Alignment and phylogenetic analysis of type II DNA topoisomerases

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Abstract. DNA topoisomerases have been evolved to solve the topological problems of DNA during replication, transcription, recombination and segregation. Discovery of several new enzymes and their characterization has necessitated this compilation. This analysis shows the distinct evolutionary relatedness of type II DNA topoisomerases. A striking feature is the absence of a contiguous stretch of about 160 amino acids in one of the subunits of prokaryotic type II enzymes, which might have important implications to their structure and function.

Keywords. Type II DNA topoisomerases; DNA gyrase; multiple alignment; phylogenetic trees.

1. Introduction

DNA topoisomerases catalyse topological interconversions: supercoiling-relaxation, catenation-decatenation and knotting-unknotting of DNA. These topological events occur during important cellular processes such as replication, transcription, recombination and chromosome segregation. Thus, the enzymes are essential for the cell survival, and hence are ubiquitous. The topoisomerases are classified into two distinct subclasses based on the mechanistics of the reaction (Wang 1985; Maxwell and Gellert 1986). The type I topoisomerases break one strand of DNA and pass the other strand through the nick created and change the linking number in steps of one. On the other hand, type II enzymes cleave both strands of DNA and pass the duplex through the 'DNA gate' resulting in the change of linking number in steps of two. All known topoisomerases form a transient covalent intermediate with DNA through a phosphotyrosine linkage and reseal after strand passage. Both prokaryotes and eukaryotes have been shown to possess multiple topoisomerases, possibly evolved to provide division of labour and in certain cases as backup strategies to take care of important cellular functions. The bacterium, *Escherichia coli* contains two type II topoisomerases besides two type I enzymes; the yeast, *Saccharomyces cerevisiae* has two type I activities (Wallis *et al* 1989), and in humans, two isozymes of topoisomerase II have been reported (Jenkins *et al* 1992). Amongst all type II topoisomerases, only DNA gyrase has the ability to introduce negative supercoils into DNA in an ATP driven reaction (Gellert *et al* 1976). The heteromeric enzyme has been the subject of extensive study (Reece and Maxwell 1991). The second bacterial type II enzyme, topoisomerase IV, has strong decatenation and weak relaxation activities. Both the bacterial type II topoisomerases have a similar architecture and also, share considerable sequence similarity.

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In this compilation, we have focused our attention on type II topoisomerases. This is due to the wealth of information available on these enzymes, their indispensability and the degree of conservation amongst the genes from variety of organisms. On the other hand, type I topoisomerases are less conserved and only few genes are characterized. Since an elaborate discussion of sequence conservation of topoisomerases has earlier been presented (Caron and Wang 1993), we have emphasized on the evolutionary relationship.

2. Sequence alignment and generation of evolutionary trees

The topoisomerase II genes have been characterized from several bacteria, yeast, protozoans and higher animals. Table 1 summarizes the source and the length of the derived polypeptides. The polypeptide sequences were aligned by MACAW ver 2.0.3 using BLOSSUM62 (Schuler *et al* 1991; Lawrence *et al* 1993). This software allows manual editing of the alignment. Also, the GyrB and GyrA polypeptide sequences were fused manually and aligned with eukaryotic type II topoisomerases using Multalin (Corpet 1988). The multiply aligned sequences were subjected to PHYLIP analysis (Felsenstein 1989). The distance matrix was generated by PROTDIST of PHYLIP employing Kimura-2 parameter. The output was then analysed by NEIGHBOR applying Neighbor-joining method. The UPGMA method was used for NEIGHBOR analysis of gyrase and eukaryotic topoisomerase II alignment. The unrooted trees were generated using DRAWGRAM and DRAWTREE.

3. Results and discussion

This compilation and alignment of type II topoisomerases is an attempt to compile complete sequences, identify subclasses and determine the extent of phylogenetic relationships. Sequence information on DNA gyrase and eukaryotic type II topoisomerase genes has been accumulating in the databank. These reports show conservation of amino acid sequence in gyrase and also its partial homology with eukaryotic type II topoisomerases. Hence, we have presented the alignment of all deduced polypeptide sequences of type II topoisomerases in figures 1 and 2. In order to avoid errors in alignment and phylogeny analyses, we have omitted partial sequences. The alignment of A subunits of gyrase and topoisomerase IV, given in figure 1A, shows the high sequence conservation predominantly in the amino terminal region. The DNA breakage-reunion site of subunit A has the sequence AAMRYTE common to all the members. The residue Tyr-122 of *E. coli* GyrA, present in this sequence gets covalently attached to DNA through phosphodiester bond. On the other hand, the C-terminal region does not show such extensive conservation. The dot matrix analysis, however, showed repeated sequences within this region in all GyrA sequences (Madhusudan and Nagaraja 1995). The C-terminal 33 kDa domain of *E. coli* GyrA has been shown to bind DNA (Reece and Maxwell 1991).

The subunit B of bacterial type II topoisomerases shows identical patches of amino acids scattered through out the sequence. The N-terminal 43 kDa fragment of *E. coli* GyrB is known to retain ATPase activity, a characteristic of all type II topoisomerases. The crystal structure of this domain complexed with ADPNP has revealed the direct interaction between the protein and the cofactor (Wigely *et al* 1991). These contact

Table 1. Polypeptide sequences of type II DNA topoisomerases.

Protein	Acronym	length	SwissProt/ *EmBL Acc. no.	Reference
GyrA				
<i>Escherichia coli</i>	EcoA	875	P09097	Swanberg and Wang 1987
<i>Klebsiella pneumoniae</i>	KpnA	876	P14829	Dimri and Das 1990
<i>Campylobacter jejuni</i>	CajA	863	Q03470	Wang <i>et al</i> 1993
<i>Rickettsia prowazekii</i>	RprA	905	P41080	Wood and Waite 1994
<i>Bacillus subtilis</i>	BsuA	821	P05653	Moriya <i>et al</i> 1985
<i>Staphylococcus aureus</i>	SauA	889	P20831	Margerrison <i>et al</i> 1992
<i>Streptomyces coelicolor</i>	ScoA	864	P35885	Calcutt 1994
<i>Haloferax Aa2.2</i>	HalA	858	*X60178	Holmes and Dyll-Smith 1994
<i>Mycobacterium tuberculosis</i>	MtbA	838	*L27512	Takiff <i>et al</i> 1994
<i>Mycobacterium smegmatis</i>	MsmA	854	*X84077	Madhusudan and Nagaraja 1995
GyrB				
<i>Escherichia coli</i>	EcoB	803	P06982	Yamagishi <i>et al</i> 1986; Adachi <i>et al</i> 1987
<i>Neisseria gonorrhoeae</i>	NgoB	781	P22118	Stein <i>et al</i> 1991
<i>Pseudomonas putida</i>	PpuB	806	P13364	Parales and Harwood 1990
<i>Bacillus subtilis</i>	BsuB	638	P05652	Moriya <i>et al</i> 1985
<i>Staphylococcus aureus</i>	SauB	640	P20832	Margerrison <i>et al</i> 1992
<i>Haloferax</i> sps.	HalB	639	P21558	Holmes and Dyll-Smith 1991
<i>Mycoplasma pneumoniae</i>	MpnB	650	P22447	Colman <i>et al</i> 1990
<i>Spiroplasma citri</i>	SciB	640	P34031	—
<i>Streptomyces sphaeroides</i> (novobiocin resistant)	SspBr	677	*Z17304	Thiara and Cundliffe 1993
<i>Streptomyces sphaeroides</i> (novobiocin sensitive)	SspBs	684	*Z17305	Thiara and Cundliffe 1993
<i>Streptomyces coelicolor</i>	ScoB	676	P35886	Calcutt 1994
<i>Mycobacterium tuberculosis</i>	MtbB	675	*X78888	Madhusudan <i>et al</i> 1994
<i>Mycobacterium smegmatis</i>	MsmB	675	*X84077	Madhusudan and Nagaraja 1995
ParC				
<i>Escherichia coli</i>	EcoC	752	P20082	Kato <i>et al</i> 1990
<i>Salmonella typhimurium</i>	StyC	752	P26973	Luttinger <i>et al</i> 1991
<i>Staphylococcus aureus</i>	SauE	800	*L25288	Ferrero <i>et al</i> 1994
ParE				
<i>Escherichia coli</i>	EcoE	630	P20083	Kato <i>et al</i> 1990
<i>Salmonella typhimurium</i>	StyE	630	P31598	Springer and Schmid 1991
<i>Staphylococcus aureus</i>	SauE	663	*L25288	Ferrero <i>et al</i> 1994
Eukaryotic topoisomerase II				
ASF Virus (BA71V)	Top2_AS	1192	Q00942	Garcia-Beato <i>et al</i> 1992
ASF Virus (Malawi)	Top2_AF	1191	P34203	Baylis <i>et al</i> 1992
<i>Crithidia fasciculata</i>	Top2_CR	1239	P27570	Pasion <i>et al</i> 1992
<i>Trypanosoma brucei</i>	Top2_TR	1221	P12531	Strauss and Wang 1990
<i>Trypanosoma cruzi</i>	Top2_TY	1232	P30190	Fragoso and Goldberg 1992
<i>Caenorhabditis elegans</i>	Top2_CA	1198	P34534	Wilson <i>et al</i> 1994
<i>Homo sapiens</i> (A)	TopA_HU	1530	P11388	Tsai-Pflugfelder <i>et al</i> 1988
<i>Homo sapiens</i> (B)	TopB_HU	1626	Q02880	Jenkins <i>et al</i> 1992
<i>Mus musculus</i> (Mouse)	Top2_MO	1528	Q01320	Adachi <i>et al</i> 1992
<i>Drosophila melanogaster</i>	Top2_DR	1447	P15348	Wykoff <i>et al</i> 1989
<i>Plasmodium falciparum</i>	Top2_PL	1398	P41001	Ridley and Kilbey 1994
<i>Saccharomyces cerevisiae</i>	Top2_YE	1429	P06786	Giaever <i>et al</i> 1986
<i>Schizosaccharomyces pombe</i>	Top2_SC	1431	P08906	Uemura <i>et al</i> 1986

MsmA
McbA

MsmA
McbA

Figure 1A.

Figure 1B.

TOP2_AS	neafseisaftebakkkswagalnkvtsiglmvfytededlmalp	92
TOP2_AF	neafseisaftebakkkswagalnkvtsiglmvfytededlmalp	92
TOP2_CA	mydthdlrlwafftkvvykagdeltwydytgdqatattqltchgsenctgrllkkaakvkspehvlrrldpytjgvmvredolwlrdsenkmakvtytpollkifidelvln	142
TOP2_CR	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	92
TOP2_DR	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	92
TOP2_DL	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	92
TOP2_PL	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	92
TOP2_MO	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	92
TOP2_SC	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	92
TOP2_TR	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	92
TOP2_TY	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	92
TOP2_XE	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	92
TOP2_YE	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	92
TOPB_HU	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	92
TOPB_HU	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	92
TOP2_AS	neafseisaftebakkkswagalnkvtsiglmvfytededlmalp	344
TOP2_AF	neafseisaftebakkkswagalnkvtsiglmvfytededlmalp	344
TOP2_CA	mydthdlrlwafftkvvykagdeltwydytgdqatattqltchgsenctgrllkkaakvkspehvlrrldpytjgvmvredolwlrdsenkmakvtytpollkifidelvln	344
TOP2_CR	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	344
TOP2_DR	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	344
TOP2_DL	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	344
TOP2_PL	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	344
TOP2_MO	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	344
TOP2_SC	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	344
TOP2_TR	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	344
TOP2_TY	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	344
TOP2_XE	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	344
TOP2_YE	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	344
TOPB_HU	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	344
TOPB_HU	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	344
TOP2_AS	neafseisaftebakkkswagalnkvtsiglmvfytededlmalp	499
TOP2_AF	neafseisaftebakkkswagalnkvtsiglmvfytededlmalp	499
TOP2_CA	mydthdlrlwafftkvvykagdeltwydytgdqatattqltchgsenctgrllkkaakvkspehvlrrldpytjgvmvredolwlrdsenkmakvtytpollkifidelvln	499
TOP2_CR	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	499
TOP2_DR	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	499
TOP2_DL	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	499
TOP2_PL	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	499
TOP2_MO	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	499
TOP2_SC	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	499
TOP2_TR	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	499
TOP2_TY	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	499
TOP2_XE	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	499
TOP2_YE	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	499
TOPB_HU	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	499
TOPB_HU	-----IHRDHC PALLKIFIDELVNAVDHERACHSKTKVYIKISFDKGVFS	499

TOP2_AS
TOP2_AF
TOP2_CA
TOP2_CR
TOP2_DR
TOP2_MO
TOP2_PL
TOP2_SC
TOP2_SR
TOP2_TY
TOP2_VE
TOPA_HU
TOPB_HU

TOP2_AS
TOP2_AF
TOP2_CA
TOP2_CR
TOP2_DR
TOP2_MO
TOP2_PL
TOP2_SC
TOP2_SR
TOP2_TY
TOP2_VE
TOPA_HU
TOPB_HU

TOP2_AS
TOP2_AF
TOP2_CA
TOP2_CR
TOP2_DR
TOP2_MO
TOP2_PL
TOP2_SC
TOP2_SR
TOP2_TY
TOP2_VE
TOPA_HU
TOPB_HU

Figure 1. Alignment of type II topoisomerases. (A) Subunit A of bacterial type II topoisomerases. The conserved tyrosine residues involved in DNA breakage-reunion reaction is marked by an asterisk (corresponding to *E. coli* sequence). (B) Subunit B of bacterial type II topoisomerases. The asterisks mark the residues involved in contacting the nucleotide cofactor. All the positions correspond to *E. coli* sequence. (C) Eukaryotic type II topoisomerases. The sequence in the upper case represent the region with high conservation.

points, Tyr-5, Asn-46, Asp-73, Lys-103, Tyr-109, Gln-335 and Lys-337 are positionally conserved in all the members, except *Spiroplasma citri* wherein Lys-337 is replaced by Asn. The two important residues, Glu-42 and His-38 (Jackson and Maxwell 1994) implicated in ATPase activity of the subunit B of the *E. coli* enzyme are also present in all the other B sequences examined. A very significant difference is the absence of a long stretch of amino acids (158–163 amino acids) in GyrB proteins of Gram positive bacteria and *Mycoplasma* (figure 2B). The same stretch of amino acids is also absent in ParE sequences (B subunits of topoisomerase IV) of both Gram negative bacteria and *S. aureus*. It should also be noted here that gyrase and topoisomerase IV differ not only in their supercoiling ability but also in their potency of decatenation.

The subunits of bacterial type II topoisomerases were further analysed to understand the evolutionary relatedness. The unrooted trees are shown in figures 3 and 4. The ParC proteins share a branch with GyrA of higher bacteria (figure 3). The ParE proteins are located closer to GyrB polypeptides of Gram negative bacteria (figure 4) in spite of sharing a common character (the absence of a long stretch of amino acids) with Gram positive bacteria and *Mycoplasma* (figure 2B). The B subunit of bacterial type II topoisomerases shows a distinct feature. All the GyrB proteins of *Mycoplasma* and Gram positive bacteria form a monophyletic group while other GyrB polypeptides and ParE sequences diverge into another group (figure 4). In case of A subunits, no such clear separation could be observed (figure 3).

The eukaryotic type II topoisomerases also show primary conservation to some extent. Although the sequences have diverged, functionally significant domains (like

Figure 2A.

Figure 2. Schematic representation of multiple alignment derived from figure 1. The panels (A) (B) and (C) correspond to that of figure 1. The thick sequences correspond to blocks sharing significant similarity with the shaded regions representing the conserved sequence. The thin blocks represent the regions of the sequence not sharing statistically significant homology in the multiple alignment.

Figures 3 and 4. Evolutionary relationship among bacterial topoisomerase II subunits. Unrooted phylogenetic trees produced from the alignment of A subunits (3) in figure 1A and B subunits (4) in figure 1B, using DRAWTREE.

ATPase and DNA breakage-reunion regions) have retained the residues important for the activities. The crystal structure of 92 kDa domain of yeast DNA topoisomerase II at 2.7 Å has been reported recently (Berger *et al* 1996). Whereas the N-terminal 409 amino acids constitute ATPase domain that shares homology with bacterial DNA gyrase subunit B ATPase region, this domain has been implicated in DNA cleavage and strand passage reactions required for the topological interconversion (Berger and Wang 1996). This

Figure 5. Phylogenetic tree of type II topoisomerases. The bacterial type II topoisomerase subunits were fused prior to the alignment. The abbreviations correspond to those given in the table 1, with AB representing fused sequences.

domain contains GyrB and GyrA like sequences (Caron and Wang 1993). Also, this region shows high conservation among eukaryotic type II DNA topoisomerases (figures 1C and 2C). Beyond this region, the amino acid sequences of eukaryotic type II DNA topoisomerases display less conservation. The cluster analysis of all type II topoisomerases, presented in figure 5, shows the monophyletic separation of bacterial sequences from the distinct diphyletic groups of eukaryotic enzymes.

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