A new human topoisomerase III that interacts with SGS1 protein

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ABSTRACT

Eukaryotic DNA topoisomerase III was first identified by studying the hyper-recombination and slow growth phenotypes of yeast mutants. Topoisomerase III interacts with DNA helicase SGS1 and the two proteins are involved in DNA recombination, cellular aging and maintenance of genome stability. A human homolog of topoisomerase III has previously been identified. Here we report the identification of cDNAs and the determination of gene structure for a second human topoisomerase III gene. This novel gene expresses three alternatively spliced transcripts, which encode gene products different in the putative DNA-binding C-termini. The largest gene product of the novel topoisomerase III was expressed and shown to interact with SGS1 protein and partially rescue the slow growth defect of a yeast topoisomerase III mutant. The presence of more than one human topoisomerase III is reminiscent of mammalian topoisomerase II, which has two genetically distinct isoforms with different expression patterns and probably different functions in mammalian cells.

INTRODUCTION

DNA topoisomerase III belongs to the type IA subfamily of topoisomerases, which catalyze the removal of negative DNA supercoils by cleaving single-strand DNA and forming a 5'-phosphotyrosine intermediate for the passage of another DNA strand. Type IA topoisomerases include yeast topoisomerase III, Escherichia coli topoisomerase I and topoisomerase III, and reverse gyrase from the thermophilic archa and bacteria (reviewed in 1,2). Deletion of the topoisomerase III gene (TOP3) in yeast displays phenotypes of slow growth, hyper-recombination between repetitive DNA elements and inability to sporulate (3). Cells that lack topoisomerase III also have shortened telomeres and a much higher sequence fluidity in the subtelomeric regions (4). Unlike top1 and top2 yeast mutants that have elevated levels of recombination only in the ribosomal DNA tandem array (5), the top3 mutant also exhibits elevated recombination between unlinked genes (6), suggesting that topoisomerase III may have a more significant role in maintaining genomic stability.

The effects of TOP3 null mutation on recombination are dependent on the presence of intact DNA repair genes RAD1 and RAD52 (6,7). RAD1 is the yeast homolog of the human excision repair gene ERCC4/XP-F; a DNA repair endonuclease defective in xeroderma pigmentosum group F individuals (8). RAD52 is involved in gene conversion and repair of double-strand breaks by homologous recombination (9). Although the exact mechanism is still largely unknown, both RAD1 and RAD52 proteins are likely involved in the processing of DNA lesions that accumulate in cells lacking topoisomerase III activity. For example, it is postulated that failure to remove negative superhelicity due to the absence of topoisomerase III may create single-stranded regions that promote pairing, an initial step in recombination (3). RAD52 protein has been shown to bind single-stranded DNA and stimulate DNA strand exchange by RAD51 protein, an E.coli RecA homolog, in recombination and DNA repair (10–12).

The yeast SGS1 (slow growth suppressor) protein, a member of the DNA helicase family of proteins which includes the human Bloom’s syndrome (13) and Werner’s syndrome gene products (14), was found to interact physically with the yeast topoisomerase III protein (15). Evolutionary conservation of this interaction is demonstrated by the discovery of reverse gyrase from hyperthermophiles, in which a type I DNA topoisomerase and a helicase-like domain are fused in a single polypeptide (16). Functionally, mutations of SGS1 can suppress both the growth defect and increased genomic instability of top3 mutants (15). Mutations of SGS1 alone can cause a shortened lifespan and aging-induced sterility in yeast mother cells and redistribution of the SIR3 silencing protein complex from telomeres to the nucleolus (17). To this end, the phenotypes of sgs1 mutations are very similar to the phenotypes of mutations in the human Werner’s syndrome gene, which are associated with premature aging and shortened lifespan (14). It is likely that topoisomerase III may function as a complex with the SGS1 family proteins to maintain genome stability and regulate cellular aging (18,19).

A human cDNA (hTOP3α) encoding a protein (topoisomerase IIIα) homologous to yeast topoisomerase III has been identified and located at 17p11.2–12 by Hanai et al. (20). Overexpression of a truncated form or an antisense construct of hTOP3α was found to inhibit spontaneous and radiation-induced apoptosis in human ataxia telangiectasia (AT) fibroblasts, suggesting that a recombinogenic topoisomerase III may be responsible for the hyper-recombination phenotype of AT cells.

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Human topoisomerase IIIα has high amino acid sequence homology to yeast topoisomerase III (20). However, a closer examination of the overall organization of the topoisomerase IIIα sequence suggests that the C-terminal sequence is more similar to the E. coli topoisomerase I sequence than the yeast topoisomerase III sequence. We rationalized that there may exist another human topⅢ gene and have conducted a search for the new gene, which resulted in the discovery of a topoisomerase IIIβ gene (hTOP3β), the second human topoisomerase III.

MATERIALS AND METHODS

Cloning of hTOP3β cDNA

The cDNA library was constructed from RNA obtained from human ovarian surface epithelial primary cultures with the Marathon cDNA amplification kit (Clontech). The cDNAs thus constructed have Marathon cDNA adaptors ligated to both ends. The hTOP3β cDNA was directly amplified from the Marathon cDNA library according to the manufacturer’s instructions. The first round of PCR was performed with the gene-specific primer 5'-CATTTGCCAGTGCCCCGAAACTGGAG-3' and adaptor primer 1 from the kit. The second round of PCR was performed with the nested gene-specific primer 5'-GGACCGAGACAG-TGAAGACTGTCG-3' and adaptor primer 2 of the kit. The PCR fragment was cloned into TA cloning vector pCR2.1 (Invitrogen). DNA sequencing was performed using the T7 Sequenase Quick-Denature plasmid sequencing kit (Amersham Life Science). Sequence analyses and sequence alignment were performed with MacVector and Lasergene Navigator sequence analysis software.

Northern blot analysis

The multiple-tissue northern blot membranes were purchased from Clontech and the hybridization conditions were according to the manufacturer’s instructions. The 2.8 kb hTOP3β cDNA was labeled using the T7 QuickPrime Kit (Pharmacia) and [α-32P]dCTP and was employed for the detection of hTOP3β expression. The membranes were then stripped and rehybridized with the hTOP3α cDNA probe, a gift from Dr James Wang. To estimate the RNA loading, the same membranes were also hybridized with a 0.6 kb GAPDH probe, obtained by PCR with primers purchased from Continental Laboratory Products, Inc.

5'- and 3'-RACE

The RACE reactions were performed with adaptor primer 1 and gene-specific primers according to the manual of the Marathon cDNA amplification kit (Clontech). The gene-specific primer for the 5'-RACE was 5'-AAAGTTTCTCAGGATGGGTGTTG-3', while the primer for the 3'-RACE was 5'-AGAGACCCACCAC-TACCCGAG-3'. The purified PCR products were then cloned into TA cloning vector pCR2.1 (Invitrogen) and sequenced.

Yeast two-hybrid assay

PCR was performed with the 2.8 kb hTOP3β cDNA as template, using the Expand™ high fidelity PCR system (Boehringer Mannheim) and 5'-AACGAAATTC(EcoRI)ATGAGAAGACTGTGC-TCATGGTT-3' and 5'-CTCGGATCC(BamHI)TCATCATACAA-AGTGGCGGC-3' as forward and reverse primers, respectively. The amplified fragment was ligated as an EcoRI–BamHI fragment into the yeast GAL4 DNA-binding domain vector pGBT9 (Clontech). The coding sequence for the first 511 amino acid residues of SGS1, which has been shown to interact with yeast topoisomerase III (15), was amplified from yeast DNA by PCR using the primers 5'-AGGCGGTAAATTGTTGACG-3' and 5'-TATTAAATGCTCCAGCATTGTA-3' and cloned into the pCR2.1 vector. To clone into the yeast-two-hybrid vector, the SGS1 DNA was amplified by the Expand™ high fidelity PCR system using 5'-ATCCCGGGG(Smal)AAATGGAGAAGCAT-GCTA-3' and 5'-TCGCTCGAG(XhoI)ATGCTCAGCCT-ATTGTAG-3' as primers. The fragment was ligated to the Smal/SalI-digested GAL4 activation domain vector pGAD424 (Clontech) as a Smal–XhoI insert. These constructs and the negative control plasmid, pGAD53m (Clontech), were used to transform the Y190 yeast strain either singly or in combination, and were selected for the presence of plasmids by the respective selection media according to the Clontech manual. Isolated colonies were assayed for reporter activity by growth on histidine drop-out minimal medium.

Immunoprecipitation assay

The 0.8 kb DNA fragment encoding a FLAG tag fused to the N-terminus of topoisomerase IIIβ was synthesized by PCR from the 2.8 kb cDNA using the primers 5'-GCCATGGAACAAAGAC-GACGATGACAAAGATGAGACTGTGC-3' and 5'-AGGA-GAGATC(Tαtg)G-3' as primers. The fragment was first cloned into the pCR2.1 vector and then used to replace the 5'-end of the 2.8 kb cDNA in pBlueScript vector as a SpeI–BglII fragment. The whole FLAG tag-linked hTOP3β cDNA was excised from the pBlueScript vector by SpeI/XhoI digestion, blunt-ended at the SpeI site and subsequently cloned into the EcoRV/XhoI-digested mammalian expression vector pcDNA3 (Invitrogen). The HA-tagged SGS1 cDNA was synthesized from the pCR2.1-SGS1 plasmid by PCR with the primers 5'-GCCATGTA-CCCTACGACTGCCCAGACATCGGTAAGGAAACCTGACCG-CTAC-3' and 5'-TCGCTCGAG(XhoI)ATGCTCAGCCT-ATTTGTAG-3', cloned into the pCR2.1 vector. The cDNA was excised from the pCR2.1 vector and cloned into the mammalian expression vector pcDNA3 as an EcoRV–XhoI fragment. Both constructs were co-transfected into COS7 cells using the LipofectA-MINE Plus reagent (Gibco BRL). Thirty-six hours after transfection, cells were lysed in 1 ml of co-immunoprecipitation buffer (20 mM Tris, pH 7.4, 150 mM NaCl, 1 mM MgCl2, 1% NP-40, 10% glycerol, 1 mM Na3VO4, 1 mM NaF, 1 mM ZnCl2, 10 mM tetrasodium pyrophosphate, 1 mM PMSF and 4 µg/ml each of aprotinin, leupeptin and pepstatin). Lysates were cleared by centrifugation and incubated overnight with the precipitating anti-HA antibody (Santa Cruz Biotechnology) at 4°C. Protein G–Sepharose beads (Gibco BRL) were added and further incubated for 2 h at 4°C. Beads were collected and washed three times with washing buffer (20 mM HEPES, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 1 mM Na3VO4, 1 mM NaF, 10 mM β-glycerophosphate, 5 mM tetrasodium pyrophosphate, 1 mM PMSF and 4 µg/ml each of aprotinin, leupeptin and pepstatin). Lysates were cleared by centrifugation and incubated overnight with the precipitating anti-HA antibody (Santa Cruz Biotechnology) at 4°C. Protein G–Sepharose beads (Gibco BRL) were added and further incubated for 2 h at 4°C. Beads were collected and washed three times with washing buffer (20 mM HEPES, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 1 mM Na3VO4, 1 mM NaF, 10 mM β-glycerophosphate, 5 mM tetrasodium pyrophosphate, 1 mM PMSF and 4 µg/ml each of aprotinin, leupeptin and pepstatin). Bound proteins were eluted with Laemmli loading buffer and separated by 10% SDS–PAGE and western blotted with anti-FLAG antibody (Sigma).
Figure 1. Alignment of the amino acid sequences for human topoisomerase IIIβ, topoisomerase IIIα, yeast topoisomerase III and E.coli type IA topoisomerases. Identical residues and conservative changes across the sequences are boxed. ‡, the putative active site tyrosine; ●, the cysteines at the C-terminal segment of the human topoisomerase IIIβ sequence (the first five marked cysteines are conserved in human topoisomerase IIIα and E.coli topoisomerase I); †, the position of intron 17 after codon 702 of the topoisomerase IIIβ sequence. Alternative splicing at this position gives rise to truncated gene products which omit the sequence after codon 702.

Expression of hTOP3β in yeast and growth rate determination

The FLAG tag-linked hTOP3β cDNA SpeI–XhoI fragment was cloned into a 2 µm-based inducible yeast expression vector p423 GALL (22), a kind gift from Dr Michael Stanbrough. The resulting plasmid was transformed into the Δtop3– yeast strain U568 (see below) and transformants were selected for growth on histidine drop-out minimal medium and characterized by western blot analysis using anti-FLAG antibody. To partially purify the protein, the yeast strain harboring the FLAG-hTOP3β plasmid was grown overnight in 100 ml of histidine drop-out minimal medium supplemented with 2% glucose and then shifted to medium supplemented with 2% galactose and 1% raffinose as carbon source for 5 h. Cells were pelleted and lysed with glass beads according to the standard protocol (23). The lysate was incubated with anti-FLAG M2 affinity gel resin (Sigma), washed with Tris-buffered saline and eluted by competition with FLAG peptide, according to the Sigma protocol. For growth rate determination, the isogenic wild-type yeast strain W303-1A (MATα ade2-1 can1-100 his3-11, 15 leu2-3, 112 trp1-1 ura3-1) and yeast strains U568, which contains a TOP3 deletion (MATα SUP4-o::URA3 top3-3Δ::LEU2), W645-7B, which contains an SGS1 mutation (MATα SUP4-o::URA3 sgs1-1Δ::LEU2), and J464, which contains TOP3/SGS1 double mutations (MATα SUP4-o::URA3 sgs1-1Δ::HIS3), have been described elsewhere (15) and were kindly provided by Dr Rodney Rothstein. These yeast strains and the hTOP3β-transformed U568 strains were counted and serial dilutions were prepared. They were spotted onto YPD agar and agar medium containing galactose, respectively. The
sizes of the colonies were observed after 2 days growth. After the experiment, colonies of the hTOP3β-transformed strains were inoculated for growth in histidine drop-out minimal medium to ensure that the expression plasmid has not been lost during growth on YPD medium.

RESULTS

Molecular cloning of human topoisomerase IIIβ cDNA

Segments of the yeast topoisomerase III amino acid sequence were used to search homologous open reading frame sequences in the GenBank Expressed Sequence Tag database using the Blast algorithm (24). A 462 nt entry (H30621) was identified to code for a polypeptide of 104 amino acids with a consensus translational initiation signal. Immediately following the initiation methionine residue, the stretch Val-Leu-Met-Val-Ala-Glu-Lys-Pro-Ser-Leu-Ala contains a sequence which has six out of seven residues identical to the most conserved N-terminal sequence Val-Leu-X-Val-Ala-Glu-Lys among all the type IA DNA topoisomerases. In addition, the Pro-Ser-Leu-Ala sequence is identical to that of the E.coli topoisomerase III sequence (the sequences mentioned here can be recognized in the sequence alignment of all type IA topoisomerase sequences shown in Fig. 1). It appears that the H30621 sequence is the 5'-end of a putative TOP3 cDNA sequence.

PCR was performed using two gene-specific oligonucleotide primers based on this region of the H30621 sequence and adaptor primers to directly amplify possible cDNA fragments from a Marathon cDNA library prepared from primary ovarian epithelial cell RNA, as described in Materials and Methods. A poly(A)-tailed 2.8 kb fragment was specifically amplified from the library. Sequencing of this fragment revealed an open reading frame of 862 amino acids. Alignment of the amino acid sequence with those of human topoisomerase IIIα, yeast topoisomerase III and both sequences of E.coli type IA topoisomerases have indicated extensive homology among the sequences (Fig. 1). Based on the significant sequence homology to human topoisomerase IIIα, the gene product for this novel sequence is named human DNA topoisomerase IIIβ. Tyr336 of the topoisomerase IIIβ sequence, as with the other corresponding tyrosine residues from other sequences, aligned to Tyr319 of E.coli topoisomerase I, which has been shown to be the active site residue in the breakage and rejoicing of DNA (25). For the region present in all eukaryotic sequences (topoisomerase IIIα amino acids 2–609), human topoisomerase IIIβ has the highest percentage of identical residues and conservative changes to yeast topoisomerase III (55%) than to topoisomerase IIIα (48.5%). The percentage of identical residues and conservative changes between topoisomerase IIIβ and yeast topoisomerase III is 45%. The C-terminal parts of all the sequences show significant divergence. The C-terminal parts of yeast topoisomerase III and E.coli topoisomerase III are shorter, whereas the mammalian enzymes and E.coli topoisomerase I share a similar overall organization and contain unusually high contents of positively charged amino acids, suggesting that the human enzymes might have adopted similar structural features to the C-terminus of E.coli topoisomerase I. Tse-Dinh et al. have reported that the C-terminal segment of E.coli topoisomerase I protein contains three ‘tetracycisteine motifs’, which bind Zn(II) atoms and are required for DNA relaxation activity (26). The C-terminal segment of the human topoisomerase IIIα sequence has six cysteines which match the N-terminal proximal six cysteines in the tetracycisteine motif of E.coli topoisomerase I (20). Five of these cysteine residues are conserved in the topoisomerase IIIβ sequence. In addition, the extreme C-terminus of the topoisomerase IIIβ sequence contains several cysteine residues at non-random positions in the form Cys-X2-Cys-X18–25-Cys-X2-Cys, which are distinct from the ‘tetracycisteine motifs’ of E.coli topoisomerase I, but resemble the C4-type zinc finger motifs of several nucleic acid-binding proteins (27–29). Any probable DNA-binding function of these zinc finger-like motifs awaits further characterization.

Tissue-specific expression of multiple hTOP3β transcripts

To analyze the tissue distribution of multiple hTOP3β expression, a multiple tissue northern blot analysis was performed (Fig. 2A). Two transcripts with respective sizes of 4 and 2.8 kb were observed. Although the intensities of the GAPDH signals indicate some variation in the RNA loading, the relative expression of the two transcripts can still be compared within the same tissue. Relative to the 4 kb transcript, expression of the 2.8 kb transcript was more prominent in testis, heart and skeletal muscle. On the other hand, relative expression of the 4 kb transcript was high in thymus, kidney and pancreas. Northern blot analysis using a hTOP3α probe on the same blot revealed a different expression pattern. Three transcript signals with sizes of 4, 6 and 7 kb, respectively, were observed (Fig. 2B). Relative expression of the 4 kb transcript was particularly high in testis, heart, skeletal muscle and pancreas.
Figure 3. Organization of the human TOP3β gene. (A) A scheme for the 5′- and 3′-RACE of hTOP3β is shown on the left. A poly(A)-tailed Marathon cDNA is shown here with the active site tyrosine indicated by Y. The filled boxes at both ends are the adaptors added to the cDNA during the construction of the library. The arrows indicate the positions of the oligonucleotide primers used for the RACE reactions. On the right are the results of the 5′- and 3′-RACE. The DNA marker used was a λ DNA Bst/EII digest ladder (New England Biolabs). The amplified cDNA fragments are indicated by arrows. (B) Exon–intron structure of the human TOP3β gene. On top is the scale for the genomic DNA, with the exon–intron structure for the transcript shown below. For the transcripts, the horizontal lines represent the intronic sequences, whereas the open boxes represent the non-coding exons and the filled boxes represent the coding exons.

To delineate the origins of the 4 and 2.8 kb transcripts of hTOP3β, 5′- and 3′-directing primers based on the hTOP3β sequence at the most conserved tyrosine active site region were used in 5′- and 3′-RACE together with an adaptor primer using the Marathon cDNA library (Fig. 3). The 5′-RACE reaction gave rise to a 1.2 kb fragment, which after sequencing was confirmed to have the same 5′-sequence of the 2.8 kb hTOP3β cDNA. The 3′-RACE reaction gave rise to two amplified fragments with sizes of 2.6 and 1.6 kb, respectively. Ethidium bromide staining of the 2.6 kb fragment was much weaker than that of the 1.6 kb fragment, which may reflect the expression levels of the two transcripts in ovarian surface epithelial cells. To minimize the chance of creating mutations when using Taq polymerase, we did not re-amplify the PCR fragments and the fragments were cloned into TA cloning vector. Sequencing of the 1.6 kb fragment revealed the same poly(A)-tailed 3′-sequence of the 2.8 kb hTOP3β cDNA and, together with the 1.2 kb 5′-RACE product, they should represent the 2.8 kb hTOP3β cDNA transcript. Cloning of the 2.6 kb fragment gave rise to clones with the expected 2.6 kb inserts and one clone with a 2.3 kb insert. Sequencing of the inserts from the 5′-ends gave the same hTOP3β cDNA sequence starting from the 3′-RACE primer, but the 3′-end sequences of the 2.3 and 2.6 kb inserts are different. Together with the 1.2 kb 5′-RACE segment, the composite transcript sizes will be 3.5 and 3.8 kb, respectively. The identities of these two inserts are described below.

**Organization of the hTOP3β gene**

Analysis of the hTOP3β cDNA for homologous sequences in the GenBank database identified an entry of a 1 Mb genomic nucleotide sequence of the human immunoglobulin λ locus located at 22q11 (30). We have determined all the hTOP3β cDNA and 3′-RACE sequences within the published genomic sequence. The fact that sequences of our PCR products conformed to the published genomic sequence and all the exon–intron boundaries are flanked by consensus donor and acceptor sequences support the authenticity of our cDNA PCR products. The organization of the hTOP3β gene and the predicted mRNA species are presented in Figure 3B. The hTOP3β gene spans a genomic region of ~29 kb. The predicted 2.8, 3.5 and 3.8 kb composite transcripts have the same 5′-end sequence, with the non-coding first exon being separated from the second exon by a 6.9 kb intron. The 2.8 kb hTOP3β cDNA transcript comprises the first 18 exons and represents the 2.8 kb signal detected in the northern blot analysis. The 3.8 kb transcript is composed of the same 18 exons. It contains, however, the unspliced 896 bp intron 17 and may represent the 4 kb signal detected in northern blot analysis. The predicted 3.5 kb transcript contains the first 17 exons and an alternatively spliced 1238 bp exon 19. Since only one 2.3 kb clone was obtained in the 3′-RACE, the 3.5 kb transcript may not be a predominant species for hTOP3β transcription. Interestingly, both 3.5 and 3.8 kb hTOP3β transcripts produce gene products that have C-terminal truncations after codon 702 and lack the C4-type zinc finger-like motifs (Figs 1 and 4). After codon 702, the 3.5 kb gene product has a unique extension of five amino acid residues, while the 3.8 kb gene product has 28 additional amino acids (Fig. 4). Differential expression of the hTOP3β transcripts with different 3′-end sequences in different tissues suggests that the C-terminal portions of the various gene products determine their tissue-specific functions.
The largest gene product of *hTOP3β* can interact with yeast SGS1 protein and partially complement the yeast *top3* mutant

The newly identified *hTOP3β* gene may have a similar function to yeast *TOP3*. It has been shown that yeast topoisomerase III protein functionally and physically interacts with the yeast DNA helicase SGS1 protein (15). To investigate if the *hTOP3β* gene product interacts with DNA helicase, a yeast two-hybrid assay was performed. The 2.8 kb cDNA encoding the largest gene product of *hTOP3β* was subcloned in-frame into the GAL4 DNA-binding domain vector pGBT9. Likewise, *SGS1* cDNA was subcloned in-frame into the GAL4 activation domain vector pGAD424. Both constructs were transformed into the yeast strain Y190 and the results of the assay demonstrate that topoisomerase IIIβ interacts specifically with SGS1 protein to activate the HIS3 reporter for growth on histidine drop-out minimal medium (Fig. 5A). To confirm the two-hybrid data, DNA sequences encoding the epitope tags FLAG and HA were fused to *hTOP3β* cDNA and *SGS1* cDNA, respectively, in a mammalian expression vector. The resulting plasmids were co-transfected into COS cells and an immunoprecipitation assay was performed with the cell lysates (Fig. 5B). The results show that the FLAG-tagged topoisomerase IIIβ protein was co-immunoprecipitated with HA-tagged SGS1 protein by anti-HA antibody. In conjunction with the two-hybrid data, the results of the co-immunoprecipitation assay demonstrate that topoisomerase IIIβ can interact with the yeast helicase SGS1 protein.

To test the function of *hTOP3β* on cell growth, the FLAG-tagged *hTOP3β* cDNA was cloned into an inducible yeast expression vector under a GAL1 promoter and transformed into the *top3* deletion mutant U568. Recombinant topoisomerase IIIβ protein was produced and purified to near homogeneity by anti-FLAG affinity chromatography (Fig. 6A). Two smaller bands detected in the western blot analysis are probably degradation products of the full-length protein. The growth rates of *hTOP3β* cDNA-transformed strains were compared with the growth rates of U568 and other isogenic yeast mutants in Figure 6B. While mock-transformed strains did not show any change in growth rate (data not shown), the *hTOP3β* cDNA-transformed strains demonstrate a partial suppression of the slow growth phenotype of the parental *top3* deletion mutation upon induction by galactose. There was also a slight increase in growth of the *hTOP3β*-harboring strains growing in glucose-supplemented media, probably because the GAL1 promoter used in this study was not tightly repressed by glucose. Interestingly, the growth pattern of the *hTOP3β*-harboring strains was very similar to that of the *sgs1* mutant W645-7B. Taken together, our data suggest that topoisomerase IIIβ can interact with yeast DNA helicase and can partially complement the growth defect of a yeast *top3* deletion mutation.

![Figure 5](image_url)

**Figure 5.** Interaction of human topoisomerase IIIβ with the yeast SGS1 protein. (A) Yeast two-hybrid assay. Clones obtained by transformation of various DNA constructs into the yeast strain Y190 were streaked on histidine drop-out minimal medium. The constructs transformed were: 1, pGBT9-*hTOP3β*; 2, pGAD424-*SOS1*; 3, negative control plasmid pGAD53m; 4, a clone obtained by co-transformation of pGBT9-*hTOP3β* and pGAD424-*SOS1*; 5, a second clone obtained by co-transformation of pGBT9-*hTOP3β* and pGAD424-*SOS1*; 6, co-transformation of pGBT9-*hTOP3β* and negative control pGAD53m. (B) Co-immunoprecipitation assay. Shown is the western blot analysis using anti-FLAG antibody. The protein molecular weight ladders are shown on the left. The first two lanes are transfected and non-transfected lysates, respectively. The third and fourth lanes are products immunoprecipitated by anti-HA antibody of non-transfected and transfected lysates, respectively. The position of the FLAG-tagged topoisomerase IIIβ signals detected by the anti-FLAG antibody is marked by an arrow. The other two smaller signals are the heavy chain and light chain of the antibody.
DISCUSSION

We have cloned and characterized a second member of human topoisomerase III, hTOP3β. Unlike single TOP3 genes in prokaryotic and lower eukaryotic cells, the presence of more than one TOP3 gene in human and probably also in other mammalian cells is reminiscent of mammalian topoisomerase II, which has two isoforms derived from different genetic loci (31). Both mammalian topoisomerase II isoforms have similar catalytic activity and are the targets of many antineoplastic agents (32).

However, their expression patterns are different in various tissue types and across the cell cycle (33–35). The expression of topoisomerase IIα is associated with cell proliferation and the protein is associated with chromosomes during mitosis (33,36). In contrast, the expression of topoisomerase IIβ is unaltered across the cell cycle and the protein diffuses into the cytosol during mitosis (33,36).

Our characterization of hTOP3β has delineated three different cDNAs which can encode three gene products differing at the C-termini. Three different transcript sizes were detected in northern blot analysis of hTOP3α, which may imply the presence of three gene products of hTOP3α. It is intriguing that there are so many variant forms of human topoisomerase III, as there are many human homologs of SGS1 (18). The differential expression patterns of the topoisomerase III variant forms may suggest that they have different physiological functions, especially given the fact that while E.coli TOP3 and yeast TOP3 are dispensable (3,37), targeted disruption of the mouse TOP3α gene is lethal (38), suggesting that a particular function of topoisomerase IIIα cannot be substituted by topoisomerase IIIβ or other topoisomerases.

The different variant forms of topoisomerase III may have distinct catalytic activities towards different DNA substrates. This postulation is based on the finding that the major differences in all type IA topoisomerases, including the various alternatively spliced forms of topoisomerase IIIβ, are in the C-termini (Figs 1 and 4). The functions of C-terminal segments of type IA topoisomerase proteins have been extensively studied in the E.coli enzymes. In E.coli, topoisomerases I and III share very striking amino acid sequence homology for the first 600 amino acids of both polypeptides, but they demonstrate strikingly different enzymatic activities (39). Topoisomerase III has minimal activity in the relaxation of negatively supercoiled DNA. However, it exhibits very potent decatenation activity in resolving DNA replication intermediates (40). Unlike topoisomerase III, E.coli topoisomerase I is very efficient in the relaxation of supercoiled DNA, but is incapable of catalyzing decatenation (39). Deletion of the Lys/Arg-rich C-terminus of E.coli topoisomerase III resulted in a drastic reduction in the ability of the enzyme to bind to single-stranded DNA and RNA substrates, without a loss of cleavage site specificity (41). Fusion of the C-terminus of E.coli topoisomerase I to the truncated topoisomerase III molecule reversed the relaxation
activity, but not the ability to resolve DNA replication intermediates (42). Therefore, the C-termini of the type IA topoisomerases may be intimately involved in determining the catalytic properties and probably also the physiological functions of the enzymes. Recently, a plasmid-borne traE gene product was shown to have extensive protein sequence similarity to \textit{E.coli} topoisomerase III. The traE protein, however, contains in the C-terminus two potential zinc finger motifs similar to that of topoisomerase I and suggests specific activity one-third that of topoisomerase III (43).

Thus, it appears that there are multiple species of topoisomerase III homologs and tissue-specific targeting of individual \textit{TOP3} gene function will facilitate the understanding of the new members of this expanding subclass of topoisomerases.

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