DNA Knotting Abolishes in Vitro Chromatin Assembly*

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Topological knots can be formed in vitro by incubating covalently closed double stranded DNA and purified topoisomerase II from the yeast Saccharomyces cerevisiae in an ATP-dependent reaction. Knotting production requires a starting enzyme/DNA mass ratio of 1. Analysis of knotted DNA was carried out by using both one- and two-dimensional agarose gel electrophoresis. The knots generated are efficiently untied, and give relaxed DNA rings, by catalytic amounts of topoisomerase II, but not by topoisomerase I. Time course analysis shows the knotting formation over relaxed and supercoiled DNA. When supercoiled DNA was used as a substrate, knots appear immediately whereas no transient relaxed rings were observed. The cell-free extract from Xenopus oocytes S-150 cannot assemble nucleosomes on knotted DNA templates as revealed by topological and micrococcal nuclease analysis. Nevertheless, the presence of knotted DNA templates does not inhibit the assembly over the relaxed plasmid. Finally, a pretreatment of knotted DNA with trace amounts of topoisomerase II before the addition of the S-150 yields a canonical minichromosome assembled in vitro. Taking into account these results, I suggest a mechanism of chromatin assembly regulation directed by topoisomerase II.

DNA topoisomerases are a kind of enzymes which, by changing the topological structure of the DNA by means of transient single (in class I) or double (in class II) stranded breaks, produce different topological forms called topoisomers (1, 2). These enzymes can easily knot/unknot, catenate/decatenate, and supercoil/relax DNA molecules in vitro (3–6). Their critical role in processes as replication, transcription, recombination, repair, and chromosome condensation have been established in vivo (2, 7–10). Protein \( \omega \) was the first DNA topoisomerase discovered (11); this bacterial enzyme falls into the class I. The class II-enzyme bacterial gyrase is, until now, the only topoisomerase capable to introduce negative supercoiling over a covalently closed relaxed DNA in an ATP-driven process (6, 12, 13).

A broad range of organisms and sources, including viruses, show activities that change the topological state of the DNA. Concomitantly with the fact of breaking and rejoicing one or two strands in the DNA molecule, topoisomerases II change the linking number in steps of two (3, 14), whereas class I alters this topological parameter in steps of one (15).

Knotting of either single or double stranded DNA rings can be carried out by both classes of topoisomerases (3, 14, 16) by breaking and rejoining one or two strands. The ATP dependence on the knotting activity is controversial: topoisomerase II knotting of circular duplex DNA, using purified enzyme from Drosophila embryos, is greatly enhanced by ATP (14). However, topoisomerase II from T4 bacteriophage can knot either covalently closed or nicked DNA rings without ATP (3, 17). Nevertheless, either the viral or the eukaryotic enzyme require in the presence of ATP or not, to be present in larger amounts (3, 14, 17, 18) than necessary to achieve DNA relaxation (4, 5).

Recently, an ATP-dependent enzymatic mechanism for yeast topoisomerase II has been reported (19, 20; reviewed in Ref. 21). Briefly, the enzyme, a homodimer of a 170-kDa polypeptide (4), forms an open clamp which traps a DNA duplex; then, a second DNA duplex enters the open gate, which is closed when ATP is bound to the protein. Simultaneously to the double strand breakage of the first DNA duplex, the second duplex crosses the break and exits through a second protein gate opposite to the entrance gate. The process finishes after the resealing of the broken duplex, which exits through the first gate depending on ATP hydrolysis. As class II DNA topoisomerases are evolutionary related, the mechanism reported can be extensive to all such enzymes.

In this study I show that the eukaryotic DNA topoisomerase II purified from Saccharomyces cerevisiae can efficiently knot any topologically closed DNA template, either relaxed or supercoiled. This ATP-driven reaction depends on the addition of stoichiometric amounts of enzyme. When topoisomerase II is employed at a catalytic level, both unknotting and relaxing activities over knotted or supercoiled DNA templates are achieved.

Finally, the addition of the cell-free extract S-150, capable of assembly of chromatin over any topologically linked DNA template (22, 23), fails to form periodically arranged nucleosomes when the DNA template is knotted. Nonetheless, a pretreatment with trace amounts of topoisomerase II before the onset of the chromatin assembly process permits the nucleosome formation over knotted DNA. These results suggest that DNA topoisomerase II may modulate, via knotting/unknotting, the assembly of chromatin.

MATERIALS AND METHODS

Enzymes and DNA—Plasmid DNA (pUC19) was purified following standard procedures including CsCl buoyant density gradients. SV40 DNA was kindly provided by Dr. F. Azorín (CID-CSIC). Yeast DNA topoisomerase II was a generous gift from Dr. J. Roca (Harvard University); the enzyme had a purity degree >99% and activity >70%.

A working stock solution was made dissolving 1 \( \mu \)g (containing 7 \( \mu \)g) of enzyme stock (kept at –70 °C) in 70 \( \mu \)l of 50 mM Tris-HCl, pH 8.0, 500 mM KCl, 5 mM dithiothreitol, 100 \( \mu \)g/ml bovine serum albumin, and 50% glycerol (18). Calf thymus DNA topoisomerase I was purchased from GIBCO-BRL.

Knotting Reactions—Knotting reactions were carried out in a mixture containing 20 mM HEPES pH 7.5, 2 mM magnesium chloride, 0.1 mM EDTA, 0.2 mM ATP, 20 mM KCl, and a topoisomerase II/DNA mass ratio ranging from 1 to 3. The final concentration of closed circular DNA (either relaxed or supercoiled) was currently 10 ng/ml and the incubation temperature was always 30 °C. The time of reaction varies between

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1 h and overnight; reactions were stopped by addition of one-fourth of the mixture volume of 2.5% Sarkosyl, 100 mM EDTA; samples were deproteinized using SDS, proteinase K, and phenol extraction.

**Unknotting / Relaxing Reactions**—Unknotting / relaxing reactions with topoisomerase II were performed at the same ionic condition than the knotting reaction but using a topoisomerase II/DNA ratio from one-tenth to one-fifteenth and a KCl concentration of 150 mM.

Electrophoresis—1% agarose-TBE gels were performed in horizontal gel slabs immersed in the electrophoresis buffer either in one or two dimensions.

Electrophoresis in the first dimension was usually performed for 16 h at 60–65 V. For the second dimension the gel was soaked in the TBE buffer plus an aliquot of a fresh solution of chloroquine (10 μg/μl), giving a final concentration of 1.4 mM, and equilibrated for 6–8 h. The equilibrated gel was shifted 90° from its original position in the first dimension and re-electrophoresed during 16 h at 65 V. In order to remove chloroquine, the gel was extensively washed with deionized water. Finally it was blotted and hybridized with the appropriate radiolabeled probe.

Chromatin Assembly Reactions—Chromatin assembly reactions were carried out with the cell-free extract S-150 (21, 22). In the experiment described in Fig. 5, 3 μg of either relaxed or relaxed plus knotted pUC19 DNA were added to a mixture containing 20 mM HEPES pH 7.5, 5 mM magnesium chloride, 1 mM EDTA, 10 mM glycerophosphate, and 600 μl of S-150 in a final volume reaction of 1 ml. Samples were incubated at 37°C; at 15, 30, 60, 120, and 240 min of reaction, one-fifth of the starting reaction was removed and stopped by addition of 50 μl of 2.5% Sarkosyl, 100 mM EDTA, followed by deproteinization with SDS (final concentration: 0.2%) and proteinase K (1 μg/μl, final concentration). After a 1 h incubation at 65°C, a phenol extraction was performed, and DNA was purified and electrophoresed.

The experiment shown in Fig. 6 was performed with 800 ng of knotted pUC19 DNA; 400 ng of knotted DNA were untied in a mixture containing 20 mM HEPES pH 7.5, 2 mM magnesium chloride, 0.1 mM EDTA, 0.2 mM ATP, 150 mM KCl, and about 20 ng of topoisomerase II in a 30-μl final volume reaction. In the mock-incubated reaction with 400 ng of knotted DNA, topoisomerase II was the only reagent omitted. After 1 h at 30°C, concentrated solutions of HEPES pH 7.5, magnesium chloride, EDTA, EGTA, ATP, creatine phosphate, creatine kinase, and glycerol were added to raise the ionic conditions described above, together with 150 μl of S-150 in 250 μl of final reaction volume. Those mixtures were incubated 6 h at 37°C. For the topological assay, two aliquots of each reaction were removed and processed as described above. The remaining aliquots were digested with 50 units of micrococcal nuclease (Boehringer Mannheim) in 3 mM calcium chloride for the indicated times at room temperature. Aliquots were removed and processed as described.

**RESULTS**

When a relaxed pUC19 plasmid DNA was incubated with increasing amounts of yeast topoisomerase II and the extensively deproteinized samples were electrophoretically resolved, an emergent distribution of bands with progressively higher mobilities appears (Fig. 1). At topoisomerase II/DNA mass ratio of 1–2, the head of this ladder runs faster than the mobilities appears (Fig. 1). At topoisomerase II/DNA mass ratio of 1–2, the head of this ladder runs faster than the mobilities appears (Fig. 1). At topoisomerase II/DNA mass ratio of 1–2, the head of this ladder runs faster than the mobilities appears (Fig. 1).

Electrophoretically resolved DNA was knotted by addition of stoichiometric amounts of topoisomerase I. Purified topoisomerase I was added to the knotting reaction containing 200 ng of relaxed pUC19, incubated at 30°C for 1 h, deproteinized, and the DNA was purified and loaded in 1% agarose-TBE slab gel. Lanes 2–5, incubation made with 0, 0.07, 0.14, 0.21, and 0.42 μg of topoisomerase I, respectively; lane 1, 200 ng of relaxed pUC19; lane 6, 50 ng of linearized pUC19; lane 7, 200 ng of supercoiled pUC19; lane 1, II, and III denotes the electrophoretic mobilities of supercoiled, relaxed, and linearized forms of pUC19, respectively. White bar depicts the knot distribution.

**FIG. 1.** Relaxed DNA is knotted by addition of stoichiometric amounts of topoisomerase I. Purified topoisomerase I was added to the knotting reaction containing 200 ng of relaxed pUC19, incubated at 30°C for 1 h, deproteinized, and the DNA was purified and loaded in 1% agarose-TBE slab gel. Lanes 2–5, incubation made with 0, 0.07, 0.14, 0.21, and 0.42 μg of topoisomerase I, respectively; lane 1, 200 ng of relaxed pUC19; lane 6, 50 ng of linearized pUC19; lane 7, 200 ng of supercoiled pUC19; lane 1, II, and III denotes the electrophoretic mobilities of supercoiled, relaxed, and linearized forms of pUC19, respectively. White bar depicts the knot distribution.

When a relaxed pUC19 plasmid DNA was incubated with increasing amounts of yeast topoisomerase II and the extensively deproteinized samples were electrophoretically resolved, an emergent distribution of bands with progressively higher mobilities appears (Fig. 1). At topoisomerase II/DNA mass ratio of 1–2, the head of this ladder runs faster than the covalently closed, supercoiled DNA (Fig. 1, lanes 4, 5, and 7), showing a higher compactness. According to previous results (3, 17), each rung of the ladder corresponds to a knot with a different number of crosses (nodes). It is noteworthy that poor staining was shown by knots with a high number of crosses when ethidium bromide was employed; on the opposite, this effect does not appear when a radiolabeled probe was used instead of the colorant. Likely, the high degree of compaction can act as a barrier for the dye intercalation (compare Figs. 1 and 4).

Under these experimental conditions, the knotting reaction is strictly dependent on ATP since in its absence, I did not obtain a detectable presence of those DNA forms (not shown), in good agreement with previous data (14).

The topological state of the knots was further assessed by two-dimensional agarose gel electrophoresis (24). Electrophoresis in the second dimension was carried out after the equilibration of the gel in the electrophoresis buffer containing chloroquine. Fig. 2 illustrates the two-dimensional electrophoresis for knotted pUC19 DNA. Several sets of spots can be distinguished. The topoisomerons are resolved as discrete spots along a regular curve (depicted with asterisks in the scheme of Fig. 2B) while knots display two different distributions, a perfect diagonal containing the nicked population of knots (18) and a curved distribution which corresponds to the covalently closed knots (diagrammed as dark and white circles, respectively, in Fig. 2B). Both distributions converge at the right end of the diagonal because the capability of the chloroquine to discriminate between both nicked and covalently closed knot distributions decreases progressively as their node number increases.

In order to gain further insight into the characterization of knots, I performed the experiment shown in Fig. 3, where equal amounts of knotted pUC19 DNA (Fig. 3, lane 1) were treated in two different reactions with topoisomerase I and II under the experimental conditions described (see "Materials and Methods") together with supercoiled SV40 DNA (Fig. 3, lane 1) added at the onset of the reaction as relaxation internal control. Both topoisomerase I and II were working properly, since their were able to relax the negatively supercoiled SV40 DNA (Fig. 3, lanes 4 and 5, respectively). However, while topoisomerase II can relax the supercoiled SV40 DNA, and unlike the knotted pUC19, topoisomerase I is able to relax the internal control but fails to unknot the knotted pUC19. These results exclude the possibility that any topological form other than knots might have been formed when topoisomerase I and circular DNA were incubated at a mass ratio close to 1.

Previous data have suggested that knotting formation using T4 bacteriophage topoisomerase II requires a supercoiled DNA as substrate rather than the nicked or relaxed forms (3, 17). However, with large amounts of topoisomerase II from Drosophila embryos, knotted DNA can be generated over either covalently closed circular DNA or its nicked, relaxed form (14). Fig. 4 displays the topological forms observed during the time course of two different knotting reactions with relaxed (Fig. 4, lanes 1-5 and lane 12, relaxed substrate before the knotting reaction) or supercoiled (Fig. 4, lanes 7-11 and lane 6, supercoiled substrate before the knotting reaction) pUC19 DNA.

Knot formation is very fast; in the experiment shown in Fig. 4, the earliest sampling, 15 min after the onset of the reaction, already shows a knot ladder almost indistinguishable from samples removed after longer incubation times, especially over the relaxed template rather than those obtained over the supercoiled form. A small, but significant, amount of supercoiled
FIG. 2. Electrophoretic characterization of knotted DNA using two-dimensional gels. A. 1 μg of covalently closed relaxed pUC19 was knotted as described under "Materials and Methods." After careful purification, the DNA sample was electrophoresed in 1% agarose-TBE gel at 65 V during 16 h, soaked in the running buffer with 1.4 μM chloroquine for 9–8 h, shifted 90° from its original position, and re-electrophoresed in the second dimension for 16 h at 65 V. Blotted to a filter and hybridized with a pUC19 probe. B. Scheme from A. Indicated are nicked circle (star), nicked knots (black dots), covalently closed knots (white dots), and supercoils (asterisks); for illustrative purposes, drawings of three node knots (trefoil) are shown.

FIG. 3. Enzymatic characterization of knotted DNA by topoisomerase I and II. 300 ng of relaxed pUC19 were knotted and, after DNA purification, the sample was divided into three identical aliquots. Knotted DNA (100 ng) was incubated, in two separate reactions, with either commercial topoisomerase I from calf thymus (10 units/μl) or yeast topoisomerase II (10 units/μl) together with 100 ng of supercoiled SV40 DNA as internal control of relaxation. After incubation, the samples were deproteinized and the DNA was purified and electrophoresed in 1% agarose-TBE gel. After running, the gel was blotted and hybridized with a SV40-pUC19 radiolabeled probe. Lane 1, supercoiled SV40 DNA; lane 2, relaxed pUC19 DNA; lane 3, knotted pUC19 DNA; lane 4, supercoiled SV40 DNA + knotted pUC19 DNA after topoisomerase I treatment; lane 5, the same mixture as in lane 4 but after relaxation with topoisomerase II; lane 6, supercoiled pUC19 DNA, I, II, and III denotes the supercoiled, nicked, and linear forms of each DNA, respectively. Bracket depicts the position of knots. Observe that only topoisomerase II is able to unknot double stranded DNA whereas both topoisomerases I and II relax the DNA with equal efficiency.

DNA appears to be resistant to knotting even after 4 h of incubation (Fig. 4, lane 11). It should be noted that the knotted form over supercoiled DNA is as fast as over the relaxed form. Moreover, partially supercoiled DNA topoisomers are not seen (Fig. 4, lanes 6–11), suggesting that supercoils become knots in one step without relaxed intermediates (see "Discussion").

Extracts from Xenopus eggs and oocytes can form regularly spaced nucleosomes on circular, topologically linked DNA in an ATP-driven process (22, 23, 25, 26): when a relaxed DNA is added to those extracts it becomes supercoiled concomitantly to the assembly of nucleosomes (25, 27). Thus, the measurement of the superhelix density reflects the actual number of nucleosomes loaded on the DNA ring (26).

To test if the knotted DNA could assemble periodically arranged nucleosomes, equal amounts of either relaxed or knotted (but containing a fraction of relaxed DNA) pUC19 DNA were incubated in two separated chromatin assembly reactions with the cell-free extract from Xenopus oocytes S-150. Fig. 5 depicts the topological changes during the time. While both relaxed (Fig. 5, lanes 1–6) and unknotted (relaxed) fraction contained in the knotted mixture (Fig. 5, lanes 9–14, white circles) become progressively supercoiled by nucleosome loading, the knotted DNA (white dashes), which runs electrophoretically in between the topoisomers and remains unaltered after the assembly process. The experiment addresses how the knotted fraction is resistant to assembly but does not inhibit this process over the unknotted (relaxed) fraction.

It is remarkable the very low levels of endogenous topoisomerase II activity detected in crude oocyte extracts which is pelleted during the preparation of the S-150 (23, 28, 29) and, in contrast, the strong topoisomerase I activity of this extract which relaxes completely in a few minutes after a supercoiled DNA exogenously was added at the beginning of the chromatin assembly; over this endogenously relaxed template occurs the nucleosome deposition, giving a negatively supercoiled DNA after the deproteinization of the sample (not shown) (Ref. 25 and references therein).

An additional question is whether the negative effect of knotted DNA substrate on chromatin assembly could be reversed or not by untying the template with trace amounts of topoisomerase II immediately before the addition of the S-150. Fig. 6 (top) depicts the design of the experiment. Supercoiled pUC19 DNA was knotted by means of stoechiometric amounts of topoisomerase II under standard conditions; the deproteinized sample was then divided into two halves and incubated at 30°C for 1 h in two different reactions, either with a trace...
amount of topo II or, in a mock incubated reaction, with buffer alone before the addition of the S-150. Six hours later, the chromatin assembly products were processed for both topological and micrococcal nuclease digestion analysis. Pretreatment with catalytic amounts of topoisomerase II prior to the assembly process can reverse the inability of the knotted DNA template (Fig. 6, lane 3) to assemble nucleosomes as revealed both by its topological state (Fig. 6, lane 8) and the presence of a series of DNA fragments which are multiples of approximately 160–170 base pairs (Fig. 6, lanes 9–11) obtained upon partial micrococcal nuclease digestion. In contrast, the topoisomerase II mock-incubated half (Fig. 6, lane 4) shows, after the micrococcal nuclease digestion, a mixture of DNA fragments without a clear nucleosomal periodicity (Fig. 6, lanes 5–7). This pattern of bands may be explained by the partial assembly over the unknotted species contained in the knotted mixture (see Fig. 5). It should also be noted the higher sensitivity against the micrococcal nuclease digestion shown by the topoisomerase II mock-incubated reaction (compare Fig. 6, lanes 6 and 10) which, again, reveals the assembly only over the unknotted template. Therefore, the previous untying of knotted DNA template seems to be critical for the correct nucleosome loading.

**DISCUSSION**

In this paper I show how the topoisomerase II from *S. cerevisiae* can introduce knots in a plasmidic DNA when the reaction is performed at an enzyme/DNA mass ratio equal or higher than 1, always in the presence of ATP. This process needs a topologically linked DNA substrate regardless its form.

DNA knotting, topoisomerase class II-mediated, has been described by means of purified enzymes from *T4* bacteriophage (3, 17), *Drosophila* (14), and yeast (18). In those cases knotting is achieved at an enzyme/DNA mass ratio quite higher than necessary to produce relaxation/unknotting decatenation reactions (5, 14, 18). Under the experimental conditions described in this paper, I estimate that about 15 molecules of topoisomerase II per pUC19 DNA molecule are needed to produce a highly compacted knotting, while in the relaxation reaction using topoisomerase II from *Drosophila* one molecule of enzyme, working in a processive fashion, can relax about 15 molecules of supercoiled DNA (5). Two different conditions have been found to promote the knotting production by both the prokaryotic and eukaryotic enzymes. The knotting process requires ATP hydrolysis or the addition of a nonhydrolyzable analog of ATP such as AMPPNP when a eukaryotic enzyme is employed (14, 18 and this report) but not when the T4 enzyme is used (3, 17). Furthermore, whereas the eukaryotic enzyme can operate over a DNA ring regardless of its topological form, the viral enzyme holds DNA with a linking number deficit much more efficiently (17).

It has been suggested that the juxtaposition of the DNA helices in the plectonemic supercoiling is the general feature for the spatial recognition of the DNA topology by topoisomerases (3, 14, 30). In addition, yeast topoisomerase II promotes the stabilization of crossings of DNA duplexes, acting as a barrier for the relaxation with topoisomerase I and, on the other hand, promoting the knotting formation strongly over several topological forms (18). Therefore, the finding that the enzyme can form knots over DNA rings without any superstructure (see Fig. 4) may account for the ability of topoisomerases.

\[^1\] The abbreviation used is: AMPPNP, 5'-adenyl-β,γ-imidodiphosphate.
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ase II to promote those crosses if the DNA molecule is wrapped around the enzyme as in the DNA-bacterial gyrase complexes observed in vitro (31–33).

Knots have several well defined characteristics: 1) they show the highest compaction degree as revealed by their electrophoretic mobilities: the knots containing more nodes run electro- phoretically ahead of the supercoiled form (Figs. 1, 3, 4, and 6). 2) In part because of its compactness, the knots are less well stained than the other topological forms when ethidium bro- mide is employed; nonetheless, when a radiolabeled probe was used instead of ethidium bromide such differences were not observed (compare Figs. 1 and 4). 3) Two-dimensional gel analysis displays the peculiar electrophoretic mobility shown by the nicked knots, which run in diagonal. When the second dimen- sion is performed in the presence of chloroquine, a curved distribution of covalently closed knots appears converging to the tip of the diagonal, as the number of crosses increases (18) (Fig. 2). 4) The knots can only be untied, without endonucleo- lytic linearization, by topoisomerase II at an enzyme/DNA ratio much lower than the necessary for the knotting formation and in the presence of 150 mKCl (data not shown).

Under the experimental conditions reported here, the level of KCl is critical: the knotting process requires low levels of KCl (20 mKCl) probably because the high ionic strength (150 mKCl) can affect, by shielding of charged groups, the binding between the DNA and the large number of topoisomerase II molecules required to produce knots (18). In the relaxation/unknotting/ decatenation processes, where the enzyme must be present at a catalytic level, this effect is present at a lesser extent. The increase of the ionic strength alters the nature of the relaxation reaction from processive to distributive by changing the binding rate between DNA and the topoisomerase II from Drosophila (5). This could explain the different requirements of KCl observed for both relaxation and knotting process.

Time course analyses over either relaxed or supercoiled DNA show that after 15 min of incubation the knotting distribution is already the same as observed 4 h later, especially when the substrate is a covalently closed relaxed DNA (Fig. 4, lanes 1–5). The knotting over negatively supercoiled DNA occurs without detectable DNA relaxation (Fig. 4, lanes 7–11), and is easily distinguishable in well resolved gels, because the knots (Fig. 4, asterisks) migrate between the topoisomers (Fig. 4, white circles), according to other reports (3, 14).

When the knotting is carried out on supercoiled DNA, a small fraction keeps this topological form throughout the time. It could be explained by considering a potentially very short step of relaxed state, which I did not see, between both the supercoiled and the knotted form. Nonetheless, the level of KCl, optimal for knotting but not for relaxation, would explain why such a supercoiled fraction does not reach the knotted state (Fig. 4, lanes 7–11).

By using the cell-free extract S-150 from Xenopus oocytes as an in vitro chromatin assembly system (22, 23) is shown by means of both topological and micrococcal nuclease digestion analysis that the knotted DNA is the only topologically linked, single ring unable to assemble nucleosomes. Interestingly, the treatment of 800 ng of knotted substrate with 20 ng of topoi- somerase II before the onset of the chromatin assembly leads to a competent substrate that allows the ordered deposition of histones in a reaction mixture containing about 400 μg of total protein, and yields a minichromosome undistinguishable from those obtained on competent substrates (Figs. 5 and 6, lanes 8–11). This effect appears to be more dramatic than the observed using the same S-150 system in the remodeling of the minichromosome by the exogenous addition of histone H1 (34). The knotted species present in the chromatin assembly reac- tion does not inhibit this process over other unknotted forms; as Fig. 5 shows, the unknotted template reaches progressively a negative supercoiled state in a very similar fashion than that obtained on the relaxed plasmids. In contrast, no nucleosomes are formed over the knotted population.

Topoisomerase II is, among the nonhistone proteins, one of the most abundant components of the mitotic chromosome. It seems to play an important role in the organization of the chromosome structure (Refs. 35–38 and references therein). This protein binds very preferentially to the so-called matrix- or scaffold-associated regions anchoring chromatin loop domains in vivo (Ref. 36 and references therein). Furthermore, it has been observed in living embryonic cells from Drosophila that the topoisomerase II appears to be localized at precise sites in the nucleus, in a temporally regulated fashion (39). This in vivo redistribution has also been reported during the induction of heat-shock genes (10).

This report documents the ability of the eukaryotic topoi- somerase II to induce very different topological structures over DNA rings depending on the enzyme concentration. Thus, when the enzyme/DNA mass rate is high, knots are produced; on the opposite, the decrease of this ratio leads to a typical relaxation/unknotted/decatenation activity.

The spatially and temporally regulated distribution of topoi- somerase II in nuclei, observed in vivo, could reflect the local variation of the enzyme concentration during the cell cycle. It would not be unreasonable to suppose that this local variation in the topoisomerase II concentration would promote the knot- ting of specific regions or domains of DNA, which could impede the regular nucleosome assembly, favoring the genetic activity of those domains. When the transcription process is finished, the enzyme, via unknotting, may open up these regions toward the regular chromatin recondensation.

These results suggest that topoisomerase II may be essential in the topological transitions undergone by the eukaryotic chro- mosome during the cell cycle.

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