Published online April 19, 2004

Nucleic Acids Research, 2004, Vol. 32, No. 7 ***2087–2092***
DOI: 10.1093/nar/gkh525

Thirteen-exon-motif signature for vertebrate nuclear and mitochondrial type IB topoisomerases

Hongliang Zhang, Ling-Hua Meng, Drazen B. Zimonjic[1], Nicholas C. Popescu[1] and Yves Pommier*

Laboratory of Molecular Pharmacology and [1]Laboratory of Experimental Carcinogenesis, Center for Cancer Research, National Cancer Institute, NIH, DHHS, Bethesda, MD, USA

Received February 6, 2004; Revised and Accepted March 11, 2004

DDBJ/EMBL/GenBank accession nos+

ABSTRACT

DNA topoisomerases contribute to various cellular activities that involve DNA. We previously identified a human nuclear gene that encodes a mitochondrial DNA topoisomerase. Here we show that genes for mitochondrial DNA topoisomerases (type IB) exist only in vertebrates. A 13-exon topoisomerase motif was identified as a characteristic of genes for both nuclear and mitochondrial type IB topoisomerases. The presence of this signature motif is thus an indicator of the coexistence of nuclear and mitochondrial type IB DNA topoisomerases. We hypothesize that the prototype topoisomerase IB with the 13-exon structure formed first, and then duplicated. One topoisomerase specialized for nuclear DNA and the other for mitochondrial DNA.

INTRODUCTION

DNA topoisomerases play important roles in cellular activities that involve DNA and have been classified as either type I or type II enzymes (1–3). The type I enzymes break and reseal one strand of duplex DNA at a time, whereas the type II enzymes break and reseal both strands in concert. Topoisomerases are further divided into types IA, IB, IIA and IIB. A total of six human DNA topoisomerases has been identified to date, including two type IA enzymes (topoisomerases IIIα and IIIβ), two type IB enzymes [topoisomerase I (nuclear) and mitochondrial DNA topoisomerase I] and two type IIA enzymes (topoisomerases IIα and IIβ).

In eukaryotic cells, mitochondrial DNA (mtDNA) constitutes extranuclear genetic material. A typical mammalian cell contains ~1000 mitochondria, with each of these organelles containing five to ten copies of covalently closed circular mtDNA of 16–18 kb. Human mtDNA consists of a circular DNA duplex of 16 569 bp and encodes 22 tRNAs, 13 mRNAs, and 12S and 16S rRNAs (4,5). Unwinding of mtDNA in human cells is mediated by a specific type IB enzyme encoded by a nuclear gene (Hs-*TOP1mt*) (6). A minor proportion of topoisomerase IIIα (a type IA enzyme) molecules is also present in mitochondria (7).

We have now investigated the existence of *TOP1mt* genes in other species. After finding that such genes are restricted to and conserved among vertebrates, we compared the structures of *TOP1mt* and *TOP1* (nuclear) genes. We show that the *TOP1mt* and vertebrate *TOP1* genes consist of 13 exons at the end of the genes in the conserved regions. This terminal 13-exon structure thus appears to be a common signature of both mitochondrial and nuclear topoisomerases I, and the fact that this signature exists only in vertebrates suggests that both genes arose from the duplication of a common ancestor gene.

MATERIALS AND METHODS

Cloning of Top1mt

DNA manipulation, PCR and DNA sequencing were performed according to standard protocols. We obtained clone BF139529 from Incyte Genomics (St Louis, MO), IMAGE clone 2601221 from ATCC (Manassas, VA) and clone pgf2n.pk002.c13 from Delaware Biotechnology Institute (Newark, DE), and sequenced them on a 377 DNA sequencer using ABI Prism Big Dye Terminator (PE Applied Biosystems). The missing 5′ end portions of *TOP1mt* genes were amplified using a GeneRacer kit (Invitrogen, Carlsbad, CA). The 5′ end was joined to the corresponding clones to generate full-length *TOP1mt*. All oligonucleotide sequences used for cDNA identification are available upon request.

Fluorescence microscopy, FISH localization, DNA relaxation assays and DNA cleavage assays

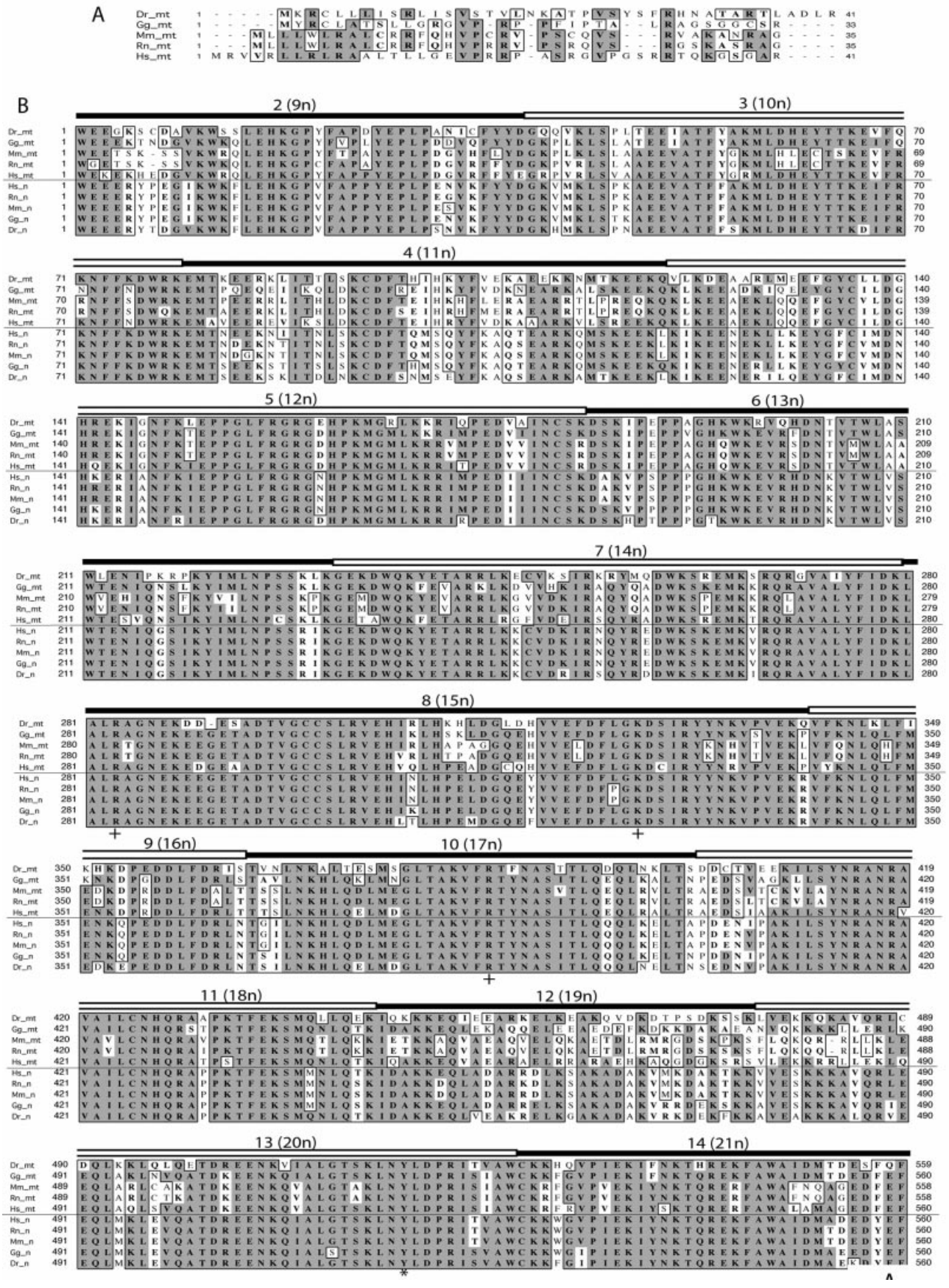
These procedures were carried out as described previously (6).

Database searches and alignment

We identified putative homologous genes using the discontiguous Mega BLAST (http://www.ncbi.nlm.nih.gov) to search all available NCBI databases. We aligned DNA sequences and corresponding amino acid sequences with available *TOP1* and *TOP1mt* genes using the ClustalW in MacVector (Accelrys, San Diego, CA).

*To whom correspondence should be addressed. Tel: +1 301 496 5944; Fax: +1 301 402 0752; Email: pommier@nih.gov

+AF362952, AF503620, AY429654, AY429655 and TPA BK001786


A
Dr_mt
Gg_mt
Mm_mt
Rn_mt
Hs_mt
B
2 (9n)
3 (10n)
4 (11n)
5 (12n)
6 (13n)
7 (14n)
8 (15n)
9 (16n)
10 (17n)
11 (18n)
12 (19n)
13 (20n)
14 (21n)
Dr_mt
Gg_mt
Mm_mt
Rn_mt
Hs_mt
Hs_n
Rn_n
Mm_n
Gg_n
Dr_n

RESULTS

Identification of a mouse mitochondrial topoisomerase I gene (Mm-*TOP1mt*)

Screening of the NCBI database with the BLAST search engine and human mitochondrial topoisomerase I (Hs-top1mt) as the bait yielded a mouse cDNA sequence (DDBJ/EMBL/GenBank accession no. BF139529). Sequencing of BF139529 revealed an open reading frame encoding a polypeptide with high homology to Hs-top1mt. The GeneRacer protocol (Invitrogen, Carlsbad, CA) was used to determine the sequence of the 5′ end of the gene. The combination of both approaches yielded a 2011 bp cDNA sequence that encodes a 593 amino acid protein. This protein, which we have designated Mm-top1mt, shares 73% sequence identity and 84% similarity with Hs-top1mt (6) (Fig. 1).

We next designed two sets of PCR primers based on the 5′ and 3′ ends of the Mm-*TOP1mt* cDNA for the purpose of screening a mouse genomic library. A bacterial artificial chromosome clone containing the full-length Mm-*TOP1mt* gene was obtained. We then used this clone as a probe to determine the chromosomal location of Mm-*TOP1mt* by fluorescence *in situ* hybridization. In two independent experiments with biotin- or digoxigenin-labeled probes, most metaphase spreads with informative signals and minimal nonspecific background fluorescence yielded symmetrical fluorescent spots on a small chromosome. Furthermore, 27 out of a total of 30 labeled spreads recorded in the two experiments exhibited a specific signal at the same site, bands E2–E3, on both chromosomes 15, to which we therefore assign Mm-*TOP1mt* (Fig. 2). This region of mouse chromosome 15 is homologous to human chromosome 8q24.3 (8), the site of Hs-*TOP1mt* (6).

Both Hs-*TOP1mt* and Mm-*TOP1mt* are positioned between locus H of the lymphocyte antigen 6 complex and the rhophilin (Rho GTPase binding protein 1) gene. The region of the mouse genome containing Mm-*TOP1mt* is thus syntenic to that of the human genome containing Hs-*TOP1mt*, suggesting that these regions share a common ancestor.

To determine the structure of Mm-*TOP1mt*, we sequenced the 5′ end of the gene and combined the resulting sequence with that available in the NCBI database. Like Hs-*TOP1mt*, Mm-*TOP1mt* contains 14 exons. This 14-exon structure is also shared by other *TOP1mt* genes (Table 1; see below). All *TOP1mt* genes also exhibit the same intron phases (Table 1). Furthermore, the corresponding introns of the human and mouse *TOP1mt* genes are similar in size, with the exception of intron 7 which is larger in human (*Homo sapiens*) than in mouse (*Mus musculus*) (Table 1).

TOP1mt genes are present only in vertebrates

We examined the available eukaryotic DNA sequences to determine which species possess genes for both mitochondrial and nuclear topoisomerases I. With Hs-top1mt and Mm-top1mt as baits, we detected *TOP1mt* genes in all the vertebrate genomes: zebra fish (*Danio rerio*) (Dr), chicken (*Gallus gallus*) (Gg) and rat (*Rattus norvegicus*) (Rn).

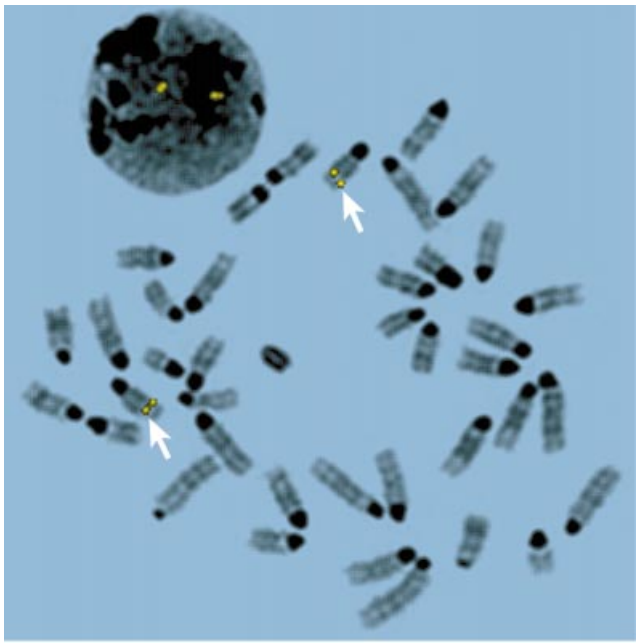


Figure 2. Fluorescence *in situ* hybridization analysis demonstrating the location of the Mm-*TOP1mt* gene at bands E2–E3 on chromosomes 15. Both chromosomes with symmetrical FITC signals on sister chromatids are identified by arrows.

For chicken top1mt, we derived most of the sequence from a cDNA clone (clone ID, pgf2n.pk002.c13) and used GeneRacer to obtain the remaining 5′ sequence. For zebra fish, the cDNA sequence was directly derived from a single clone (IMAGE clone ID, 2601221). Expression experiments revealed that both the recombinant chicken (Gg-top1mt) and zebra fish (Dr-top1mt) proteins possess topoisomerase I activity. Cleavage assays (6) also confirmed that Gg-top1mt is a type IB topoisomerase, given that it forms a covalent bond with the 3′ end of the cleaved DNA (data not shown).

The sequences and structures of the rat, chicken and zebra fish *TOP1mt* genes were derived from the recently released databases (NCBI). The rat (Rn-*TOP1mt*) and chicken (Gg-*TOP1mt*) genes, like the human and mouse genes, comprise 14 exons (Table 1). For the zebra fish gene (Dr-*TOP1mt*), we were able to compile only 11 exons from the incomplete genomic sequence (Table 1). The exon sizes for these five vertebrate *TOP1mt* genes vary for the first exon but are identical for the remaining 13 exons, with the minor exception that exons 2 and 13 of the rodent genes are 3 bp shorter (corresponding to deletion of one amino acid and likely a characteristic of the common rodent ancestor).

Figure 1. Alignment of the amino acid sequences of top1mt proteins. (**A**) Alignment of the sequences encoded by the first exon of the genes for the five identified mitochondrial topoisomerases I. (**B**) Alignment of the sequences encoded by the last 13 exons of the genes for the five mitochondrial (mt) and corresponding nuclear (n) topoisomerases I. Residues encoded by each exon are marked by solid or open bars above the sequences; exon numbers for the mitochondrial and nuclear enzymes are outside and inside the parentheses, respectively. The catalytic tyrosine (Y) is marked with an asterisk and the critical basic amino acids (RKR) are marked with plus signs.

Table 1. Structure of the genes for mitochondrial topoisomerases I (*TOP1mt*)

Exon no.	Exon size					Intron phase	Intron size	
	Hs	Rn	Mm	Gg	Dr		Hs	Mm
1	**122 (141)**	**104 (137)**	**104 (138)**	**98 (200)**	**122 (153)**	2	3400	2521
2	116	**113**	**113**	116	116	1	1752	1605
3	122	122	122	122	122[a]	0	3005	>4000
4	123	123	123	123	123	0	688	587
5	188	188	188	188	188	2	716	516
6	145	145	145	145	145	0	342	665
7	144	144	144	144	–[b]	0	2612	216
8	186	186	186	186	–[b]	0	3114	1098
9	69	69	69	69a	–[b]	0	180	551
10	115	115	115	115	115	1	1596	1444
11	128	128	128	128	128[a]	0	177	564
12	95	95	95	95a	95	2	5509	5526
13	150	**147**	**147**	150	150	2	524	469
14	103 (184)	103 (175)	103 (180)	103 (238)	103 (221)			

For the sizes of exons 1 and 14, the first numbers correspond to the coding region and those in parentheses include the noncoding region. Bold indicates differences in exon size. The dashed line separates the conserved last 13 exons. Hs, *H.sapiens*; Rn, *R.norvegicus*; Mm, *M.musculus*; Gg, *G.gallus*; Dr, *D.rerio*. Exon and intron sizes are in base pair units.

[a]Exon size deduced from neighbors.

[b]Exon sizes could not be determined from the available sequence databases.

The NH_2-terminal portion of Hs-top1mt encoded by exon 1 contains the mitochondrial localization signal (6). Alignment of the corresponding NH_2-terminal regions of the vertebrate top1mt polypeptides revealed that they share little sequence homology (Fig. 1A). To verify that the newly identified top1mt proteins are indeed mitochondrial enzymes, we transfected M059J human neuroblastoma cells with expression vectors for either Mm-top1mt or Gg-top1mt tagged at their COOH-termini with green fluorescent protein (GFP) and then examined the transfected cells by fluorescence microscopy, as previously described for Hs-top1mt (6). Both of the GFP fusion proteins localized to mitochondria (data not shown), demonstrating the presence of a functional mitochondrial targeting sequence in both mouse and chicken top1mt. We also examined whether, despite their low homology, the amino acid sequences encoded by exon 1 of the various *TOP1mt* genes might function as mitochondrial leader sequences with the use of the Mitoprot program (http://www.mips.biochem.mpg.de/cgi-bin/proj/medgen/mitofilter). The probability of mitochondrial targeting was high for all five identified top1mt proteins: 92, 98, 99, 99 and 98% for zebra fish, chicken, mouse, rat and human top1mt, respectively. When the sequences encoded by the first exons were removed, however, low scores were obtained for all five proteins, indicating that the mitochondrial-targeting sequences are located in the regions encoded by exon 1 of the *TOP1mt* genes.

The terminal 13-exon motif is present in all vertebrate *TOP1* genes and is highly conserved between *TOP1* and *TOP1mt* genes

The conservation of the terminal 13-exon structure among *TOP1mt* genes as well as the human gene for nuclear topoisomerase I (Hs-*TOP1*) (6) led us to investigate whether this structure was common to other type IB topoisomerases. All the vertebrate *TOP1* genes examined (rat, mouse, chicken and zebra fish) consist of 21 exons, of which the last 13 exons (exons 9–21) are conserved with regard to size and phase (Table 2).

Alignment of the amino acid sequences encoded by the last 13 exons of both nuclear and mitochondrial topoisomerases I revealed a high degree of conservation between the nuclear and mitochondrial enzymes (Fig. 1B). The catalytic residues, including the critical basic amino acids (RKR, marked with plus signs) and tyrosine residue (Y, marked with an asterisk), are all preserved.

Pairwise comparisons of the 13-exon motifs revealed high homology among the 10 topoisomerases examined (Table 3). At the nucleotide level, the *TOP1* genes exhibited a higher level of identity (83.43 $\pm$ 6.87%) than did the *TOP1mt* genes (73.98 $\pm$ 7.91%); the level of identity between the *TOP1* and *TOP1mt* genes was lower (67.72 $\pm$ 2.08%).

Both the 13-exon motif and the presence of two type IB topoisomerase (mitochondrial and nuclear) genes are restricted to vertebrates

We next investigated the existence of genes for type IB topoisomerases in nonvertebrate eukaryotes. The 13-exon topoisomerase motif was not detected in budding yeast (*Saccharomyces cerevisiae*), fission yeast (*Schizosaccharomyces pombe*), fruit fly (*Drosophila melanogaster*), nematode (*Caenorhabditis elegans*), rice (*Oryza sativa*) or thale cress (*Arabidopsis thaliana*). In budding yeast, the *TOP1* gene contains no introns. The fission yeast *TOP1* gene contains two introns at its 5′ end. The fruit fly *TOP1* gene consists of eight exons, and the nematode *TOP1* gene comprises five exons. Both the rice and the two thale cress *TOP1* genes share a common 15-exon structure unrelated to the vertebrate 13-exon topoisomerase motif (not shown). The existence of a distinct but shared (in length and phase) 15-exon structure in these plant species indicates that they are derived from a common ancestor.

The sea squirt (*Ciona intestinalis*) (Ci) has a single *TOP1* gene that is markedly similar to those of vertebrates (Table 2).

Table 2. Structure of the genes for nuclear topoisomerases I (*TOP1*)

Exon no.	Exon size (vertebrates)					Exon size	Intron phase		Intron size	
	Hs	Rn	Mm	Gg	Dr	Ci	Ci	Vert.	Hs	Mm
1	33 (280)	33	33 (246)	33[a]	33 (143)	–[b]	–[b]	0	330	345
2	25	25	25	25[a]	25	–[b]	–[b]	1	31 938	23 467
3	97	**103**	**103**	**103**	97	**104**	**0**	2	14 680	12 448
4	124	124	124	**127**	**121**	**131**	**2**	0	1287	1673
5	56	56	56	**59**	**47**	**40**	**0**	2	2447	3144
6	96	96	96	96	**90**	**44**	2	2	984	2533
7	76	76	76	76	**82**	**79**	0	0	3221	3343
8	107	107	107	**98**[a]	**98**	**45**	**0**	2	7903	4321
9	116	116	116	116	116	**160**	1	1	4632	4462
10	122	122	122	122	122	122	0	0	873	748
11	123	123	123	123	123	123	0	0	1718	1088
12	188	188	188	188	188	188	2	2	965	206
13	145	145	145	145	145	145	0	0	11 428	7323
14	144	144	144	144	144	144	0	0	1044	1292
15	186	186	186	186	186	186	0	0	1215	779
16	69	69	69	69	69	69	0	0	838	598
17	115	115	115	115	115	115	1	1	1776	1559
18	128	128	128	128	128	**131**	0	0	3399	3064
19	95	95	95	95	95	**174**	0	2	215	253
20	150	150	150	150	150	**71**	2	2	1039	375
21	103 (1298)	103	103 (1310)	103 (1411)	103 (130)	103				

For exons 1 and 21, the first numbers correspond to the coding region and those in parentheses include the noncoding region. Bold indicates differences in exon size. The dashed line separates the conserved last 13 exons. The intron sizes of human and mouse were derived from the NCBI and our own sequence data. Vert., vertebrates; Hs, *H.sapiens*; Rn, *R.norvegicus*; Mm, *M.musculus*; Gg, *G.gallus*; Dr, *D.rerio*; Ci, *C.intestinalis*. Exon and intron sizes are in base pair units.

[a]Exon size deduced from neighbors.

[b]Exon sizes and intron phases for Ci could not be determined from the available sequence data.

Table 3. Comparison of the nucleotide and predicted amino acid sequences of the last 13 exons of the genes for vertebrate mitochondrial (mt) and nuclear (n) type IB topoisomerases

(**A**) cDNAs (% identity)

Dr-mt	100									
Gg-mt	69.8	100								
Mm-mt	65.8	72.5	100							
Rn-mt	66.1	73.0	92.6	100						
Hs-mt	68.8	74.4	78.0	78.8	100					
Hs-n	67.7	71.2	66.0	66.6	69.4	100				
Rn-n	67.2	70.1	64.9	66.1	67.7	89.9	100			
Mm-n	67.3	70.5	64.2	65.7	67.5	89.9	96.9	100		
Gg-n	67.7	71.0	65.5	66.2	68.9	84.1	82.2	82.2	100	
Dr-n	67.9	71.8	65.9	66.6	69.6	77.3	76.2	76.5	79.1	100
	Dr-mt	Gg-mt	Mm-mt	Rn-mt	Hs-mt	Hs-n	Rn-n	Mm-n	Gg-n	Dr-n

(**B**) Peptides (% identity/similarity)

Dr-mt	100									
Gg-mt	73/83	100								
Mm-mt	64/78	73/86	100							
Rn-mt	64/78	73/86	95/98	100						
Hs-mt	71/86	77/89	75/87	77/88	100					
Hs-n	70/83	75/86	66/82	67/82	71/87	100				
Rn-n	07/83	75/85	66/82	66/82	70/86	98/99	100			
Mm-n	70/83	75/85	66/82	66/82	69/86	98/99	99/99	100		
Gg-n	70/83	75/86	76/82	67/82	71/87	95/97	94/97	94/97	100	
Dr-n	69/83	75/85	67/82	67/82	71/86	88/95	87/95	76.587/94	89/95	100
	Dr-mt	Gg-mt	Mm-mt	Rn-mt	Hs-mt	Hs-n	Rn-n	Mm-n	Gg-n	Dr-n

Hs, *H.sapiens*; Rn, *R.norvegicus*; Mm, *M.musculus*; Gg, *G.gallus*; Dr, *D.rerio.*

We determined the structure of exons 3–21 of Ci-*TOP1*, assuming that the gene consists of 21 exons (Table 2). For exons 3–8, the homology with vertebrate *TOP1* genes is low. In contrast, the homology (in terms of size and phase) is high for exons 10–18 and for exon 21 of Ci-*TOP1* and vertebrate *TOP1* genes. Moreover, the cumulative length of exons 19 and

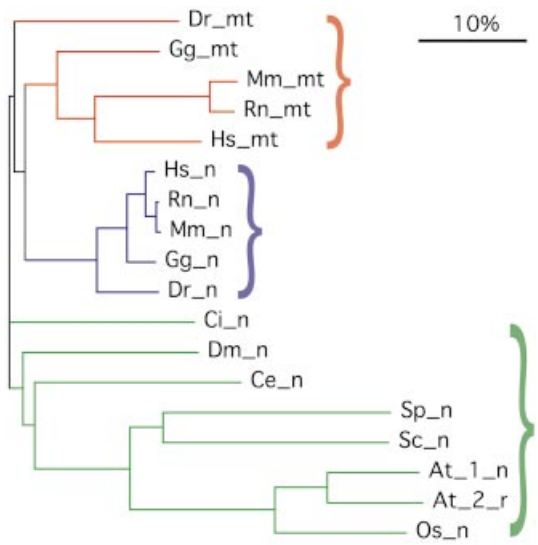


Figure 3. Cluster analysis of the mitochondrial and nuclear topoisomerases I. The amino acid sequences encoded by the 13-exon topoisomerase motif of vertebrate genes for nuclear (blue bracket) and mitochondrial (red bracket) enzymes (Fig. 1B) were used for this analysis. For the other enzymes (green bracket), the corresponding regions, based on sequence alignment and size, were used. Hs, *H.sapiens*; Rn, *R.norvegicus*; Mm, *M.musculus*; Gg, *G.gallus*; Dr, *D.rerio*; Ci, *C.intestinalis*; Dm, *D.melanogaster*; Ce, *C.elegans*; Sp, *S.pombe*; Sc, *S.cerevisiae*; At, *A.thaliana*; Os, *O.sativa*. The bar indicates the scale for 10% dissimilarity between amino acid sequences.

20 of Ci-*TOP1* (174 + 71 = 245 bp) is equal to the total size of the corresponding exons in vertebrates (95 + 150 = 245 bp), and exon 18 of Ci-*TOP1* is only 3 bp longer than that of vertebrate *TOP1* genes. Thus Ci-*TOP1* resembles the vertebrate *TOP1* genes in its terminal 12 exons.

Finally, we compared the common portions of the vertebrate type IB topoisomerases encoded by the last 13 exons of their genes and the corresponding sequences of other type IB topoisomerases (Fig. 3). Three main clusters, corresponding to the vertebrate mitochondrial enzymes, the vertebrate nuclear enzymes and the nonvertebrate enzymes, were obtained, with the sea squirt topoisomerase being positioned between the vertebrate and other nonvertebrate enzymes.

DISCUSSION

From zebra fish to human, all the vertebrate type IB topoisomerases examined possess a common terminal 13-exon motif, suggesting that this motif is characteristic of vertebrates. This 13-exon motif, encoding the topoisomerase activity, corresponds to the portion of human topoisomerase I resolved by crystallography (9). Interestingly, the exon–intron boundaries do not occur at the boundaries of the domains identified in the crystal structures of human topoisomerase I.

Given that the organisms with this 13-exon motif possess nuclear genes for both a nuclear and a mitochondrial topoisomerase IB, it is likely that both genes evolved from a common ancestor gene. During evolution, gene duplication might thus have resulted in the emergence of one gene for an enzyme targeted to nuclear DNA and of another gene for an enzyme targeted to mitochondrial DNA. The common topoisomerase I catalytic domain is encoded by the last 13 exons of each gene, and the targeting sequences are encoded by the first exon of the genes for the mitochondrial enzymes and by the first eight exons of the genes for the nuclear enzymes. The structure of the sea squirt *TOP1* gene shares similarities with the vertebrate genes in its last 12 exons, suggesting that this gene might share a common ancestor with an early precursor of the vertebrate *TOP1* and *TOP1mt* genes, but failed short of vertebrates.

The absence of a specific *TOP1mt* gene in the other eukaryotic species raises the question of how these organisms perform mitochondrial DNA metabolism functions. It is possible that other topoisomerases (types II or IA) perform such functions in these species. However, the only specific mitochondrial topoisomerase enzymes identified to date are type IB enzymes. We cannot exclude the possibility that other types of topoisomerase contain mitochondrial targeting sequences that are short and not readily recognizable. Alternatively, a single gene might encode two polypeptides that are targeted either to the nucleus or to mitochondria. The human *TOP3*α gene, for example, contains two start codons that yield two distinct enzymes, one for the nucleus and the other for mitochondria (7).

The sequence data from this study have been submitted to GenBank under the following accession numbers. Mm-*TOP1mt* cDNA, AF362952; the 5′ end of Mm-*TOP1mt* gene, AF503620; Gg-*TOP1mt* cDNA, AY429654; Dr-*TOP1mt* cDNA, AY429655; Rn-*TOP1mt* cDNA, TPA BK001786.

ACKNOWLEDGEMENT

We wish to thank Dr Kurt W. Kohn for insightful discussions.

REFERENCES

1. Champoux,J.J. (2001) DNA topoisomerases: structure, function and mechanism. *Annu. Rev. Biochem.*, **70**, 369–413.
2. Krogh,B.O. and Shuman,S. (2002) A poxvirus-like type IB topoisomerase family in bacteria. *Proc. Natl Acad. Sci. USA*, **99**, 1853–1858.
3. Wang,J.C. (2002) Cellular roles of DNA topoisomerases: a molecular perspective. *Nat. Rev. Mol. Cell. Biol.*, **3**, 430–440.
4. Anderson,L. (1981) Identification of mitochondrial proteins and some of their precursors in two-dimensional electrophoretic maps of human cells. *Proc. Natl Acad. Sci. USA*, **78**, 2407–2411.
5. Bibb,M.J., Van Etten,R.A., Wright,C.T., Walberg,M.W. and Clayton,D.A. (1981). Sequence and gene organization of mouse mitochondrial DNA. *Cell*, **26**, 167–180.
6. Zhang,H., Barcelo,J.M., Lee,B., Kohlhagen,G., Zimonjic,D.B., Popescu,N.C. and Pommier,Y. (2001) Human mitochondrial topoisomerase I. *Proc. Natl Acad. Sci. USA*, **98**, 10608–10613.
7. Wang,Y., Lyu,Y.L. and Wang,J.C. (2002) Dual localization of human DNA topoisomerase IIIalpha to mitochondria and nucleus. *Proc. Natl Acad. Sci. USA*, **99**, 12114–12119.
8. DeBry,R.W. and Seldin,M.F. (1996) Human/mouse homology relationships. *Genomics*, **33**, 337–351.
9. Redinbo,M.R., Stewart,L., Kuhn,P., Champoux,J.J. and Hol,W.G. (1998) Crystal structures of human topoisomerase I in covalent and noncovalent complexes with DNA. *Science*, **279**, 1504–1513.