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Functional characterization of *Caenorhabditis elegans* DNA topoisomerase IIIα

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ABSTRACT

To investigate the function of a DNA topoisomerase III enzyme in *Caenorhabditis elegans*, the full-length cDNA of *C.elegans* DNA topoisomerase IIIα was cloned. The deduced amino acid sequence exhibited identities of 48 and 39% with those of human DNA topoisomerase IIIα and *Saccharomyces cerevisiae* DNA topoisomerase III, respectively. The overexpressed polypeptide showed an optimal activity for removing negative DNA supercoils at a relatively high temperature of 52–57°C, which is similar to the optimum temperatures of other eukaryotic DNA topoisomerase III enzymes. When topoisomerase IIIα expression was interfered with by a cognate double-stranded RNA injection, pleiotropic phenotypes with abnormalities in germ cell proliferation, oogenesis and embryogenesis appeared. These phenotypes were well correlated with mRNA expression localized in the meiotic cells of gonad and early embryonic cells.

INTRODUCTION

DNA topoisomerase III is a type IA DNA topoisomerase and has the catalytic activity of removing negative but not positive DNA supercoils (1). The enzyme supports chain elongation and decatenation of daughter DNA molecules in an *in vitro Escherichia coli* replication system and *E.coli* cells deficient for this enzyme display an increase in spontaneous DNA deletions (2,3). The null mutation in *Saccharomyces cerevisiae* causes hyper-recombination between repeated sequences, destabilizes telomere structure, slows cell growth and inhibits sporulation (4–6). In contrast to the mutant slow growth phenotype of *S.cerevisiae* cells, the null mutation ultimately causes blockage of mitotic growth in *Schizosaccharomyces pombe* cells and early embryos of mice (7,8). Even though the catalytic activity and the mutant phenotypes are known, the pathway connecting the loss of enzymatic activity to the phenotypes has not been explicitly revealed. The *Sgs1* gene, encoding a RecQ homolog with DNA helicase activity, was discovered as a suppressor of DNA topoisomerase III mutation in *S.cerevisiae* and an interaction between Sgs1 and DNA topoisomerase III proteins was shown in the yeast two-hybrid system (9–11). In mammals, there are two isozymes (α and β) of DNA topoisomerase III, which share an identity of 36% in their amino acid sequences (12–15). The two human isozymes show distinct tissue specificities, the α form being predominantly expressed in the testes and the β form in the ovaries (15). Interestingly, it was recently found that human DNA topoisomerase IIIβ interacts with yeast Sgs1 (15).

To investigate the function of DNA topoisomerase IIIα in *Caenorhabditis elegans*, the cDNA clone was obtained and its enzymatic activity was characterized using the overexpressed polypeptide. To assess the *in vivo* role of the enzyme, gene expression was inhibited by double-stranded RNA interference (dsRNAi) (16,17) and the resultant *C.elegans* phenotypes were analyzed.

MATERIALS AND METHODS

Amplification of the cDNA fragment of *C.elegans* DNA topoisomerase IIIα by RT–PCR

mRNA was isolated from *C.elegans* worms using a Fast Track 2.0 kit (Invitrogen) with minor modifications in the tissue lysis procedure as follows. Frozen *C.elegans* of mixed stages (1 g) were crushed under liquid nitrogen with a mortar and pestle and then sonicated in a solution consisting of 1 ml of phenol, 1 ml of chloroform and 3 ml of buffer (0.2 M Tris–HCl, pH 7.5, 0.5 M NaCl, 10 mM EDTA, 1% SDS, 0.07% β-mercaptoethanol). The cell lysate was cleared by centrifugation and was extracted with phenol and chloroform. mRNA was separated from the cleared lysate using oligo(dT)–cellulose (Invitrogen).

Two conserved peptides, AHPPIHP and GIDTDAT, in the amino acid sequences of human (α form) and *S.cerevisiae* DNA topoisomerase III were selected to design degenerate primers (KOMA Biotech, Korea). cDNA synthesis was in a reaction mixture (50 µl) containing the isolated *C.elegans* mRNA (1 µg), a degenerate deoxyribonucleotide primer (5′-GTNGCRTCNGTNCCDATNCC where N is G, A, T or C, R is A or G and D is G, A or T, 50 pmol) corresponding to the GIGTDAT peptide and Superscript reverse transcriptase (200 U; Gibco BRL) at 42°C for 1 h. First cDNA strand synthesis was terminated by heat treatment at 70°C and then the template RNA was degraded with RNase H (2 U; Takara) at 55°C for 15 min. A cDNA region was amplified using the same primer (20 pmol) as in first cDNA strand synthesis, a degenerate primer encoding the AHPPIHP peptide

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(5′-GCNCAYCCNCCNATHCAYCC where H is A, T or C, 20 pmol) and a premix-PCR kit (Bioneer, Korea). The amplified cDNA fragment was cloned into the pGEM-T vector (Promega) and the sequence was determined using the ABI PRISM dye terminator cycle sequencing kit (Perkin Elmer) and an ABI 310 automatic sequencer (Perkin Elmer). The cDNA fragment of 0.4 kb was 45 and 40% identical in sequence to human DNA topoisomerase IIIα and *S.cerevisiae* DNA topoisomerase III, respectively.

Caenorhabditis elegans cDNA library screening

The 0.4 kb cDNA clone was labeled radioactively with random primers (Promega), Klenow fragment (Takara) and [α-^{32}P]dCTP (Amersham-Pharmacia) to be used as a probe in the screening of a *C.elegans* embryonic cDNA library (λUniZAP XR vector; Stratagene). Among ~5×10^5 recombinant phage plaques, one positive plaque was identified and a recombinant pBluescipt SK(–) plasmid was excised from the positive phage DNA using Exassist helper phage (Stratagene). The positive cDNA clone of 1.7 kb (CeTop3αA) was sequenced using dideoxy-nucleotides, [α-^{35}S]dCTP and T7 sequenase (Amersham-Pharmacia) or using the ABI PRISM dye terminator cycle sequencing kit (Perkin Elmer) in combination with an ABI 310 automatic sequencer (Perkin Elmer).

Amplification of the 5′-end cDNA of *C.elegans* DNA topoisomerase IIIα

To obtain the genuine 5′-end cDNA of *C.elegans* DNA topoiomerase IIIα, the first cDNA strand was synthesized using a primer of sequence 5′-TATACAACTTCTCAG-CCACT (complementary to nt 994–975 in the complete cDNA sequence reported later, GenBank accession no. AF057032), obtained from the CeTop3αA cDNA clone. PCR was performed on the first cDNA strand using primer SL1 (5′-GG-TTTAATTACCCAAGTTTGAG) and a nested primer (5′-CAT-TGTCTGCTTAGCCGACA, complementary to nt 972–953 in the complete cDNA sequence). The amplified DNA product was electrophoresed in a 1% agarose gel and then eluted from the gel using a Gene Clean kit II (BIO101). The purified cDNA fragment, named CeTop3αSL1, was inserted into the pGEM-T vector and sequence determination was done as described above.

Northern blot analysis

N2 *C.elegans* (1 g) at mixed stages were crushed under liquid nitrogen and sonicated in a lysis buffer containing 1% β-mercaptoethanol. Total RNA was isolated from the lysate according to the procedure of the RNeasy total RNA kit (Qiagen). Briefly, cell debris was removed from the lysate by centrifugation and the supernatant was transferred to an RNeasy spin column. After centrifugation, the flow-through was discarded and the RNA eluted from the column by addition of DEPC-treated distilled water and subsequent centrifugation. The isolated total RNA (15 µg) was electrophoresed in a 1% formaldehyde–agarose gel (40 mM MOPS, pH 7.4, 10 mM sodium acetate, 1 mM EDTA). RNA in the gel was capillary transferred to a nitrocellulose membrane (Schleicher & Schuell) in 20× SSPE solution and then crosslinked to the membrane by UV irradiation. The membrane was incubated in hybridization solution (50% formamide, 5× SSPE, 5× Denhardt's solution, 0.5% SDS, 100 µg/ml salmon sperm DNA) containing a probe DNA which had been prepared from the cDNA fragment (nt 1439–2079 in the complete cDNA sequence, GenBank accession no. AF057032) by random primed labeling as described above. The hybridized membrane was washed in 2× SSPE and 0.5% SDS and then in 0.2× SSPE and 0.5% SDS for 10 min each at 60°C.

Expression of *C.elegans* DNA topoisomerase IIIα in *E.coli*

To construct a full-length cDNA of *C.elegans* DNA topoisomerase IIIα, the CeTop3αSL1 cDNA fragment was excised from the recombinant pGEM-T and cloned between the *Bcl*I and *Pst*I sites of recombinant pBluescript SK(–) DNA containing the CeTop3αA cDNA. The full-length cDNA clone was named CeTop3α (*C.elegans* DNA topoisomerase IIIα), nearly all of which (nt 25–2307) was cloned between the *Bam*HI and *Eco*RI sites in pGEX-5X-2 plasmid DNA (Amersham-Pharmacia). *Escherichia coli* XL1-Blue cells harboring the recombinant pGEX-5X-2/CeTop3α plasmid were cultured at 30°C until the cell density reached 0.4 $OD_{600\ nm}$ and then isopropyl-thio-β-D-galactoside (IPTG; Calbiochem) was added to the culture at a final concentration of 0.5 mM. After further incubation for 5 h, the cells were harvested and sonicated in binding buffer [1× phosphate-buffered saline, 1 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride]. The cell lysate was cleared by centrifugation at 12 000 *g* for 30 min (4°C) and then fractionated by glutathione–agarose column chromatography. The lysate was further purified through a heparin–agarose column, from which the overexpressed polypeptide was eluted in a buffer solution (20 mM Tris–HCl, pH 8.0, 1 mM DTT) containing 250 mM KCl. The cell lysate and its fractions were electrophoresed in two 7% SDS–polyacrylamide gels in duplicate. One of the gels was stained with Coomassie Blue and the other was electrotransferred to a nitrocellulose membrane. The membrane was incubated with monoclonal anti-glutathione *S*-transferase (GST) antibody, obtained from Dr E. Y. Choi (Hallym University), and then with goat anti-mouse horseradish peroxidase-conjugated IgG (Jackson Immunoresearch Laboratory), followed by color development using 4-chloro-1-naphthol.

The catalytic activity of DNA topoisomerase IIIα was assayed by reacting negatively supercoiled pBluescript SK(–) plasmid DNA (200 ng) with the recombinant protein in buffer solution (50 mM HEPES–KOH, pH 7.6, 1 mM $MgCl_2$) for 15 min at 37°C or at the indicated temperatures. After being extracted with phenol, the DNA products were separated in a 0.8% agarose electrophoresis gel either in the presence or absence of 5 µg/ml chloroquine.

Interference with *C.elegans* DNA topoisomerase IIIα expression by double-stranded RNA microinjection

The CeTop3α plasmid DNA was linearized using *Xba*I or *Xho*I restriction enzymes to be used as a template for *in vitro* transcription. Antisense RNA was synthesized using the *Xba*I-digested plasmid DNA (2 µg), T7 RNA polymerase (5 U; Stratagene), all four ribonucleoside triphosphates (0.4 mM each) and RNasin (5 U; POSCO, Korea) in reaction buffer (40 mM Tris–HCl, pH 8.0, 8 mM $MgCl_2$, 2 mM spermidine, 50 mM NaCl, 18 mM DTT) at 37°C for 2 h. Sense RNA was synthesized using the *Xho*I-treated DNA (2 µg) and T3 RNA polymerase (5 U; Stratagene) instead of the *Xba*I-digested plasmid DNA and T7 RNA polymerase. The CeTop3αΔ*Sac*I

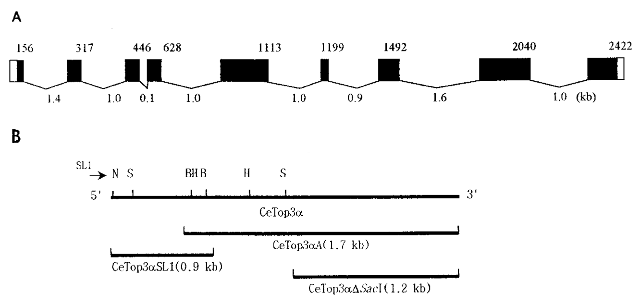

Figure 1. The gene structure of *C.elegans* DNA topoisomerase IIIα. (**A**) Genomic DNA structure. Exons are marked by rectangles and 3′-ends of the exons are numbered as in the reported cDNA sequence (GenBank accession no. AF057032). An open reading frame (nt 13–2292) is marked by filled rectangles. (**B**) cDNA clones. The CeTop3αA cDNA clone (nt 628–2422 in the reported cDNA sequence) was obtained from a *C.elegans* cDNA library and CeTop3αSL1 was synthesized by gene-specific RT–PCR. The full-length CeTop3α cDNA clone was generated by connecting the CeTop3αA and CeTop3αSL1 clones at the *Bcl*I site. BH, *Bam*HI; B, *Bcl*I; H, *Hin*dIII; S, *Sac*I; N, *Nde*I.

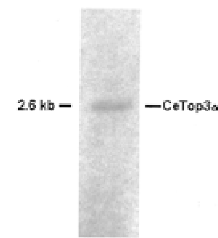

Figure 2. Northern analysis of *C.elegans* DNA topoisomerase IIIα. The total RNA was electrophoresed in a 1% agarose gel containing formaldehyde and then transferred to a nitrocellulose filter. The membrane was probed with a radiolabeled cDNA fragment (nt 1439–2079 in the complete cDNA sequence of *C.elegans* DNA topoisomerase IIIα).

clone (Fig. 1B), derived from CeTop3αA by deleting the 5′-end cDNA segment using *Sac*I restriction enzyme, was also used to prepare the antisense and sense RNAs. After RNA synthesis, RQ1 RNase-free DNase (2 U; Promega) was added to the reaction mixture and then phenol (pH 4.5) extraction and ethanol precipitation were carried out. Equivalent amounts of the sense and antisense RNAs were mixed and the RNA mixture (1 µg/µl) was microinjected into the gonads of young adult *C.elegans* worms of the N2 strain. Microinjected *C.elegans* were placed on a NGM plate with an *E.coli* OP50 lawn and transferred to a new plate after 12 h. The total numbers of embryos born during the next 24 h were counted and the P0 worms and their F1 progeny were observed under a stereomicroscope (Tritech Research) or a microscope with Nomarski optics (Carl Zeiss, Axioplan 2).

In situ RNA hybridization

In situ RNA hybridization of gonads was performed as described by Jones *et al.* (18). About 100 adult *C.elegans* of wild-type N2 strain (or of F1 progeny from *C.elegans* which had been microinjected with double-stranded RNA) were killed to extrude the gonads, which were subsequently fixed in 3% formaldehyde, 0.25% glutaraldehyde, 0.1 M K_2HPO_4, pH 7.2. Gonads were treated with proteinase K (50 µg/ml; Sigma) for 30 min and then with fixation solution. Hybridization proceeded at 48°C for 36 h in hybridization solution (100 µg/ml salmon sperm DNA, 50 µg/ml heparin, 0.1% Tween 20, 50% formamide, 5× SSC) containing a sense or an antisense digoxigenin-labeled probe (50 µg/ml). A cDNA fragment of *C.elegans* DNA topoisomerase IIIα (nt 1715–2080 in the complete cDNA sequence, GenBank accession no. AF057032) was used as a DNA template to prepare a digoxigenin-11-dUTP-labeled (La Roche) single-stranded DNA probe by multiple repeated primer extensions as described by Seydoux and Fire (19). After the hybridization, gonads were incubated with anti-digoxigenin antibody conjugated to alkaline phosphatase (1:2500 dilution; La Roche) at 4°C overnight and then in a buffer solution (100 mM Tris–HCl, pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$, 0.1% Tween 20, 1 mM levamisole) containing 4-nitroblue tetrazolium chloride (0.23 mg/ml) and 5-bromo-4-chloro-3-indolyl-phosphate (0.18 mg/ml). The stained *C.elegans* were observed using a microscope with Nomarski optics (Axioplan 2; Carl Zeiss). *In situ* hybridization of the embryos was for 12 h with a cDNA probe (50 µg/ml) according to the freeze-cracking method of Seydoux and Fire (19).

RESULTS

cDNA cloning of *C.elegans* DNA topoisomerase IIIα

A cDNA fragment of *C.elegans* DNA topoisomerase IIIα was obtained by reverse transcription of *C.elegans* mRNA followed by PCR (RT–PCR) using primers encoding peptide sequences conserved in eukaryotic DNA topoisomerase III enzymes. The reverse transcription was performed using degenerate oligodeoxynucleotides encoding the GIGTDAT peptide and the synthesized cDNA strands were amplified using a pair of degenerate oligodeoxynucleotides encoding GIGTDAT and AHPPIHP, as described in Materials and Methods. An amplified cDNA fragment of 0.4 kb was cloned and the cDNA sequence was determined to be similar to those of other DNA topoisomerase III enzymes (4,12). Using the cDNA fragment as probe, a *C.elegans* embryonic cDNA library was screened to obtain a cDNA clone of 1.7 kb (Fig. 1B). The exact 5′-end cDNA clone was produced by reverse transcription using a gene-specific primer and then PCR using a nested primer and the SL1 primer of the splice leader sequence (20). The amplified 5′-end cDNA sequence was aligned with the 1.7 kb cDNA sequence to obtain the complete cDNA sequence of 2.4 kb (GenBank accession no. AF057032). The northern analysis in Figure 2 shows a 2.6 kb mRNA, which agrees in length with the full-length cDNA including a poly(A) tail. The cDNA sequence contained an open reading frame of 758 amino acids, which shared 48 and 39% congruity in the amino acid sequence with human DNA topoisomerase IIIα and *S.cerevisiae* DNA topoisomerase III, respectively.

While our cDNA sequence determination was in progress, the *C.elegans* genomic DNA sequencing consortium reported

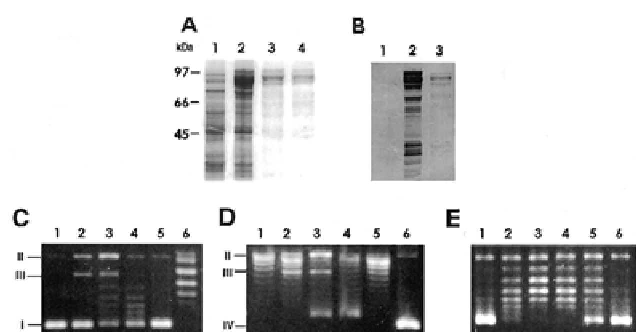

Figure 3. Catalytic activity of overexpressed *C.elegans* DNA topoisomerase IIIα. (**A**) Overexpression and purification of *C.elegans* Top3α fused with GST. Lane 1, crude extract (–IPTG); lane 2, crude extract (+IPTG); lane 3, glutathione–agarose column fraction of lane 2; lane 4, heparin–agarose column fraction of lane 3. (**B**) Western blot of (A) using monoclonal anti-GST antibody. (**C** and **D**) Relaxation of negative DNA supercoils by the overexpressed *C.elegans* Top3α. Forms I, II, III and IV are supercoiled, nicked, linear and relaxed plasmid DNAs, respectively. Lane 1, no protein; lane 2, GST (1 µg) after glutathione–agarose column chromatography; lane 3, *C.elegans* Top3α (50 ng) after glutathione–agarose column chromatography; lane 4, *C.elegans* Top3α (10 ng) after heparin–agarose column chromatography; lane 5, as lane 3 but without Mg^{2+}; lane 6, human DNA topoisomerase I (Topogen, 4 U). (**E**) Relaxation of negative DNA supercoils was carried out using *C.elegans* Top3α (5 ng) after heparin–agarose column chromatography at different temperatures. Lane 1, 42°C; lane 2, 47°C; lane 3, 52°C; lane 4, 57°C; lane 5, 62°C; lane 6, 67°C. Electrophoresis was without chloroquine in (C) and (E) and with chloroquine (5 µg/ml) in (D).

a genomic YAC clone sequence (Y56A3A positioned on chromosome III) containing the *C.elegans* DNA topoisomerase IIIα gene. The reported genomic DNA sequence exactly matched our cDNA sequence except for a nucleotide designated no. 2360 in the 3′-untranslated region. By comparison of our cDNA sequence with the genomic DNA sequence, a gene structure of nine exons spread over a 10.3 kb genomic DNA was revealed (Fig. 1A). However, the gene structure in Figure 1A is different in the length of the first exon from the gene structure predicted by the *C.elegans* genomic DNA sequencing consortium, which relied on the program Genefinder.

Catalytic activity of *C.elegans* DNA topoisomerase IIIα fused with GST

The two cDNA clones of *C. elegans* DNA topoisomerase IIIα obtained were linked together as described in Materials and Methods and the full-length polypeptide was overexpressed in *E.coli* cells as a fusion protein with GST as shown in Figure 3A. The overexpressed fusion polypeptide of ~100 kDa was purified by glutathione–agarose (Fig. 3A, lane 3) and then by heparin–agarose column chromatography (Fig. 3A, lane 4). The main polypeptide of 100 kDa in the purified fractions (Fig. 3A) was confirmed to be the fusion polypeptide by western blot analysis (Fig. 3B). Eluates from the two columns were used to measure the catalytic activity of the overexpressed protein (Fig. 3C, lanes 3 and 4). The protein relaxed negatively supercoiled plasmid DNA in the presence of Mg^{2+} (Fig. 3C, compare lanes 3 and 5), but not as completely as human DNA topoisomerase I (Fig. 3C, compare lanes 3, 4 and 6). An eluate from the glutathione–agarose column, onto which the cleared lysate of *E.coli* cells harboring pGEX-5X-2 vector DNA had been loaded, did not show any DNA supercoil relaxing activity (Fig. 3C, lane 2). This, together with the western blot analysis of Figure 3B, supports the idea that the DNA relaxation activity in the purified fractions (Fig. 3C, lanes 3 and 4) was exerted by the overexpressed *C.elegans* DNA topoisomerase IIIα and not by endogenous *E.coli* DNA topoisomerase I or III. The extent of DNA relaxation by the overexpressed protein was again manifest in agarose gel electrophoresis in the presence of chloroquine, as shown in Figure 3D. The relaxing activity of the overexpressed DNA topoisomerase IIIα was not as efficient as that of human DNA topoisomerase I (Fig. 3D, compare lanes 4 and 5). The catalytic activity was optimum at a temperature of 52–57°C (Fig. 3E) and a KCl concentration of 50–100 mM. Mg^{2+} could be replaced by Mn^{2+} but not by Ca^{2+} (data not shown). Heparin–agarose column chromatography was effective in removing a nuclease which co-purified with the overexpressed polypeptide until the glutathione–agarose column chromatography step (Fig. 3C and D, compare lanes 3 and 4).

Analysis of inhibition of *C.elegans* DNA topoisomerase IIIα gene expression

To analyze the *in vivo* functions of *C.elegans* DNA topoisomerase IIIα, we used the dsRNAi technique, which produces loss-of-function phenotypes for various genes in *C.elegans* (16,17). When double-stranded RNA derived from a cDNA sequence is introduced into *C.elegans*, corresponding endogenous gene expression is disrupted and the interference effects can be propagated for a few generations. Antisense and sense RNAs were synthesized by *in vitro* transcription from the full-length cDNA clone or the CeTop3αΔ*Sac*I clone (Fig. 1B) and an equivalent mixture of the antisense and sense RNAs was microinjected into the gonads of young adult *C.elegans* worms. All the injected P0 worms (34 for the full-length dsRNA and 18 for the CeTop3αΔ*Sac*I dsRNA) appeared normal and showed no difference in the aging progress compared to the uninjected ones. The embryos born 12–36 h after microinjection showed normal development until the young adult stage, at which time sterile phenotypes appeared in some of the F1 worms (Table 1 and Fig. 4B). Gonad arms of the RNAi phenotype were shorter than those of the wild-type worms and and the oocytes were deformed, although a normal number of sperm were present. When the gonad of a sterile hermaphrodite was observed under a fluorescence microscope after chromosomal staining, the number of nuclei in one gonad arm was about 400, which is much less than that of a wild-type young adult (about 1000) (Fig. 4C and D). The gonad was also distinctive in the expanded transition zone compared with the wild-type gonad. Besides that, oocytes in the proximal arm of the gonad showed abnormal chromosomal condensation. Gonadogenesis in the RNAi phenotype worm was much slower (0.6 times) compared to the wild-type, although other parts of the body showed normal growth. F1 progeny of the RNAi phenotypes showed a normal lifespan, but were slow in movement a week after birth. In a small fraction of the F1 progeny apparently normal oocytes developed into abnormal embryos stalled in development (data not shown).

Table 1. Effects of RNAi on the DNA topoisomerase IIIα gene

Targeted region of *C.elegans* DNA topoisomerase IIIα gene	F1 progeny with degenerate gonad arms[a]
Exons 1–9	317/1434 (22%)[b]
Exons 6–9	87/1001 (9%)

[a]F1 progeny from embryos born 12–36 h after dsRNA microinjection were scored for sterile phenotypes.
[b]The percentage was increased to 39% (193/496) for embryos born 36–60 h after dsRNA microinjection.

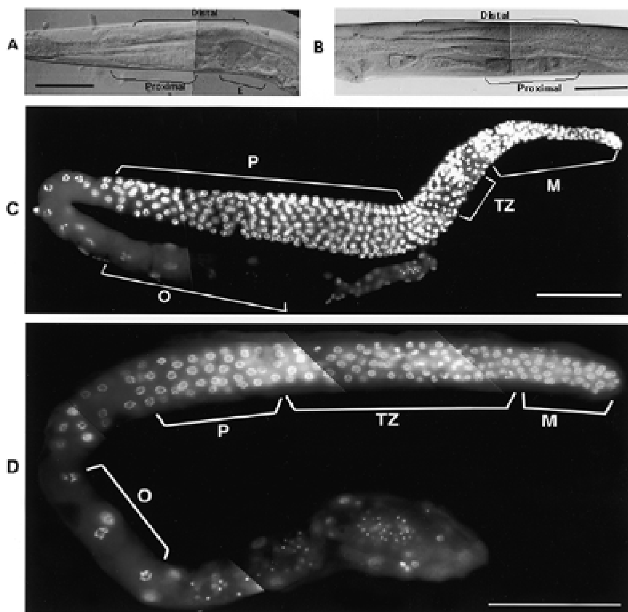

Figure 4. Adult stage F1 progeny of *C.elegans* worms which had been microinjected with double-stranded RNA of the CeTop3α clone. Worms in (**A**) and (**B**) were observed under Nomarski optics (scale bars 50 µm). Gonads in (**C**) and (**D**) were stained with Hoechst 33342 (scale bars 20 µm). (A and C) Wild-type; (B and D), RNAi phenotype. M, mitotic region; TZ, transition zone; P, pachytene stage; O, oocytes; E, embryos.

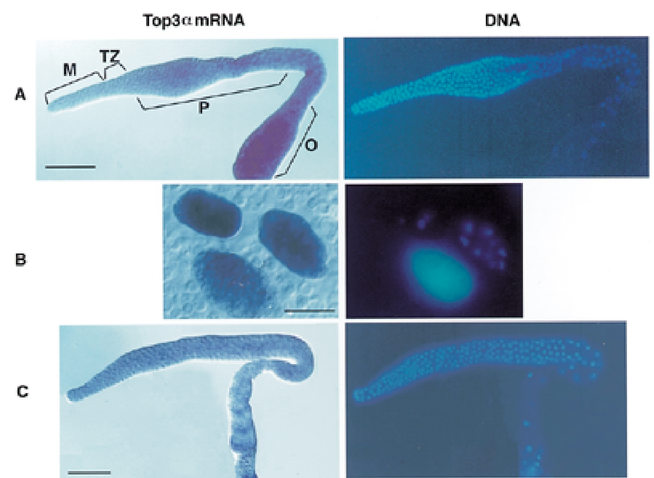

Figure 5. DNA topoisomerase IIIα mRNA expression in an adult hermaphrodite and its absence in the RNAi phenotype. The gonad and embryos were hybridized with the antisense probe of *C.elegans* Top3α and stained with Hoechst 33342. (**A**) Top3α expression in the wild-type gonad. (**B**) Top3α expression in wild-type embryos. (**C**) Inhibited Top3α expression in the gonad of the RNAi phenotype. Scale bars 20 µm.

mRNA localization of the DNA topoisomerase IIIα gene

The effects of the pleiotropic phenotypes produced by RNAi on the DNA topoisomerase IIIα gene suggest crucial actions of the enzyme during germ cell proliferation, oogenesis and embryogenesis. To probe mRNA expression during these developmental processes, *in situ* RNA hybridization of the gonads and embryos was carried out using a digoxigenin-labeled antisense cDNA probe as described in Materials and Methods. In the gonads of wild-type hermaphrodites, a mid-to-proximal region including pachytene stage cells and oocytes was strongly stained (Fig. 5A). This strong expression was retained in early embryos (Fig. 5B) and decreased gradually after the 200 cell stage until complete disappearance at the 2-fold stage (data not shown). The sense strand probe gave little or no staining in the gonads nor the embryos, supporting the idea that the staining in Figure 5 represented DNA topoisomerase IIIα mRNA expression (data not shown). The staining almost disappeared in the gonads of F1 progeny affected by dsRNAi (Fig. 5C), which supported the idea that the RNAi phenotypes above were most likely due to inhibition of *C.elegans* DNA topoisomerase IIIα gene expression.

DISCUSSION

In this study we have obtained the full-length cDNA sequence of *C.elegans* DNA topoisomerase IIIα and characterized the functions of the protein both *in vitro* and *in vivo*. The Top3α cDNA sequence was compared with the reported genomic DNA sequence of clone Y56A3A mapped to chromosome III. This comparision yielded the Top3α gene structure consisting of nine exons with a *trans*-splicing site in the first exon, as illustrated in Figure 1A. The deduced open reading frame of 758 amino acids has a length between those of human Top3α (976 amino acids) and *S.cerevisiae* Top3 (656 amino acids), and Tyr334 is the putative catalytic residue. The C-terminal tail of about 150 amino acids in *C.elegans* Top3α has a high composition of Pro and Gly residues, which is also true for the C-terminal tail of human Top3α (12). Whether these polypeptide tails have any cellular function is an intriguing question.

The overexpressed CeTop3α polypeptide was shown to relax negatively supercoiled plasmid DNA in the presence of Mg^{2+}. The relaxation activity was optimal at a moderately high temperature (52–57°C), which was also observed for human topoisomerase IIIα and yeast topoisomerase III (5,21). The peculiar temperature dependence of the catalytic activity implies that the protein conformation at higher temperature

may be close to that induced by interaction with other proteins, such as the RecQ homologs (22–25).

Since mammalian topoisomerase III isoforms are known to be highly expressed in the testis and ovary (13–15), we focused on the mRNA expression pattern in the gonads of a hermaphrodite and embryos. mRNA expression began at the end of the transition zone in the gonads, continued in the oocytes and early embyos (Fig. 5) and then decreased gradually until it completely disappeared at the 2-fold stage (data not shown). Interestingly, the expression pattern in the gonad is similar to that of *gld-1* mRNA (18), but it is not germline specific, because the mRNA was present in all cells of early embryos. In the human, even though Top3α mRNA is abundant in such reproductive tissues as testis and ovary, it is also found in heart, skeletal muscle and pancreas (15).

The high mRNA expression in the hermaphrodite germline cells and early embryos provided a clear explanation for the pleiotropic phenotypes produced by dsRNAi in germ cell proliferation, oogenesis and embryogenesis. The abnormalities in germ cell proliferation and oogenesis in *C.elegans* agree well with the phenotype of *top3* null mutation in *S.cerevisiae* blocked in sporulation (6). It is remarkable that the number of cells in the gonad was much reduced and the transition zone was relatively expanded by dsRNAi, which may reflect a difficulty in entering meiotic prophase I. However, this conjecture is contrary to a recent report (6) that suggests a role of *S.cerevisiae* Top3 in completing meiotic recombination involving double-strand breaks initiatied by Spo11 (26). The RNAi phenotype blocked in the middle of *C.elegans* embryogenesis does not necessarily mean that Top3α is needed during embryogenesis and could have originated from abnormal oocytes. However, the fact that Top3α is essential for the progression of embryogenesis in mice supports the possibility of Top3α having an essential role in the embryogenesis of *C.elegans* as well (8). In the reported *C.elegans* genomic DNA sequence (clone Y48C3A, chromosome II), a Top3β gene is found, which shared 53% identity in amino acid sequence with human DNA topoisomerase IIIβ. The fact that the effects of RNAi on DNA topoisomerase IIIα expression resulted in the obvious phenotypes suggests that the two DNA topoisomerase III enzymes in *C.elegans* probably play distinct roles *in vivo*.

In conclusion, the RNAi phenotypes suggest that *C.elegans* DNA topoisomerase IIIα is needed for germ cell proliferation and oogenesis and very likely also for embryogenesis. However, the specific step in meiotic progression where Top3α plays an essential role remains to be elucidated.

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