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Inhibition of Topoisomerase II Does Not Inhibit Transcription of RNA Polymerase I and II Genes

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Injection of VM-26 (teniposide) into *Xenopus* oocytes inhibits the activity of topoisomerase II but does not inhibit transcription by RNA polymerases I and II. A specific assay for topoisomerase II, resolution of catenated DNA molecules into product rings, was used to quantitate VM-26 inhibition in vivo. When catenanes were injected without VM-26, about 60% of them were separated into product rings in the first 5 min after injection, and decatenation of the remainder was complete within 15 min. When VM-26 was coinjected, 60% of the catenanes were separated into product rings in the first 5 min after injection, but the remaining 40% were stable over the next 40 min. At 1 h after injection catenanes were no longer detected in the gel analysis, but the increasing numbers of linear product rings indicated that topoisomerase II continued to be inhibited by VM-26. These results suggest that a short lag of approximately 5 min is required for VM-26 to inhibit topoisomerase II and that after this initial period topoisomerase II is inhibited by more than 90%. There was no detectable decrease in transcription of injected rRNA and thymidine kinase (TK) genes or in the activity of the rRNA enhancer when these transcription templates were coinjected with VM-26. The time required for assembly of injected DNA into chromatin doubled in the presence of VM-26.

The superhelical density in procaryotes is known to influence the promoter activity of a variety of genes, and supercoiling is controlled by the opposing actions of gyrase and topoisomerase I (25). Despite differences in the activities of topoisomerases and in chromatin structure between eucaryotes and procaryotes, a link between supercoiling and transcription in eucaryotes is an attractive idea. Experiments to investigate this link have generally correlated changes in topology or the activities of topoisomerases with transcriptional activity. Results with *top1* and *top2* yeast mutants indicate that topoisomerase I and II can substitute for each other in all cellular functions except chromosome segregation, which requires a double-strand break (1, 23, 24). Transcription proceeds at a reasonable rate in the *top1 top2* double mutant, indicating that neither topoisomerase I nor topoisomerase II is essential for transcription. Genetic data for higher eucaryotes are not available, but the chromosomal and cellular localizations of topoisomerases I and II suggest that they have different roles in transcription. Topoisomerase I maps to positions inside the transcribed region of both *Drosophila* heat shock genes and *Xenopus* rRNA genes (5, 7), so that it could be used to relieve supercoils generated during the process of transcription elongation (6, 27). In contrast, topoisomerase II is found in the nontranscribed 5′ flanking regions of genes (19, 28) and associated with the DNA at sites termed matrix attachment regions, which often occur in 5′-flanking DNA of genes (4).

Experiments on supercoiling and transcription in eucaryotes use systems such as simian virus 40, yeast 2μm DNA, and plasmids injected into *Xenopus* oocytes, in which a circular DNA molecule can be analyzed (2, 6, 9, 15, 16). Injection into oocytes is a particularly attractive experimental system, since transcription can be studied in the absence of replication and templates can be perturbed by the injection of drugs and enzymes. Harland et al. (9) showed that linearization of DNA injected into *Xenopus* oocytes disrupted transcription even if the DNA was cleaved after transcription was established. Similar results were obtained when injected *Xenopus* rRNA genes were linearized (16), and injection of ethidium bromide into oocytes affects transcription from the spacer promoters as well as the major promoter of the rRNA genes (17). These results suggest that supercoiling (or at least circularity) is important for eucaryotic transcription. Although injection of the topoisomerase II inhibitor novobiocin dramatically reduced transcription of injected 5S genes in oocytes (20), it is now clear that the observed transcriptional inhibition does not result from inhibition of topoisomerase II (8). Furthermore, addition of the inhibitors camptothecin and VM-26 (teniposide) to in vitro transcription reactions at levels that inhibit topoisomerases I and II, respectively, does not affect transcription of 5S genes (8). These in vitro results for RNA polymerase III genes are consistent with the finding that linearized RNA polymerase III templates are actively transcribed when they are injected into oocytes (11). However, the effect of topoisomerase II inhibition on transcription of RNA polymerases I and II genes has not been determined.

I have examined the effect of topoisomerase II inhibition on the transcription of RNA polymerase I and II genes by injecting VM-26 into *Xenopus* oocytes. VM-26 inhibits eucaryotic topoisomerase II during the breakage-reunion reaction (3). The drug does not intercalate into the DNA and does not inhibit topoisomerase I. The in vivo inhibition of topoisomerase II was measured by monitoring the kinetics of decatenation when multiply intertwined catenanes were injected into oocytes. Since the removal of these interlinks requires that a double-strand break be made, analysis of decatenation specifically assays topoisomerase II activity. Injection of VM-26 into *Xenopus* oocytes inhibits topoisomerase II activity by more than 90%. There is no concomitant decrease in transcription of injected rRNA or thymidine kinase (TK) templates with topoisomerase II inhibition, and the activity of the rRNA enhancer is not affected. The time required for assembly of injected DNA into chromatin is increased by approximately a factor of 2 in the presence of

VM-26, raising the possibility that topoisomerase II is involved in the process of chromatin assembly.

MATERIALS AND METHODS

Preparation of injection materials. VM-26 was obtained either as a gift from Leroy Liu or directly from Bristol-Myers. It was dissolved in anhydrous dimethyl sulfoxide (Aldrich Gold Label) at 10 mM and stored in small aliquots at −80°C. Aliquots were thawed immediately prior to use and discarded after each injection experiment. During the course of these experiments, three different lots of VM-26 were tested for inhibition of decatenation and transcription. The first lot of VM-26 that I tested by oocyte injection inhibited topoisomerase II at a concentration of 100 μM, one-fifth the concentration of VM-26 used in the experiments reported here. The second lot that I tested had very little effect on decatenation. The data reported here were collected by using a third batch of VM-26, which was effective in inhibiting topoisomerase II at 500 μM. Neither VM-26 nor diluted dimethyl sulfoxide affected oocyte viability over a 24-h period.

Catenanes of pPDψ40 were prepared by in vitro reaction of the plasmid with bacteriophage λ integrase and *Escherichia coli* integration host factor (14). After in vitro recombination, the reaction was phenol extracted and the DNA was precipitated twice with ethanol. All plasmid DNAs used for injection were suspended in 10 mM Tris hydrochloride (pH 7.8)–1 mM EDTA (TE) and diluted with the same buffer for injection. However, dimethyl sulfoxide was added to a concentration of 5% in control injections to parallel its concentration in VM-26 injections.

For chromatin assembly experiments, a relaxed template of pXlrψ40 was prepared by reaction of the supercoiled plasmid with wheat germ topoisomerase I (a gift from Chris Boles). The DNA was phenol extracted, ethanol precipitated, and suspended in TE for injection.

Oocyte injection. An ovary was surgically removed from female *Xenopus laevis* and then treated with collagenase in Barth buffer for 2 to 4 h to dissociate the oocytes. Approximately 20 nl was injected into the oocyte nucleus in all cases; when double-injection protocols were followed, both injection volumes were 20 nl. The concentrations of VM-26 stated in the figure legends are those of the injection solution. DNA (1 to 2 ng) was injected into each oocyte as stated in the figure legends. Between 60 and 100 oocytes were injected with DNA, and survival after overnight incubation was 80% or better. From these injected oocytes, pools of five were harvested at appropriate times for analysis of DNA. At time points when transcription was assayed, at least 20 oocytes were pooled and harvested.

Oocytes were harvested by being placed in microcentrifuge tubes, having all but the interstitial buffer removed, and being homogenized in 50 mM Tris (pH 8.0)–100 mM NaCl–50 mM EDTA–1% sodium dodecyl sulfate (SDS). Proteinase K was added to 0.1 mg/ml, and the samples were incubated at 37°C for 30 min. The samples were then phenol extracted, and the nucleic acids were ethanol precipitated and suspended in sterile TE.

Analysis of decatenation and chromatin assembly. To analyze decatenation of the DNA that was recovered from the injected oocytes, the oocyte samples were nicked by reaction with 50 μg of DNase I in the presence of 500 μg of ethidium bromide per ml at 30°C for 30 min. Nicked catenanes containing different numbers of interlinks can be resolved easily from each other and from the nicked, unrecombined plasmid by electrophoresis (22). The nicking reactions were stopped by phenol extraction, and portions of the samples were electrophoresed through a 0.8% agarose gel in 10 mM Tris hydrochloride (pH 7.5)–10 mM sodium acetate –0.1% SDS (22). The gel was run for 18 to 22 h at 40 V with buffer recirculation. After electrophoresis, the DNA was acid depurinated and blotted onto nitrocellulose. The blot was hybridized with the labeled probes indicated in the figure legends.

For analysis of chromatin assembly, the DNA from injected oocytes was purified as described above. A portion was electrophoresed on a 1% agarose gel in Tris-borate buffer containing 15 μg of chloroquine per ml to separate the topoisomers. The DNA was depurinated and blotted onto nitrocellulose.

Analysis of transcription. Oocyte samples were prepared as described above. Templates for rRNA contain either a 40- or 52-base insert so that their transcripts can be biochemically distinguished from the transcripts originating from the endogenous genes (17) by using specific S1 probes. Probes were prepared by first cutting pXlrψ40 and pXlrψ52 with *Bam*HI at the end of the 40- or 52-base-pair insertion. The DNA was treated with calf alkaline phosphatase, digested with *Bgl*I at −61, and then reacted with T4 polynucleotide kinase and [γ-^{32}P]ATP to label the *Bam* end. The DNA was then run on a 6% polyacrylamide–7 M urea–Tris-borate gel to separate the single-stranded end-labeled probe from its complement. The nucleic acid from four or five injected oocytes was hybridized with an excess of S1 probe in 30 μl of 0.25 M NaCl–10 mM Tris hydrochloride (pH 7.5)–10 mM EDTA at 80°C for 2 h. The samples were diluted 10-fold with 20 mM NaCl–30 mM sodium acetate (pH 4.5)–1 mM $ZnSO_4$–5% glycerol and digested with 45 U of S1 nuclease (Bethesda Research Laboratories, Inc.) for 30 min at 37°C. The nucleic acid was ethanol precipitated, suspended in 99% formamide, and loaded onto an 8% acrylamide–7 M urea–Tris-borate gel. Gels were dried and exposed to preflashed Kodak X-AR film at −80°C with intensifying screens. Transcriptional signals were quantitated by densitometry of appropriate autoradiographic exposures.

Transcription of injected TK genes was assayed by primer extension as described by McKnight et al. (13). For TK transcription the equivalent of one oocyte was assayed.

RESULTS

Measurement of the in vivo inhibition of topoisomerase II by VM-26. A decatenation assay was used to measure topoisomerase II activity in vivo. Decatenation of intertwined DNA circles, i.e., catenanes, requires that a double-strand break be made in one of the rings. This assay is therefore specific for topoisomerase II activity. Catenanes are injected into the oocytes, and the DNA is recovered at various times after injection. The DNA is nicked and then analyzed by using a gel electrophoresis system that separates catenanes on the basis of interlink number (22). The DNA is then visualized by Southern blotting. Nicking the DNA prior to electrophoresis is essential for resolving the catenanes, and also simplifies the analysis of decatenation by eliminating topoisomers of catenanes that result from in vivo relaxation of the injected DNA and subsequent assembly into nucleosomes. The effect of the topoisomerase II inhibitor VM-26 can therefore be directly measured by the decatenation assay.

A dimeric, multiply intertwined catenane that also contains a transcription template was prepared by in vitro

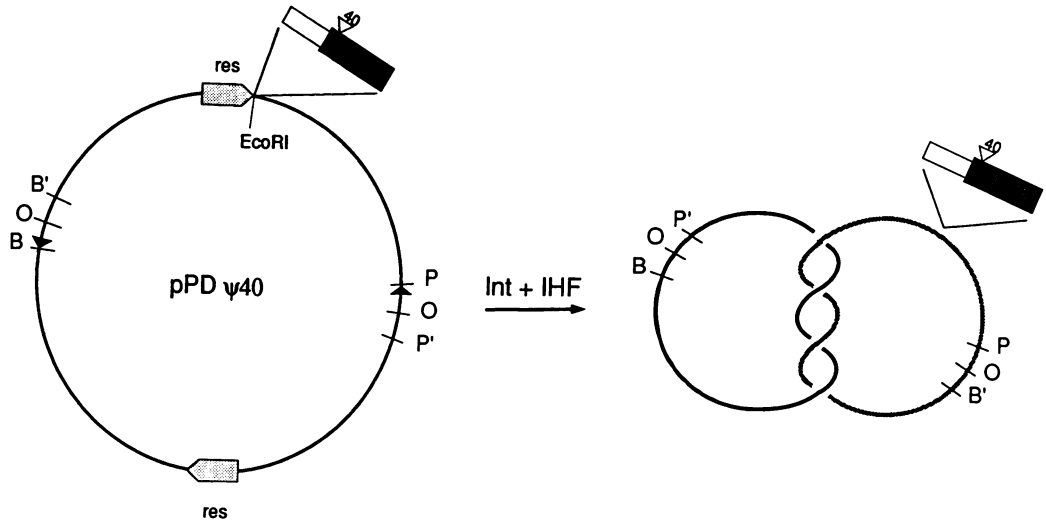

FIG. 1. Recombination products of pPDψ40. Schematic representation of the products resulting from in vitro recombination with phage λ integrase and *E. coli* integration host factor. The ψ40 minigene consists of a promoter (□) with gene body constructed from the first 115 base pairs (■) and final 200 base pairs (▩) of the *X. laevis* 40S rRNA precursor. The gene body contains a 40-base pair insertion so that its transcript can be distinguished from the endogenous rRNA transcripts by an S1 nuclease assay (18).

recombination of pPDψ40 (Fig. 1). This plasmid was constructed by inserting the rRNA minigene ψ40 (18) into pAB7.0d (26), which contains direct repeats of the recombination sites for the Tn*3* resolvase and phage λ integrase recombination systems. Recombination of pPDψ40 in vitro with integrase in conjunction with *Escherichia coli* integration host factor produces a family of catenanes having multiple interlinks that are exclusively right-handed (21).

The gel analysis of decatenation of pPDψ40 catenanes after injection both with and without 500 μm of VM-26 is shown in Fig. 2. When the catenanes were injected without topoisomerase inhibitors (lanes 2 to 4), decatenation was complete within 15 min after injection. In contrast, when the catenanes were coinjected with VM-26, multiply interlinked catenanes containing up to seven interlinks were still present 45 minutes after injection (lanes 5 to 8). Thus, injection of VM-26 inhibits topoisomerase II. The accumulation of linear molecules as well as the disappearance of catenanes is significant in evaluating VM-26 inhibition of topoisomerase II. VM-26 inhibits topoisomerase II during the breaking-rejoining reaction (3) by stabilizing a cleavable complex. When the injected oocytes were harvested and homogenized in buffers containing SDS, inhibited topoisomerase II became covalently linked to each 5′ end of the double-strand break and released the 3′ end to create a broken DNA molecule. Therefore, VM-26 inhibition of topoisomerase II directly results in the accumulation of linear molecules.

To evaluate the extent of inhibition of topoisomerase II by injection of VM-26, the data in Fig. 2 were quantitated by densitometry. In Fig. 3A the time course of decatenation is plotted for injections with and without VM-26. When the catenanes were injected without the drug, 68% of them were resolved into product rings within 5 min. Decatenation was complete within 15 min after injection of the DNA. When 500 μM VM-26 was coinjected with the catenanes, 62% of the catenanes were decatenated within the first 5 min, which follows the same kinetics as when no inhibitor was injected. However, the remaining catenanes were stable over the next 40 min after injection, increasing the half-life of the remaining catenanes by more than ninefold. The rapid decatenation observed in the first 5 min is likely to reflect the time required for VM-26 to inhibit topoisomerase II.

At 45 min and 2 h after injection, the accumulation of linear product ring 1 can be clearly seen in the gel analysis (Fig. 2, lanes 7 and 8). The linear and nicked circle forms of product ring 2 were not separated by this gel system. The accumulation of linear molecules and the kinetics of decatenation are each plotted as a function of time after injection in Fig. 3B. The two plots are mirror images of each other, indicating that the number of catenanes decreases as inhibited topoisomerase II becomes associated with them and causes linearization after homogenization in SDS-containing buffers. This decrease in the number of catenanes is inevitable, since a catenane having inhibited topoisomerase II associated with it will appear in the analysis as one linear and one circular product ring. Topoisomerase II inhibition can be accurately measured only when intact catenanes are detected in the gel analysis. Therefore, topoisomerase II inhibition by VM-26 cannot be measured precisely at 2 h, when no catenanes are detected in the gel analysis. Nevertheless, the accumulation of linear molecules is consistent with continued inhibition of topoisomerase II over the time course of the oocyte injection experiment.

To try to eliminate this initial decatenation prior to inhibition of topoisomerase II by VM-26, an injection protocol in which the drug is injected prior to the injection of transcription templates was used (Fig. 2, lanes 9 to 12). Injecting the drug before injecting the catenanes inhibited topoisomerase II and increased the half-life of the catenanes, but did not eliminate the initial decatenation of template. Even when excess plasmid vector DNA was coinjected with VM-26 to trap inhibited topoisomerase II and catenanes were injected 1 h later, decatenation of the rings still occurred (data not shown). Indeed, I find that VM-26 is more effective at inhibiting decatenation when the drug is coinjected with the catenanes.

Injection of VM-26 does not inhibit RNA polymerase I or II. The effect of VM-26 injection on transcription of both RNA polymerase I and II genes was tested by coinjecting VM-26 with rRNA and herpesvirus TK transcription templates.

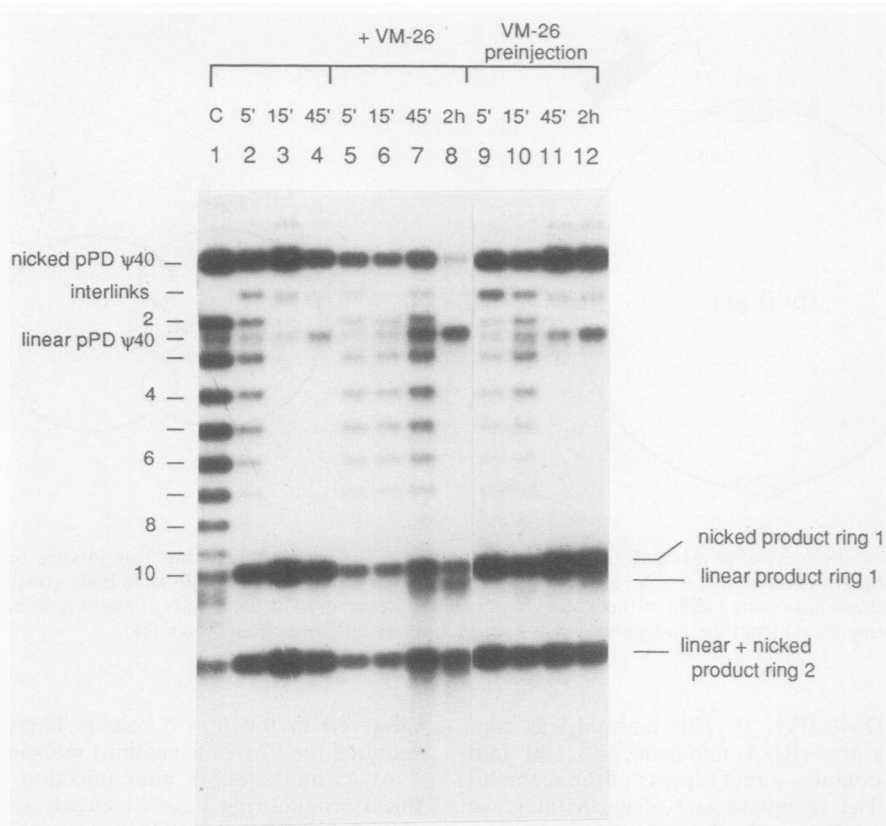

FIG. 2. Effect of VM-26 on decatenation of injected catenanes. Oocytes were injected with catenanes of pPDψ40 prepared by in vitro recombination of the plasmid with λ phage integrase and *E. coli* integration host factor. Injected oocytes were collected at the indicated times after injection of 1 ng of pPDψ40 catenanes, and the nucleic acid was prepared as described in Materials and Methods. DNA samples from the injected oocytes were then nicked with DNase I in the presence of ethidium bromide, electrophoresed as described by Sundin and Varshavsky (22), and blotted. The blot was probed with a fragment containing the site for resolvase recombination, which hybridizes with both rings. The interlink number of the catenanes, as well as linear and nicked unrecombined plasmid, are given on the left of the figure. The products of decantenation are indicated on the right. The linear and nicked forms of product ring 2 comigrate in this gel system. Lanes: 1, catenanes used for injection; 2 to 4, catenanes alone; 5 to 8, catenanes coinjected with 500 μM VM-26; 9 to 12, injection of 500 μM VM-26 30 min prior to injection of catenanes.

Coinjection of VM-26 with the rRNA transcription template pPDψ40 had no inhibitory effect on transcription (Fig. 4A) when transcripts were allowed to accumulate overnight. I have performed this experiment more than 10 times with three different preparations of VM-26 and have never observed any inhibition of transcription. Indeed, there is frequently a small stimulation of transcription when VM-26 is injected, but this stimulation is no more than twofold.

Theoretically, VM-26 could inhibit transcription indirectly. Since VM-26 inhibits topoisomerase II in the breaking-rejoining reaction (3), inhibited molecules of topoisomerase II on the transcription template could block RNA polymerase. To determine whether this was a large effect, a second injection protocol was used in which pUC13 DNA was coinjected with VM-26 to serve as a trap for inhibited topoisomerase II, and the transcription template was injected 30 min later (Fig. 4A). No increase in transcriptional activity was observed when the trapping DNA was present.

The effect of VM-26 injection on the function of the rRNA enhancer was tested (Fig. 4B). The activity of the enhancer in oocytes is assayed by injecting two competing transcription templates; one of these templates has an enhancer, and the other contains only a promoter (18). Under these conditions in which the two plasmids compete for activation, the transcriptional advantage of the plasmid having the enhancer can be expressed as the ratio of the two transcription signals. Figure 4B shows the results of an experiment in which pXlr521 (promoter plus enhancer) is competed against pXlrψ40 (promoter only). In a parallel injection these two plasmids were coinjected with 500 μM VM-26 (Fig. 4B, lanes 2a and b). The ratio between the two transcription signals is not affected by VM-26. Therefore, inhibition of topoisomerase II by VM-26 does not affect enhancer function.

Transcription of rRNA templates was also measured at shorter intervals after coinjection of the DNA with VM-26 (Fig. 5). The injections were done as usual, but rather than allowing the transcripts to accumulate to steady-state levels, the oocytes were harvested at the indicated times. No dramatic differences in transcription were observed when the DNA was coinjected with VM-26. The small difference observed at the 1-h time point is within the variation observed in oocyte injection experiments.

To extend these studies on topoisomerase II inhibition and transcription to RNA polymerase II genes, I employed the same injection protocols and used the thymidine kinase promoter from herpesvirus (12). Coinjection of VM-26 with the TK template did not affect transcription (Fig. 6). These results suggest that topoisomerase II activity is not strictly required for transcription of RNA polymerase I and II genes.

Injection of VM-26 retards bulk chromatin assembly. The possibility that topoisomerase II is involved in chromatin assembly has been suggested on the basis of novobiocin

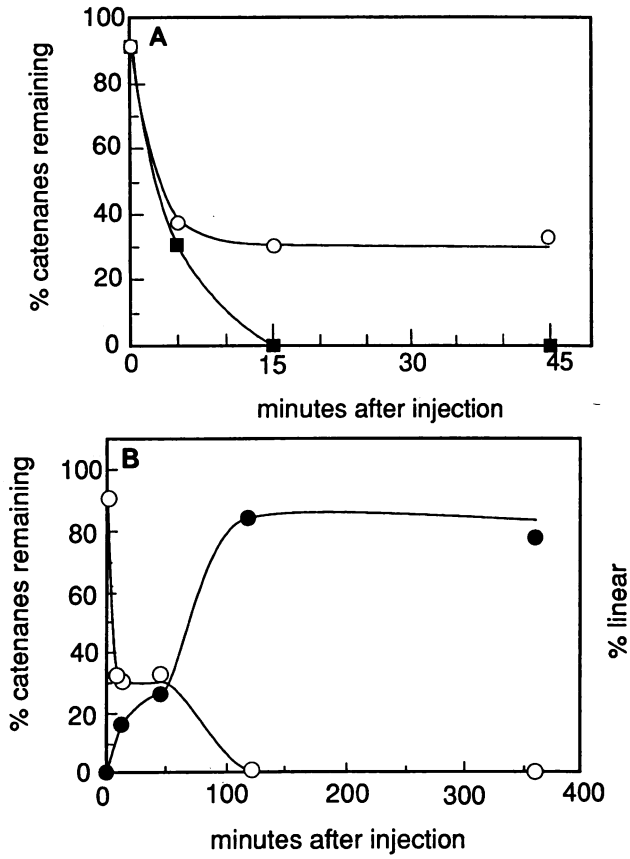

FIG. 3. Quantitation of decatenation. The amounts of both catenanes and linear molecules from the data in Fig. 2 were quantitated by densitometry of appropriate exposures of the autoradiogram. Samples for the 6-h time point were analyzed on a second gel (data not shown). (A) The percentage of catenanes remaining was calculated as the amount of catenanes detected on the blot divided by the total material present either as catenanes or as product rings. The zero time point was obtained from using uninjected catenanes that were mixed with uninjected oocyte homogenate, so that these catenanes experienced the entire procedure necessary to analyze the DNA after injection. The catenanes were considered to be a pool without regard to the number of interlinks. Symbols: ■, catenanes alone (Fig. 2, lanes 2 to 4); ○, catenanes coinjected with 500 μM VM-26 (Fig. 2, lanes 5 to 8). (B) The decrease in the number of catenanes and the increase in the number of linear molecules in the presence of VM-26 (Fig. 2, lanes 5 to 8, and data not shown) is plotted as a function of time after injection. For quantitation of linear DNA molecules, both the unrecombined pPDψ40 and linear product ring 1 were used. In both these cases the linear molecule is sufficiently resolved from the nicked circle to obtain quantitative data for both. The linear and nicked-circle forms of product ring 2 are not resolved in this gel system, and therefore were not used in the analysis of linear molecules. Symbols: ○, percentage of catenanes remaining; ●, percentage of linear molecules.

inhibition of in vitro chromatin assembly (20). To determine the effect of VM-26 on bulk chromatin assembly, pXlrψ40 was injected with and without VM-26, and the process of assembly into nucleosomes was assayed by the change in linking number reflected by the topoisomer distribution of the injected plasmid. To eliminate any complexities in the analysis as a result of differences in the initial relaxation of a supercoiled plasmid, pXlrψ40 was relaxed in vitro with wheat germ topoisomerase I before injection.

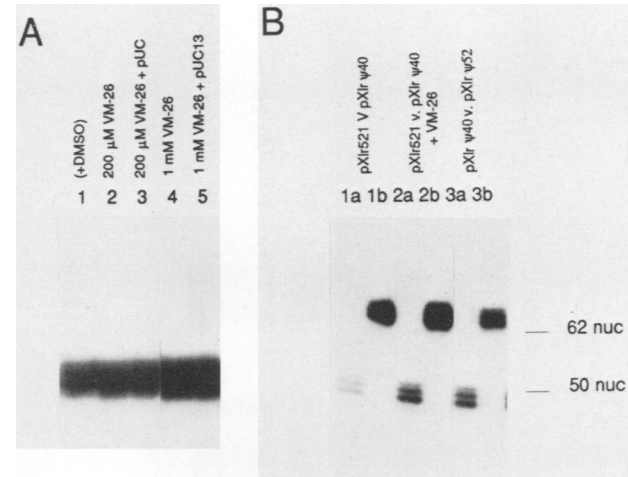

FIG. 4. Effect of VM-26 on transcription of injected rRNA gene templates (A) pPDψ40 catenanes were injected into oocytes alone (lane 1) or with either VM-26 (lanes 2 and 3) or VM-26 plus pUC13 plasmid (lanes 4 and 5). Transcripts were allowed to accumulate overnight and were detected by an S1 nuclease assay. (B) The effect of VM-26 on rRNA enhancer activity was assayed by coinjection of two competing templates (18) with and without VM-26. Lanes: 1a and b, pXlr521 versus pXlrψ40 (enhancer/promoter versus promoter); 2a and b, pXlr521 versus pXlrψ40 plus 500 μM VM-26; 3a and b, pXlrψ40 versus pXlrψ52 (promoter versus promoter). DMSO, Dimethyl sulfoxide.

Figure 7 shows the analysis of chromatin assembly in the presence and absence of VM-26. When only the relaxed plasmid was injected, most of the injected plasmid was found in topoisomers corresponding to a fully supercoiled or assembled molecule within 1 h after injection. However, when the plasmid was coinjected with 500 μM VM-26, complete assembly was delayed until more than 2 h after injection. The time required to assemble the plasmid into chromatin varies somewhat between batches of oocytes, but delayed assembly is consistently observed when plasmids

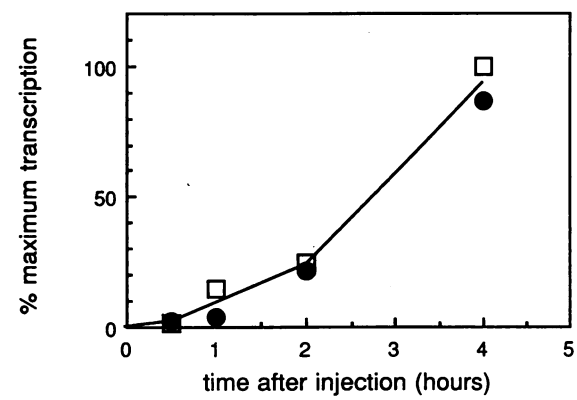

FIG. 5. Time course of rRNA transcription in the presence of VM-26. Oocytes were injected with 1 ng of pXlrψ40 alone or with 500 μM VM-26. The injected oocytes were harvested at the times indicated, and the transcripts were measured by S1 assay. The experiment was assayed in triplicate, transcription results were quantitated by densitometry, and the results were averaged. Transcription levels are expressed as the percentage of the maximum level observed in the experiment. Symbols: □, pXlrψ40 alone; ●, pXlrψ40 coinjected with 500 μM VM-26.

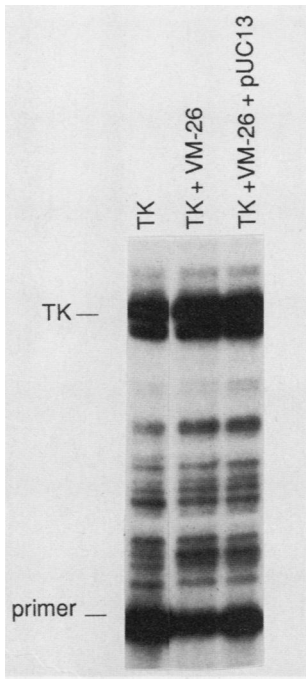

FIG. 6. Effect on VM-26 on transcription of injected TK templates. Plasmids bearing the herpesvirus TK genes were injected into oocytes, and their transcripts were assayed by primer extension. VM-26 and plasmid DNA (pUC13) were injected prior to injection of the transcription template.

are coinjected with VM-26. These results suggest that topoisomerase II is involved in the chromatin assembly process.

DISCUSSION

I have directly measured the in vivo inhibition of topoisomerase II by the drug VM-26. Previous studies (3, 19) have correlated DNA breakage with VM-26 exposure, but the experimental systems have not permitted a direct measurement of topoisomerase II activity in vivo. When VM-26 is coinjected with multiply intertwined catenanes into *Xenopus* oocytes, there is an initial 5-min period of topoisomerase II activity, but the remaining interlinked DNA molecules are relatively stable over the next 40 min. The half-life of these remaining catenanes is increased more than ninefold compared with the half-life of catenanes injected without VM-26. This corresponds to a >90% inhibition of topoisomerase II. The initial rapid decatenation during the first 5 min is likely to reflect the time required for VM-26 to bind to topoisomerase II and cause its inhibition. At more than 45 min after VM-26 and template injection, only a very small number of singly interlinked catenanes are detected. Because VM-26 inhibition creates a cleavable complex, the DNA having inhibited topoisomerase II associated with it will be linearized when the oocytes are homogenized. Therefore, if a catenane has inhibited topoisomerase II associated with it in vivo, the catenane will appear in the gel analysis as one linear and one nicked product ring. Because of the mechanism of VM-26 action, there is some uncertainty in the extent of topoisomerase inhibition after 45 min, when no catenanes are detected in the gel analysis. However, since a proportionate increase in the number of linear molecules is observed as the number of catenanes decreases, the data suggest that the reduction of catenanes is due primarily to VM-26 induced cleavage rather than decatenation. The high proportion of linear DNA molecules, both from unrecombined plasmid and from product ring 1, is consistent with continued topoisomerase II inhibition.

One remaining question concerning the inhibition of topoisomerase II by VM-26 in oocytes is why injection of VM-26 prior to injection of the catenanes does not eliminate the initial rapid decatenation. One explanation is that VM-26 does not diffuse rapidly after injection into the oocyte. The injection solution of VM-26 contains 10% dimethyl sulfox-

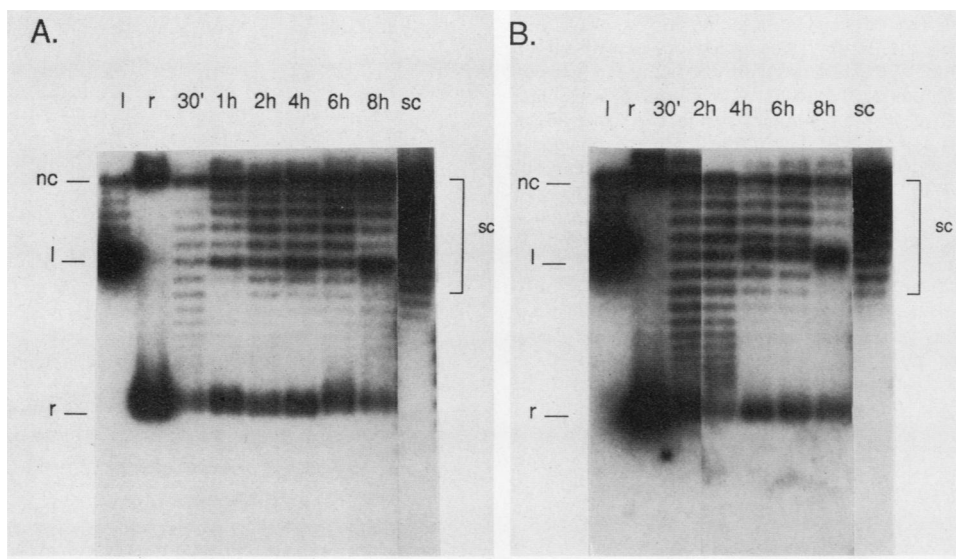

FIG. 7. Effect of VM-26 on assembly of bulk chromatin. Plasmid pXlrψ40 was relaxed by treatment with wheat germ topoisomerase I in vitro. Approximately 1 ng of the relaxed plasmid DNA was injected into oocytes, the oocytes were harvested at the indicated times, and total nucleic acid was prepared as described in Methods and Materials. The topoisomers that are created by in vivo assembly were separated on a 1% agarose gel containing 15 μg of chloroquine per ml. The gels were blotted and hybridized with a probe that is specific for the injected plasmid DNA. Markers: nc, nicked circle; l, linear; r, relaxed circle; sc, supercoiled. (A) Relaxed pXlrψ40 injected alone. (B) Relaxed pXlrψ40 coinjected with 500 μM VM-26.

ide, and when VM-26 is further diluted in water, the drug begins to come out of solution. Precipitation of VM-26 may also occur when VM-26 is injected into the oocyte. If so, this would result in a high concentration of VM-26 at the site of injection, but lower concentrations throughout the remainder of the oocyte. Therefore, the local concentration of VM-26 in the vicinity of the catenanes may be higher when the drug and DNA are coinjected. Even when VM-26 was injected with a plasmid to trap inhibited topoisomerase II molecules before the catenanes were injected, initial decatenation was not eliminated. However, since topoisomerase II is involved in the resolution of catenanes after replication (1, 10, 24), the enzyme may have a preference for catenated DNA. If so, plasmids coinjected with catenanes may not be effective substrates for trapping topoisomerase II.

In contrast to the inhibition of topoisomerase II, VM-26 has no detectable inhibitory effect on transcription. Although there is a greater than 90% inhibition of topoisomerase II activity in the presence of 500 μM VM-26, there is no decrease in transcription of injected rRNA and TK transcription templates. These results do not support the notion that topoisomerase II is strictly required for transcription and are consistent with the results in yeasts (1, 23, 24), indicating that *top2* is essential only for chromosome segregation. My results also show that the linear molecules that are observed in the Southern blot analysis must be circular inside the oocyte, since linear templates do not support transcription of RNA polymerase I or II genes (9, 15). This DNA cleavage in the presence of SDS buffers used to homogenize the oocytes is characteristic of VM-26 inhibition.

In contrast to the result that VM-26 injection does not inhibit transcription, assembly of DNA into nucleosomes required twice as long in the presence of VM-26, which suggests that topoisomerase II is involved in chromatin assembly. Notably, the topoisomerase II inhibition did not parallel the inhibition of chromatin assembly. A likely explanation is that topoisomerase I is able substitute for topoisomerase II in assembly.

Although the results of this study suggest that topoisomerase II is not strictly required for transcription, these studies do not rule out the participation of topoisomerase II in gene regulation, nor do they eliminate the possibility that topoisomerase II normally participates in transcription. The results from yeast *top1* and *top2* mutants suggest that there is some overlap in the functions of topoisomerase I and II, and so it is possible that topoisomerase I could assume the role usually played by topoisomerase II in transcription. An advantage of these oocyte injection experiments is that the effect of topoisomerase II inhibition on transcription can be examined separately from any role that the enzyme has in replication or cell division. Therefore, these experiments do not address whether topoisomerase II may have some role in the coordination of transcription with other cellular processes, perhaps by tethering active genes to cellular structures in the case of normal eucaryotic cells (4). Nevertheless, the inhibitor studies reported here indicate that topoisomerase II is not strictly required for transcription in vertebrates.

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