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Cloning and characterization of DNA topoisomerase I gene *Top1* from maize (*Zea mays* L.)

Yi-Jun Wang *, De-Xiang Deng, Yun-Long Bian, Qin Xie, Yan-Ping Lv

Key Laboratory of Ministry of Education for Plant Functional Genomics, Key Laboratory of Jiangsu Province for Crop Genetics and Physiology, Agricultural College, Yangzhou University, Yangzhou 225009, China

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ABSTRACT

DNA topoisomerase I is a ubiquitous and essential enzyme in nature. Despite the biological importance, the molecular cloning of *Top1* has not yet been reported in cereal crops. Herein, we presented the isolation and characterization of *Top1* in maize. A total of 15 exons and 14 introns spanned 5325 bp genomic region of the maize topoisomerase I gene. The full-length 3265 bp cDNA of maize *Top1* contained an ORF of 2715 bp encoding 904 amino acids with four characterized regions. The deduced amino acid sequence showed homology with Topo I proteins from other plants. Based on the human Topo I information, 3D structure of maize topoisomerase I was built. 169 putative *cis*-elements that are involved in hormone and light signalling responses, abiotic stress adaption were identified in the promoter region of *Top1*. Subcellular location *in silico* showed the position of Topo I in maize nuclei. Expression pattern analysis using MPSS method indicated maize *Top1* expressed constitutively in all tissues and organs examined, with higher transcript levels in meristems, endosperm and anther.

1. Introduction

DNA topoisomerase I (Topo I) plays a vital role in many cellular and developmental processes, including replication, transcription, repair, recombination, chromatin condensation and segregation, *etc*. Topo I cleaves single DNA strand and allows the single or double strand(s) to go through the nick before filling it. It relaxes the negative supercoil in an ATP-independent manner during DNA metabolism [1–3]. Based on the different structure and enzymatic mechanism, Topo I can be divided into some subfamilies, including Topo IA, Topo IB and Topo IC. Topo IA only relaxes the negative supercoil. In contrast, Topo IB and Topo IC can unlink both positive and negative strains. Topo IA tends to link 5′ end of the broken strand. Topo IB and Topo IC link 3′ end of the gaps. Topo IA looses the DNA strand by enzyme-bridged mechanism, that is, enzyme cleaves one DNA strand and assists the other strand to pass through. Topo IB and Topo IC relink the strand by rotating DNA strands. Although Topo IB and Topo IC share many characters, core domains of Topo IB and Topo IC are different. Moreover, Topo IC as an orphan protein is discovered only in one species so far. Topo IB exsits in eukaryotes, some bacteria and poxviruses. Topo IA is widely distributed in nature [2,4].

Due to the physiological importance, the function of *Top1* gene has been extensively investigated in unicellular and multicellular organisms. In unicellular cycle, Topo I is necessary for circle plasmid pairing *in vitro* [5]. In the presence of RNA polymerase II, *Homo sapiens* Topo I is required to trigger the transcription process [6]. Although Topo I is not vital to viability of *Escherichia coli* and yeast, the Topo I deficiency mouse cannot survive at the early stage of embryogeny [7]. Results also showed Topo I is essential to the larva and pupa growth, ovum genesis in *Drosophila melanogaster* [8]. Additionally, the role of Topo I has been studied in plants. In tobacco, Topo I confers stress tolerance [9]. *Top1* is also related to the morphogenesis of *Arabidopsis* [10]. The carrot Topo I takes part in cell proliferation and programmed cell death processes [11,12]. The above results imply that Topo I plays different roles during organism growth and development, not just loosing and relinking the DNA strand.

DNA topoisomerase I gene *Top1* has been isolated in many species, including *Nicotiana tabacum*, *Daucus carota*, *Arabidopsis thaliana*, *Pisum sativum*, *E. coli*, *Schizosaccharomyces pombe*, *Homo sapiens* and *Fugu rubripes*, but no *Top1* gene was cloned from cereal crops so far [9,11,13–18]. The canonical Topo I consists of four characterized regions, that is, disordered NH_2-terminal and linked region, conserved core region and COOH-terminal. The non-conserved N terminal and linked region are not necessary for

Abbreviations: Topo I, topoisomerase I; *Top1*, topoisomerase I gene; RT-PCR, reverse transcription PCR; LD-PCR, long distance PCR; FLcDNA, full-length cDNA; RACE, rapid amplification of cDNA ends; MPSS, massively parallel signature sequencing; *cis*-elements, *cis*-acting regulatory DNA elements; EST, expressed sequence tag; ORF, open reading frame; UTR, untranslated region.

* Corresponding author. Fax: +86 514 8799 6817.
E-mail address: yjwang61@163.com (Y.-J. Wang).

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enzyme activity. The core region and COOH-terminal are adequate for catalytic reaction. The catalytic site tyrosine (Tyr) lies in C terminal [19]. Phosphorylation has little effect on the enzymatic activity of tobacco Topo I. Contrarily, pea Topo I activity is switched by phosphorylation [9,20]. Besides, pea Topo I activity is stimulated by DNA helicase [21]. *Top1* gene is characteristic of basal constitutive expression. Moreover, the transcription of *Top1* is modulated by endogenous and exogenous stimuli [9,14].

Recently, a special issue of Nucleic Acid Research containing the program of Topo2008 meeting highlights the significance of topoisomerase research [22]. During past, topoisomerase I investigation in cereal crops was limited in purifying enzyme and studying its activity. In this study, we aimed at cloning and characterizing of *Top1* gene encoding maize topoisomerase I. The FLcDNA sequence of *Top1* was achieved using RACE in combination with RT-PCR method. Genomic organization, phylogenesis, 3D structure, *cis*-elements and subcellular location of maize *Top1* gene were analyzed by several bioinformatics tools. Finally, MPSS method was adopted to display the expression pattern of maize *Top1* gene. To our knowledge, this marks the first report in which cereal *Top1* gene cloning has been demonstrated. It also provides a window for the genomic research of *Top1* gene in cereal crops.

2. Materials and methods

2.1. Total RNA preparation and cDNA synthesis

Total RNA was extracted from young leaves of maize inbred line Mo17 using RNAiso Plus (Takara, Dalian, China) according to the manufacture's instruction, with some modifications in the purification step. All RNA samples were treated with DNase I free of RNase activity (Takara, Dalian, China). The quality of RNA was checked by 1% denaturing agarose gel electrophoresis and the UV spectrophotometer (Thermo, USA). The cDNA synthesis kit (Takara, Dalian, China) was employed to synthesize cDNA for the following RT-PCR. The mRNA used in RACE reaction was isolated by PolyA Tract® mRNA Isolation System III (Promega, USA).

2.2. RT-PCR

10 μl volume of RT-PCR contained cDNA template, 1× PCR buffer, 200 μM dNTPs, 0.2 μM forward primer, 0.2 μM reverse primer, 0.5 U Taq DNA polymerase (Takara, Dalian, China) and appropriate count of distilled water. The mixture was predenatured at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, extension at 72 °C for 1 min. The final extension step was at 72 °C for 10 min. Targeted PCR products were purified from gel with the agarose gel DNA purification kit (Takara, Dalian, China) and ligated into pGEM-T vector (Promega, USA) for sequencing. Primers were designed by the primer3 tool (http://frodo.wi.mit.edu/). Primer synthesis and DNA sequencing were performed at Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China).

2.3. FLcDNA cloning

The FLcDNA sequence was obtained using RT-PCR in conjunction with RACE technique. Conserved 3′ terminal of maize *Top1* gene was amplified directly by RACE strategy using the BD SMART™ RACE cDNA Amplification Kit (Clontech, USA). The diversified 5′ terminal was achieved step by step. First, sequence of 3′ terminal was used as seed to query homologies in the NCBI database (http://www.ncbi.nlm.nih.gov/). Then, the most similar sequence was examined by RT-PCR. The validated sequence was adopted in the next round of data mining. By query and validation cycle, the FLcDNA sequence of maize *Top1* gene was finally obtained. Purification and sequencing of the amplified products were performed as described above.

2.4. Promoter sequence analysis and subcellular location

2 kb of genomic DNA sequences upstream of initiation codon ATG were retrieved from the maize B73 sequencing database (http://www.maizesequence.org/index.html). The PLACE website (http://www.dna.affrc.go.jp/PLACE/) was subjected to identify *cis*-elements in the promoter regions [23]. Online PLOC (http://www.genome.jp/SIT/plocdir/) and WoLF PSORT (http://wolfpsort.org/) were scanned for subcellular location of maize Topo I [24,25].

2.5. Expression pattern investigation

MPSS technique is used for investigating the expression profile in-depth. By counting the number of unique 17 bp or 20 bp signature of each cDNA, the transcript level of every gene was quantified. Commonly, the 17 bp probe beginning with nucleotides "GATC" was used as tag of MPSS technique [26]. In this study, transcript levels of *Top1* gene were checked in various maize tissues and organs from different vegetative and reproductive stages by MPSS method. The relative expression level of *Top1* expression was quantified by ppm (parts per million) value. All MPSS expression data were normalized by sample size.

2.6. Bioinformatics analysis

EST information was obtained from the NCBI dbEST (http://www.ncbi.nlm.nih.gov/dbEST/). The ORF was identified by the ORF Finder tool (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Genomic sequences of *Top1* gene were retrieved from the maize B73 sequencing database. Positions of exons and introns were determined by the GSDS utility (http://gsds.cbi.pku.edu.cn/index.php) [27]. 3D structure of conserved residues of maize Topo I was conducted in the SWISS-MODEL workspace using the automatic modelling mode (http://swissmodel.expasy.org//SWISS-MODEL.html) [28]. The obtained 3D structure was embellished by the PyMol software (http://www.pymol.org/). Basic parameters of maize Topo I were calculated by the ProtParam tool (http://www.expasy.org/tools/protparam.html). Multiple alignment of Topo I proteins was performed using the Clustal X tool with default parameters (http://www.ebi.ac.uk/Tools/clustalw2/index.html) [29]. Phylogenic tree was built by the Clustal X tool with neighbor-joining (N-J) method and 1000 replicates. MEGA software was adopted to display the dendrogram [30]. The catalytic domain of Topo I was featured by online WebLogo 3 (http://weblogo.berkeley.edu/) [31].

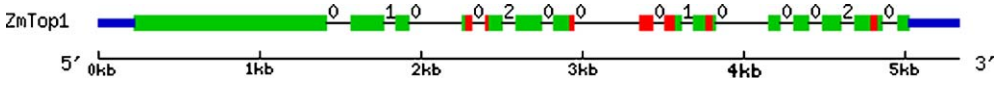

Fig. 1. Schematic structure of maize *Top1* gene. Exons and introns were showed by filled boxes and single lines, respectively. Four conserved domain I, II, III and IV were marked in red. UTRs were displayed by thick blue lines at both ends. Intron phases were indicated by numbers 0, 1 and 2 above the single lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)