7, two fractions of 0.24 M KCl step eluate; 8, 0.32 M KCl step eluate. (*C*) ATP-dependent supercoiling of DNA template. The 0.32 M KCl column fraction from the DE-52 chromatographic step was used as 7S-particle source. Lanes: 1, pXbs201 DNA control without bacterial topoisomerase I or 7S particle; 2, 330 ng of 7S particles and RNase A at 150 μg/ml; 3–14, all contained 2 units of *E. coli* DNA topoisomerase I plus the following in lanes: 3, 330 ng of 7S particles; 4–8, RNase A at 150 μg/ml, and 0, 0.33, 3.3, 33, and 330 ng of 7S particles, respectively; 9–14, 330 ng of 7S particles plus RNase A at 0, 0.15, 15, 150, and 1500 μg/ml, respectively. NC, nicked-circular DNA; L, linear DNA; SC, supercoiled DNA.

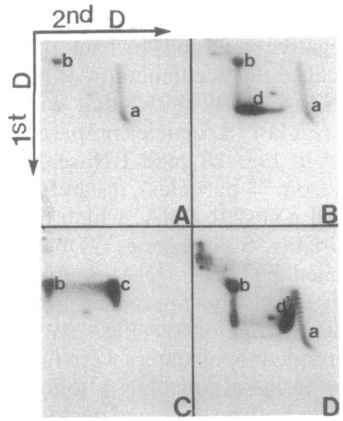

FIG. 2. Characterization of the product of the supercoiling reaction. The reaction product (pXbs201 DNA) was purified and mixed with negatively supercoiled pXbs201 DNA, which served as an internal control. The mixed DNAs were then treated with either eukaryotic or prokaryotic DNA topoisomerase I. Topoisomerase-treated DNAs were analyzed by two-dimensional gel electrophoresis with the second dimension containing 12.5 μM chloroquine (4). (*A*) Product of the supercoiling reaction. DNA product was the same DNA sample shown in lane 8 of Fig. 1*C*. (*B*) Mixed DNAs: 25 ng each of negatively supercoiled pXbs201 DNA and of the reaction product. (*C*) Mixed DNAs treated with HeLa DNA topoisomerase I. (*D*) Mixed DNAs treated with *E. coli* DNA topoisomerase I. Different forms of pXbs201 DNA are denoted as follows: a, positively supercoiled DNA; b, nicked-circular DNA; c, relaxed closed-circular DNA; d and d', negatively supercoiled and partially relaxed DNA, respectively.

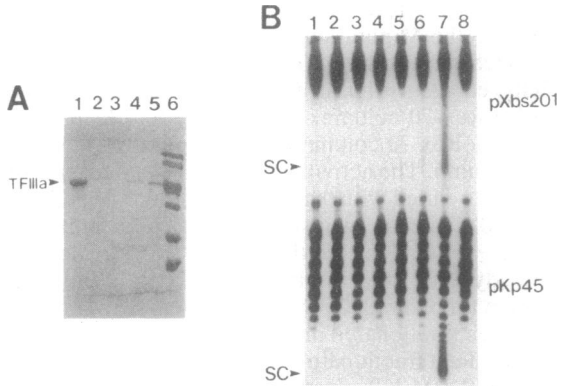

FIG. 3. Purified TFIIIA is inactive in the supercoiling assay but can complement 7S-particle preparation. (*A*) SDS/PAGE of TFIIIA-containing fractions. Various fractions from BioRex 70 column chromatography were analyzed. Lanes: 1, fraction from 0.5 M KCl step elution; 2 and 3, fractions from 0.3 M KCl step elution; 4, flow-through fraction; 5, pooled TFIIIA-containing fractions from DE-52 column (0.32 M KCl step eluate) for loading onto the BioRex 70 column; 6, molecular weight markers. (*B*) Purified TFIIIA complements 7S-particle preparation in the positive supercoiling assay. The supercoiling reaction was done as described, except that RNase A was omitted, and a mixture of relaxed pXbs201 and pKP45 DNAs (12 ng each) was used. Lanes 1, control, DNA only; 2, 250 ng of TFIIIA and *E. coli* topoisomerase I but no 7S particles; 3–7, each reaction contained 330 ng of 7S-particle preparation (0.32 M KCl fraction from DE-52 column) and *E. coli* DNA topoisomerase I plus 0, 2, 10, 50, and 250 ng of TFIIIA, respectively; 8, 330 ng of 7S-particle preparation and 250 ng of TFIIIA but no *E. coli* topoisomerase I. SC, supercoiled DNA form.

Identification of Two Protein Factors Responsible for ATP-Dependent Supercoiling Activity in the 7S-Particle Preparation. To further test whether TFIIIA was responsible for ATP-dependent supercoiling activity in the 7S-particle preparation, TFIIIA was purified to homogeneity (12, 15). Purified TFIIIA (Fig. 3*A*, lane 1) was completely inactive in the supercoiling assay containing ATP and bacterial DNA topoisomerase I (Fig. 3*B*, lane 2). This unexpected result prompted us to search for another factor or factors in the 7S-particle preparation. The requirement for RNase A in the supercoiling reaction (Fig. 1*C*, lanes 9–14), however, suggested that TFIIIA might be a necessary component in the 7S-particle preparation. Therefore, purified TFIIIA was used to complement the other possible factor(s) in the 7S-particle preparation by using the positive supercoiling assay. Indeed, when purified TFIIIA (in large molar excess to DNA) was added to the 7S-particle preparation, positive supercoiled plasmid DNA was produced in the absence of RNase A (Fig. 3*B*, lanes 4–7). These results indicated that ATP-dependent supercoiling activity in the 7S-particle preparation was from the combined action of TFIIIA and another factor(s). The requirement for TFIIIA in the supercoiling reaction appeared nonspecific; neither the TFIIIA-supplemented nor the RNase A-treated 7S-particle preparation exhibited a requirement for the 5S RNA gene sequence in the supercoiling reaction (Fig. 3*B*, lanes 4–7; data not shown). In addition, a large molar excess of TFIIIA to template DNA was required.

TFIIIA-Complementing Activity in the 7S-Particle Preparation Is a DNA-Dependent ATPase. The TFIIIA-complementing activity was purified from a *X. laevis* 7S-particle preparation by a procedure involving five chromatographic steps (Fig. 4*A*), using the supercoiling assay in which TFIIIA, bacterial DNA topoisomerase I, and ATP were included. Active fractions from the final ATP-agarose column were pooled and analyzed by glycerol-gradient sedimentation. The activity profile of the gradient fractions is shown in Fig. 4*B*. The peak fraction from the glycerol gradient contained a number of polypeptides between 45 kDa and 56 kDa, as revealed by SDS/PAGE (Fig. 4*C*). Gel filtration and glycerol-gradient analysis of the purified protein gave a Stokes' radius of 27.3 Å and a sedimentation coefficient of 4.4 S ($s_{20,w}$). The calculated native molecular mass based on an assumed partial specific volume of 0.725 cm^3/g (16) is 50 kDa. The asymmetric ratio is 1.13. The native molecular mass of the protein, however, was significantly larger when the peak fraction from DE-52 column was analyzed. While a prominent contaminating DNA-independent ATPase was present in the 7S-particle preparation and partially copurified with the TFIIIA-complementing activity in the early chromatographic steps, it was completely separated from the TFIIIA-complementing activity by the ATP-agarose chromatographic step. The only ATPase activity that copurified with the TFIIIA-complementing activity in the final step of purification was a DNA-dependent ATPase, which converted ATP to ADP and orthophosphate (Fig. 5*B*). Furthermore, purified TFIIIA had no detectable ATPase activity and had no effect on the ATPase activity of the purified protein (data not shown). No detectable topoisomerase, nuclease, or DNA ligase activity was present in the purified DNA-dependent ATPase preparation.

Studies of the nucleoside triphosphate requirement provided further support that the DNA-dependent ATPase is responsible for the ATP-dependent helix-tracking activity in the 7S-particle fraction; ATP, dATP, adenosine [α-thio]-triphosphate, UTP, and CTP (in decreasing order of effectiveness), but not GTP, dGTP, dCTP, dTTP, adenosine [γ-thio]triphosphate, and adenosine 5'-[β,γ-imido]triphosphate, supported the positive supercoiling reaction in the presence of bacterial DNA topoisomerase I and TFIIIA (Fig. 5*A*). Consistent with the supercoiling assay, the DNA-dependent ATPase activity, as assayed by the hydrolysis of [α-^{32}P]ATP, was strongly inhibited by excess unlabeled ATP, dATP, UTP, and CTP, but not by GTP, dGTP, dCTP, and dTTP (Fig. 5*B*). These results suggest that the DNA-dependent ATPase is responsible for the positive supercoil-

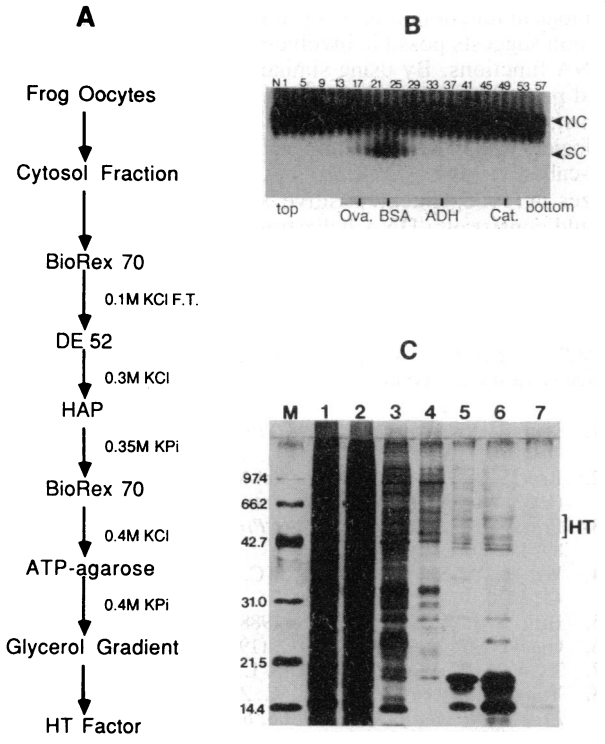

FIG. 4. Purification of TFIIIA-complementing activity from *Xenopus* oocytes. (*A*) Purification of TFIIIA-complementing activity is shown as diagrammed. F.T., flow through; HAP, hydroxyapatite; HT, helix-tracking activity. (*B*) Activity profile of the TFIIIA-complementing protein fractionated in 15–35% glycerol gradient. Each fraction was assayed with 250 ng of TFIIIA, 2 units of *E. coli* topoisomerase I, and 25 ng of relaxed pXbs201 DNA. Fractions 1–57 were assayed. N, DNA only, no fractions added. Ova, ovalbumin; BSA, bovine serum albumin; ADH, alcohol dehydrogenase; Cat, catalase; NC, nicked-circular DNA; SC, supercoiled DNA. (*C*) SDS/PAGE of TFIIIA-complementing factor. Proteins (20 μl) from various purification steps were electrophoresed in SDS/12.5% PAGE and were then visualized by silver-staining. Lanes containing protein fractions: 1, oocyte extract; 2, BioRex 70 flow-through; 3, DE-52, 0.3 M KCl eluate; 4, hydroxyapatite, 0.35 M KP_i, pH 7.0 eluate; 5, second BioRex 70, 0.4 M KCl eluate; 6, ATP-agarose, 0.4 M KP_i eluate; and 7, glycerol-gradient fraction 23. HT, protein bands possibly responsible for the DNA helix-tracking activity.

ing activity assayed in the presence of bacterial DNA topoisomerase I.

DISCUSSION

Our results showed that an ATP-dependent DNA helix-tracking process could be detected in 7S-particle preparations from *Xenopus* oocytes. Through enzyme purification, we have identified two proteins, a DNA-dependent ATPase and TFIIIA, which together could fully reconstitute the supercoiling activity in the 7S-particle preparations. The partially purified proteins can mediate ATP-dependent positive supercoiling of DNA in the presence of bacterial DNA topoisomerase I, which specifically relaxes negative supercoils. By analogy to RNA transcription and the helix-tracking activity of SV40 large tumor antigen, TFIIIA and the DNA-dependent ATPase together may produce twin-supercoiled domains as a result of protein tracking along the DNA helix.

The DNA-dependent ATPase is most likely the DNA helix-tracking component. Why TFIIIA is required in the supercoiling reaction is not clear. Under our supercoiling reaction conditions, TFIIIA was not required for its sequence-specific binding to the 5S RNA gene sequence; plasmid DNAs (e.g., pKP45, pSV2.cat, and pJL0), which do

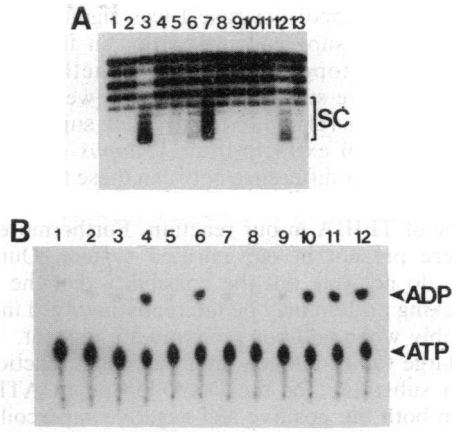

FIG. 5. Purified TFIIIA-complementing factor is a DNA-dependent ATPase. (*A*) The nucleoside triphosphate requirement in the positive supercoiling reaction. Lanes: 1, DNA only (250 ng of relaxed pKP45 DNA); 2–13, each contained (*i*) 250 ng of TFIIIA, (*ii*) 2 units of *E. coli* DNA topoisomerase I, (*iii*) 100 ng of TFIIIA-complementing factor (fractions pooled from the ATP-agarose chromatographic step), (*iv*) 250 ng of relaxed pKP45, and (*v*) different nucleoside triphosphates (4 mM); 2, (*vi*) no nucleoside triphosphate; 3, (*vi*) ATP; 4, (*vi*) GTP; 5, (*vi*) CTP; 6, (*vi*) UTP; 7, (*vi*) dATP; 8, (*vi*) dGTP; 9, (*vi*) dCTP; 10, (*vi*) dTTP; 11, (*vi*) adenosine 5′-[β,γ-imido]triphosphate; 12, (*vi*) adenosine (α-thio)triphosphate; and 13, (*vi*) adenosine (γ-thio)triphosphate. SC, supercoiled DNA. (*B*) ATPase assay. ATPase assays were done as described (17) with slight modifications. Assay buffer was the same as the supercoiling reaction buffer except that unlabeled ATP was replaced with 5 μCi of [α-^{32}P]ATP (1 Ci = 37 GBq). Lanes: 1, buffer only; 2, 25 ng of pKP45 DNA and buffer; 3, 100 ng of TFIIIA-complementing activity; 4–12, each contained (*i*) 100 ng of TFIIIA-complementing activity, (*ii*) 25 ng of pKP45 DNA, and (*iii*) different unlabeled nucleosides triphosphates (4 mM each); 4, (*iv*) no unlabeled nucleoside triphosphate; 5, (*iv*) ATP; 6, (*iv*) GTP; 7, (*iv*) CTP; 8, (*iv*) UTP; 9, (*iv*) dATP; 10, (*iv*) dGTP; 11, (*iv*) dCTP; and 12, (*iv*) dTTP.

not contain the 5S RNA gene sequence, were also efficiently supercoiled. Furthermore, a high molar ratio of TFIIIA to the DNA template (500:1 in most reactions) was necessary to support the supercoiling reaction, whereas specific binding of TFIIIA to the somatic 5S RNA gene sequence occurs at a near equimolar ratio. The finding that TFIIIA can be partially substituted for by spermidine in the supercoiling reactions suggests that TFIIIA may be required to coat or aggregate the DNA template and that the DNA-dependent ATPase is the DNA helix-tracking protein. The supercoiling induced by a DNA helix-tracking protein on a closed-circular DNA could be affected by at least two parameters: the rotational frictional coefficient of the helix-tracking protein and the rate of rotational diffusion of the supercoils generated along the duplex DNA template. Aggregation of the DNA template by TFIIIA could affect both parameters. Because the native molecular mass of the purified DNA-dependent ATPase is only ≈50 kDa, template supercoiling would be greatly enhanced if the helix-tracking protein could anchor to another DNA-bound protein (e.g., another DNA-bound helix-tracking protein). The aggregation of the DNA templates by TFIIIA or spermidine could facilitate such an interaction. This type of anchored translocation of a protein along the DNA helix is not unprecedented. The search for the cutting sites by *Eco*K restriction endonuclease has been proposed to act in such a manner (18).

When bacterial DNA topoisomerase I was replaced with eukaryotic DNA topoisomerase I in the supercoiling assay that uses purified DNA-dependent ATPase and TFIIIA (or spermidine), ATP-dependent negative supercoiling of the DNA template was also seen (unpublished results). However, the negative supercoiling reaction in the presence of

eukaryotic DNA topoisomerase I was significantly weaker than the positive supercoiling reaction in the presence of bacterial DNA topoisomerase I. Whether the ATP-dependent negative supercoiling reaction we observed with purified proteins is related to the negative supercoiling reaction seen in S-150 extracts from *Xenopus* oocytes (10) is unclear. The major difference between these two reactions is the high stoichiometry of TFIIIA and the lack of sequence specificity of TFIIIA in our reaction. Furthermore, no histones were present in our purified system. Our results, however, do not rule out the possibility that the *Xenopus* helix-tracking protein may be indirectly involved in chromatin assembly when additional factors are present.

SV40 large tumor antigen under identical reaction conditions can substitute for the DNA-dependent ATPase and TFIIIA in both the positive and negative supercoiling reactions (8, 19; unpublished results). Because of their similar activity in our supercoiling assays, we expected that our DNA-dependent ATPase, like SV40 large tumor antigen, might be a DNA helicase. However, we have been unable to detect the helicase activity in our ATPase preparations using the strand-displacement assay. One possible explanation is that the DNA-dependent ATPase we purified may translocate along the grooves of DNA without disrupting the base pairs. Alternatively, the DNA helicase activity might be too weak for detection under our assay conditions. Regardless of the possible helicase activity, use of closed-circular DNA in our assays suggests that the DNA-dependent ATPase must be able to enter duplex DNA without the requirement of an end or a single-stranded tail. So far, the only known DNA helicases that can enter closed-circular duplex DNA and affect subsequent ATP-dependent translocation is the SV40 large tumor antigen and *DnaB* with the help of other proteins involved in the initiation of replication (20). Although SV40 large tumor antigen does not show any sequence specificity in the supercoiling reactions under our assay conditions (data not shown), its entry into duplex DNA in the replication reaction or extensive unwinding reaction is origin-specific. Similarly, the entry of *DnaB* into duplex DNA is specific for the origins of replication (ori-C or γ-ori) and requires other protein complexes for assistance (20). The apparent nonspecific entry of our purified DNA-dependent ATPase into duplex DNA, therefore, does not preclude the possibility that its entry into duplex DNA *in vivo* may be sequence specific and require other protein complexes for assistance.

Identification of a helix-tracking protein that can enter and translocate along the duplex DNA suggests another means by which DNA conformation can be modified. Although the biological function(s) of this protein is unknown, its mode of action suggests possible involvement in several fundamental DNA functions. By using similar assays, we have detected and partially purified a similar protein from HeLa cells and yeast. Such an activity appears to be ubiquitous. The physiological role of this protein awaits further studies. Also the so-called reverse gyrase from Archaebacteria, which catalyzes ATP-dependent positive supercoiling of DNA (21), could consist of a DNA helix-tracking component similar to our purified DNA-dependent ATPase and a DNA conformation-specific bacterial DNA topoisomerase I.

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