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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 λ 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 λgt11 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 ATP-dependent 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 double-stranded 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 λgt10 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. 1*a* 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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‖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).

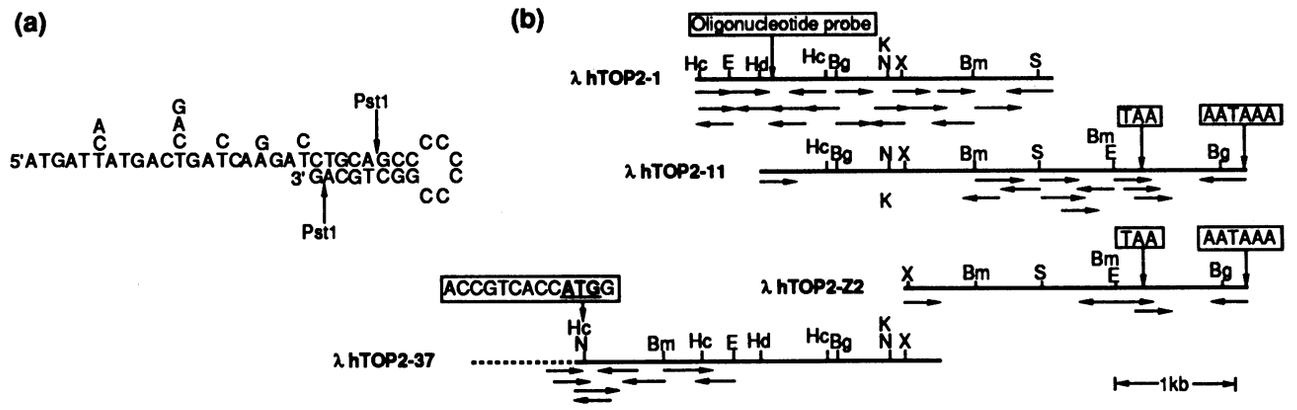


FIG. 1. (*a*) The hairpinned mixed oligonucleotide probe. (*b*) cDNA inserts of human TOP2 clones. Selected restriction sites are indicated: Bm, *Bam*HI; Bg, *Bgl* II; E, *Eco*RI; Hc, *Hin*cII; Hd, *Hin*dIII; K, *Kpn* I; N, *Nco* I; X, *Xba* I. All fragments shown are bounded by *Eco*RI sites introduced 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 λhTOP2-37, 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 λgt10 with the mixed probe yielded a clone, λhTOP2-1 (Fig. 1*b*). Partial sequencing of the subcloned *Eco*RI fragments of the 2.9-kb human DNA segment in λhTOP2-1 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, λhTOP2-11 and λhTOP2-37, are depicted in Fig. 1*b*.

In a second approach, a human HepG2 cDNA library in λgt11 was screened by the use of rabbit antibodies specific to human DNA topoisomerase II, following the procedures of Young and Davis (23). A positive, λhTOP2-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 λhTOP2-11 (see Fig. 1*b*).

Coding Sequence of the Human *TOP2* Gene. Sequencing results of subclones derived from clones λhTOP2-1, -11, and -37 are depicted in Fig. 2*a*. 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. 2*b*, 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 carboxyl-terminal 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.

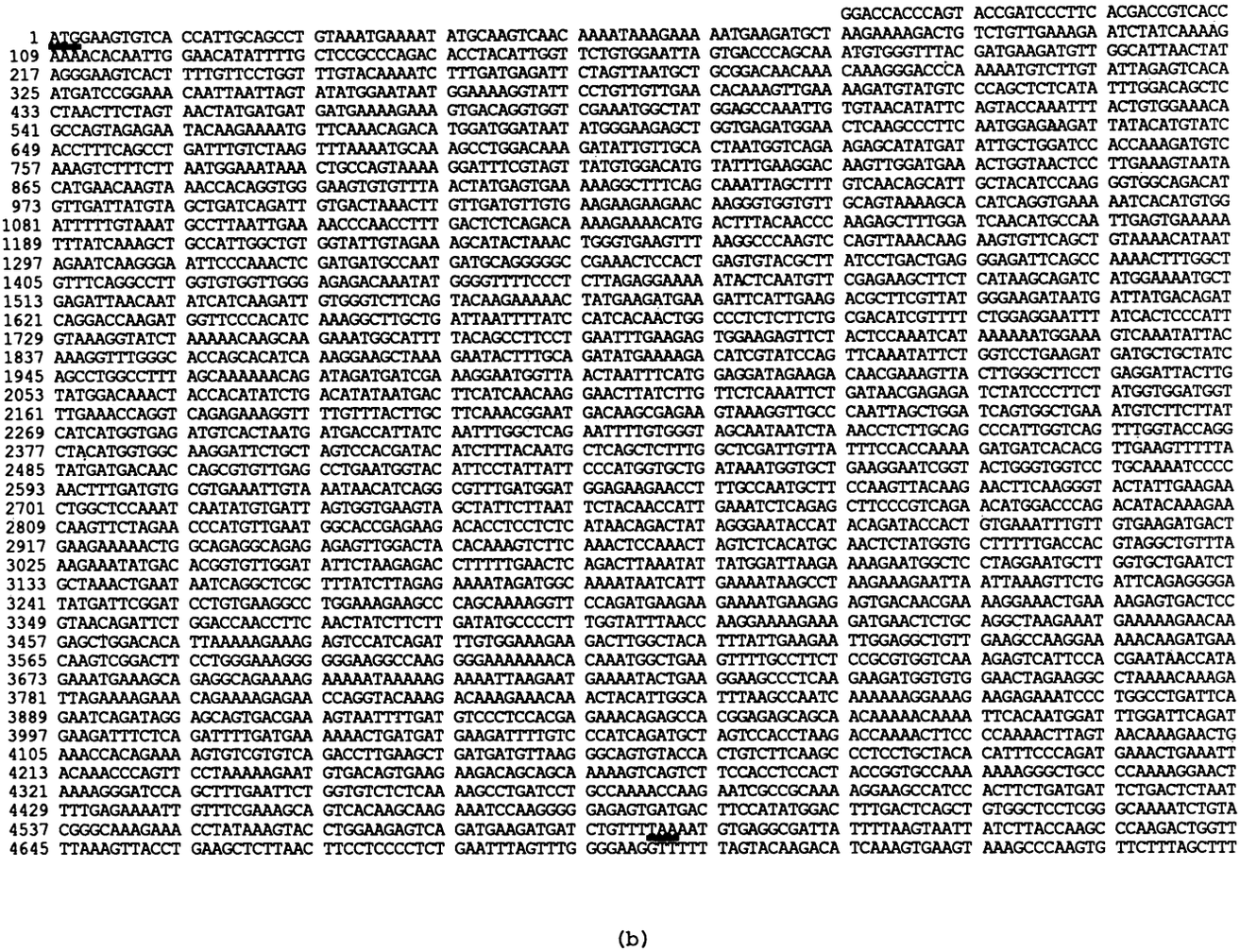
Human *TOP2* Gene Is a Single-Copy Gene Encoding a 6.2-kb mRNA. Blot hybridization (25) of HeLa cell cytoplasmic mRNAs resolved by formaldehyde/agarose gel electrophoresis with the ^{32}P-labeled 1.8-kb *Eco*RI fragment of the λhTOP2-Z2 clone reveals a single band of 6.2 ± 0.2 kb (mean ± SD; results 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 ^{32}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 ^{3}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 *Eco*RI digests of DNA samples isolated from a group of 20 mouse–human hybrid cell lines (27–31), in which different sets of the human chromosomes had been lost, was carried out with a ^{32}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 mouse–human 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→q21) and Hox-2 (17q21) but not the flanking markers p53 (17p13) and Her-2/Neu (17q12→q21) on one side and PKc-α (17q22→q24) on the other side; line c19 retains the human chromo-

(a)



(b)

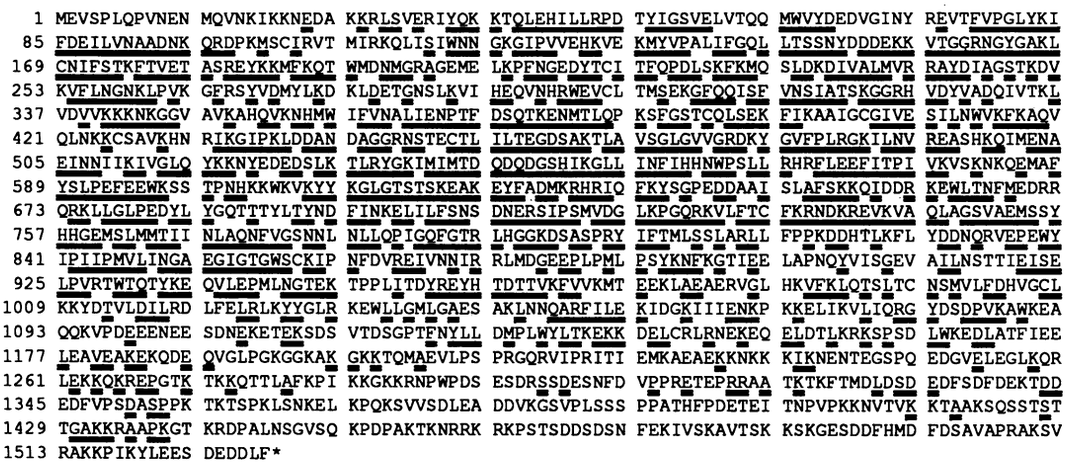


FIG. 2. Human DNA topoisomerase II cDNA sequence (*a*) and the amino acid sequence derived from it (*b*). The nucleotide sequence is numbered from the putative ATG start; amino acids are doubly underlined if they are identical with those at the corresponding positions in the *Drosophila* DNA topoisomerase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

some 17 regions q11→q12 and q21→qter, as evidenced by the presence of markers Her-2/Neu, Hox-2, and PKc-α and the absence of markers p53 and NGFR (27); line 275s retains human chromosome 17 markers NGFR, Hox-2, and PKc-α 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^+ chromosome (17qter→17p13::22q11→qter) (31), and line SKBR3 is derived from a human mammary carcinoma and contains an amplified region on chromosome 17q includ-

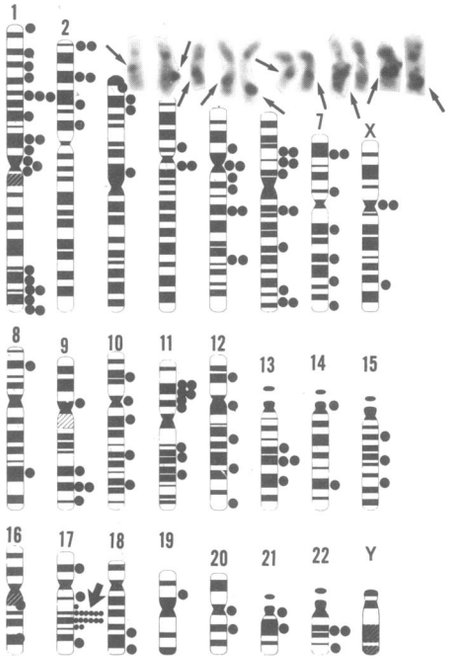


FIG. 3. Mapping of the chromosomal location of the human *TOP2* gene by *in situ* hybridization to metaphase chromosomes. The solid circles represent all chromosomal grains observed in 40 metaphases on the schematic representation of fluorodeoxyuridine-synchronized Wright-stained metaphase chromosomes. 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

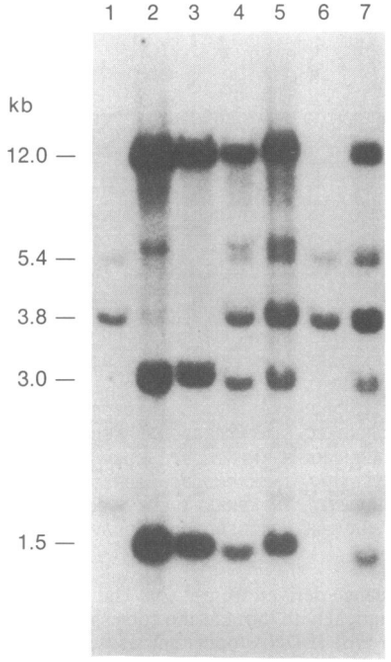


FIG. 4. Mapping of the chromosomal location of the human *TOP2* gene by blot hybridization (25). Each lane contained ≈10 μg of DNA from the following sources: lane 1, mouse; lane 2, human T-cell line; lane 3, human mammary carcinoma cell line SKBR3; lane 4, mouse–human hybrid c19; lane 5, mouse–human hybrid N9; lane 6, mouse–human hybrid 275s; lane 7, mouse–human hybrid GL-5. ^{32}P-labeled plasmid DNA containing the 1.8-kb *Eco*RI λhTOP2-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. 2*b*, 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 ACCGTCACCATGG, 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. 1*b*, 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 λhTOP2-11 and -Z2 (Fig. 1*b*) 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 ± 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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