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Developmental Regulation of *Caenorhabditis elegans* DNA Topoisomerase I Expression

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The developmental regulation of *Caenorhabditis elegans* DNA topoisomerase I expression was examined using synchronized *Caenorhabditis elegans* cultures. Variations of the relative mRNA and protein levels of the enzyme during their development were measured by Northern and Western analyses, respectively. The mRNA level was the highest at the embryonic stage, decreasing rapidly to the one tenth level at the L1 stage, and then increasing by a few fold at the L4 and young adult stages. The protein level was the highest at the L1 stage, with gradual decreasing at the following stages until it showed a slight increase at the young adult stage. Based on our results of the expressional regulation, the possible roles of DNA topoisomerase I in the development of *C. elegans* are discussed.

Keywords: *C. elegans*, Development, Gene expression, Topoisomerase.

Introduction

The eukaryotic nuclear DNA topoisomerase I represses basal transcription and acts as a cofactor of transcriptional activation (Kretzschmar *et al.*, 1993; Merino *et al.*, 1993; Shykind *et al.*, 1997) and as a swivelase during transcriptional elongation (Zhang *et al.*, 1988). In accordance with the involvement of the enzyme in the transcriptional processes, the enzyme level is a few fold higher in proliferating eukaryotic cells than in stationary cells, although some contradicting observations have been reported (Hsiang *et al.*, 1988; Baker *et al.*, 1995). The mRNA level is increased by a serum addition to human fibroblast cells (Romig and Richter, 1990a), an adenovirus-5 infection in HeLa cells (Romig and Richter, 1990b), and a PHA stimulation in human lymphocytes (Hwong *et al.*, 1993). The specific enzyme activity is increased by phosphorylation (Kordiyak *et al.*, 1994) and decreased by poly(ADP)-ribosylation of the protein (Boothman *et al.*, 1994). The mRNA and protein levels, and phosphorylation of the protein, vary with the progression of the cell cycle (Baker *et al.* 1995; D'Arpa and Liu, 1995; Meyer *et al.*, 1997). Recently, its subnuclear distribution was observed to be dependent on cell proliferation, that is, the enzyme is mostly in the nucleoli of growing cells, while it shows perinuclear distribution in stationary cells (Baker *et al.*, 1995). In the promoter of human DNA topoisomerase I, several regulatory regions were found, and the gene was shown to be regulated by NF-IL6 (Heiland and Knippers, 1995). Although the enzyme is dispensable in *Saccharomyces cerevisiae* (Goto and Wang, 1985), it is essential in the multicellular organism of *Drosophila melanogaster* (Lee *et al.*, 1993). In *D. melanogaster*, the mRNA and protein levels of DNA topoisomerase I change during the development, maximizing in both levels at the embryonic stage (Lee *et al.*, 1993).

Previously, a *C. elegans* DNA topoisomerase I cDNA sequence of 3.2 kb had been determined using three overlapping cDNA clones obtained from two *C. elegans* cDNA libraries and 5′-end cDNA clones obtained by RACE (rapid amplification of cDNA ends) (Kim *et al.*, 1996; Lee *et al.*, 1998; EMBL/GenBank X96762). The gene structure containing 5 exons was delineated by comparing the cDNA sequence with the genomic DNA sequence reported by the *C. elegans* genomic DNA sequencing consortium (Lee *et al.*, 1998; EMBL/GenBank Z93385). By reanalyzing the DNA topoisomerase I cDNA clones obtained by RT-PCR (reverse transcription-polymerase chain reaction) and those from the cDNA libraries, an mRNA missing the second exon by an alternative splicing was found to be the major form (Lee *et al.*, 1998). As a first step to study the role of DNA topoisomerase I in the development of *C. elegans* and its regulation of the gene expression, we examined how the

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DNA topoisomerase I mRNA and protein levels vary during the development.

Materials and Methods

Synchronization of *C. elegans* growth N2 *C. elegans* worms were grown on NGM plates covered with *E. coli* OP50 cells at 20°C (Sulston and Hodgkin, 1988). The avid worms at mixed stages were collected by washing the plates with M9 buffer. After having been cleared of the M9 buffer, the worms were lysed in 10 vol of a 1% NaOCl and 0.5 M NaOH solution for several minutes with occasional vortexing. Eggs were precipitated from the lysate by a centrifugation at $140 \times g$ for 1 min and were hatched into L1 stage worms on NGM plates without *E. coli* OP50 lawn. The L1 stage worms were incubated on NGM plates covered with *E. coli* OP50 cells at 20°C. Worms at the L2, L3, L4, and young adult stages were harvested by washing the plates with M9 buffer at 15, 24, 34, and 46 h lapses, respectively, after the transfer of the L1 stage worms to the *E. coli* lawn. *C. elegans* worms were cleared of the M9 buffer and were separated from the *E. coli* as described previously (Park and Koo, 1994).

Northern blot Frozen *C. elegans* eggs and worms (0.1 g) at different developmental stages were pulverized under liquid nitrogen with a mortar and pestle, and then were homogenized in 1 ml of buffer solution (4 M guanidinium thiocyanate, 25 mM sodium citrate, pH 7.0, 0.5% sodium sarkosyl, 0.1 M β-mercaptoethanol). The cell debris were precipitated from the lysate by a centrifugation, and the supernatant was processed using a total RNA isolation kit (Qiagen). The total RNAs (30 μg per lane) from *C. elegans* at different developmental stages were electrophoresed in a 1% agarose gel (2.2 M formaldehyde), and the Northern blot hybridization was followed using the same cDNA fragment of *C. elegans* DNA topoisomerase I as described in a previous article (Kim *et al.*, 1996). The membrane was exposed to an X-ray film, and the relative band intensities were measured using the Eagle Eye II gel documentation system (Stratagene). The hybridization was repeated using a 0.5 kb *Hind*III–*Xba*I DNA fragment of the pPD33.24 plasmid (kindly provided by A. Fire) containing the *C. elegans* ribosomal protein 21 gene (Spieth *et al.*, 1991) as a probe.

Expression of a C-terminal polypeptide fragment of *C. elegans* DNA topoisomerase I in *E. coli* cells The 2.0 kb long *Cla*I–*Hind*III DNA fragment of pBluescript/TOPO1 plasmid DNA (Kim *et al.*, 1996), which contained a 2.5 kb 3′-end cDNA fragment of *C. elegans* DNA topoisomerase I, was inserted into the *Nsp*V–*Hind*III restriction site of pET-32a(+) expression vector (Novagen). *E. coli* BL21(DE3) cells harboring the recombinant expression vector were treated with 1.5 mM IPTG in the exponential growth phase, harvested, and then stirred for 1 h in 5 ml of the lysis buffer (6 M guanidine·Cl, 0.1 M sodium phosphate, 0.01 M Tris·Cl, pH 8.0) per gram of the cells. After centrifuging the cell lysate at $10{,}000 \times g$ at 4°C for 15 min, the supernatant was taken and run through a Ni-NTA resin (Qiagen) column. The column was washed with buffer solution (8 M urea, 0.1 M sodium phosphate, 0.01 M Tris·Cl, pH 6.3). The DNA topoisomerase I polypeptide fused with thioredoxin-tag and His-tag was eluted in buffer solution (8 M urea, 0.1 M sodium phosphate, 0.01 M Tris·Cl, pH 4.5).

Antibody production against a C-terminal polypeptide fragment of *C. elegans* DNA topoisomerase I The purified C-terminal polypeptide fragment (50 kDa) of *C. elegans* DNA topoisomerase I tagged with thioredoxin and histidine was electrophoresed in a 10% SDS polyacrylamide gel, and then the gel slice containing the polypeptide of 65 kDa was cut out. The gel slice was crushed in PBS (phosphate buffered saline) solution that was added to make up the concentration of the polypeptide to 1 mg/ml. The suspension (2 ml) was mixed with the same volume of complete Freund's adjuvant and then injected into the hyperdermis of rabbits. After two more injections of the gel suspension, given at 2 week intervals, blood was collected by a heart puncture. The blood was left at 4°C overnight, and the supernatant was taken. The obtained antiserum was buffered by adding Tris·Cl (pH 8.0) to the concentration of 0.1 M and loaded onto a protein-A column. After washing the column with 10 mM Tris·Cl (pH 8.0), immunoglobulins were eluted from the column using 100 mM glycine (pH 3.0), and then the pH of the eluate was quickly adjusted to 8.0.

Western blot *C. elegans* eggs and worms at different developmental stages (0.1 g) were mixed with two volumes of PBS. After adding phenyl methyl sulfonylfluoride to the concentration of 1 mM, the *C. elegans* samples were sonicated, and then Triton X-100 was added to 1%. The lysate was gently shaken for 30 min at room temperature and then centrifuged at $5000 \times g$ for 10 min. The supernatant was taken, and the protein concentration was measured using a bicinchonic acid assay.

Cleared *C. elegans* lysates (30 μg of protein for each developmental stage) were electrophoresed in a 10% SDS polyacrylamide gel, and the proteins in the gel were then electro-blotted to a nitrocellulose membrane. The membrane was treated with the antibody against the C-terminal polypeptide fragment of *C. elegans* DNA topoisomerase I (1:500 dilution) and then with anti-rabbit alkaline phosphatase-conjugated IgG (1:5000 dilution, Sigma). Alkaline phosphatase activity on the membrane was visualized using nitro blue tetrazolium chloride and 5-bromo-4-chloro-indolyl phosphate, followed by the quantitation using the Eagle Eye II gel documentation system (Stratagene).

Results and Discussion

In order to study the developmental regulation of *C. elegans* DNA topoisomerase I expression, *C. elegans* growth was synchronized as described in Materials and Methods. Total RNAs prepared from *C. elegans* at different developmental stages were electrophoresed in a 1% agarose gel and transferred to a nitrocellulose membrane. The DNA topoisomerase I mRNA level in the membrane was probed using the radioactively labeled 1.25 kb *Eco*RI–*Eco*RI cDNA fragment of *C. elegans* DNA topoisomerase I, as shown in Fig. 1A. The membrane was reprobed using a cDNA fragment of *C. elegans* ribosomal protein 21, as shown in Fig. 1B, in order to show the almost constant expression of the ribosomal protein 21 as a control for developmental variations of the DNA topoisomerase I expression (Spieth *et al.*, 1991). The equivalence in the amount of total RNA loaded in each

lane of the agarose gel was confirmed by measuring the band intensities of ribosomal RNAs in Fig. 1C. The DNA topoisomerase I mRNA level was the highest at the embryonic stage and showed a sudden decrease at the L1 stage to one tenth of the maximum level. At the following developmental stages, the mRNA level was more or less constant except for a few fold increase later in the developmental stage.

Relative protein levels of the DNA topoisomerase I at different *C. elegans* developmental stages were measured as shown in the Western blot of Fig. 2A. Crude lysates of *C. elegans* at different developmental stages were electrophoresed in a 10% SDS-polyacrylamide gel, and proteins in the gel were blotted onto a nitrocellulose membrane. The membrane was probed using the antibody against the 50 kDa C-terminal DNA topoisomerase I polypeptide fragment. The polypeptide with a molecular weight of 76 kDa, marked in the Western blot of Fig. 2, is thought to be the DNA topoisomerase I, although the size is a little smaller than expected for the DNA topoisomerase I consisting of 734 amino acids (Kim *et al.*, 1996; Lee *et al.*, 1998; EMBL/GenBank X96762, Z93385). The equivalence in the total amount of proteins loaded in each lane was verified by Coomassie-staining of a duplicate gel, as shown in Fig. 2B. The 76 kDa polypeptide level was the highest at the L1 stage unlike the mRNA level and gradually decreased in the following stages until it showed a slight increase at the young adult stage. Changes of the protein level followed on those of the mRNA level at a rather slow and smooth pace. In *Drosophila melanogaster*, the mRNA and protein levels of DNA topoisomerase I vary during the development (Lee *et al.*, 1993). The protein level is maximal at the 6–12 h embryonic stage and gradually decreases through the larval and pupal stages. There are two mRNAs of different lengths, which encode the same polypeptide (Hsieh *et al.*, 1992). The shorter mRNA, which is abundant at the early embryonic stage, is of maternal origin and decreases in the level during the development, while the longer mRNA peaks to a maximum at the 6–12 h embryonic stage and again at the pupal stage to one fifth of the maximal level.

In *C. elegans*, the mRNA level of DNA topoisomerase I was maximal at the embryonic stage coinciding with the observation in *D. melanogaster* (Fig. 3). Some portion of the DNA topoisomerase I mRNA in *C. elegans* embryos is

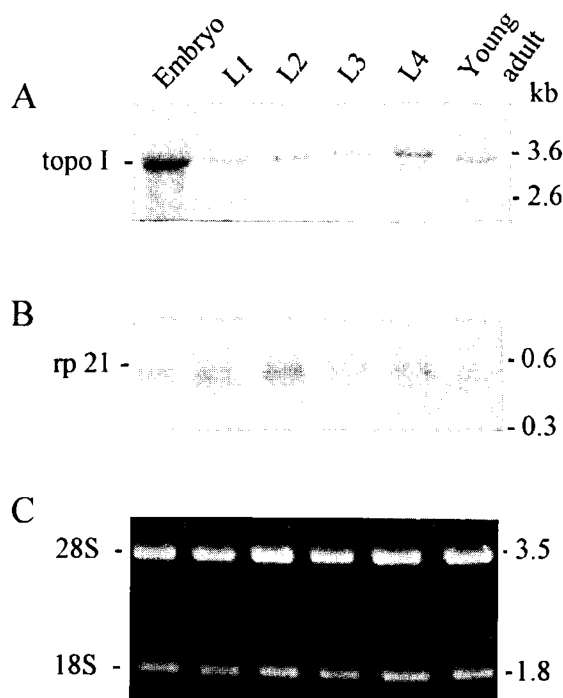

Fig. 1. Developmental Northern blot analysis of *C. elegans* DNA topoisomerase I. A. Total RNAs (30 μg) isolated from *C. elegans* at different developmental stages were electrophoresed in a 1% agarose gel (2.2 M formaldehyde) and the *Eco*RI–*Eco*RI cDNA fragment of *C. elegans* DNA topoisomerase I was used as a probe. B. The blot in panel A was deprobed and then reprobed with a *C. elegans* ribosomal protein 21 cDNA fragment. Topo I and rp 21 stand for topoisomerase I and ribosomal protein 21, respectively. The positions of RNA size markers (Promega) are indicated at the right side of the blots. C. Total RNAs (2 μg) were electrophoresed as described for panel A, and then the 18S and 28S ribosomal RNAs were visualized by ethidium bromide staining.

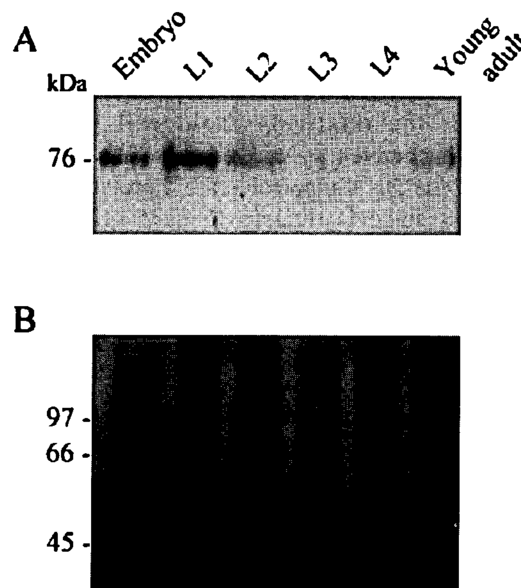

Fig. 2. Developmental Western blot analysis of *C. elegans* DNA topoisomerase I. A. The lysates (30 μg of protein) of *C. elegans* at different developmental stages were electrophoresed in a 10% SDS-polyacrylamide gel and then reacted with the antibody against the 50 kDa C-terminal *C. elegans* DNA topoisomerase I polypeptide fragment. B. The lysates (3 μg of protein) were electrophoresed as described for panel A, and the proteins were stained with Coomassie brilliant blue R-250. The positions of molecular weight standards (Bio-Rad) are marked at the left side of the gel.

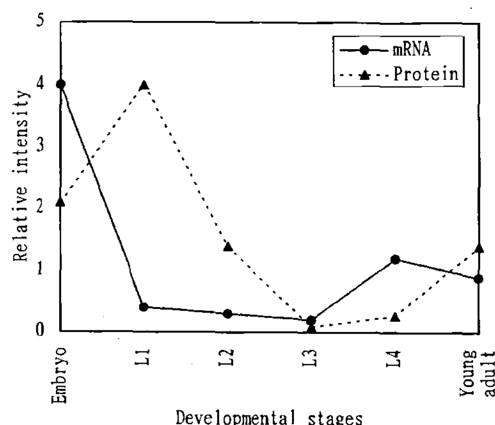

Fig. 3. Variations in the mRNA and protein levels of *C. elegans* DNA topoisomerase I with the development. The mRNA and protein band intensities in the blots of Figs. 1A and 2A, respectively, were integrated over each band area and the backgrounds were subtracted from the integrals. The integrated band intensities were plotted, arbitrarily taking the maximal intensity as 4 in each plot.

likely to be of maternal origin, as observed for the *D. melanogaster* DNA topoisomerase I mRNA (Lee *et al.*, 1993). However, the protein level was the highest at the L1 stage in *C. elegans*, unlike the maximal level at the embryonic stage in *D. melanogaster*. Somatic cell divisions are more active at the L1 stage than at the following larval and adult stages in *C. elegans* (Sulston *et al.*, 1988). The high level of DNA topoisomerase I may be needed for the relatively rapid cell growth with accompanying active transcription at the L1 stage. A slight increase of the DNA topoisomerase I protein level at the young adult stage is thought to have originated from oogenesis. For a better understanding of the function of DNA topoisomerase I in the multicellular organism, tissue specific expressions of the enzyme and the null mutant phenotypes need to be investigated.

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