Human cells express two differentially spliced forms of topoisomerase II β mRNA

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ABSTRACT

Screening of a human B-cell cDNA library with a topoisomerase II β gene-specific probe revealed the presence of two distinct forms of topoisomerase II β cDNA. One form (designated topoisomerase II β -1), representing the majority of the clones, would encode the topoisomerase II β amino acid sequence reported recently [Jenkins, J.R. *et al.* (1992) *Nucleic Acids Res.*, 20, 5587–5592]. The second form (designated topoisomerase II β -2) would encode a protein containing an additional 5 amino acids inserted after Valine-23 of the topoisomerase II β -1 protein sequence. The topoisomerase II β -1 and β -2 mRNAs were both widely expressed in human cell lines and tissues. Topoisomerase II β -2 mRNA was expressed at a lower level than that of the β -1 form, but the relative expression of the two forms varied in different cell types. Analysis of genomic DNA clones revealed that the two forms of topoisomerase II β mRNA arose via differential splicing. These data indicate that in addition to the closely related topoisomerase II α and β isozymes, there are two forms of topoisomerase II β mRNA widely expressed in human cells.

INTRODUCTION

DNA topoisomerases are essential enzymes in both prokaryotic and eukaryotic cells. There are two classes of topoisomerase enzyme, designated type I and type II, which alter the linking number of supercoiled DNA in steps of one and two, respectively. The type II enzymes catalyse the transient breakage and religation of both strands of a DNA molecule and subsequently change the topology of DNA by passing an intact duplex through the broken DNA molecule (reviewed in 1–3).

Studies with conditional lethal top2 mutants of both fission and budding yeast have revealed an essential role for topoisomerase II in the segregation of newly replicated chromosomes at mitosis (4–7). Topoisomerase II has also been implicated in chromosome condensation, DNA replication, and possibly transcription (8–14). Analysis of yeast mutants also indicated that the mitotic functions of topoisomerase II are performed by a single enzyme.

In contrast, human cells express two structurally similar topoisomerase II isozymes, designated topoisomerase II α and β , which are genetically and biochemically distinct (15,16), being encoded on chromosomes 17q21–22 and 3p24, respectively (17,18).

It has not been possible to assign the many possible roles of topoisomerase II to one or other human isozyme, primarily because of a lack of human cell mutants defective in topoisomerase II function. The strong proliferation-dependence of topoisomerase II α protein expression suggests a cell division-specific role for this isozyme (19). Expression of the β isozyme is not as strongly associated with cell proliferation and this isozyme is present at detectable levels in quiescent cells in culture (19). Comparison of the predicted amino acid sequences of the human isozymes with those of lower eukaryotic topoisomerase II enzymes, indicates that neither the α nor the β isozyme can be considered the generic topoisomerase II protein in human cells (18).

We reported recently the isolation of full length cDNA clones encoding the human topoisomerase II β protein (18). Subsequent sequencing of independent clones revealed the presence of a second form of topoisomerase II β cDNA differing by only 15 base pairs within the coding region of the topoisomerase II β protein. Here, we have characterised this variant form of topoisomerase II β mRNA and show that the two forms of topoisomerase II β mRNA are widely expressed in human cells and appear to arise by differential splicing.

MATERIALS AND METHODS

Isolation of topoisomerase II β cDNAs

cDNAs encoding the topoisomerase II β protein were isolated from a library constructed from normal human B-cell poly (A)⁺ RNA as described previously (18).

DNA sequencing

Double stranded cDNA or genomic DNA clones were sequenced using Sequenase (United States Biochemical Corp). Multiple oligonucleotide primers were synthesised and used to sequence both strands of the DNA.

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PCR amplification of topoisomerase II β cDNA from libraries

Primers were made to regions flanking the 15 base pair insert in the topoisomerase II β -2 cDNA. Sequences of the primers are given below:-

5' primer-5' CGGCGGCAACGGGGCACT 3'
3' primer-5' TCCACTGACCCAATATATGT 3'

The 5' primer included a (CUA)₄ tail at its 5' end, and the 3' primer contained a (CAU)₄ tail for cloning into the pAMP vector and use in the CloneAmp system (Gibco/BRL). PCR products were generated from human cDNA libraries constructed from mRNA isolated from normal B-cells, lymphokine activated killer (LAK) cells or HL60 promyelocytic leukaemia cells. The amplified cDNA was cloned into the pAMP vector, and DNA transformed into *E.coli* TG1 cells. Plasmid DNA from transformed cells was prepared by standard procedures and the nucleotide sequence determined as described above using primers flanking the 15 base pair insert.

RNase protection probe

The RNase protection probe was generated by amplifying a 296 base pair fragment of the topoisomerase II β -2 cDNA using the polymerase chain reaction. The primers used were as follows:

5' - CTGCGGCGCGGGAGCCGGCG (5' primer)
5' - ATCTTGATATAACCTGGCACA (3' primer)

The 5' primer began at nucleotide 18 of the previously published topoisomerase II β cDNA (18,20) and the 3' primer ended at nucleotide 299. The remaining 15 base pairs of the fragment were accounted for by the 15 base pair insert within the topoisomerase II β -2 cDNA (see Results). The amplified product was cloned into the SmaI site of pUC18, excised using KpnI and BamHI, and ligated into pBluescript II (Stratagene), previously digested with KpnI and BamHI.

To generate an antisense RNA probe, the Bluescript plasmid containing the topoisomerase II β cDNA fragment was digested with BamHI and radiolabelled transcripts synthesised *in vitro* using T3 RNA polymerase and [α -³²P]-CTP by the method outlined in Ausubel *et al.* (21). Samples were equalised for loading on gels by spectrophotometric measurement of total RNA concentration. Each gel lane was loaded with 50 μ g of total RNA. Conditions for annealing and digestion of topoisomerase II β mRNAs were as described previously (18).

Analysis of genomic DNA encoding topoisomerase II β protein

DNA from a recombinant λ phage carrying a portion of the human topoisomerase II β gene (kindly provided by Dr P.D'Arpa, Johns Hopkins University, Baltimore, USA) was digested with HindIII and a 1.8 kb fragment isolated from a 1% agarose gel using GeneClean (Stratagene Scientific). This fragment was identified as carrying a region close to the 5' end of the topoisomerase II β gene by hybridisation with a PCR-derived cDNA fragment generated using primers (below) representing base pairs 1-18 and 210-230 of the coding region of the topoisomerase II β -1 cDNA (18,20).

5' - ATGGCCAAGTCGGGTGGC
5' - TCCACTGACCCAATATATTGT

The genomic DNA fragment was subcloned into HindIII-digested pBluescript and sequenced as described above.

RESULTS**Identification of cDNA clones encoding a variant form of topoisomerase II β protein**

A human B-cell cDNA library in the vector pCDM8 was screened using a PCR-generated fragment of known topoisomerase II β sequence as probe. Several positive clones were isolated and sequenced. Although the majority of the clones had a protein coding region of sequence identical to that of the previously reported topoisomerase II β cDNA (18,20), one clone contained an insertion near the 5' end of the protein coding region. The sequence of this 15 base pair insertion and the amino acids (3 letter code) that it would encode are shown below:

ACT	CTT	TTT	GAT	CAG
Thr	Leu	Phe	Asp	Gln

The additional amino acids would be inserted between residues valine 23 and asparagine 24 (18). The variant form of the topoisomerase II β cDNA containing the 15 base pair insertion was designated topoisomerase II β -2 to distinguish it from the previously published form, which will hereafter be termed topoisomerase II β -1.

Analysis of genomic DNA for the topoisomerase II β gene

To assess the mechanism by which the two forms of topoisomerase II β mRNA were generated, analysis of the topoisomerase II β gene was undertaken. A recombinant λ phage carrying a portion of the topoisomerase II β gene was digested with HindIII and a 1.8 kb DNA fragment identified which hybridized to the 5' end of the topoisomerase II β cDNA. This fragment was sequenced on both strands (EMBL accession number X71911). By comparison with the sequences of the topoisomerase II β -2 cDNA and the topoisomerase II β -1 cDNA reported previously (18,20), it was possible to determine the intron/exon structure of this region of the topoisomerase II β gene (Figure 1).

This analysis revealed the presence of two potential splice acceptor sites separated by the 15 base pairs evident in the topoisomerase II β -2 cDNA.

The topoisomerase II β -2 protein sequence

The 15 base pair insert present in the topoisomerase II β -2 mRNA would encode 5 amino acid residues (Thr-Leu-Phe-Asp-Gln). As this additional short amino acid stretch could fulfil one of at least two functions; such as the disruption of an important domain which is functional in the topoisomerase II β -1 protein, or the introduction of key residues conferring an additional function on the topoisomerase II β -2 protein, we scanned the SWISSPROT protein database (release 24.0; 1/93) for similar sequences. The portion of the topoisomerase II β -1 sequence 'interrupted' by the 5 amino acid insert shows significant similarity to a region of the plastocyanin amino acid sequence, with a run of eight conserved residues of which six are identical and two structurally similar (Figure 2). The 5 amino acid insert present in the topoisomerase II β -2 protein exists in the putative *pol* protein encoded by a *Drosophila* transposable genetic element (22). Indeed, the *pol* and topoisomerase II β -2 proteins share a run of six identical amino acids (Figure 2).

Expression of the topoisomerase II β -1 and β -2 mRNAs in human cells

To assess the extent to which the topoisomerase II β -1 and β -2 mRNAs were expressed in human cells of different origin, two

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ccattaaaaaagaaacccatgctgtgtgtccatactcat
tgtctacccttgtaggtattttaaattgattttttgg

ttaaattttg ACT CTT TTT GAT CAG N N A 26
          ACT CTT TTT GAT CAG AAC AAT GCT

A K K E E S E T A N K 37
GCA AAA AAA GAA GAG TCA GAA ACT GCC AAC AAA

N D S S K K L S V E R 48
AAT GAT TCT TCA AAG AAG TTG TCT GTT GAG AGA

V Y Q K K T Q L E H I 59
GTG TAT CAG AAG AAG ACA CAA CTT GAA CAC ATT

L L R P D T Y I G S V 70
CTT CTT CGT CCT GAT ACA TAT ATT GGG TCA GTG

E P L T Q 75
GAG CCA TTG ACG CAG gtaattattgctaaaattgatt
gaaattaggagatgtgcatctatatttactttttaatatgtt
gataaaattattgttaattacttgccttggaaattt

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Figure 1. Nucleotide sequence of a portion of the human topoisomerase II β gene. Introns are shown in lower case letters and the exon in upper case letters. The exon sequence is arranged in coding triplets with the corresponding amino acid written above each codon in the single letter code. The proposed alternately used splice acceptor sequences (AG) are in bold type and underlined. The numbering of the amino acids (shown on the right) corresponds to the topo II β -1 protein sequence reported previously (18). The 15 base pairs specific for the topoisomerase II β -2 mRNA are boxed. The start of the exon common to both forms of topoisomerase II β cDNA beginning after the 15 base pairs corresponds to nucleotide 70 of the previously reported topoisomerase II β -1 cDNA sequence (20).

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Topo II  $\beta$ -1      A L T W V N N A A K
                . * * * * *
Plastocyanin    T V T W V N N A G F

Topo II  $\beta$ -2      W V T L F D Q N
                * * * * *
Retroviral pol  T V T L F D Q T

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Figure 2. Comparison of the topoisomerase II β -1 and β -2 deduced amino acid sequences, in the region of the inserted five residues in the β -2 form, with protein sequences in the SWISSPROT database. Identical and similar amino acids are indicated by an asterisk and a dot, respectively. The arrow indicates the position of the insertion.

approaches were undertaken. Firstly, the polymerase chain reaction was used to amplify a 171 base pair (or 186 in the case of the β -2 form) region of topoisomerase II β cDNA from a series of cDNA libraries and the amplified fragments sequenced. Thirty clones carrying topoisomerase II β cDNA were isolated from either a LAK cell or an HL60 cell cDNA library. Screening by PCR revealed that one clone from each library contained the 15 base pair insertion (confirmed by DNA sequencing), while the remainder lacked the insertion (data not shown).

In the second approach, a region of the topoisomerase II β -2 cDNA encompassing the 15 base pair insert was cloned into pBluescript and an antisense RNA probe generated *in vitro* using T3 polymerase and [α - 32 P]-CTP. This probe was used in the RNase protection assay to quantify the level of expression of the topoisomerase II β -1 and β -2 mRNAs.

As the single topoisomerase II β probe used in this experiment spans the region containing the 15 base pair insertion, it can

Figure 3. RNase protection analysis of topoisomerase II β -1 and β -2 mRNA levels in human cells. Tracks: 1, tRNA (negative control); 2, biopsy from a kidney tumour; 3, biopsy from a head and neck tumour; 4, embryonal teratoma cell line; 5, placenta; 6, promonocytic leukaemia cell line; 7, immortalised normal breast cell line; 8, cervical carcinoma cell line. The positions of the topoisomerase II β -1 and β -2 protected fragments are indicated on the right. The sizes (in base pairs) of molecular weight standards (lane M) are indicated on the left. Densitometric scanning of autoradiograms to quantify expression levels was performed when each signal was within the linear range.

Table 1.

Cell Type	Topo II β -1 mRNA level ^a	Topo II β -2 mRNA level ^a	Ratio of β -1 and β -2 ^b
Kidney tumour	19	0.90	21
Head and neck tumour	38	1.0	37
Teratoma	14	2.2	6.2
Placenta	14	0.67	22
Promonocytic leukaemia	89	4.5	18
Immortalized normal breast	26	1.1	24
Cervical carcinoma	69	6.6	11

^a Integrated optical density from scanning of autoradiograms.

^b Relative topo II β -1 and β -2 mRNA levels based on integrated optical density values taken from a single representative experiment.

hybridize to both forms of mRNA, but will give rise to RNA fragments, protected from RNase digestion, of different sizes. The fragment protected from digestion following annealing to topoisomerase II β -2 mRNA would be expected to be full length at 296 base pairs. In contrast, the probe would be endonucleolytically cleaved at the site of the 15 base pair insert when annealed to the topoisomerase II β -1 mRNA, producing a protected fragment of 228 base pairs (and a 53 base pair fragment not visible on the low percentage acrylamide gels used to resolve the 296 and 228 base pair fragments).

RNase protection analysis was performed on total cellular RNA from 4 cell lines, 2 tumour biopsies and 1 normal tissue (placenta), all of human origin. Figure 3 shows that protected fragments of the size expected for topoisomerase II β -1 and β -2 mRNAs were present in all of the samples tested.

In four cases, a greater than 20-fold higher level of topoisomerase II β -1 mRNA than of topoisomerase II β -2 mRNA was observed (Table 1). In the teratoma cell line the difference in the level of expression of the two mRNAs was less pronounced, but even in this case the ratio of β -1 to β -2 mRNA was approximately 6.

DISCUSSION

The screening of a cDNA library for clones encoding topoisomerase II β protein revealed the presence of two forms of cDNA differing only by the presence or absence of a 15 base pair stretch located near to the 5' end of the coding sequence. We have previously reported (18,20) the sequence of the form lacking the 15 base pair insert (herein designated topoisomerase II β -1). Here, we have shown that a variant form of the topoisomerase II cDNA (designated topoisomerase II β -2), which includes the 15 base pair insert, is expressed widely in human cells. This result was confirmed by both PCR amplification from existing cDNA libraries and analysis of mRNA expression in several different human cell types.

The protein encoded by the topoisomerase II β -2 mRNA would include an additional 5 amino acids (TLFDQ—in the single letter amino acid code) inserted following Valine-23 of the previously published protein sequence (18). Our current work has not revealed a role for this additional run of amino acids, although a 6 residue block including the TLFDQ motif is found in a retroviral *pol* protein (22). To determine whether this is coincidental or not requires further analysis.

The region in which the additional 5 amino acids lies is at the extreme N-terminus of the topoisomerase II β protein. Although the N-terminal ATPase domain of type II topoisomerases has been highly conserved throughout evolution, the extreme N-terminal segment where this insertion lies is not conserved. The sequence of this region has diverged not only between the human topoisomerase II β protein and other eukaryotic topoisomerase II enzymes, but also between the human topoisomerase II α and β isozymes (18). It seems unlikely, therefore, that this region plays a direct catalytic function, although modulation of the catalytic activity of the different enzymes, such as by acting as a target for post-translational modification, cannot be discounted. It is also possible that this region plays a role in determining subcellular localisation of the topoisomerase II β protein, or interactions that the protein may have with other cellular proteins. It is also possible that the insertion has no functional significance, as it lies outside the catalytic core of the topoisomerase II β protein.

Immunohistochemical studies of topoisomerase II β protein expression in human cells have provided somewhat confusing data concerning its precise subcellular localisation. A location either exclusively in the nucleolus (23,24), or in both the nucleoplasm and the nucleolus (25) have been seen using different antisera in different cell types. Whether the relative levels of expression of the topoisomerase II β -1 and β -2 forms in any way accounts for the different patterns of staining seen with monoclonal and polyclonal antisera can now be addressed by raising peptide antisera specific for either the topoisomerase II β -1 or the β -2 form.

The reported location of the topoisomerase II β protein in the nucleolus in human cells (23–25) may indicate a role in ribosomal RNA synthesis or in the suppression of rDNA recombination, which is abnormal in yeast top2 mutants (26,27). Whether the topoisomerase II β -2 protein, with its homology to the retroviral *pol* protein (or the topoisomerase II β -1 protein), is involved in these processes is the subject of ongoing studies.

In all cases studied, and in the head and neck tumour sample in particular, the topoisomerase II β -1 mRNA was expressed at a relatively higher level than that of the topoisomerase II β -2 mRNA. Whether these differences in mRNA expression are

reflected in the levels of topoisomerase II β -1 and β -2 proteins can only be ascertained by the use of specific antibodies. Although comparison of the relative levels of two mRNAs from different genes can be problematical using the RNase protection assay, we can in this case be confident about the quantification of levels of topoisomerase II β -1 and β -2 mRNAs in that a single antisense RNA probe was used to detect both forms of message simultaneously.

The two forms of topoisomerase II β mRNA appear to be generated by differential splicing. Sequencing of genomic DNA in the appropriate region of the topoisomerase II β gene revealed the existence of two consensus splice acceptor sites, the alternate use of which would generate the topoisomerase II β -1 and β -2 forms. The finding that the relative expression of the β -1 and β -2 mRNAs varies in different human cell types indicates that the choice of splice site is unlikely to depend solely upon the primary sequence of the unspliced message.

In summary, we have identified two forms of topoisomerase II β mRNA, apparently arising via differential splicing, and shown that both of these forms are widely expressed in human cells of different origin. Future analyses of topoisomerase II β protein function in human cells will have to take account of the existence of these two closely related and widely expressed forms of topoisomerase II β mRNA, which may encode proteins that perform specialized roles in DNA structure or function. We are currently overexpressing the two topoisomerase II β proteins in order to assess their respective roles in DNA metabolism in human cells.

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