Subject: Biotechnology

Paper No.: 04 Genetic engineering and Recombinant DNA technology
Module: 05 DNA Topoisomerase

<table>
<thead>
<tr>
<th>Principal Investigator:</th>
<th>Dr Vibha Dhawan, Distinguished Fellow and Sr. Director The Energy and Resources Institute (TERI), New Delhi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co-Principal Investigator:</td>
<td>Prof S K Jain, Professor, of Medical Biochemistry Jamia Hamdard University, New Delhi</td>
</tr>
<tr>
<td>Paper Coordinator:</td>
<td>Dr Mohan Chandra Joshi, Assistant Professor, MCARS, Jamia Millia Islamia, New Delhi</td>
</tr>
<tr>
<td>Content Writer:</td>
<td>Dr Mohan Chandra Joshi, Assistant Professor, MCARS, Jamia Millia Islamia, New Delhi</td>
</tr>
<tr>
<td>Content Reviewer:</td>
<td>Samer Singh, Ph.D., Assistant Professor &amp; Ramalingaswami Fellow, Department of Microbial Biotechnology, Panjab</td>
</tr>
</tbody>
</table>
James Wang demonstrated that an enzyme (first report of DNA topoisomerase) can change supercoiling (negative) of λ DNA of bacteriophage. The change in negative supercoiling was measured using sedimentation rate of DNA in presence/absence of the enzyme. Subsequently, researchers from different groups reported discovery of similar enzymes with capability of altering the DNA topology.

DNA topoisomerases are ubiquitous enzymes found in all kingdoms of life. These enzymes play an important role in maintaining DNA supercoiling and dynamics. These enzymes play major roles in various cellular DNA metabolism processes. For example, the role of DNA topoisomerases during transcription and replication is well documented. During these processes, the replication complex or transcription machinery need to access the DNA, which is highly compacted and condensed, topoisomerases modulate the compaction/supercoiling to allow access to DNA. Furthermore, role of topoisomerase in eukaryotic cell-cycle is also well established as topo-II plays an important role in cohesion loss and DNA condensation. In prokaryotes, particularly in E. coli, biochemical studies have documented role of these enzymes in various enzymatic reactions post-cell cycle however, recent studies have shown greater role of topoisomerase in replication and segregation (primarily during cohesion).

**Type of Topoisomerases:** Topoisomerases are enzymes that introduce a cut to DNA molecule to relieve torsional stress. Based upon the number of cut that are introduced by topoisomerase, enzymes are classified into two categories: (a) Type-I Topoisomerases; and (b) Type-II topoisomerases. Type-I topoisomerases are enzyme that induce a single cut to release the torsional stress, while Type-II topoisomerase induce cut at both strands of DNA to release the torsional stress.

**Type-I Topoisomerase:** These enzymes induce single cut in a DNA strand to relax DNA supercoiling. Majority of topoisomerases doesn’t require ATP as an energy source to
complete the reaction. Instead, these enzymes utilize energy that is stored in compact and supercoiled DNA. However, it remains poorly understood that how enzyme recognizes these structure and utilizes the stored energy. Reverse gyrase is an exception this as it utilizes ATP as a source of energy to carry out a reaction. Type-I topoisomerases can relax positively and negatively supercoiled DNA either one by one or simultaneously except reverse gyrase that can introduce positive supercoils. Type-I DNA topoisomerases modulate linking number between two DNA strands, which results in change the number of superhelical turn. Moreover, these enzymes form a covalent bond with broken end of the DNA strand (either with 5’or 3’end). Type-I topoisomerases can further sub-classified into three subgroups; (a) type-IA, (b) type-1B, and (c) type-1C.

Table-1: Topoisomerases across kingdom (source: Andrew D. Bates, Anthony Maxwell, DNA Topology, Oxford University Press, 2005 - Science - 198 pages)*

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Type</th>
<th>Source</th>
<th>Subunit size (kDa)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial topoisomerase I</td>
<td>IA</td>
<td>Bacteria (e.g. E. coli)</td>
<td>97 Monomer</td>
<td>Cannot relax positive supercoils</td>
</tr>
<tr>
<td>Eukaryotic topoisomerase I</td>
<td>IB</td>
<td>Eukaryotes (e.g. human)</td>
<td>91 Monomer</td>
<td>Can relax both positive and negative supercoils</td>
</tr>
<tr>
<td>Vaccinia virus topoisomerase I</td>
<td>IB</td>
<td>Vaccinia virus</td>
<td>37 Monomer</td>
<td>ATP stimulates topoisomerase activity</td>
</tr>
<tr>
<td>Topoisomerase IIIa</td>
<td>IA</td>
<td>Bacteria (e.g. E. coli)</td>
<td>73 Monomer</td>
<td>Potent decatenating activity</td>
</tr>
<tr>
<td>Reverse gyrase</td>
<td>IA</td>
<td>Thermophilic Archaea (e.g. Sulfolobus acidocaldarius)</td>
<td>143 Monomer</td>
<td>Can introduce positive supercoils into DNA (ATP-dependent)</td>
</tr>
<tr>
<td>DNA gyrase</td>
<td>IIA</td>
<td>Bacteria (e.g. E. coli)</td>
<td>97 and 90 A₂B₂</td>
<td>Can introduce negative supercoils into DNA (ATP-dependent)</td>
</tr>
<tr>
<td>T4 topoisomerase</td>
<td>IIA</td>
<td>Bacteriophage T4</td>
<td>58, 51, and 18 2 copies of each subunit</td>
<td>Can relax, but not supercoil, DNA (ATP-dependent)</td>
</tr>
<tr>
<td>Eukaryotic topoisomerase II</td>
<td>IIA</td>
<td>Eukaryotes (e.g. human topoisomerase IIx)</td>
<td>174 Homodimer</td>
<td>Can relax, but not supercoil, DNA (ATP-dependent)</td>
</tr>
<tr>
<td>Topoisomerase IVa</td>
<td>IIA</td>
<td>Bacteria (e.g. E. coli)</td>
<td>84 and 70 C₂E₂</td>
<td>Can relax, but not supercoil, DNA, potent decatenase (ATP-dependent)</td>
</tr>
<tr>
<td>Topoisomerase VI</td>
<td>IIB</td>
<td>Archaea (e.g. Sulfolobus shibatae)</td>
<td>45 and 60 A₂B₂</td>
<td>Can relax, but not supercoil, DNA (ATP-dependent)</td>
</tr>
</tbody>
</table>

Type-1A Topoisomerase: This class of topoisomerases is primarily found in bacteria that show sequence, structural as well as mechanistic similarity. Reverse gyrase, type-IA is an exception to this subgroup that utilizes ATP and divalent dependent enzyme to carry out its function, while rest namely, bacterial and archaean topoisomerase I and topoisomerase III perform its action independent of ATP. Structurally, these enzymes contain four domains (I-IV), in which active site (catalytic tyrosine residue) lies in domain-III. Fig.1 shows structure of Topo-III from E. coli, in which domain-III is shown in purple, which lies in close proximity of...
domain-I (Brown) and domain-IV (green), with domain-II positions itself at 90 degree angle to rest of the domains. Substrate (Single stranded DNA) binds to domain III and I that allows active sites to access DNA to make a cut, which results in a transient bond between 5’ ends of DNA with tyrosine. Domain-II acts as hinge to separate broken strand to allow passage of second strand. Once strand passage is completed domain III & I is closed and strand is reannealed, thus allowing strand passage.

Fig.1. E. coli topoisomerase-III (source: Nicole M. Baker, Rakhi Rajan, & Alfonso Mondragón. Structural studies of type I topoisomerases. Nucleic Acids Res. 2009 Feb; 37(3): 693–701.)*

Strand passage/relaxation reaction can be classified into intramolecular (same supercoiled DNA duplex) or intermolecular (Independent DNA molecule) reactions. These reaction differ in terms of its substrate and compared to intramolecular reaction, intermolecular reaction is reversible (Fig.2.).

Type-1B topoisomerase: In comparison to type-1A, this subgroup of enzymes relax supercoiling (both negative as well as positive) through rotation. The enzyme wraps itself around substrate (DNA) and creates a transient bond with 3’end of DNA with tyrosine group of active site. This results in free 5’end, which rotates itself around other strand. Subsequently, the DNA is reannealed to relieve the supercoiling. These subgroups of enzyme are found in bacteria, eukaryotes as well as in poxviruses. Type-1B enzymes are structurally and functionally similar that contains N-terminal domain, active site and a capping domain and a C-terminal domain (Fig.3.). N-terminal domain is dispensable for enzymatic activity, core domain and C-terminal together can constitute a functional enzyme.

Fig.2. Intra and Intermolecular reaction (source: Zhiyu Li, Alfonso Mondragon, Russell J DiGate, The Mechanism of Type IA Topoisomerase-Mediated DNA Topological Transformations Molecular cell Volume 7, Issue 2, 2001, 301-307)*
Type-IC Topoisomerase: DNA topoisomerase-V has been recently classified as type-1C, though functionally it has similarity with type-IB but show no sequence or structural similarity. Topo-V is isolated from a thermophile "Methanopyrus kandleri". Similar to type-IB enzyme, it cleaves single DNA strand to create a transient bond between 3’ end of phosphate of broken DNA and tyrosine and allows relaxing the supercoiling by rotation. As mentioned earlier, these reactions are resulted similar outcome with no significant structure similarity. As shown in fig.4. Structure differs from type-IB (fig.3.), as well as the spatial position of active between two enzymes, also it contains acidic residue at its active site, which is absent in type-IB. It is fascinating that two sequentially and structurally different molecules result in similar outcome with a common substrate. Further investigation will reveal how nature evolves this configuration to maintain functional similarity.

Type-II topoisomerase: The Type-II topoisomerases are enzyme that introduce cut at both the strands of DNA to allow strand passage. These enzymes change the DNA linking number by a factor of two. These enzymes are found in bacteria, Eukaryotes as well as in Archaea. Well known type-II topoisomerase in E. coli is Topo-IV and gyrase; this enzyme can be further classified as, (a) Type-IIA; and (b) Type-IIB enzyme. Bacterial DNA gyrase, Topoisomerase-IV, and human topoisomerase-II (Topo-II) belongs to type-IIA; while topoisomerase VI belongs to type-IIB. Type-IIB are structurally and biochemically distinct enzyme and are mostly found in archaea or higher plants.
Biotechnology

Genetic engineering and Recombinant DNA technology

DNA Topoisomerase

Type-II topoisomerases involve the following steps during decatenation/catenation or relaxation, knotting/unknotting (Fig. 6);

Step 1. Enzyme binds to double stranded DNA molecule and introduces a cut on to the opposite strand.
Step 2. Enzyme attaches itself to free 5’ end DNA through a phosphoryl-tyrosine bond.
Step 3. This results in a conformational change of enzyme, termed as G-Segment DNA, creating a N-Gate.
Step 4. Depending upon the reaction, whether Decatenation/Catenation or Knotting/Unknotting, either different DNA double strand from other molecule or other duplex DNA from same molecule is allowed to enter through N-Gate (termed as T-Segment).
Step 5. Utilizing ATP, DNA gate is closed and passage of strands is allowed.
Step 6. Once strand passage is complete the DNA is released via C-Gate.

Type-IIA and IIB topoisomerases differ in the following;

(a) IIA enzyme breaks DNA duplex resulting in four base pair overhang, while IIB enzyme results in two base pair overhang; and
(b) IIA enzyme can simplify DNA topology below the equilibrium but IIB enzyme cannot simplify DNA topology below the equilibrium. This represents evolution of enzyme depending upon the system requirement. Therefore, DNA topoisomerases represent an intriguing example of enzyme evolution under selection pressure that results in altered structural property but retains its enzymatic capabilities/potential.
Reactions undertaken by Topoisomerase: As mentioned earlier, topoisomerases play an important role in cellular DNA metabolism. These enzymatic reactions are categorized as: (a) Catenation; (b) Decatenation; (c) Knotting; (d) Unknotting; and (e) Relaxing or introducing supercoiling. Figure-1 schematically represents the reactions. Catenation is defined as introduction of linkage between DNA molecules, while removing these linkages is defined a decatenation. Supercoiling can be introduced in a relaxed DNA molecule by introducing linkages as well as supercoiling can be relaxed by reducing the linking number. Knots are complex DNA topology that includes complex intertwining of DNA resulting either post-replication or transcription (Fig.7.). Reaction carried out by Type-II topoisomerases are reversible, which means it can relax as well as introduce linkages, while reaction mediated by type-I topoisomerases are one direction, except, knotting/unknotting and Catenation decatenation (Fig.7A and B).

DNA Topoisomerase as a drug target: Topoisomerases are evolutionary conserved across kingdom; thereby make an excellent target for cytotoxic drugs. Both classes of topoisomerases (type-I and Type-II) are being used to generate inhibitors. For example type-II topoisomerases in bacteria (Topo-IV and well as gyrase) are targeted by antibiotics such as Quinolones (ciprofloxacin) and Coumarins (nalidixic acid). In Eukaryotes, Topo-I is used a target for chemotherapeutic drugs. These inhibitors bind to enzyme and lock the conformation that either prevents either re-annealing of DNA Strand or prevents forward movement of DNA. Subsequently, these inhibitors result in DNA double strand break and induction of either SOS response or check point activation, resulting in cell-cycle arrest.

D) Summary:
- Topoisomerases are evolutionary conserved and are crucial for Cellular DNA metabolism
• These enzymes are classified as type-I and type-II, based upon number of cuts it makes in a DNA strand.
• Topoisomerases is critical for: (a) accessing DNA; (b) removing DNA supercoils; (c) strand passage during recombination; (d) chromosome condensation; and (e) Decatenation/Catenation, Unknotting/Knotting of intertwined DNA.
• These enzyme show varying degree of structure and sequence similarities, however undertake similar reaction with great efficiency.
• Being evolutionary conserved enzyme, Topoisomerases are being used a drug target, as an antibiotics as well as a chemotherapeutic agent.
• However it remains challenging for researchers to answer that (a) how these enzymes show such specificity toward topology preference, (b) what is the interplay between these enzymes during DNA metabolism; and (c) how kinetics of enzymatic reaction differs for each enzyme based upon DNA topology.