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Localization of a high molecular weight form of DNA topoisomerase I in amphibian oocytes

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ABSTRACT ***Xenopus* oocytes express a 165 kDa variant of DNA topoisomerase I (topo I) as opposed to the canonical 110 kDa form of somatic cells (Richard and Bogenhagen, *Dev. Biol. 146:* 4-11, 1991). By immunofluorescence microscopy using variant-specific antibodies we show that this high molecular weight form is associated with lampbrush chromosome loops and the inner regions of the amplified nucleoli. Inhibition of topo I-activity by either Camptothecin-treatment or microinjection of neutralizing antibodies resulted in loop retraction and the condensation of chromosomes and amplified nucleoli. These data indicate that the oocyte-specific 165 kDa form of topo I is involved in transcriptional processes mediated by RNA polymerase I and II and is therefore functionally equivalent to the somatic cell 110 kDa counterpart.**

KEY WORDS: *DNA topoisomerase I, lampbrush chromosomes, nucleolus, oocytes, camptothecin*

Introduction

During amphibian oogenesis large amounts of mRNPs, proteins, and ribosomes are accumulated. The stored material is used during early embryonic development when rapid cleavages and almost no transcription of the embryonic genome occur (Newport and Kirschner, 1982a,b). The unusually high rate of ribosome biosynthesis in amphibian oocytes is supported by rDNA amplification which results, in *Xenopus laevis*, in the assembly of roughly one thousand extra nucleoli (Brown and Dawid, 1968). Accumulation of mRNPs and proteins is a result of increased transcriptional activity rather than gene amplification. The cytological equivalent of this high transcriptional activity are the well known lampbrush chromosomes of amphibian oocyte nuclei. These chromosomes consist of a chromosomal axis from which numerous loops radiate laterally. The axis represents a linear array of chromomeres containing highly condensed chromatin, while the lateral loops consist of decondensed, transcriptionally active chromatin covered with numerous nascent transcripts (for a review see Callan, 1986). Thus, amphibian lampbrush chromosomes offer the opportunity to study transcriptional active genes *in situ* in an almost native state.

Which molecular components maintain the particular lampbrush chromosome organization? Recently, several monoclonal and polyclonal antibodies have been successfully used for the identification of lampbrush chromosome-associated proteins. For example, it has been shown that core histones are constituents of the chromomeres of lampbrush chromosomes (Scheer *et al.*, 1979; Hock *et al.*, 1993; Sommerville *et al.*, 1993). In contrast, histone H1, the H1-like protein B4 (now termed H1M, Dworkin-Rastl *et al.*, 1994), and DNA topoisomerase II were virtually absent from *Xenopus* lampbrush chromosome axes as shown by immunofluorescence studies (Fischer *et al.*, 1993; Hock *et al.*, 1993). Bellini *et al.* (1993) have identified a 70 kDa putative zinc finger protein associated with the lampbrush chromosome loops of *Pleurodeles waltl*. Nucleoplasmin (Moreau *et al.*, 1986), several snRNP- and hnRNP proteins (Gall and Callan, 1989; Piñol-Roma *et al.*, 1989; Roth and Gall, 1989; Roth *et al.*, 1990, 1991), and the non-snRNP splicing factor SC 35 (Gall, 1992) have all been localized to lampbrush chromosome loops.

Previous studies have shown that the enzyme DNA topoisomerase I (topo I) is required for transcription of protein-coding as well as rRNA genes and is associated with transcriptionally active regions of the genome (Brill *et al.*, 1987; Egyhazi and Durban, 1987; Zhang *et al.*, 1988; Kretzschmar *et al.*, 1993). Therefore it is reasonable to assume that topo I is also associated with the transcriptionally active lampbrush chromosome loops. However, experimental proof is as yet lacking. Recently, Richard and Bogenhagen (1989) purified a 165 kDa form of topo I from *Xenopus laevis* ovaries. Subsequently they showed that this high molecular weight form is an oocyte-specific variant of the enzyme. Topo I isolated from somatic tissues such as *Xenopus* liver or cultured kidney epithelium cells revealed the usual molecular mass of 110 kDa (Richard and Bogenhagen, 1991). By using variant-specific antibodies these authors demonstrated that the somatic 110 kDa form is virtually absent from oocyte nuclei, and that the oocyte-specific 165 kDa variant is rapidly lost during progesterone-induced maturation.

Abbreviations used in this paper: AMD, actinomycin D; CPT, camptothecin; DMSO, dimethyl sulfoxide; FITC, fluorescein isothiocyanate; PBS, phosphate buffered saline; topo I, DNA topoisomerase I.

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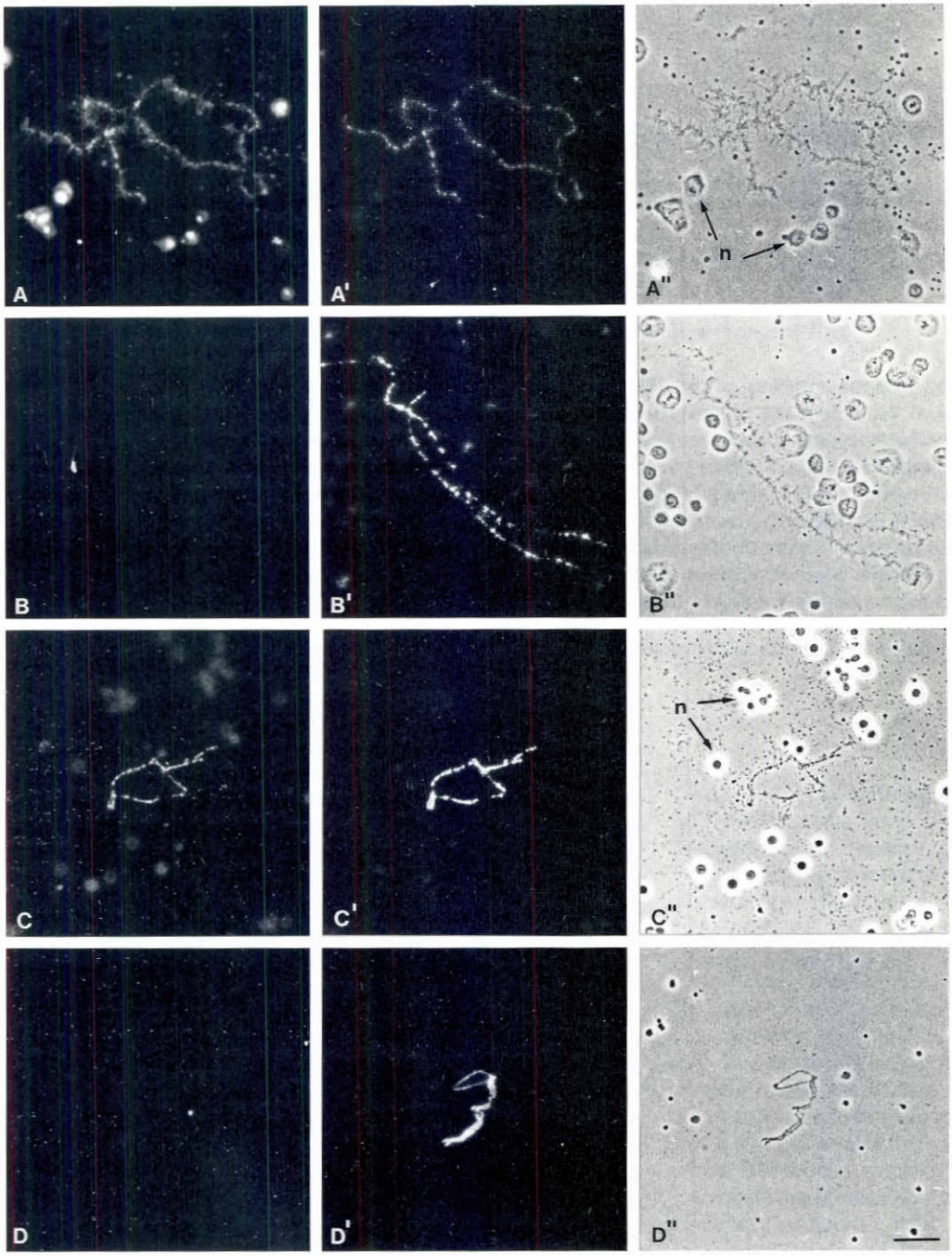

Fig. 1. Immunofluorescence localization of topo I on spread *Xenopus* lampbrush chromosomes and amplified nucleoli. *Spread nuclear contents of stage IV oocytes were incubated with topo I antiserum* **(A,C,D)** *or the corresponding preimmune serum* **(B)**. *After incubation with topo I antiserum lampbrush chromosomes fluoresce in controls* **(A)** *and after CPT-treatment* **(C)**, *but not in AMD-treated oocytes* **(D)**. *The inner regions of the amplified nucleoli (n) which appear as dense cores in phase contrast* **(A′′)** *are intensely stained by the topo I antiserum* **(A)**. *The corresponding Hoechst staining* **(A′-D′)** *and phase contrast images* **(A′′-D′′)** *are shown. Bar, 20 μm.*

Here we report that the 165 kDa form of topo I is associated with lampbrush chromosome loops and the amplified nucleoli in *Xenopus* oocytes. This localization suggests that the 165 kDa variant of topo I is part of the transcriptional machineries of RNA polymerase I and II in oocyte nuclei.

Results

In order to study the intranuclear localization of the oocyte-specific 165 kDa variant of topo I we performed immunofluorescence experiments with antibodies that have previously been characterized in detail (Richard and Bogenhagen, 1991). When spread nuclear contents (i.e. lampbrush chromosomes and amplified nucleoli) were incubated with anti-topo I, lampbrush chromosomes fluoresced (Fig. 1A). In contrast, they were negative when probed with the corresponding preimmune serum (Fig. 1B). Topo I seemed to be concentrated at the lampbrush chromosome axis where lateral loops are anchored, but a weak fluorescence was also observable along the larger loops. The relatively strong axial fluorescence most likely reflects the presence of numerous small loops which, under the preparation conditions used, collapse on to the chromosome axes (also see below). In addition, a bright fluo-

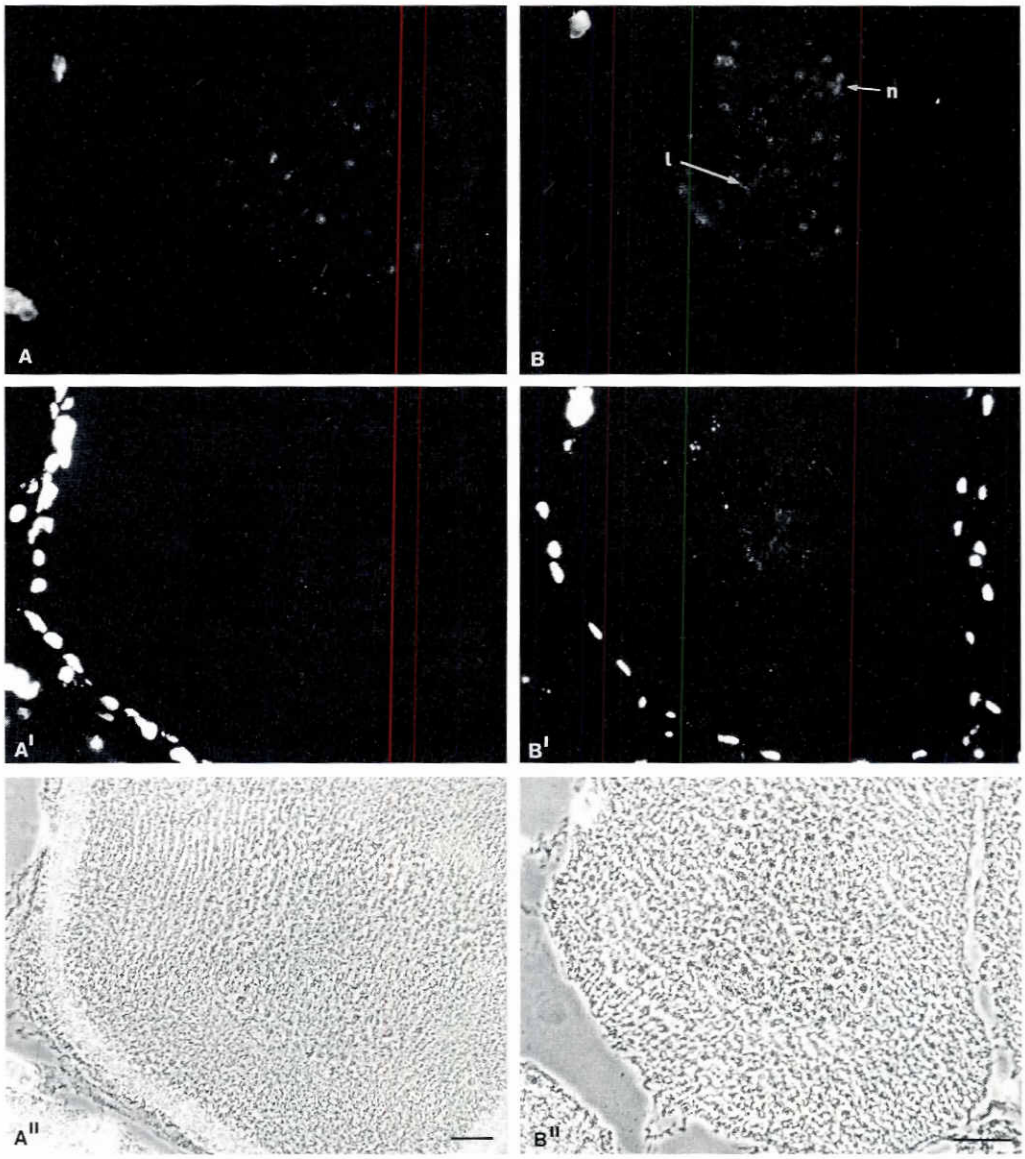

Fig. 2. Immunofluorescence microscopy of paraffin sections of freeze-substituted *Xenopus* oocytes. *Sections of control* **(A)** *or CPT-treated* **(B)** *pieces of ovary were stained with topo I antiserum. In both cases the amplified nucleoli (n) fluoresce after incubation with the antiserum. Fluorescence of the lampbrush chromosome portions included in the sections is recognized only after CPT-treatment (l in* **B***) (see text for explanation). Bars, 30 μm.*

rescence of the amplified nucleoli was noted (Fig. 1A). Topo I seemed to be particularly enriched in the internal component of the nucleoli which contains the rRNA genes (Miller and Beatty, 1969).

When oocytes were incubated with the topo I-inhibitory drug camptothecin (CPT), lampbrush chromosome loops retracted, chromosomes condensed and foreshortened, and nucleolar morphology changed considerably (Fig. 1C´´). Retraction of the lateral loops was accompanied by the premature release of the nascent transcripts as indicated by immunofluorescence microscopy with antibodies directed against transcript-associated proteins (Zinger, 1991). Within two hours after drug administration, the fluorescent pattern changed completely from a bright lateral loop staining to virtually no staining at all. CPT is known to inhibit topo I activity by stabilizing the "cleavable complex", the transient covalent bond between the enzyme and its target DNA (Liu, 1989). In agreement with this mode of drug action, topo I was still detectable at the axes of CPT-treated lampbrush chromosomes (Fig. 1C). When transcription was blocked by incubation of the oocytes with actinomycin D (AMD), essentially the same morphological changes were observed (Fig. 1D´´). However, in contrast to CPT-treated oocytes, topo I was no longer detectable on lampbrush chromosomes (Fig. 1D).

Surprisingly, immunostaining of the nucleoli in nuclear spreads of CPT-treated oocytes was not as bright as in the control specimens (compare Fig. 1C with Fig. 1A). This phenomenon might be due to the condensed structure of CPT-treated nucleoli which could limit the accessibility of antibodies. In support of this hypothesis is the finding that other antibodies (for example antibodies reacting with DNA) also fail to stain CPT-treated nucleoli (data not shown). In order to make the nucleolar interior accessible to the antibodies, we used sections of paraffin-embedded ovaries. Immunofluorescence experiments revealed topo I in the nucleoli of both control (Fig. 2A) and CPT-treated (Fig. 2B) oocytes. Due to the condensation of chromosomes in CPT-treated oocytes, topo I was even seen in the lampbrush chromosome portions included in the sections (Fig.

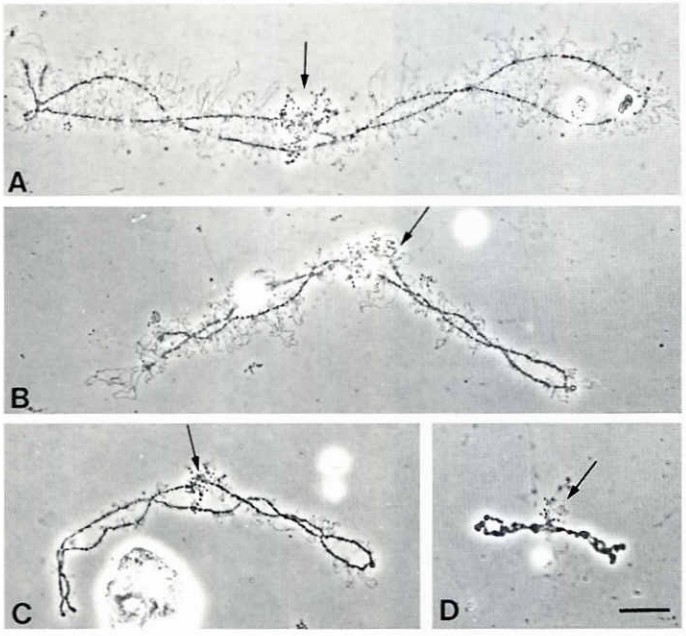

Fig. 3. Effect of affinity-purified topo I antibodies on lampbrush chromosomes after microinjection into Pleurodeles oocyte nuclei. *Stage IV oocytes were microinjected into the nuclei with 40 ng of affinity-purified topo I antibodies* **(B,C,D)** *or the same amount of non-immune rabbit immunoglobulins* **(A)**. *Lampbrush chromosomes were prepared 1 h* **(B)**, *2 h* **(C)** *and 4 h* **(A,D)** *after injection and photographed with an inverted microscope using phase contrast optics. All micrographs show the heteromorphic lampbrush chromosome bivalent number IV with the diagnostic globular loop (arrows). Injection of topo I antibodies causes loop retraction and shortening of lampbrush chromosomes. Bar, 20 μm.*

2B), whereas in control oocytes the concentration of topo I in the lampbrush chromosome loops was below the limits of detection (Fig. 2A). The latter finding clearly indicates that, under normal conditions, topo I is part of the lateral loops and absent from the chromosome axes.

Because the antibodies against the oocyte-specific 165 kDa variant of topo I do not neutralize enzyme activity (Richard and Bogenhagen, 1991), they could not be used for functional assays such as microinjection experiments. Therefore, the following experiment was performed with a different topo I-specific antibody which was raised against calf thymus topo I (Oddou *et al.*, 1988; Rose *et al.*, 1988). Since this antibody inhibits *in vitro* the topo I activity of *Xenopus* oocyte nuclei (Richter and Scheer, unpublished data), it apparently recognizes an epitope common to both the 165 kDa and the 110 kDa variant of topo I. For the microinjection experiments, oocytes of the newt *Pleurodeles waltl* were chosen because lampbrush chromosome loops of this species are considerably larger than those of *Xenopus*. Microinjection of affinity-purified anti-topo I immunoglobulins resulted in a time-dependent retraction of most of the lateral loops and a condensation of the chromosomal axis (Fig. 3). Some landmark loops (globular loops) were more or less resistant to the antibody-induced retraction. This result indicates that transcription of most of the genes located on lampbrush chromosome loops is dependent on the enzymatic activity of topo I. Preliminary experiments indicate that an oocyte-specific variant of topo I with a high molecular weight also exists in *Pleurodeles*. In immunoblot experiments with the neutralizing antiserum against topo I a 100 kDa protein was detected in somatic nuclei. In contrast, several protein bands ranging from 180 kDa to 40 kDa were stained in oocyte nuclei, some probably representing degradation products. A 100 kDa form of topo I was apparently absent from *Pleurodeles* oocyte nuclei (data not shown).

Discussion

Recently it has been shown that *Xenopus* oocytes express a high molecular weight (165 kDa) form of topo I (Richard and Bogenhagen, 1991). Up until now it was unknown why a tissue-specific variant of topo I is synthesized during oogenesis and what the detailed functions of this variant might be. Richard and Bogenhagen (1991) discussed several possibilities. 1) Since topoisomerases have been implicated in the maintenance of genome stability (Wang, 1990), it has been suggested that a specialized form of topo I is needed to allow rDNA excision and amplification during early oogenesis. 2) The rapid rates of rRNA synthesis in oocytes may require a topo I variant with qualities different from the somatic form. 3) It may be that a high ratio of DNA topoisomerase II to topo I is needed to enable the rapid cycles of chromosome condensation and decondensation during the cleavage stages of *Xenopus* embryogenesis. Consequently, the oocyte-specific topo I variant may contain domains which target the enzyme for developmentally regulated degradation.

As the knowledge of the intracellular location of a protein may provide clues as to its function, we decided to perform immunolocalization studies using an antiserum against the 165 kDa variant of topo I. In this report we show that the 165 kDa form of topo I is not only a constituent of the lampbrush chromosome loops, but also of the inner regions of the amplified nucleoli. This localization suggests that the oocyte-specific variant of topo I is involved in transcriptional processes mediated by RNA polymerase I as well as RNA polymerase II. In correspondence with the localization data is our finding that inhibition of topo I activity by the drug CPT, or by microinjection of appropriate topo I-antibodies into oocyte nuclei, resulted in loop retraction and condensation of chromosomes and nucleolar chromatin. These are effects which are typical for transcriptional inhibition (Scheer, 1987; Moreau *et al.*, 1994). Our data suggest that the functions of topo I are the same in oocytes and somatic cells. Thus, the hypothesis that the 165 kDa form of topo I is a variant specifically targeted for rapid degradation during progesterone-induced oocyte maturation seems to be the most plausible one.

Cloning of cDNAs coding for the oocyte-specific form of topo I is required for the identification of those domains which differ from the somatic form of topo I and give rise to oocyte-specific characteristics of the enzyme. Moreover, determination of genomic sequences should contribute to our understanding of how the expression of the two variants of topo I is regulated during development.

Materials and Methods

Animals

Female *Xenopus laevis* were purchased from the South African Snake Farm (Fish Hoek, Cape Province, South Africa). Female

Pleurodeles waltl were kindly provided by Peter Eichhorn (German Cancer Research Center, Heidelberg, Germany).

Antibodies

The rabbit-derived antiserum against the oocyte-specific 165 kDa variant of topo I has been described in detail (Richard and Bogenhagen, 1991). Antiserum and the corresponding preimmune serum were generously provided by Dr. D. Bogenhagen (Stony Brook, New York, USA). Affinity-purified rabbit antibodies raised against calf thymus topo I were a kind gift of Dr. A. Richter (Universität Konstanz, Germany). For a detailed characterization of these antibodies see Oddou *et al.* (1988) and Rose *et al.* (1988).

Drug treatment

Camptothecin lactone (NSC-94600) was a gift of the Drug Synthesis and Chemistry Branch of the National Cancer Institute (Bethesda, MD, USA). A stock solution of camptothecin (CPT) was prepared (10 mM in DMSO) and stored at -20°C. Actinomycin D (AMD) was purchased from Serva (Heidelberg, Germany). The stock solution (100 μg/ml in PBS) was kept at 4°C in the dark. Small pieces of ovary were incubated in modified Barth's medium (Peng, 1991) supplemented with either CPT (final concentration 100 μM), AMD (final concentration 10 μg/ml), or, as a control, with DMSO (final concentration 1%). Oocytes were exposed to the drugs for 3-4 h.

Preparation of lampbrush chromosomes and paraffin sections

Oocytes were staged according to Dumont (1972). *Xenopus* and *Pleurodeles* lampbrush chromosomes were prepared according to Callan *et al.* (1987) or Scheer *et al.* (1979), respectively. Freeze-substitution and paraffin-embedding of small pieces of ovary were performed as described previously (Hock *et al.*, 1993).

Immunofluorescence microscopy

Lampbrush chromosome preparations and paraffin sections were processed for immunofluorescence microscopy as described previously (Fischer *et al.*, 1993; Hock *et al.*, 1993). Topo I antiserum or preimmune serum were used at 1:100 dilution in PBS. For lampbrush chromosome staining, FITC-conjugated goat anti-rabbit immunoglobulins (Dianova, Hamburg, Germany; diluted 1:75 in PBS) were used as secondary antibodies. In the case of paraffin sections, Texas red-conjugated anti-rabbit immunoglobulins (Dianova, Hamburg, Germany; diluted 1:150 in PBS) were used. All photographs were taken with a Zeiss Axiophot (Carl Zeiss, Oberkochen, Germany) equipped with epifluorescence optics and the appropriate filter sets.

Microinjection

Oocyte nuclei were injected with 15 nl affinity purified topo I antibodies or non-immune rabbit immunoglobulins (2.6 mg/ml in 20 mM HEPES, pH 7.5) following a previously published protocol (Scheer *et al.*, 1979).

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