## Cloning and sequencing of cDNA encoding human DNA topoisomerase II and localization of the gene to chromosome region 17q21-22

(hairpinned oligonucleotide probe/single-copy gene/in situ hybridization/mouse-human hybrids)

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ABSTRACT Two overlapping cDNA clones encoding human DNA topoisomerase II were identified by two independent methods. In one, a human cDNA library in phage  $\lambda$  was screened by hybridization with a mixed oligonucleotide probe encoding a stretch of seven amino acids found in yeast and Drosophila DNA topoisomerase II; in the other, a different human cDNA library in a  $\lambda$ gt11 expression vector was screened for the expression of antigenic determinants that are recognized by rabbit antibodies specific to human DNA topoisomerase II. The entire coding sequences of the human DNA topoisomerase II gene were determined from these and several additional clones, identified through the use of the cloned human TOP2 gene sequences as probes. Hybridization between the cloned sequences and mRNA and genomic DNA indicates that the human enzyme is encoded by a single-copy gene. The location of the gene was mapped to chromosome 17q21-22 by in situ hybridization of a cloned fragment to metaphase chromosomes and by hybridization analysis with a panel of mouse-human hybrid cell lines, each retaining a subset of human chromosomes.

Eukaryotic DNA topoisomerase II is a ubiquitous ATPdependent type II topoisomerase (reviewed in refs. 1–3). The enzyme mediates the transient breakage of a pair of complementary strands in a double-stranded DNA to form a gate for the passage of duplex DNA. The passage of a DNA segment through a temporarily opened gate in the same DNA molecule or in a different DNA molecule can lead to interconversions between topological isomers (topoisomers) of doublestranded DNA rings. Unlike their counterpart in bacteria, DNA gyrase, the eukaryotic and phage T4 type II DNA topoisomerases cannot utilize the free energy of ATP hydrolysis to supercoil DNA. Nucleotide sequencing of the genes encoding the enzymes reveals, however, that all type II DNA topoisomerases are structurally and evolutionarily related.

Genetic studies of two distantly related yeasts, Saccharomyces cerevisiae and Saccharomyces pombe, show that eukaryotic DNA topoisomerase II is an essential enzyme (reviewed in ref. 4). Its essentiality correlates with its irreplaceable role in the extrication of pairs of intertwined, newly replicated chromosomes (5–7). In addition, the enzyme normally participates in a number of vital processes involving DNA, including replication, transcription, and the condensation of mitotic chromosomes (see reviews cited and ref. 8). A structural role of the enzyme in chromosomal organization has also been postulated (9–12). Identification of the dimeric enzyme as the target of a number of antitumor agents further accentuated the biological importance of eukaryotic DNA topoisomerase II (13–17). The biological and clinical importance of the enzyme led us to embark on the characterization of the *TOP2* gene encoding human DNA topoisomerase II. We report here the identification of cDNA clones of this enzyme, the determination of the entire coding sequence of the gene, and the localization of the single-copy gene to the q21-q22 region of chromosome 17.

## MATERIALS AND METHODS

Materials. A HeLa cDNA library in  $\lambda gt10$  was kindly provided by R. Tjian (University of California at Berkeley). The mixed oligonucleotide probe used in the screening of the human gene was synthesized by the use of an automated instrument (Pharmacia). Peripheral blood from normal volunteers was used in the preparation of metaphase chromosomes. Cell lines used in the chromosomal localization experiments are described in the relevant references.

Methods. Nucleotide sequencing was done by the Sanger dideoxynucleotide method (18), using either single-stranded or double-stranded (19) templates. *In situ* chromosome mapping was performed according to the procedures described by Harper and Saunders (20).

## RESULTS

Identification of cDNA Clones Encoding Human DNA Topoisomerase II. In one approach, amino acid sequences deduced from the nucleotide sequences of the TOP2 genes encoding the yeast S. cerevisiae and Drosophila melanogaster enzymes (21, 22) were compared, and stretches of identical sequences were identified. One of these, a 7-amino acid stretch, Met-Ile-Met-Thr-Asp-Gln-Asp, appeared to be particularly attractive because of the low degeneracy of the nucleotide sequences encoding it. A mixture of oligonucleotides of the structure shown in Fig. 1a was synthesized. The hairpinned structure was designed for the synthesis of a highly radioactive copy of the mixture by Escherichia coli DNA polymerase I. Following the extension of the 3' ends of the oligonucleotides by the polymerase, the product was digested with Pst I and the labeled strand was separated from its unlabeled complement, which is 4 nucleotides longer, by preparative gel electrophoresis. The hairpinned probe was

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<sup>&</sup>lt;sup>II</sup>The sequence reported in this paper is being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J04088).

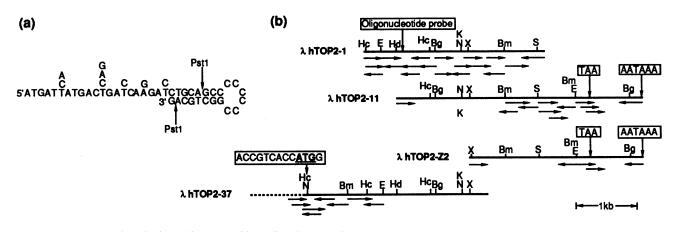


FIG. 1. (a) The hairpinned mixed oligonucleotide probe. (b) cDNA inserts of human TOP2 clones. Selected restriction sites are indicated: Bm, BamHI; Bg, Bgl II; E, EcoRI; Hc, HincII; Hd, HindIII; K, Kpn I; N, Nco I; X, Xba I. All fragments shown are bounded by EcoRI sites introduced during cDNA cloning. The arrows indicate the region and direction of sequencing of various subclones obtained by the cloning of various restriction fragments or DNA resected with BAL-31 nuclease. In most cases, each subclone was sequenced twice simultaneously, using the large fragment of E. coli DNA polymerase I in one and T7 DNA polymerase in the other set of reactions. The beginning 800 base pairs (bp) of  $\lambda$ hTOP2-37, shown as a dotted line, is absent in the mature mRNA and was probably introduced artifactually in the construction of the cDNA or during passages of the library (unpublished data of Z.-M. Tu and J.C.W.). The positions of ATG translational initiation (underlined), TAA translational termination, the oligonucleotide probe sequence, and a potential poly(A) consensus sequence AATAAA are depicted in boxes above the maps of the clones. kb, Kilobase.

also used directly in several screens following  $^{32}P$  labeling at its 5' end.

Screening of a HeLa cDNA library in phage  $\lambda$ gt10 with the mixed probe yielded a clone,  $\lambda$ hTOP2-1 (Fig. 1b). Partial sequencing of the subcloned *Eco*RI fragments of the 2.9-kb human DNA segment in  $\lambda$ hTOP2-1 showed that the segment shared extensive homologies with the yeast and the *Drosophila TOP2* genes. The 5' and 3' proximal sequences of the 2.9-kb insert were then used in the search of additional cDNA clones. Two of the clones obtained,  $\lambda$ hTOP2-11 and  $\lambda$ hTOP2-37, are depicted in Fig. 1b.

In a second approach, a human HepG2 cDNA library in  $\lambda gt11$  was screened by the use of rabbit antibodies specific to human DNA topoisomerase II, following the procedures of Young and Davis (23). A positive,  $\lambda h TOP2-Z2$ , was obtained; mapping with several restriction endonucleases and sequencing of several stretches of the cloned insert showed that the human cDNA insert in this clone was identical to the last 3 kb of that in  $\lambda h TOP2-11$  (see Fig. 1b).

Coding Sequence of the Human TOP2 Gene. Sequencing results of subclones derived from clones  $\lambda$ hTOP2-1, -11, and -37 are depicted in Fig. 2a. Human DNA topoisomerase II is encoded by an open reading frame of 1530 amino acids, with a calculated molecular weight of 174,000. A comparison of the human enzyme amino acid sequence with those deduced from TOP2 gene sequences of S. cerevisiae (21), S. pombe (24), and Drosophila (22) shows clearly that human DNA topoisomerase II shares extensive sequence homologies with the other eukaryotic type II topoisomerases. As indicated in Fig. 2b, for the first 1000 amino acids 63% are identical between the human and Drosophila enzymes. The two sequences are colinear with very few insertions or deletions of amino acids in this region: relative to the Drosophila sequence, the human sequence has 1 fewer amino acid between Asp-65 and Glu-74, 1 fewer between Ile-105 and Ser-116, 2 fewer between Val-968 and Glu-973, and 1 extra amino acid between Gln-419 and Lys-425. The carboxylterminal one-third of the amino acid sequences of eukaryotic DNA topoisomerase II from yeast, Drosophila, and human are more divergent, and colinearity of the sequences is less stringent.

Human TOP2 Gene Is a Single-Copy Gene Encoding a 6.2kb mRNA. Blot hybridization (25) of HeLa cell cytoplasmic mRNAs resolved by formaldehyde/agarose gel electrophoresis with the <sup>32</sup>P-labeled 1.8-kb *Eco*RI fragment of the  $\lambda$ hTOP2-Z2 clone reveals a single band of 6.2  $\pm$  0.2 kb (mean  $\pm$  SD; results not shown). This result suggests that the human *TOP2* message is encoded by a unique gene. This notion is confirmed by blot hybridization of various restriction enzyme digests of human genomic DNA with several <sup>32</sup>P-labeled cloned cDNA fragments as probes (results not shown).

Human TOP2 Gene Is Located on Chromosome 17q21-22. Two independent methods identify human chromosome 17q21-22 as the region where the TOP2 gene is located. Results obtained by *in situ* hybridization of a <sup>3</sup>H-labeled TOP2 gene probe to normal human metaphase chromosomes are summarized in Fig. 3. Of a total of 129 grains from 40 metaphase chromosomes scored on the autoradiograms, 17 or 13% were located on the long arm of chromosome 17, with 88% of these grains clustered in the area q21.2-22, according to the 1985 International System for Human Cytogenetic Nomenclature (26). Eleven examples of grains on chromosome 17 are depicted on the upper right-hand corner of Fig. 3. Increase of grains over the background level in other chromosomal locations is insignificant.

In separate experiments, blot hybridization of EcoRI digests of DNA samples isolated from a group of 20 mousehuman hybrid cell lines (27-31), in which different sets of the human chromosomes had been lost, was carried out with a <sup>32</sup>P-labeled TOP2 probe. Lanes 1 and 2 in Fig. 4 depict the patterns of hybridization to DNA from mouse and a human T-cell line, respectively. The mouse DNA gave a major band at 3.8 kb and two minor bands at 5.4 and 1.7 kb; the human DNA gave three major bands at 12, 3.0, and 1.5 kb and a minor band at 5.8 kb. For all 20 DNA samples from mousehuman hybrid cells, the human DNA hybridization pattern correlates perfectly with the presence of human chromosome 17: none of the DNA samples from cell lines lacking human chromosome 17 exhibited the human DNA pattern, whereas all DNA samples from cell lines containing human chromosome 17 did (results not shown).

Several of the human-mouse hybrid lines are particularly interesting in that they retain only parts of human chromosome 17, as determined by tests for the presence of chromosome 17-linked probes (29, 30, 32-34): line N9 retains the human chromosome 17 markers NGFR (17q12 $\rightarrow$ q21) and Hox-2 (17q21) but not the flanking markers p53 (17p13) and Her-2/Neu (17q12 $\rightarrow$ q21) on one side and PKc- $\alpha$  (17q22 $\rightarrow$ q24) on the other side; line c19 retains the human chromo-

(a)

							GGACCACCCAGT	ACCGATCCCTTC	ACGACCGTCACC
1	ATGGAAGTGTCA	CCATTCCACCCT	СТАААТСААААТ	ATCOMACTOMAC	АААЛАААТАААА	AATGAAGATGCT	AAGAAAAGACTG	TCTGTTGAAAGA	ATCTATCAAAAG
100	AAAACACAATTG	CANCATATTTC	CTCCCCCCAGAC	ACCTACATTGGT	TCTGTGGAATTA	GTGACCCAGCAA	ATGTGGGTTTAC	GATGAAGATGTT	GGCATTAACTAT
217	AGGGAAGTCACT	TTTCTTCCTCCT	TTCTACAAAATC	TTTGATGAGATT	CTAGTTAATGCT	GCGGACAACAAA	CAAAGGGACCCA	AAAATGTCTTGT	ATTAGAGTCACA
217	ATGATCCGGAAA	CAATTAATTACT	ATATCCAATAAT	GGAAAAGGTATT	CCTGTTGTTGAA	CACAAAGTTGAA	AAGATGTATGTC	CCAGCTCTCATA	TTTGGACAGCTC
323	CTAACTTCTAGT	AACTATCATCAT	CATCAAAACAAA	GTGACAGGTGGT	CGAAATGGCTAT	GGAGCCAAATTG	TGTAACATATTC	AGTACCAAATTT	ACTGTGGAAACA
5/1	GCCAGTAGAGAA	TACAACAAAATC	TTCAAACACACA	TGGATGGATAAT	ATGGGAAGAGCT	GGTGAGATGGAA	CTCAAGCCCTTC	AATGGAGAAGAT	TATACATGTATC
640	ACCTTTCAGCCT	CATTTCTCTAAC	TTTAAAATGCAA	ACCOTCGACAAA	GATATTGTTGCA	CTAATGGTCAGA	AGAGCATATGAT	ATTGCTGGATCC	ACCAAAGATGTC
757	AAAGTCTTTCTT	ANTCOMMENTAN	CTCCCACTAAAA	GGATTTCGTAGT	TATGTGGACATG	TATTTGAAGGAC	AAGTTGGATGAA	ACTGGTAACTCC	TTGAAAGTAATA
945	CATGAACAAGTA	ANCORACITCO	CAACTCTCTTTA	ACTATGAGTGAA	AAAGGCTTTCAG	CAAATTAGCTTT	GTCAACAGCATT	GCTACATCCAAG	GGTGGCAGACAT
005	GTTGATTATGTA	COTCATCACATT	CTCACTABACTT	CTTCATCTTCTC	AAGAAGAAGAAC	AAGGGTGGTGTT	GCAGTAAAAGCA	CATCAGGTGAAA	AATCACATGTGG
1001	ATTTTTGTAAAT	CCCTTANTCAN	AACCCAACCTTT	CACTOTCAGACA	ANGANACATG	ACTITACAACCC	AAGAGCTTTTGGA	TCAACATGCCAA	TTGAGTGAAAAA
11001	TTTATCAAAGCT	GCCITARITGAR	COTATTOTACAA	ACCATACTABAC	TCCCTCAACTTT	AAGGCCCAAGTC	CAGTTAAACAAG	AAGTGTTCAGCT	GTAAAACATAAT
1207	AGAATCAAGGGA	ADDICCON A ACTIC	CATCATCCCAAT	CATCOACCCCCC	CCANACTCCACT	CACTCTACCCTT	ATCCTGACTGAG	GGAGATTCAGCC	AAAACTTTGGCT
1405	GTTTCAGGCCTT	COTOTOCOTTOCC	ACACACADATAT	CCCCTTTTTCCCT	CTTACACCAAAA	ATACTCAATCTT	CCACAACCTTCT	CATAAGCAGATC	ATGGAAAATGCT
1403	GAGATTAACAAT	AUCOUCO CONTRA	AGAGACAAAIAI	TACAACAAAAAAC	TATCAACATCAA	CATTCATTCAAC	ACCONTROCTION	CCCAACATAATG	ATTATGACAGAT
1513	CAGGACCAAGAT	COMPCCCACATI	A B B C C C T T C AG	1 ACAAGAAAAAC	CATCACAACTCC	COTOTOTOTO	CCACATCCTTT	CTCCACCAATT	ATCACTCCCATT
1720	GTAAAGGTATCT	GGIICCLACAIC	CARAGGUIIGUIG	TACACCOTTCOT	CATCACAACIOG	TCGAAGAGTTCT	ACTCCAAATCAT	AAAAAATGGAAA	GTCAAATATTAC
1/29	AAAGGTTTGGGC	AAAAACAAGCAA	GAMAIGGCAIII	CARTACTTTCCA	CATATCAAAAA	CATCCTATCCAC	TTCAAATATTCT	CCTCCTCAACAT	GATGCTGCTATC
1037	AGCCTGGCCTTT	ACCAGGAGAICA	AAGGAAGCIAAA	BACCA ATCOTTA	ACTA ATTTCATC	CACCATACAACA	CAACGAAAGTTA	CTTCCCCTTCCT	CACCATTACTTC
1945	TATGGACAAACT	AGCAAAAAACAG	ATAGATGATCGA	MAGGAAIGGIIA	CARCTARTITICATO	TTCTC & & ATTCT	CARCORAGITA	TCTATCCCTTCT	ATCCTCCATCCT
2053	TTGAAACCAGGT	ACCACATATCIG	ACATATAATGAC	TICAICAACAAG	CACADOCCACA	CTARATICI	CANTRACCTCCA	TCACTCCCTCAA	ATCTCTTCTTAT
2101	CATCATGGTGAG		AMCACCAMMAN	11CAAACGGAAI	A A THE TOTOL OF THE A A THE TOTOL OF THE A A THE TOTOL OF THE A A A THE TOTOL OF THE A A A A A A A A A A A A A A A A A A A	ACCANTANCTA	AACCTCTTCCAC	CCCATTCCTCAC	TTTCCTACCACC
2269	CTACATGGTGGG	AIGICACTAAIG	AIGACCATIAIC	AAIIIGGUICAG	CTCACCTCTTTC	CONCONTON	TTTCCACCAAAA	CATCATCACACG	TTCAACTTTTTA
2311	TATGATGACAAC	AAGGATTCTGCT	AGICCACGAIAC	AICITIACAAIG	CICAGCICITIG	ATAAATCCTCCT	CAACCAATCCCT	ACTCCCTCCTCC	TCCANATCCCC
2483	AACTTTGATGTG	CAGCGIGIIGAG	ANTINATIGUTAC	ATTCCIATIATT COTTACATCOAT	CCARGIGLIG	TTOCCANTCOTT	CCAACTTACAAC	ACTOGOIGGICC	ACTATTCAACAA
2593	CTGGCTCCAAAT	CGIGAAATIGIA	AATAACATCAGG	CGTTTGATGGAT	GGAGAAGAACC1	CANATOTOLO	CURAGITACAAG	AACIICAAGGOI	ACIAIIGAAGAA
2701	CAAGTTCTAGAA	CAATATGTGATT	AGIGGIGAAGIA	GCTATICITAAT	1CIACAACCAII	ACCONTRACTOR	ACACATACCACT	CTCA & ATTTCTT	CTCNACATCACT
2809	GAAGTICTAGAA	CCCATGTTGAAT	GGCACCGAGAAG	ACACCICCICIC	ATAACAGACTAT	AGGGAAIACCAI	ACAGAIACCACI	CTTTTTCACCAC	GTACCCTCTTTA
2917	GAAGAAAAACIG	GCAGAGGCAGAG	AGAGTIGGACIA	CACAAAGICIIC	AAACICCAAACI	AGICICACAIGC	AACICIAIGGIG	CIIIIIGACCAC	COTCOTCANTOT
3025	AAGAAATATGAC	ACGGIGITGGAT	ATTCTAAGAGAC	CTITITGAACIC	AGACITAAATAT	CARAGATIAAGA	AAAGAAIGGCIC	ATTA A ACTTCTC	ATTCACACCCCA
	GCTAAACTGAAT								
	TATGATTCGGAT								
	GTAACAGATTCT								
	GAGCTGGACACA								
	CAAGTCGGACTT								
	GAAATGAAAGCA								
	TTAGAAAAGAAA								
	GAATCAGATAGG								
	GAAGATTTCTCA								
	AAACCACAGAAA								
	ACAAACCCAGTT								
4321	AAAAGGGATCCA	GCTTTGAATTCT	GGTGTCTCTCAA	AAGCCTGATCCT	GCCAAAACCAAG	AATCGCCGCAAA	AGGAAGCCATCC	ACTTCTGATGAT	TUTGACTUTAAT
	) TTTGAGAAAATT								
	CGGGCAAAGAAA								
4645	5 TTAAAGTTACCT	GAAGCTCTTAAC	TTCCTCCCCTCT	GAATTTAGTTTG	GGGAAGGTTTTT	TAGTACAAGACA	TCAAAGTGAAGT	AAAGCCCAAGTG	TICITIAGCITT

(b)

1	MEVSPLQPVNEN	MQVNKIKKNEDA	KKRLSVERIYOK	KTQLEHILLRPD	TYIGSVELVTQQ	MWVYDEDVGINY	REVTEVPGLYKI
85	FDEILVNAADNK	ORDPKMSCIRVT	MIRKQLISIWNN	GKGIPVVEHKVE	KMYVPALIFGOL	LTSSNYDDDEKK	VTGGRNGYGAKL
169	CNIFSTKFTVET	ASREYKKMEKOT	WMDNMGRAGEME	LKPFNGEDYTCI	TFOPDLSKFKMO	SLDKDIVALMVR	RAYDIAGSTKDV
253	KVFLNGNKLPVK	GFRSYVDMYLKD	KLDETGNSLKVI	HEQVNHRWEVCL	TMSEKGFOOISF	VNSIATSKGGRH	VDYVADQIVTKL
337	VDVVKKKNKGGV	AVKAHOVKNHMW	IFVNALIENPTF	DSQTKENMTLOP	KSFGSTCOLSEK	FIKAAIGCGIVE	SILNWVKFKAQV
421	QLNKKCSAVKHN	RIKGIPKLDDAN	DAGGRNSTECTL	ILTEGDSAKTLA	VSGLGVVGRDKY	GVFPLRGKILNV	REASHKOIMENA
505	EINNIIKIVGLO	YKKNYEDEDSLK	TLRYGKIMIMTD	<b>ODODGSHIKGLL</b>	INFIHHNWPSLL	RHRFLEEFITPI	VKVSKNKQEMAF
589	YSLPEFEEWKSS	TPNHKKWKVKYY	KGLGTSTSKEAK	EYFADMKRHRIQ	FKYSGPEDDAAI	SLAFSKKQIDDR	KEWLTNFMEDRR
673	ORKLLGLPEDYL	YGQTTTYLTYND	FINKELILFSNS	DNERSIPSMVDG	LKPGQRKVLFTC	FKRNDKREVKVA	QLAGSVAEMSSY
757	HHGEMSLMMTII	NLAQNEVGSNNL	NLLOPIGOFGTR	LHGGKDSASPRY	IFTMLSSLARLL	FPPKDDHTLKFL	YDDNORVEPEWY
841	IPIIPMVLINGA	EGIGTGWSCKIP	NFDVREIVNNIR	RLMDGEEPLPML	PSYKNFKGTIEE	LAPNQYVISGEV	AILNSTTIEISE
925	LPVRTWTOTYKE	QVLEPMLNGTEK	TPPLITDYREYH	TDTTVKEVVKMT	EEKLAEAERVGL	HK <u>VFKLQT</u> SLTC	NSMVLFDHVGCL
1009	KKYDTVLDILRD	LFELRLKYYGLR	KEWLLGMLGAES	AKLNNQARFILE	KIDGKIIIENKP	KKELIKVLIORG	YDSDPVKAWKEA
1093	QQKVPDEEENEE	SDNEKETEKSDS	VTDSGPTFNYLL	DMPLWYLTKEKK	DELCRLRNEKEQ	ELDTLKRKSPSD	LWKEDLATFIEE
1177	LEAVEAKEKQDE	OVGLPGKGGKAK	GKKTQMAEVLPS	PRGQRVIPRITI	EMKAEAEKKNKK	KIKNENTEGSPQ	EDGVELEGLKOR
1261	LEKKOKREPGTK	TKKOTTLAFKPI	KKGKKRNPWPDS	ESDRSSDESNFD	VPPRETEPRRAA	TKTKFTMDLDSD	EDFSDFDEKTDD
1345	EDFVPSDASPPK	TKTSPKLSNKEL	KPQKSVVSDLEA	DDVKGSVPLSSS	PPATHFPDETEI	TNPVPKKNVTVK	<b>KTAAKSQSST<u>ST</u></b>
1429	TGAKKRAAPKGT	KRDPALNSGVSQ	KPDPAKTKNRRK	RKPSTSDDSDSN	FEKIVSKAVTSK	KSKGESDDFHMD	FDSAVAPRAKSV
1513	RAKKPIKYLEES	DEDDLF*					

FIG. 2. Human DNA topoisomerase II cDNA sequence (a) and the amino acid sequence derived from it (b). The nucleotide sequence is numbered from the putative ATG start; amino acids are doubly underlined if they are identical with those at the corresponding positions in the *Drosophila* DNA topoisomerase II sequence. For homology comparisons with the other type II DNA topoisomerases, see figure 6 of ref. 22.

some 17 regions q11 $\rightarrow$ q12 and q21 $\rightarrow$ qter, as evidenced by the presence of markers Her-2/Neu, Hox-2, and PKc- $\alpha$  and the absence of markers p53 and NGFR (27); line 275s retains human chromosome 17 markers NGFR, Hox-2, and PKc- $\alpha$  but not markers p53 and Her-2/Neu (27–30). As shown in lanes 5–7 of Fig. 4, the human *TOP2* sequences on the gene probe used are found in lines c19 and N9 but not in line 275s. These results indicate that human *TOP2* resides in the q21

region, distal to the Her-2/Neu locus and proximal to the NGFR locus.

Three additional cell lines were also analyzed. Line c131 retains human chromosome 17 region pter—qter (29, 30), line GL-5 retains the human 17p13—qter region in the form of a 17 p<sup>+</sup> chromosome (17qter $\rightarrow$ 17p13::22q11 $\rightarrow$ qter) (31), and line SKBR3 is derived from a human mammary carcinoma and contains an amplified region on chromosome 17q includ-

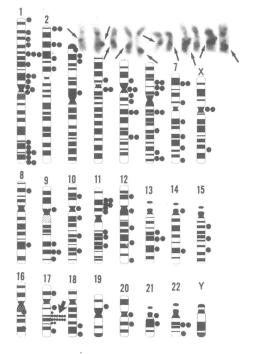


FIG. 3. Mapping of the chromosomal location of the human *TOP2* gene by *in situ* hybridization to metaphase chromosomes. The solid circles represent all chromosomal grains observed in 40 metaphases on the schematic representation of fluorodeoxyuridine-synchronized Wright-stained metaphase chromosomes. Pictured in the upper right-hand corner are 11 individual number 17 chromosomes from seven different metaphases. Arrows indicate grains at q21.2-q22.

ing the Her-2/Neu locus (35). The human *TOP2* sequence on the gene probe used is found in all three lines (Fig. 4, lanes

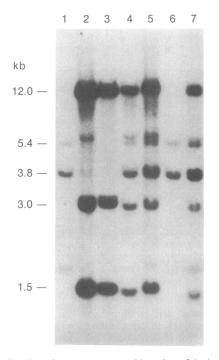


FIG. 4. Mapping of the chromosomal location of the human *TOP2* gene by blot hybridization (25). Each lane contained  $\approx 10 \,\mu g$  of DNA from the following sources: lane 1, mouse; lane 2, human T-cell line; lane 3, human mammary carcinoma cell line SKBR3; lane 4, mouse-human hybrid c19; lane 5, mouse-human hybrid N9; lane 6, mouse-human hybrid 275s; lane 7, mouse-human hybrid GL-5. <sup>32</sup>P-labeled plasmid DNA containing the 1.8-kb *Eco*RI  $\lambda$ hTOP2-Z2 fragment was used as the probe. Lengths of size markers (in kb) are indicated on the left side of the autoradiogram.

3 and 7; c131 not shown) but is not a part of the amplified region in SKBR3 (Fig. 4, compare lanes 3 and 7). Thus all results obtained by Southern hybridization analysis are in agreement with the conclusion based on *in situ* hybridization to metaphase chromosomes.

## DISCUSSION

Several clones containing the coding sequences of human DNA topoisomerase II have been obtained by the use of two different methods: one is based on the supposition that a nucleotide sequence encoding a conserved stretch of 7 amino acids found in S. cerevisiae and D. melanogaster is also present in the human TOP2 coding region, and the other is based on the presence of DNA sequences encoding antigenic determinants that are recognized by rabbit antibodies raised against human DNA topoisomerase II. The finding that clones obtained by these methods from two different human cDNA libraries share a 3-kb-long common region provides strong evidence that these clones are authentic. The extensive sequence homology between the cloned human gene and the previously determined coding sequences of S. cerevisiae. S. pombe, and D. melanogaster DNA topoisomerase II provides further evidence that the clones represent the coding sequences of the human TOP2 gene. The success in cloning the human TOP2 by the use of the mixed oligonucleotide probe suggests that the probe might be universally applicable in the cloning of TOP2 genes of other eukaryotes.

As shown in Fig. 2b, the highly conserved stretches of sequences that were previously identified in other type II DNA topoisomerases (22) are present in the human enzyme as well, including the 2 amino acids Arg-Tyr at positions 803 and 804, respectively, in the human enzyme, which most likely contains the active site tyrosine that becomes linked covalently to the DNA when the enzyme transiently breaks DNA (22, 36, 37).

The hybridization results between the cloned TOP2 gene sequences and mRNAs and genomic DNA restriction fragments indicate that the human TOP2 gene is a single-copy gene, similar to the cases reported for the other eukaryotes (5, 38-40). If a separate gene is present in the human genome encoding a different type II DNA topoisomerase, as suggested by Drake *et al.* (41), the coding sequence of that gene must have diverged significantly from the TOP2 gene sequence reported here.

Our sequence data do not exclude the possibility that the initiation codon of the human TOP2 gene is located upstream of the putative ATG start shown in Fig. 2. This seems unlikely, however, in view of the nucleotide sequence in the vicinity of the putative start site: the sequence ACCGTCAC-CATGG, in which the underlined ATG is the putative start codon, matches very well with the consensus sequence for initiation of translation in vertebrates, GCCGCC (A or G)CCATGG (42).

As shown in Fig. 1b, there is 0.95 kb of cDNA sequence downstream of the stop signal of translation. A hexameric motif AATAAA, which is usually found 10-30 bp upstream of the poly(A) site (43, 44), is present near the 3' end of the clones  $\lambda$ hTOP2-11 and -Z2 (Fig. 1b) and might be the poly(A) signal. Because the distance between this hexameric motif and the ATG start codon, about 5.5 kb, is significantly shorter than the 6.2  $\pm$  0.2 kb length of the *TOP2* mRNA, there is probably a 5' untranslated region of the message several hundred nucleotides in length.

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