Cloning and characterization of the gene for the somatic form of DNA topoisomerase I from *Xenopus laevis*

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**ABSTRACT**

Two distinct tissue-specific forms of DNA topoisomerase I with Mr of 165 and 110 kDa have been purified from oocytes and somatic cells respectively of the African frog *Xenopus laevis*. In this paper, cDNAs encoding a *Xenopus* topoisomerase I were cloned using PCR primers derived from sequences of yeast and human topoisomerase I. A polypeptide expressed from a portion of the coding sequence was recognized by an antiserum directed against the somatic topoisomerase I that had previously been shown to be unable to cross-react with the oocyte enzyme. Thus, the clone encodes the somatic cell topoisomerase I. An antiserum raised against a synthetic peptide containing the sequence surrounding the active site tyrosine of the somatic topoisomerase I reacts with the enzymes purified from both oocytes and somatic cells, indicating that the two enzymes share some limited sequence homology. RNA blot hybridization showed that oocytes contain an abundant store of somatic topoisomerase I mRNA that is not efficiently polyadenylated in oocytes. This stored RNA contains a consensus cytoplasmic polyadenylation element that is found in a variety of mRNAs that are translationally repressed in oocytes. Microinjection into oocytes of *in vitro* transcribed mRNA prepared from a Myc-tagged construct of the somatic topoisomerase I sequence is translated to yield a 110 kDa product. This suggests that the oocyte-specific 165 kDa topoisomerase I is not produced by tissue-specific post-translational modification of the somatic topoisomerase I. The oocyte enzyme appears to be produced from a minor mRNA species in oocytes that has not yet been identified.

**INTRODUCTION**

Two distinct tissue-specific forms of DNA topoisomerase I have been purified from oocytes and somatic cells of the African frog *Xenopus laevis* (1,2). These two isoforms can be distinguished by their different molecular weights and antigenic properties. Polyclonal antisera raised against the 165 kDa oocyte topoisomerase I do not cross-react with the 110 kDa enzyme purified from liver or cultured cells. Similarly, a polyclonal antiserum directed against the somatic enzyme does not cross-react with the oocyte variant.

This situation provides a rare example of a case where a key component of the replication and transcription machinery exists as a tissue-specific variant. The molecular weight of the somatic enzyme is similar to that of the type I topoisomerases purified and cloned from human cells (3), while the oocyte enzyme is unusually large. It is possible that domains present in the oocyte-specific topoisomerase I may target this protein to distinct cellular sites or activities in oocytes. The basis for this tissue-specific difference in the structure of topoisomerase I has not been explained in molecular terms.

There are several possibilities to account for the larger size of the oocyte topoisomerase I. First, *X.laevis* may contain two genes that are differentially regulated to permit tissue-specific expression of the two forms. Second, differential splicing of transcripts from a single gene may result in proteins with different domains. Third, it is possible that post-translational modification of topoisomerase I in oocytes may reduce the electrophoretic mobility of the enzyme. In this paper, we report the molecular cloning of a cDNA encoding the somatic form of topoisomerase I and studies of the relationship of this enzyme to the oocyte variant.

**MATERIALS AND METHODS**

**PCR**

PCR to isolate a fragment of the *X.laevis* topoisomerase I cDNA was performed with degenerate primers derived from protein sequences conserved among human, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* topoisomerase I sequences. Template DNA was prepared from a stage 24 *X.laevis* embryo cDNA library. Primers were designed to include restriction endonuclease cleavage sites adjacent to degenerate coding sequences. The two primer sequences were **GGAATTCCTTYG-AYTTYYTRGGNAA**, derived from the sense strand for protein
sequence DTVGCC (residues 431–436 of S.cerevisiae topoisomerase I), and GGATCCCTGRTGRTRCAYARNAT, from the antisense strand of the amino acid sequence ILCNHQ (residues 554–559 of S.cerevisiae topoisomerase I). PCR was performed in a total volume of 50 µl containing 25 pM each primer, 1 µg template DNA, 200 µM each dNTP, 1.5 mM MgCl2, 10 mM Tris–HCl, pH 8.3 at room temperature, 50 mM KCl, 5 µg gelatin and 1 U Taq DNA polymerase (Perkin-Elmer Cetus). The reaction mixture was overlaid with 20 µl mineral oil. Following an initial denaturation step at 94°C for 3 min, DNA was amplified for 50 cycles at 94°C for 10 s, 55°C for 1 min and 72°C for 2 min. Following thermal cycling, the reactions were held at 72°C for 5 min and then stored at 4°C. Ten microliters of the PCR product were analyzed on a 6% polyacrylamide gel. The gel was stained with ethidium bromide and the product sizes estimated by comparison with standard size markers of MspI-digested pBR322 and HaeIII-digested φX174 RF DNA. The amplified band was eluted from the gel and subcloned in M13 using the EcoRI and BamHI sites within the primers. M13 subclones were sequenced as described below. The PCR fragment was also subcloned into a pSK+ plasmid vector to generate plasmid pSKXT1.

cDNA library screening

Agt11 containing an oligo(dT)-primed cDNA library from stage 24 embryos was used to infect Escherichia coli Y1090 and plated onto 150 mm Petri plates. Plaques were Lifted onto nitrocellulose filters and the filters hybridized using standard procedures. A riboprobe synthesized using T7 RNA polymerase to transcribe BamHI-digested pSKXT1 was used to screen the filters. Positive plaques were purified by secondary and tertiary screening. The longest cDNA obtained from this library contained an insert of ~2 kb, which was subcloned as an EcoRI–XbaI insert into pSK+ to generate pSKXT9. Two other libraries, a λgt11 (SfiI/NotI directional library; Promega) stage 30 library (2 × 106 independent recombinants) and a λZap II egg (1 × 106 recombinants) cDNA library, were screened using a random primer-labeled 850 bp EcoRI–PstI fragment from the 5′ portion of the cDNA insert in the pSKXT9 clone.

Plate lysates were prepared from positive phage isolates. Phage were purified by CsCl gradient centrifugation and the DNA prepared as described (4). Positive isolates were analyzed by restriction digestion, subcloning and DNA sequencing. When screening for clones containing longer 5′ extensions, a PCR-based approach was used. Amplified phage lysates were prepared from single positive plaques. Ten microliters of this amplified lysate was boiled for 10 min and then cooled on ice. The lysate was spun for 5 min and 0.5 µl of the supernatant subjected to PCR with one phage-specific vector primer and the Xenopus topoisomerase I-specific internal primer. The sequence reported here is that of a single isolate from the stage 30 embryo library, designated clone no. 1.

DNA sequence analysis

The cDNAs were subcloned into M13 vectors and deletions were generated using the Cyclone-I Biosystem kit (IBI). Overlapping deletion derivatives were sequenced using the dideoxy chain termination method and fluorescently labeled primers. The sequence was analyzed on an Applied Biosystems (ABI) DNA sequencer (model 370 and 373A). Both strands of the cDNA were sequenced. In some cases the deletions were sequenced using [35S]dATP and the Sequenase 2.0 DNA sequencing kit (US Biochemicals). Overlapping sequences were assembled into a contiguous sequence using the IBI computer program.

RNA procedures

Total RNA was isolated from frog tissues using the single-step method of RNA isolation (5). Poly(A)+ RNA was then selected by oligo(dT)–cellulose chromatography using standard methods. Total and poly(A)+ RNA were resolved by electrophoresis through a 1% formaldehyde–agarose gel. The RNA was transferred to nylon membranes and fixed by UV irradiation. The filter was prehybridized at 42°C for 4 h in 50% formamide, 4x SSPE, 5x Denhardt’s solution, 0.5% SDS, 100 µg/ml denatured calf thymus DNA and 100 µg/ml tRNA. Random primer-generated [32P]dCTP-labeled DNA probe was then added to the same solution and the filter hybridized overnight at 42°C. The filter was then washed once with 2x SSC, 0.5% SDS at room temperature for 15 min followed by a wash in 0.1x SSC, 0.1% SDS at 65°C for 2 h with continuous agitation. The filter was dried and exposed for autoradiography.

In vitro transcription of T3 expression constructs

Plasmids for in vitro transcription were linearized with restriction endonuclease NotI, which cuts downstream of the insert. In vitro sense transcripts were synthesized as described (4) from the T3 promoter using T3 RNA polymerase. RNAs were capped by transcription in the presence of 5 mM capping analog m7ppp(5′)G (10-fold molar excess over GTP). Following DNase I treatment, the RNA was extracted with phenol/chloroform, ethanol precipitated and resuspended in DEPC-treated water. RNA concentrations were estimated by measuring absorbance at 260 nm. The RNA was also checked by electrophoresis through a 1.5% Tris-acetate–agarose gel along with standard RNA markers (Gibco BRL) and by in vitro translation.

Oocyte injection and analysis of translation products

Stage VI oocytes were collected by collagenase treatment as described (6). The oocytes were kept at 18°C in OR3 medium. Injections of RNA were made into the oocyte cytoplasm, as described by Gurdon and Wickens (7). Each oocyte received roughly 50 nl water containing RNA (~10–20 ng/oocyte). Following injection, the oocytes were incubated at 18°C in OR3 medium for ~48 h. Germinal vesicles (GVs) were isolated manually in a GV isolation medium as described by Roth and Gall (8). About 50–60 GVs were collected in a minimum volume of GV isolation medium and an equal volume of 2x SDS sample buffer was added. The GVs were boiled for 5 min and then cooled to room temperature, following which 0.1 vol 1 M iodoacetamide was added and the sample was alkylated in the dark for 30 min. After electrophoresis on an SDS–polyacrylamide gel (7.5% gel with 5% stacking), the proteins were electroblotted onto Immobilon-P membranes (Millipore). Blots were probed with anti-Myc monoclonal antibody 9E10 (Oncogene Science) and developed using alkaline phosphatase-conjugated anti-mouse antibody.

Immunological methods

Polyclonal antisera directed against purified oocyte topoisomerase I and liver topoisomerase I have been described (2). To generate an anti-peptide antibody directed against the sequence surrounding

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the active site tyrosine of the somatic topoisomerase I sequence reported here, a peptide with the sequence SKLNYLDPRTSVA was synthesized (Multiple Peptide Systems, San Diego, CA), coupled to keyhole limpet hemocyanin and injected into rabbits using standard techniques (9). Antibodies were purified from the immune serum by affinity chromatography on a column of peptide conjugated to bovine serum albumin and were used at 1:1000 dilution to probe immunoblots. Blots were developed using colorimetric detection following incubation with calf alkaline phosphatase-conjugated goat anti-rabbit secondary antisera.

RESULTS

cDNA cloning

Primers to permit cloning of a segment of X.laevis topoisomerase I were designed using protein sequences that were highly conserved among human, S.cerevisiae and S.pombe topoisomerase I (10). PCR was performed with these primers on DNA prepared from a λgt11 X.laevis stage 24 embryo cDNA library. A 415 bp fragment was produced with approximately the size predicted from the spacing between the relevant peptide sequences of the previously described topoisomerase I sequences. This fragment was subcloned into an M13 vector using the EcoRI and BamHI restriction sites in the primers and sequenced. The translated sequence contained a continuous open reading frame of 134 amino acids, of which 124 were identical to human topoisomerase I protein (92% identity).

This PCR-amplified fragment was used to screen the λgt11 X.laevis stage 24 embryo cDNA library. A partial cDNA clone from this library was used to screen three other libraries, resulting in identification of eight independent cDNA clones. PCR screening and preliminary sequencing identified one clone (no. 1) from a λgt11 stage 30 embryo cDNA library that contained the entire topoisomerase I protein sequence but only 111 bases of 5’ flanking sequence preceding the putative initiating ATG. An overlapping clone from the same library provided an additional 60 nt of 5’ untranslated sequence, including an in-frame stop codon. The sequence of clone no. 1 containing 4043 nt including 111 nt of 5’ untranslated sequence and 1445 nt of 3’ untranslated sequence, was deposited in GenBank (accession no. L07777). The predicted topoisomerase I protein contains 829 amino acids, assuming that translation initiates at the first methionine, i.e. at nt 112, and stops at a nonsense codon at residue 2599. The encoded polypeptide has a molecular weight of 99.48 kDa and a calculated isoelectric point of 10.2. A putative poly(A) addition signal is present at nt 4001–4007.

Xenopus laevis DNA topoisomerase I is homologous to other eukaryotic type I topoisomerases

The predicted amino acid sequence of the X.laevis topoisomerase I was compared with other eukaryotic type I topoisomerases (Fig. 1). All five eukaryotic topoisomerase I sequences contained two highly conserved domains, as noted in Figure 1 and tabulated in Figure 2. A small conserved sequence of ~50 amino acid residues surrounds the active site tyrosine near the C-terminus of each protein (residue 779 in X.laevis topoisomerase I). This is separated by a spacer sequence of variable length from a conserved central domain of ~440 residues. This spacer sequence and the N-terminal domain are not well conserved among all five topoisomerases, but do show increased relatedness within phyyla.

As shown in Figure 2, pairwise comparisons of the two yeast topoisomerase sequences and the two vertebrate topoisomerase sequences reveal conservation of both the lengths and sequences of the N-terminal and spacer regions. The greatest divergence between the X.laevis and human proteins is due to the occurrence of 10 consecutive repeats of a basic–acidic motif in the X.laevis sequence, as shown in Figure 2. These eight residue repeats may be considered as tandem copies of a four residue repeat related to the KHDK motif noted by Kunze et al. (11) in the human topoisomerase I sequence. It is interesting to note that a distinctive histidine-serine-rich domain in the Drosophila melanogaster topoisomerase I occurs in approximately the same region (12). No functional roles have been determined for either the basic–acidic repeats of the X.laevis or human sequences or the histidine-serine repeats of the D.melanogaster sequence.

The cloned cDNA encodes the somatic topoisomerase I

The full-length Xenopus cDNA sequence was cloned in a pSK plasmid and transfected into E.coli. We were able to observe topoisomerase enzymatic activity in crude cellular lysates in the presence of EDTA (S.D.Pandit, PhD thesis), which is characteristic of eukaryotic topoisomerase I (13). This established that the cloned sequence encodes an active form of topoisomerase I. We next tested whether polyclonal antisera specific for the somatic or oocyte forms of X.laevis topoisomerase I (2) would react with a fragment of the topoisomerase expressed in E.coli. We expressed a 29 kDa polypeptide fragment of the cDNA in E.coli using the T7 RNA polymerase-directed expression system (14). A protein of the predicted size was observed following induction of T7 RNA polymerase and was found to cross-react with an antisera raised against the somatic form of X.laevis topoisomerase I (S.D.Pandit, PhD thesis). Since this antisera does not cross-react with the 165 kDa oocyte-specific topoisomerase I, we conclude that the cDNA clone encodes the somatic topoisomerase I. The size of the protein predicted from the cloned cDNA sequence and the fact that the clone was obtained from an embryo cDNA library reinforce this conclusion.

Oocyte and somatic topoisomerase I contain the same sequence surrounding the active site tyrosine

The sequence of the X.laevis somatic topoisomerase I shows a close relationship to other eukaryotic topoisomerase I sequences in the region surrounding the active site tyrosine residue (Figs 1 and 2). To explore the question of whether the oocyte topoisomerase I shares this homology, we raised an anti-peptide antisera against a synthetic peptide, SKLNYLDPRTSVA, containing the sequence surrounding the active site tyrosine of Xenopus somatic topoisomerase I. Figure 3 shows that this anti-peptide antisera reacts well with both the oocyte and somatic topoisomerases I, indicating that both isoforms share this sequence. We have previously shown that other polyclonal antisera raised against intact purified somatic and oocyte topoisomerases do not cross-react (2). Taken together, the results obtained with antibody probes suggest that the somatic and oocyte topoisomerases share homology near the active site tyrosine but diverge in other regions, so that major epitopes recognized by the isozyme-specific antibodies are distinct. Since all eukaryotic type I topoisomerases studied to date contain an active site tyrosine near their C-termini, our working model for the structure of the two X.laevis topoisomerases is that these
Figure 1. Comparison of eukaryotic type I topoisomerase protein sequences.
The protein sequence of the *X.laevis* (XL) topoisomerase I is compared with those of human (3), *D.melanogaster* (12), *S.pombe* (30) and *S.cerevisiae* (13) using the program Clustal. The symbols * and . denote residues that are identical or well conserved respectively. Dashed lines show gaps in the sequence that were introduced to maximize homology. Regions of high homology are demarcated above the sequences and are described further in Figure 2.

proteins share homology near their C-termini and differ near the N-termini, where the major epitopes identified by our existing anti-protein antisera may be located.

**Topoisomerase I mRNA expression**

To determine the size and tissue-specific distribution of the *Xenopus* topoisomerase I mRNA, Northern blot analysis was done on RNA isolated from frog oocytes, liver, egg and tissue culture cells. Figure 4 shows a representative Northern blot done on total and poly(A)-selected RNA from *X.laevis* oocytes and tissue culture cells, probed with a 2.3 kb *Eco*RI fragment representing residues 770–3075 of the cloned sequence. An abundant 4.3 kb RNA consistent with the size of the cloned topoisomerase I cDNA was identified in both oocytes and tissue culture cells. This RNA was efficiently enriched by poly(A) selection of tissue culture cell RNA, but not by poly(A) selection of oocyte RNA. Figure 4B shows the results of a control experiment to rule out the possibility that the failure to enrich for the 4.3 kb oocyte RNA by poly(A) selection was due to technical problems. Re-probing the same RNA blot with a probe for TFIIIA mRNA showed that this mRNA was enriched by poly(A) selection of oocyte RNA. Therefore, we conclude that the major fraction of 4.3 kb oocyte RNA hybridizing to our probes is not efficiently polyadenylated.

The observation that oocytes contain an abundant RNA that hybridizes to a probe representing the somatic topoisomerase I was quite surprising, since oocytes contain no detectable 110 kDa topoisomerase I. We considered two possibilities to account for
Figure 2. Domain analysis of topoisomerase I protein sequences. The five eukaryotic topoisomerase I protein sequences were divided into a series of domains as noted in Figure 1. Thin lines in the diagram indicate the poorly conserved N-terminal and spacer (Sp) domains that separate the well-conserved central domain (light gray) and smaller active site domain (dark gray box labeled Y, for the active site tyrosine residue that is transiently covalently linked to DNA during the enzymatic reaction). A large portion of the N-terminal domain of the *X.laevis* sequence represents a series of 10 consecutive repeats of an alternating basic–acidic repeat (depicted as KD\(^{30}\)EKH\(^{9}\)KD\(^{30}\)E).

Figure 3. An anti-peptide antiserum directed against the active site of somatic topoisomerase I cross-reacts with oocyte topoisomerase I. An anti-peptide antiserum to the active site region of the topoisomerase I sequence SKLNYLDPRISV AC was prepared as described in Materials and Methods. Preparations of oocyte topoisomerase I purified through fraction III (1) and of *Xenopus* A6 tissue culture cell topoisomerase I purified by hydroxyapatite chromatography in the presence of SDS (2) were subjected to SDS–PAGE and blotted to Immobilon membranes. Strips from the oocyte topoisomerase I blot (A) were probed with antiserum raised against oocyte topoisomerase I (lane 1), preimmune serum from the rabbit used for the anti-peptide antibody (lane 2) or the immune anti-peptide serum (lane 3). Strips from the somatic topoisomerase I blot (B) were probed with antiserum raised against somatic topoisomerase I (lane 1), preimmune serum from the rabbit used for the anti-peptide antibody (lane 2) or the immune anti-peptide serum (lane 3). The mobilities of marker proteins are indicated in both panels.

Figure 4. Northern analysis of topoisomerase I mRNA. Total RNA was isolated from immature frog ovaries and tissue culture cells as described in Materials and Methods. Samples of ∼2 µg total RNA and 0.5 µg poly(A)\(^{+}\) RNA from each source were subjected to electrophoresis on a formaldehyde–1% agarose gel and transferred to a Hybond N\(^{+}\) filter. (A) Autoradiogram of RNAs that hybridized to a \(^{32}\)P-dCTP-labeled topoisomerase I probe (2.3 kb EcoRI fragment). Mobility markers were end-labeled fragments of λ DNA digested with HindIII. (B) Autoradiogram of RNAs identified by stripping the probe from the filter in (A) and reprobing with a \(^{32}\)P-labeled probe for *X.laevis* TFIIA (31).

Figure 3. An anti-peptide antiserum directed against the active site of somatic topoisomerase I cross-reacts with oocyte topoisomerase I. An anti-peptide antiserum to the active site region of the topoisomerase I sequence SKLNYLDPRISV AC was prepared as described in Materials and Methods. Preparations of oocyte topoisomerase I purified through fraction III (1) and of *Xenopus* A6 tissue culture cell topoisomerase I purified by hydroxyapatite chromatography in the presence of SDS (2) were subjected to SDS–PAGE and blotted to Immobilon membranes. Strips from the oocyte topoisomerase I blot (A) were probed with antiserum raised against oocyte topoisomerase I (lane 1), preimmune serum from the rabbit used for the anti-peptide antibody (lane 2) or the immune anti-peptide serum (lane 3). Strips from the somatic topoisomerase I blot (B) were probed with antiserum raised against somatic topoisomerase I (lane 1), preimmune serum from the rabbit used for the anti-peptide antibody (lane 2) or the immune anti-peptide serum (lane 3). The mobilities of marker proteins are indicated in both panels.

somatic cells. It would be unlikely, but not impossible, for a 4.3 kb RNA to encode such a large protein. This is particularly so since \(M_r\) values determined for topoisomerases based on relative mobility on SDS–PAGE tend to be overestimates. The *X.laevis* somatic topoisomerase I has a \(M_r\) of 110 kDa but a calculated mol. wt of only 99 kDa. If the 165 kDa oocyte topoisomerase has a similar anomalous gel mobility, it may contain as few as 1300 amino acid residues. Since we have reasoned that the somatic and oocyte proteins may differ at their N-termini, we repeated the experiment shown in Figure 4 with probes representing N-terminal and C-terminal portions of the somatic topoisomerase I cDNA. These experiments identified the same 4.3 kb oocyte RNA observed in Figure 4 (data not shown). We also performed experiments in which primers derived from the somatic topoisomerase I cDNA sequence were used in RT–PCR assays using oocyte RNA as template. Again, these experiments revealed only fragments characteristic of the somatic cDNA sequence (data not shown). Thus, we conclude that oocytes contain an abundant store of untranslated mRNA for somatic topoisomerase I. We have not yet identified a discrete candidate mRNA for the oocyte topoisomerase I using probes derived from the somatic topoisomerase I sequence.

The somatic topoisomerase I mRNA appears to be stored but not translated in oocytes, like several other mRNAs studied in
Our inability to identify a clear candidate for the oocyte topoisomerase I mRNA raised the possibility that the high molecular weight oocyte enzyme could be produced by post-translational modification of the 110 kDa form of the protein in oocytes. We performed a number of experiments to try to determine whether the oocyte topoisomerase I could contain specific known post-translational modifications. For example, the mobility of the protein on SDS–PAGE could be influenced by phosphorylation or poly(ADP) ribosylation, both of which have been reported as post-translational modifications of topoisomerase I (18,19). Although the enzyme is a phosphoprotein, treatment of in vivo transcribed mRNA encoding the oocyte topoisomerase I with potato acid phosphatase liberates all 32P with only a minor shift in gel mobility (R.E.Richard, PhD thesis). Thus, phosphorylation or poly(ADP) ribosylation cannot account for the slower mobility of the oocyte topoisomerase I.

We microinjected an in vitro transcribed mRNA encoding the 110 kDa topoisomerase I mRNA into oocytes to determine whether the product would be processed to a 165 kDa form when expressed in oocytes. In order to follow the expressed protein, the injected RNA was modified to add a Myc-epitope tag to the protein. Two plasmid constructs were used in this experiment, as shown in the upper part of Figure 5. In construct 1 the 5′ untranslated region of topoisomerase I along with the initiator methionine was removed and the rest of the peptide was fused in-frame at the N-terminus with 13 amino acids of a c-Myc epitope, 9E10 (20). In construct 2 most of the 3′ untranslated region of Xenopus topoisomerase I was deleted (from a unique Xbal site to the 3′-end) and replaced with the 3′ untranslated region of a heterologous clone SE5A. Clone SE5A encodes a protein found on lampbrush chromosomes in the newt Notopthalinus that is efficiently translated when injected into Xenopus oocytes (8). This substitution of untranslated sequences was done to remove the CPE of the topoisomerase I mRNA and to provide a poly(A) tail to improve the stability of messages injected into oocytes. Both cloned templates were transcribed in vitro with T3 RNA polymerase and the RNA was injected into oocyte cytoplasm. Following injections, the oocytes were incubated for 48 h, after which the germinal vesicles (GVs) were manually isolated. Total proteins from 35–40 GVs were subjected to SDS–PAGE, transferred to an Immobilon membrane and the blot probed with anti-Myc monoclonal antibody. The Western blot is shown in the bottom half of Figure 5. Lane 0 contains total proteins from GVs isolated from mock-injected oocytes. Lanes 1 and 2 contain GV proteins from oocytes injected with RNA made from constructs 1 and 2 respectively. In both lanes 1 and 2, a single band migrating at ~110 kDa was visible. Substitution of the 3′-end of the UTR of the topoisomerase mRNA with sequences from SE5A to remove the CPE was not required to obtain expression. A faint band near the 110 kDa position in lane 0 migrates a bit faster than the intense bands seen in lanes 1 and 2. This appears to be a weak background band produced by reaction with the antibody reagents in the absence of injected RNA. Thus, both Myc-tagged constructs programed the expression of a 110 kDa form of topoisomerase I and no evidence was seen for its modification to a 165 kDa form in oocytes. It is interesting to note that efficient translation was observed for the mRNA produced from construct 1, which includes the topoisomerase I CPE. Construct 2, which lacks the CPE and contains a poly(A) tail from a heterologous mRNA, was included in this experiment since we were concerned that the mRNA produced from construct 1 might be repressed. Microinjected mRNAs can escape the translational repression imposed on mRNAs synthesized in vivo and can be translated efficiently (21,22).

DISCUSSION
Cloning of the somatic cell X.laevis topoisomerase I cDNA

This paper reports the cloning of a cDNA that encodes a DNA topoisomerase I from X.laevis. Two lines of evidence suggest that...
the cloned cDNA corresponds to the 110 kDa somatic-specific DNA topoisomerase I. First, the sequence predicts a polypeptide of ~98 kDa, similar to the Mr observed on SDS–PAGE for the somatic form of the enzyme and significantly smaller than the 165 kDa oocyte topoisomerase I. Microinjection of an epitope-tagged version of this sequence into oocytes programed synthesis of a protein with an Mr corresponding to that of somatic topoisomerase I (Fig. 5). Second, expression in E.coli of a fragment of the X.laevis topoisomerase I sequence resulted in a protein that reacted only with antibodies raised against the somatic topoisomerase I and not with those raised against the oocyte form (S.D.Pandit, PhD thesis).

It was initially quite surprising to find that the message for the somatic form of the protein is present in high amounts in oocytes. However, the dormant state of this mRNA is explained by the lack of a sufficient poly(A) tail to permit efficient selection on an oligo(dT) column (Fig. 4) and by the presence of a CPE preceding the poly(A) addition signal. This phenomenon has been well-studied for other maternally deposited mRNAs in Xenopus that are translated only following cytoplasmic polyadenylation accompanying oocyte maturation. Control of translation by this mechanism has been characterized in oocytes in organisms as diverse as Drosophila and mammals (16,20,23). Although we have not identified any functional differences between oocyte and somatic topoisomerase I in Xenopus, there does appear to be tight developmental control over the expression of these two forms of the enzyme. Richard and Bogenhagen (2) showed that the 165 kDa oocyte topoisomerase is actively degraded during oocyte maturation. Cloning of the somatic topoisomerase cDNA will permit studies of the timing of activation of translation of the somatic topoisomerase I during embryonic development.

What is the relationship between oocyte and somatic topoisomerase I isoforms?

The Xenopus somatic topoisomerase I reflects a domain organization conserved throughout evolution from yeasts to humans. The active site tyrosine for Xenopus topoisomerase I is present in the phylogenetically conserved C-terminal region at amino acid 779. An antibody raised against the peptide sequence surrounding the active site tyrosine of the somatic form of the enzyme cross-reacts with the 165 kDa oocyte-specific topoisomerase, suggesting that the sequence in this region is conserved (Fig. 3). The larger central domain of the somatic topoisomerase I is also well conserved in topoisomerase I sequences from other organisms (Fig. 2). This domain in human and mouse topoisomerase I contains amino acid residues which, when mutated, confer resistance to camptothecin (24–26). Both the oocyte (1) and somatic (R.E.Richard, PhD thesis) topoisomerase I enzymes are sensitive to camptothecin. Thus, it would be quite surprising if the oocyte-specific enzyme failed to show substantial conservation of the somatic topoisomerase I sequence in these domains.

We considered the possibility that the slow electrophoretic mobility of the oocyte topoisomerase I might result from some sort of oocyte-specific post-translational modification of the somatic enzyme. There are precedents for substantial shifts in gel mobility upon protein modification, such as that induced by phosphorylation of the repetitive sequence elements in the C-terminal domain of the large subunit of RNA polymerase II (27). The large shift in mobility from Mr 110 kDa to a discrete band at Mr 165 kDa would tend to rule out many known patterns of modification. Several experiments that were designed to determine whether known specific modification events could account for this mobility shift were unsuccessful. Therefore, we performed the experiment shown in Figure 5 to express Myc-tagged somatic topoisomerase I in oocytes as a general method to detect any sort of post-translational modification. Injection of the in vitro transcribed somatic topoisomerase I cDNA into oocytes programmed synthesis of only the 110 kDa protein, with no evidence of post-translational modification to the 165 kDa form (Fig. 5).

The likelihood that the protein sequences of the two isoforms of Xenopus topoisomerase I should include some conserved domains encouraged us to attempt to use sequence information from the somatic topoisomerase I clone to isolate an oocyte-specific clone. These experiments have been unsuccessful. RNA blot hybridization experiments did not show any discrete message larger than 4.3 kb in immature or mature oocytes that might encode the 165 kDa protein. We note that the autoradiogram in Figure 4 shows some weak hybridization to regions of the blot above the major 4.3 kb band in poly(A)-selected oocyte RNA. It has been difficult to detect an authentic oocyte-specific mRNA in the presence of a large excess of stored somatic topoisomerase I mRNA.

We cannot yet distinguish between two models to explain the relationship between the oocyte and somatic isoforms of topoisomerase I. First, it is possible that the oocyte topoisomerase I may be produced by differential splicing of transcripts from a single Xenopus topoisomerase I gene. Alternatively, the oocyte-specific topoisomerase I in X.laevis may represent the product of a second chromosomal gene. Chromosomal mapping experiments have identified three loci in human DNA that hybridize to the topoisomerase I cDNA, two of which appear to be processed pseudogenes (11). It has recently been established that the human genome contains two distinct type II topoisomerase I genes (reviewed in 28) encoding two isoforms first observed at the protein level (29). We have not yet studied genomic sequences that hybridize to the somatic topoisomerase I cDNA in X.laevis.

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