Isolation of cDNA clones encoding the β isozyme of human DNA topoisomerase II and localisation of the gene to chromosome 3p24

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ABSTRACT

Topoisomerases catalyse the interconversion of topological isomers of DNA and have key roles in nucleic acid metabolism. Human cells express two distinct type II topoisomerase isozymes, designated topoisomerase II α (170 kDa form) and topoisomerase II β (180 kDa form). We have isolated cDNA clones encoding the β isozyme from a human B-cell library. The proposed coding region for the topoisomerase II β protein is 4,863 nucleotides long and would encode a polypeptide with a calculated M_r of 182,705. The predicted topoisomerase II β protein sequence shows striking similarity (72% identical residues) to that of the human α isozyme, and homology to topoisomerase II proteins from *Drosophila*, yeast and bacteria. Regions of greatest amino acid sequence divergence lie at the extreme N-terminus and over a C-terminal domain comprising ~25% of the total protein. We have quantified the level of topoisomerase II β mRNA in a panel of human tumour cell lines of different origin using an RNase protection assay, and compared the level to that of topoisomerase II α mRNA. Topoisomerase II β mRNA was expressed in haemopoietic, epithelial and fibroblast cell lines, although to different extents, with U937 cells (promonocytic leukaemia) showing a particularly high level. There was no obvious relationship in terms of level of expression between the topoisomerase II α and β genes. We have localised the gene encoding topoisomerase II β protein to chromosome 3p24 in the human genome.

INTRODUCTION

Type II topoisomerases are ubiquitous nuclear enzymes which catalyze changes in the tertiary structure of DNA *via* the introduction of transient double-strand breaks (reviewed in 1,2). Topoisomerase II enzymes in eukaryotic cells play several important roles in chromosome structure and function, such as

relief of torsional stress during DNA replication and transcription (3–6), genetic recombination (7–9), and chromosome condensation (10). Analysis of temperature sensitive *TOP2* mutants of yeast has revealed an essential role for topoisomerase II in cell cycle progression, with mutants incapable of chromosome segregation during mitotic division (11–14). In budding yeast, the topoisomerase II protein is also involved in stabilization of ribosomal DNA *via* suppression of mitotic recombination in the highly repetitive ribosomal RNA gene cluster (15,16). In addition to these catalytic functions, topoisomerase II has been implicated as a structural component of the interphase nucleus, probably linking chromatin to the nuclear matrix or scaffold (17–20).

Studies on yeast *TOP2* mutants indicated that there is only one topoisomerase II enzyme required in lower eukaryotes to carry out essential chromosome functions, such as segregation. In contrast, two distinct isozymes of topoisomerase II have been identified in mammalian cells, which are designated topoisomerase II α (170 kDa form) and topoisomerase II β (180 kDa form), the latter having been discovered only recently (21–24). The β isozyme differs from topoisomerase II α not only in apparent M_r on SDS-PAGE, but also in certain biochemical respects, such as the sequence specificity of binding to DNA (23).

Human topoisomerase II protein is a key target for many active anticancer drugs, such as etoposide, adriamycin, daunorubicin and mitoxantrone (reviewed in 25). These drugs interfere with the breakage/reunion reaction of topoisomerase II trapping the enzyme in a so-called 'cleavable complex', consisting of protein monomers bound covalently to the 5' end of double-strand breaks in DNA (26,27). The pathway by which these protein-associated DNA lesions leads to cell death is poorly characterized. The level of topoisomerase II protein in cells correlates with sensitivity to killing by these drugs, with high protein levels conferring relative sensitivity and low levels resistance (28–32). The catalytic cycle of both of the human topoisomerase II isozymes can be affected by these drugs.

Partial cDNA clones encoding regions of the human topoisomerase II β mRNA have been described previously

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(24,33). Here, we report the isolation of cDNA clones covering the complete coding region of the gene, which we have localised in the human genome to chromosome 3p24. We have also studied the relative expression of mRNAs encoding the α and β isozymes in a panel of human tumour cell lines to analyze the extent which the two isozymes are co-expressed.

MATERIALS AND METHODS

Isolation of topoisomerase II β cDNAs

DNA representing a cDNA library in vector CDM8, constructed from normal human B-cell poly (A)⁺ RNA (D. L. Simmons, unpublished), was digested overnight with 10 units *Sfi*I to linearize the plasmids. The digested DNA was run on a 0.8% agarose gel, and DNA in the size range 9–15 kb excised from the gel and purified using GeneClean II (Stratagene Scientific). The purified DNA was diluted to approximately 5 μ g/ml and self-ligated. The DNA was then phenol/chloroform extracted, ethanol precipitated, resuspended in TE buffer and transformed into *E. coli* MC1061/P3. This 'mini-library' of approximately 50,000 bacteria was plated, transferred to HyBond-N, the bacteria denatured, and the DNA hybridized with a random primed probe of topoisomerase II β cDNA using standard techniques (34). The 0.5kb probe was generated by the polymerase chain reaction (PCR) with the following primers taken from published cDNA sequences.

5'-CACCAGCATTAATTTCTGAT-3' (5' primer)
5'-AGTTCCTCAATCGGAGGAAC-3' (3' primer)

Six positive clones were isolated which were shown by restriction mapping to contain inserts ranging from 2.5 to 5.6 kb. The 5.6 kb clone, designated pt2 β -1 was taken for further study.

Additional clones were obtained from a non-size selected human bone marrow cDNA library (D.L. Simmons, unpublished). Clones were isolated and processed as described above.

DNA sequencing

Double-stranded cDNA clones were sequenced using Sequenase (United States Biochemical Corp.). Multiple synthetic oligonucleotide primers were synthesized and used to sequence both strands of any previously unreported cDNA. The sequences of regions previously published by others (24,33) were confirmed on one strand only.

RNase protection probes for topoisomerase II α and β

The topoisomerase II α probe (kindly provided by D. Hochhauser) was prepared by PCR using a topoisomerase II α cDNA as template. The region between nucleotides 4052 and 4244 (21) was amplified using 27 mer primers which contained 5' *Bam*HI and 3' *Eco*RI sites. Following digestion, the PCR product was cloned between the *Bam*HI and *Eco*RI sites of pBluescript II. To generate antisense transcripts, the T3 promoter of pBluescript was utilized, after linearization of the plasmid with *Eco*RI. For topoisomerase II β , PCR was used to amplify the region between nucleotides 18 and 299 using the pT2 β -1 cDNA as template. The product was then cloned into the *Sma*I site of pUC18. The fragment was excised using *Kpn*I and *Bam*HI and ligated into pBluescript II. The T3 promoter was utilized to generate antisense transcripts, following linearization with *Bam*HI. The topoisomerase II α - and β -specific probes, generated 215 bp and 228 bp protected fragments, respectively. In each reaction, an internal loading

control of an antisense transcript to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used. This probe, kindly provided by M. Spencer, Oxford, U.K., produced a 120 bp protected fragment.

0.5 μ g linearized topoisomerase II α or β template (together with 0.5 μ g *Hind*III-digested GAPDH template) was added to 4 μ l 5 $\times$ transcription buffer (200 mM Tris-Cl pH 8.0, 40 mM MgCl₂, 10mM spermidine, 250 mM NaCl) 10mM DTT, 0.4 mM NTP's, 5 μ l α ³²P-CTP (Amersham), 40u RNasin and 40u T3 RNA polymerase (Boehringer Mannheim), in a total volume of 20 μ l, and the mixture incubated at 37°C for 30 minutes. 35u DNase I were added and the sample incubated for a further 15 mins at 37°C. The sample was then spun through a G50 Sephadex column to remove unincorporated nucleotides. Approximately 2 $\times$ 10⁵ cpm of probe were added to 10 μ g total RNA and the mixture incubated for 16 hours at 55°C. Following annealing, RNA:RNA hybrids were digested for 30 minutes at 30°C in a solution containing 40 μ g/ml ribonuclease A and 2 μ g/ml ribonuclease T1. Reactions were stopped by addition of SDS to 0.5% and proteinase K to 125 μ g/ml. The sample was vortexed with an equal volume of phenol/chloroform (50:50 mix), ethanol precipitated, resuspended in gel loading buffer and run on a 6% polyacrylamide DNA sequencing gel. The gel was then dried down and exposed to X-ray film.

In situ hybridization

Normal male peripheral blood lymphocytes were harvested and slides prepared using standard cytogenetic techniques (35). The topoisomerase II β cDNA clone, pt2 β -1, was biotinylated (BRL Bionick), the DNA purified on a Sephadex G50 column, and co-precipitated with salmon sperm DNA and *Escherichia coli* tRNA. 200ng labeled probe was mixed with 3 μ g of human cot-1 DNA (to compete out repetitive elements) and the mixture dried in a vacuum centrifuge. *In situ* hybridization and detection using FITC-conjugated avidin were performed as described by Williams et al. (36). Chromosomes were counterstained with 0.5 μ g/ml propidium iodide and 0.05 μ g/ml DAPI (diamidino-2-phenylindole, dihydrochloride; Sigma) in Citifluor (Citifluor Ltd, London).

To localize the gene to a chromosome band, metaphase spreads were prepared from chromosomes in which BrdU was incorporated in the early phase of the cell cycle. Detection of probe signal was as stated above, except that Texas red-avidin DCS (Vector Labs) was used as the fluorochrome and an additional incubation was performed using 10 μ g/ml anti-BrdU-FITC (Boehringer). This enabled direct visualization of fluorescent signals on R-banded chromosomes. Chromosomes were counterstained with 0.05 μ g/ml DAPI.

Colour photographs were taken on Fujicolor HG400 colour print film using a Zeiss Axiophot microscope with Zeiss filter sets 02 and 09, and with a dual band pass filter set (Omega Optical) for two colour analysis.

RESULTS

Isolation of topoisomerase II β cDNAs

A cDNA library of mRNA prepared from an EBV-transformed normal human B-cell line was size fractionated by agarose gel electrophoresis, and plasmids in the size range 9–15 kb screened using a PCR-generated fragment of the known topoisomerase II β cDNA as probe. Six clones were isolated which contained cDNA inserts of up to 5.6 kb in length. The cDNA insert of the largest

TopII α 1 mevsplqpvnenmqVM kIKK neDakKzLSVERIYQKKTQLSHILL
 TopII β 1 maksggogegagvggggaltvVhnaaKkeesetankDesKLSVERVYQKKTQLSHILL
 TopII α 46 RPDYIYSVSEVLTQmVYDEDMGILREVTFFVGLYKIFDEILVMAADNRQDpKmeCkr
 TopII β 62 RPDYIYSVSEVLTQmVYDEDMGILREVTFFVGLYKIFDEILVMAADNRQDpKmeCkr
 TopII α 107 VtIDPENNIISIMNKGKIPVVEHVKEMVYPALIPQQLTSSNYDDDEKKVTOGRNGYGA
 TopII β 123 VtIDPENNIISIMNKGKIPVVEHVKEMVYPALIPQQLTSSNYDDDEKKVTOGRNGYGA
 TopII α 168 KLCNIFSTKPTVTAarEYKkaFKQTMKdNgragEmelKpPnGEDYTCTTPQDLSKPKM
 TopII β 184 KLCNIFSTKPTVTAarEYKkaFKQTMKdNgragEmelKpPnGEDYTCTTPQDLSKPKM
 TopII α 229 qeLDKDIAlMvRAYDLAGStkdvKVLINQKLPVkgFRSTYdVYIKDLSTGneLKVI
 TopII β 245 ekLDKDIAlMvRAYDLAGScrgVVKaEWKdKLPVkgFRSTYdVYIKDLSTGneLKVI
 TopII α 290 HBqVhNRWvCLTSEKGFQQLISFVNSIATeKGRHVDTVaDQIVtKLvVKKKNGKvGa
 TopII β 306 HBLaNeNRWvCLTSEKGFQQLISFVNSIATeKGRHVDTVaDQIVtKLvVKKKNGKvGa
 TopII α 351 VKahQVNNhWIFVhaLIENPTFDSQTKENNTLQPKSPGSTCQLSEKFIKAALgCGIVEI
 TopII β 367 VKpEQVNNhWIFVhaLIENPTFDSQTKENNTLQPKSPGSTCQLSEKFIKAALgCGIVEI
 TopII α 412 LAMVFKAQQLAKKCSvKhrIKGIPKLODANDAGGmStECTLILTEGDSAKtLAVSG
 TopII β 428 LAMVFKAQQLAKKCSvKhrIKGIPKLODANDAGGmStECTLILTEGDSAKtLAVSG
 TopII α 473 LGVvGRdKYGvFLPRLKILMvREASHQIMENAIHIIKIVGLQYKKnYaDeSLKTLAY
 TopII β 489 LGVIGDRYGVVFLPRLKILMvREASHQIMENAIHIIKIVGLQYKKnYaDeSLKTLAY
 TopII α 534 GKIMINTDQDQSHIGKLLINFIHBMPSLLHrFLSEPTPIVVKSKNKQmaFYSIPE
 TopII β 550 GKIMINTDQDQSHIGKLLINFIHBMPSLLHrFLSEPTPIVVKSKNKQmaFYSIPE
 TopII α 595 FeBNKestPnhKMKVYKYYKGLGTStEKAKEYFADNRHRIqPhYaGPDAAIaLAFSK
 TopII β 611 FdEMKhleqKaMKIKYYKGLGTStEKAKEYFADNRHRIqPhYaGPDAAIaLAFSK
 TopII α 656 KqIDDKEMLTWPFEDRRQRKLIGLPdyLYQctTcyLTYNDFINKELIFSNDSNERSIP
 TopII β 672 KaIDDKEMLTWPFEDRRQRKLIGLPdyLYQctTcyLTYNDFINKELIFSNDSNERSIP
 TopII α 717 SmVDGIKPOQRKVLFTCFKRNDRKREKVAQLAGSVAESaYHGBmaLMTIvILNAQNFVG
 TopII β 733 sIVDGIKPOQRKVLFTCFKRNDRKREKVAQLAGSVAESaYHGBmaLMTIvILNAQNFVG
 TopII α 778 SNNINLQPIQGFOTLHGGKDaSPRYIPTMLStLARLLFPpkDdhtLkFLYDDNQRPV
 TopII β 794 SNNINLQPIQGFOTLHGGKDaSPRYIPTMLStLARLLFPavDnLkFLYDDNQRPV
 TopII α 839 EMYIPIIMVILNAGSIGTGMCKIPNIDREIVNIRRLaDGePIMPLPaYKMFQTI
 TopII β 855 EMYIPIIMVILNAGSIGTGMCKIPNIDREIVNIRRLaDGIclPhMPLPnKMFQTI
 TopII α 900 eELapNQYvISGEvailnatTiEILePVRTVTQYKQVLEPMLANTKTPPLiDYrEY
 TopII β 916 qELgqNQYvISGEvailnatTiEILePVRTVTQYKQVLEPMLANTKTPPLiDYrEY
 TopII α 961 HTDTTVKVVNHTBEKLaAaErgLHVKFKLQTLTCHSNVLPDhVOCLEKYYdTVIIDLrd
 TopII β 977 HTDTTVKVVNHTBEKLaAaErgLHVKFKLQTLTCHSNVLPDhVOCLEKYYdTVIIDLrd
 TopII α 1022 lFeLRkLYQLRKEMILGMLGABSaKLNQARFIEKIDGKIIENhKpKkELikVLQRGY
 TopII β 1038 fPdLRkLYQLRKEMILGMLGABSaKLNQARFIEKIDGKIIENhKpKkELikVLQRGY
 TopII α 1083 dSDPVKAMKEAQkvvEdeEneEedNekeKSDvYDsgPTFNyILNpLmYLYKEKkDE
 TopII β 1099 eSDPVKAMKEAQ ekaaeE dEtqghddesDsgTPSGPFPNylLnMeLwLTYKEKvE
 TopII α 1144 LcrlRneKegELdKLRKSPDLMKEDLaFIELEaVeaKekgDeqvGlpGK gGK
 TopII β 1157 LikQRdaKgrEvdLKRKSPDLMKEDLaFIELEaVeaKekgDeqvGlpGK gGK
 TopII α 1200 aKqKKtQmaSVlPSPrgRvIPzITIEakaseeKknKKK iknentegspgedv
 TopII β 1218 pKvKKIqIEStwSPSgRrLIIPaITamkdaaskKlIKKKgdlidtaavkvfdeefesgav
 TopII α 1254 E leglqrlekKqRk EPQTtkKqtTlaFKP iKKGKKRNPW pdSES
 TopII β 1279 EgageealtpevpinkgppKpRkheEPQTtrvrKtpesgKPaKvKKRNPWdedesSES
 TopII α 1300 D ResdEanfvdppretEprraAtktktfmdldsdedfadedktD
 TopII β 1340 DleestepvvlprdelRaaEerpyctfdfeeEedddAddddndndleelkvkaapitnd
 TopII α 1345 dED FVPsD aappkttkSpklnKELKpGKS vvsdleadvkgavplassp
 TopII β 1401 gEDeFVPsDgldkdeyt fspgKskatpeKaLhdKKSqdfgnlfsfpeysqtseddsakfde
 TopII α 1395 pathfpdeteltnpvppknvtvKKT AAKqestStTgaKkraAPKgtKrdpALNS gvSg
 TopII β 1462 needsaavfspefglktqdvKpETAAKkgpseSdVpKpKAPKqKvvaVNSdesSe
 TopII α 1454 kpdPAKT knrrKRKpStSddsdanfekivakvTSKK skgeSD dPha
 TopII β 1523 fglPKTKtpkgkrgakKRKaSgSenegdynprktaktTSKKpkktSfdqSDvdIffe
 TopII α 1501 DFdaavprakevRAKpIKYIEESDE DDIf.
 TopII β 1584 DFpteppelpgtgRarKeVYfEESDEeDvdfamfn.

of these clones, designated pt2 β -1, was sequenced. This revealed that the longest open reading frame continued to the 5' vector cloning site beyond a putative ATG initiation codon. To obtain clones extending beyond the 5' limit of pt2 β -1, a human bone marrow cDNA library was screened. A clone, designated pt2 β -2, was isolated which contained this extreme 5' sequence. The sequence of pt2 β -2 revealed an in-frame stop codon lying upstream of the 5' limit of pt2 β -1. We concluded, therefore, that pt2 β -1 contains the topoisomerase II β ATG initiation codon. In support of this, the proposed start codon lies in a sequence context (see below) which conforms well to the consensus for translation initiation sites derived by Kozak (38), and is also very similar to that of the topoisomerase II α gene.

Topoisomerase II α – TCACC(ATG)G (reference 21)

Topoisomerase II β – TCGCC(ATG)G (reference 37)

Consensus – CCRCC(ATG)G (reference 38)

'R' represents a purine

The open reading frame commencing at this ATG and terminating at a TAA codon is 4,863 nucleotides long and would encode a polypeptide comprising 1,621 amino acids. The topoisomerase II β protein has a calculated M_r of 182,705, in close agreement with the size of topoisomerase II β protein estimated by SDS-PAGE (22,23), and an isoelectric point of 8.1. The nucleotide sequence encoding topoisomerase II β protein has been deposited in the EMBL library under the accession number X68060. In the regions of overlap, our predicted amino acid sequence matches exactly the previously published partial topoisomerase II β protein sequences (24,33).

Figure 1 shows an alignment of the full amino acid sequences of the human topoisomerase II β and topoisomerase II α proteins. Overall, the sequences show a striking degree of similarity, containing 72% identical residues (83% when conservative changes are included). With the exception of a short region at the extreme N-terminus, the sequences of the highly conserved N-terminal ATPase domain and the central breakage/reunion domain (see Figure 2 for details) of the topoisomerase II α and β proteins are colinear with no insertions or deletions required for alignment. In contrast, their C-terminal domains differ markedly both in size and in sequence.

Homology between eukaryotic cell topoisomerase II proteins

The domain structure and sequence similarity of five eukaryotic topoisomerase II enzymes is shown in Figure 2. Within the ATPase and breakage/reunion domains of the different topoisomerase II enzymes there is a much greater degree of sequence similarity than within their C-terminal domains (Figure 2 and reference 39). The human topoisomerase II α and β protein sequences are more highly related to each other than they are to the sequences of non-mammalian topoisomerase II enzymes. However, topoisomerase II β protein is as related to

Figure 1. Alignment of the human topoisomerase II α and β sequences. The single letter amino acid code is used. Identical residues are in upper case letters and separated by a vertical line. Non-identical residues are in lower case letters. Residue numbers are indicated on the left. The topoisomerase II α sequence is from Tsai-Pflugfelder *et al.* (21), with the amendment of residues 109–114, as indicated by Hinds *et al.* (47), and confirmed by sequencing of a cDNA clone isolated in our laboratory from the B-cell cDNA library.

Figure 2. Domain structure and sequence similarity of eukaryotic topoisomerase II proteins. Each protein (indicated on the left) is depicted as three boxes representing from left to right; the N-terminal ATPase domain, the central breakage/reunion domain and the C-terminal domain. The boundaries between domains in the topoisomerase II β amino acid sequence were placed after residues 451 and 1191. Boundaries in the other proteins were based on sequence homology to topoisomerase II β . The figures within the boxes represent % sequence identity to the corresponding domain of topoisomerase II β . The total number of residues in each protein is indicated on the right.

Figure 3. RNase protection analysis of topoisomerase II α and β mRNA levels in a panel of human tumour cell lines. Lanes: Lane 1, U937; Lane 2, Raji; Lane 3, Jurkat; Lane 4, A431; Lane 5, RT4; Lane 6, T47D; Lane 7, HeLa; Lane 8, VA13. The positions of the 228 bp topoisomerase II β and the 215 bp topoisomerase II α protected fragments (and the GAPDH internal control) are indicated on the right. The sizes of molecular weight standards in base pairs (Lane M) are shown on the left. Densitometric scanning of autoradiograms to quantify expression levels was performed when each signal was within the linear range for x-ray film.

the topoisomerase II enzymes of lower eukaryotes as is topoisomerase II α .

The C-terminal domain of topoisomerase II β contains a high percentage of charged amino acids, including several blocks of acidic residues (Figure 1). Within this domain there are also a number of sequences which closely match the consensus for sites of phosphorylation by casein kinase II, protein kinases A and C, and p34^{cdc2} kinase (reviewed in 40).

By comparison with other topoisomerase II enzymes (21,39), it is predicted that Tyr-821 would be the active site residue

Table 1. Relative expression of topoisomerase II α and β mRNAs in a panel of human tumour cell lines

Cell line	Cell type	Expression level ^a		$\alpha:\beta$ ratio ^b
		α	β	
U937	Promonocytic leukaemia	13	44	3.45
Raji	B-cell	10	2.4	47.7
Jurkat	T-cell	2.5	1.0	28.6
A431	Vulval carcinoma	1.0	1.0	11.3
RT4	Bladder carcinoma	2.7	4.4	6.87
T47D	Breast carcinoma	31	3.7	95.4
HeLa	Cervical carcinoma	18	1.3	162
VA13	Lung fibroblast	13	3.3	44.1

^a Expression levels for the topoisomerase II α and β mRNAs are shown separately. The cell line with the lowest level of expression of each form is arbitrarily given a value of 1.0. Other values are relative to this. Values are corrected for differences in the GAPDH control.

^b Values represent the relative amounts of topoisomerase II α and β mRNA based directly on densitometric scanning of gels.

forming the covalent phosphotyrosyl linkage when topoisomerase II β enzyme transiently breaks DNA.

Expression of the topoisomerase II α and β mRNAs in human tumour cell lines

To quantify the level of the topoisomerase II α and β mRNAs in a panel of human tumour cell lines, fragments of the topoisomerase II α and β cDNAs were cloned into pBluescript, and specific antisense RNA probes generated. These probes, together with a GAPDH probe acting as an internal loading control, were hybridized to total cellular RNA and the mixture digested with ribonucleases. The specificity of the probes was verified using tRNA as a negative control (data not shown). Figure 3 shows that the α and β probes respectively produce distinct 215 bp and 228 bp protected fragments. All cell lines expressed a detectable level of both topoisomerase II α and β mRNA, but at levels which differed markedly between different cell types. Table 1 shows the calculated expression levels for each cell line, based on densitometric scanning of autoradiograms. U937 cells showed by far the highest expression level for topoisomerase II β mRNA (10-fold higher than in any other sample), as well as the highest $\beta:\alpha$ mRNA ratio. By contrast, HeLa cells expressed very high levels of topoisomerase II α relative to β mRNA (Table 1). There were also very significant differences in the combined level of topoisomerase II α and β mRNA in the different cell lines, with T47D and A431 cells, respectively, showing the highest and lowest overall level of topoisomerase II mRNA expression (Figure 3 and Table 1).

Chromosomal localisation of the topoisomerase II β gene

The topoisomerase II β gene has previously been localised to chromosome 3 (41). To accurately map its location, *in situ* hybridization to 20 metaphase spreads was used. This revealed specific hybridization to both alleles of chromosome 3 located on the short arm at band 24 (Figures 4a and 4b). No other paired signals were seen at a consistent location elsewhere in the genome.

DISCUSSION

We have identified cDNA clones covering the full coding sequence for the human topoisomerase II β protein. The predicted amino acid sequence shows strong similarity to that of the α isozyme of human topoisomerase II, and highly significant

Figure 4. (a) Representative human metaphase spread showing signal (arrows) on both chromatids of each copy of human chromosome 3 located on the short arm at band 3p24. Probe was detected with FITC and counterstained with propidium iodide. (b) Idiogram of human chromosome 3 at 400 band stage showing location of the topoisomerase II β gene at band 3p24.

homology to topoisomerase II enzymes from other eukaryotes. However, a C-terminal domain shows marked sequence divergence between the different topoisomerases. Previously, only partial topoisomerase II β cDNAs had been isolated representing regions highly conserved between the α and β isozymes (24,33). The finding that topoisomerase II β is at least as highly related to topoisomerase II enzymes from lower eukaryotes as is topoisomerase II α , raises questions about which

functions associated with topoisomerase II can be assigned to the α or β isozyme in human cells.

Topoisomerase II β protein differs from topoisomerase II α in a number of important respects. Firstly, the genes encoding the topoisomerase II α and β proteins map to chromosomes 17q21–22 (21) and 3p24 (this work), respectively, and are clearly therefore distinct. Secondly, there is a difference in cell cycle regulation of topoisomerase II α and β protein levels. Following serum starvation and re-commencement of the cell cycle, topoisomerase II β protein levels are broadly constant, whereas α protein levels remain low and only rise several hours later (42). Thirdly, topoisomerase II β protein is less sensitive to inhibition by intercalating agents and epipodophyllotoxins than is the α protein (23). Thus, there are significant biochemical and pharmacological differences between the isozymes.

Staining of human cells with isozyme-specific antisera revealed a difference in the subcellular localisation of the α and β forms, almost certainly indicating some degree of specialization in their respective roles in DNA metabolism. The β isozyme is found predominantly (perhaps exclusively) in the nucleolus with topoisomerase II α more generally located in the nucleoplasm (43,44). A nucleolar localisation could obviously indicate a role for the β isozyme in ribosomal RNA transcription. Previous studies in fission yeast revealed a structural role for topoisomerase II in the nucleolus (45). It is possible that topoisomerase II β protein fulfils this role in human cells in a manner analogous to that in which the human α isozyme is thought to be a structural component of nuclear chromatin. The cDNA clone reported here encoding the full topoisomerase II β amino acid sequence will permit the design of constructs for mRNA and/or protein expression as the first stage in determining the precise role of the β isozyme in chromosome structure and function.

Our analysis has shown that the C-terminal domain of topoisomerase II β , comprising approximately 25% of the protein, has diverged significantly from that of the α isozyme. This domain has an unusual sequence composition including several stretches of charged residues, and motifs matching the consensus (reviewed in 40) for recognition sites for several protein kinases. As this domain is not conserved between the different topoisomerases and thus unlikely to be essential for catalytic activity, it seems reasonable to suggest that it fulfils a regulatory function. The C-terminal domain of at least one eukaryotic topoisomerase II, that from *S.cerevisiae*, has been shown to be a target for phosphorylation by casein kinase II (46). We have shown that topoisomerase II β protein is a phosphoprotein in human cells and is a substrate for at least three protein kinases *in vitro* (A.M.Fry and I.D.Hickson, unpublished data). However, the sites of phosphorylation have yet to be mapped. The topoisomerase II β cDNA clone reported here will allow the different domains of the protein to be overexpressed in *E.coli* or in other organisms to facilitate phosphorylation studies.

Although topoisomerase II is a ubiquitously expressed protein in eukaryotic cells, the relative expression of the α and β isozymes has not previously been studied in detail. One of the difficulties in distinguishing between the isozymes has been the strong sequence similarity and identical size of their mRNAs (21–24). To overcome the problems associated with Northern blot analysis of such closely related mRNAs, we have developed specific RNase protection probes to quantify the level of expression of the topoisomerase II α and β genes. Using a panel of human tumour cell lines of different origin, we have shown that topoisomerase II β (and α) mRNA is very widely expressed.

There was no obvious relationship between the level of expression of the topoisomerase II α and β genes, although both mRNAs were present at very low levels in A431 cells (vulval carcinoma). Similarly, there was no indication of cell type-specific regulation of expression, as, for example, the highest and lowest overall topoisomerase II mRNA levels were each found in lines of epithelial origin. It will be informative to test whether nuclear protein levels mirror these observed differences in steady-state mRNA levels. By far the highest expression level for topoisomerase II β was found in U937 cells, representative of an early monocyte progenitor cell. U937 cells may prove to be a good source for preparation of the topoisomerase II β protein, particularly considering the ease of large scale culture of leukaemic lines. It is interesting to note that HeLa cells, a commonly used source for purification of human topoisomerase II protein, showed a high level of topoisomerase II α , but not of β , mRNA. Because of the wide variation in expression levels of the topoisomerase II α and β mRNAs, we would conclude that it is unlikely that strong coordinate regulation of the two topoisomerase II genes occurs in human tumour cell lines under normal growth conditions.

In summary, we have isolated cDNA clones encoding the full coding region for the human topoisomerase II β protein and have studied expression of the topoisomerase II α and β genes in a range of human cell lines. It will now be possible to carry out a number of expression and functional studies on the topoisomerase β isozyme in order to clarify its role in DNA metabolism in human cells.

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