IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 2, March 2022
Impact Factor: 6.252

Topoisomerase: An Overview

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Abstract: *The structure of DNA is a double-stranded helix, where the four bases are paired and stored in the center of this helix. The two strands of DNA are intertwined and this would require the two strands to be untwisted in order to access the information stored. Topoisomerases catalyze and guide the unknotting of DNA by creating transient breaks in the DNA using a conserved Tyrosine as the catalytic residue. Two classes of Topoisomerses are identified yet. Since the overall chemical composition and connectivity of the DNA does not change, the tangled and untangled DNAs are chemical isomers, differing only in their global topology, hence the enzymes are named as Topoisomerases. The insertion of viral DNA into chromosomes and other forms of recombination also require the action of topoisomerases. Topoisomerase inhibitors are agents designed to interfere with the action of topoisomerase enzymes, which control the changes in DNA structure by catalyzing the breaking and rejoining of the phosphodiester backbone of DNA strands during the normal cell cycle. Thus they are found to be important tools for treatment of cancer..*

Keywords: Cancer, DNA, Irinotecan, Topoisomerases

I. INTRODUCTION

Topoisomerases (type I: EC 5.99.1.2, type II: EC 5.99.1.3) are enzymes that unwind and wind DNA, in order for DNA to control the synthesis of proteins and to facilitate DNA replication. The enzyme is necessary due to inherent problems caused by the double-helical nature of DNA, as first noted by Watson and Crick (Nature, May (171) 1953) The structure of DNA is a double-stranded helix, where the four bases, adenosine, thymidine, guanidine and cytosine are paired and stored in the center of this helix. While this structure provides a stable means of storing the genetic code, Watson and Crick noted that the two strands of DNA are intertwined and this would require the two strands to be untwisted in order to access the information stored. However they also foresaw that there would be some mechanism to overcome this problem. In order to help overcome these problems caused by the double helix, topoisomerases bind to either single-stranded or double stranded DNA and cuts the phosphate backbone of the DNA. This intermediate break allows the DNA to be untangled or unwound and at the end of these processes, the DNA is reconnected again. Since the overall chemical composition and connectivity of the DNA does not change, the tangled and untangled DNAs are chemical isomers, differing only in their global topology, thus their name. Topoisomerases are isomerase enzymes that act on the topology of DNA.

II. FUNCTION

The double-helical configuration that DNA strands naturally reside in, makes them difficult to separate and yet they must be separated by helicase proteins if other enzymes are to transcribe the sequences that encode proteins, or if chromosomes are to be replicated. In so-called circular DNA, in which double helical DNA is bent around and joined in a circle, the two strands are topologically linked, or knotted. Otherwise identical loops of DNA having different numbers of twists are topoisomers and cannot be interconverted by any process that does not involve the breaking of DNA strands. Topoisomerases catalyze and guide the unknotting of DNA by creating transient breaks in the DNA using a conserved Tyrosine as the catalytic residue. The insertion of viral DNA into chromosomes and other forms of recombination can also require the action of topoisomerases.

III. TOPOLOGICAL PROBLEMS

There are three main types of topology: supercoiling, knotting and catenation. Outside of the essential processes of replication or transcription, DNA needs to be kept as compact as possible and these three states help this cause. However when transcription or replication occur, DNA needs to be free and these states seriously hinder the processes. In addition,

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during replication, the newly replicated duplex of DNA and the original duplex of DNA become intertwined and need to be completely separated in order to ensure genomic integrity as a cell divides.As a transcription bubble proceeds, DNA ahead of the transcription fork becomes overwound, or positively supercoiled, while DNA behind the transcription bubble becomes underwound, or negatively supercoiled. As replication occurs, DNA ahead of the replication bubble becomes positively supercoiled, while DNA behind the replication fork becomes entangled forming precatenanes. One of the most essential topological problem occurs at the very end of replication, when daughter chromosomes must be fully disentangled before mitosis occurs. Topoisomerase IIA plays an essential role in resolving these topological problems.

3.1 Classes

Topoisomerases can fix these topological problems and are separated into two types separated by the number of strands cut in one round of action: Both these classes of enzyme utilize a conserved tyrosine. However these enzymes are structurally and mechanistically different.

- **Type I** topoisomerase cuts one strand of a DNA double helix, relaxation occurs and then the cut strand is reannealed. Type I topoisomerases are subdivided into two subclasses: type IA topoisomerases which share many structural and mechanistic features with the type II topoisomerases and type IB topoisomerases, which utilize a controlled rotary mechanism.[7] Examples of type IA topoisomerases include topo I and topo III. Historically, type IB topoisomerases were referred to as eukaryotic topo I, but IB topoisomerases are present in all three domains of life. Interestingly, type IA topoisomerases form a covalent intermediate with the 5' end of DNA, while the IB topoisomerases form a covalent intermediate with the 3' end of DNA. Recently, a type IC topoisomerase has been identified, called topo V. While it is structurally unique from type IA and IB topoisomerases, it shares a similar mechanism with type IB topoisomerase.
- **Type II** topoisomerase cuts both strands of one DNA double helix, passes another unbroken DNA helix through it and then reanneals the cut strand. It is also split into two subclasses: type IIA and type IIB topoisomerases, which share similar structure and mechanisms. Examples of type IIA topoisomerases include eukaryotic topo II, E. coli gyrase and E. coli topo IV. Examples of type IIB topoisomerase include topo VI.

Both type I and type II topoisomerases change the linking number of DNA. Type IA topoisomerases change the linking number by one, type IB and type IC topoisomerases change the linking number by any integer, while type IIA and type IIB topoisomerases change the linking number by two.

3.2 Topoisomerase Inhibitor

Topoisomerase inhibitors are agents designed to interfere with the action of topoisomerase enzymes (topoisomerase I and II), which are enzymes that control the changes in DNA structure by catalyzing the breaking and rejoining of the phosphodiester backbone of DNA strands during the normal cell cycle. In recent years, topoisomerases have become popular targets for cancer chemotherapy treatments. It is thought that topoisomerase inhibitors block the ligation step of the cell cycle, generating single and double stranded breaks that harm the integrity of the genome. Introduction of these breaks subsequently lead to apoptosis and cell death Topoisomerase inhibitors can also function as antibacterial agents. Quinolones have this function.

IV. CLASSIFICATION

Topoisomerase inhibitors are often divided according to which type of enzyme it inhibits.

- Topoisomerase I inhibitors: irinotecan, topotecan, camptothecin and lamellarin D all target type IA topoisomerases.
- Topoisomerase II inhibitors: etoposide, doxorubicin.

4.1 Compounds that Target Type II Topoisomerase

These inhibitors are split into two main classes: topoisomerase poisons, which target the topoisomerase-DNA complex and topoisomerase inhibitors, which disrupt catalytic turnover.[13]

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A. Topo II Poisons

Examples of topoisomerase poisons include the following:

- Eukaryotic type II topoisomerase inhibitors (topo II): amsacrine, etoposide, etoposide phosphate, teniposide and doxorubicin. These drugs are anti-cancer therapies.
- Bacterial type II topoisomerase inhibitors (gyrase and topo IV): fluoroquinolones. These are antibacterials and include such fluoroquinolones as ciprofloxacin.

Some of these poisons encourage the forward cleavage reaction (fluoroquinolones), while other poisons prevent the re-ligation of DNA (etoposide and teniposide).Interestingly, poisons of type IIA topoisomerases can target prokaryotic and eukaryotic enzymes preferentially, making them attractive drug candidates. Ciprofloxacin targets prokaryotes in excess of a thousandfold more than it targets eukaryotic topo IIs. The mechanism for this specificity is unknown, but drug-resistant mutants cluster in regions around the active site.

B. Topo II Inhibitors

Examples of topoisomerase inhibitors include ICRF-193. These inhibitors target the N-terminal ATPase domain of topo II and prevent topo II from turning over. The structure of this compound bound to the ATPase domain was solved by Classen showing that the drug binds in a non-competitive manner and locks down the dimerization of the ATPase domain.

4.2 Topoisomerases I & II

DNA Topoisomerases are enzymes that catalyze the unlinking of the DNA strands by making transient DNA strand breaks and allowing the DNA to rotate around these breaks. Both Topoisomerase I and Topoisomerase II can remove DNA supercoiling by catalyzing DNA swiveling and relaxation. There are many top2 inhibitors and a large number of them, including the anthracyclines had been used as anticancer agents before identification of top2 as their target. The antitumor top2 inhibitors presently used in the clinic poison the enzyme by stabilizing cleavable complexes, presumably by inhibiting DNA religation and preventing enzyme catalytic activity. It has been found that removal of the 4-methoxy group in the newer anthracyclines such as Idarubicin exhibit greater potency against purified top2 than daunorubicin. Anthracyclines induce mostly top2-mediated DNA double strand breaks. The development of a method to align and analyze DNA sequences around topoisomerases cleavage sites enabled a demonstration that drug differences were correlated with base sequence preferences and to propose the stacking model. Interaction of RNA transcription with cleavable complexes may play a role in the activity of top2 inhibitors.Top2-mediated DNA damage may be responsible for the chromosomal translocations associated with etoposide-induced secondary malignancies.

4.3 Structure of Topoisomerase

During replication, the unwinding of DNA may cause the formation of tangling structures, such as supercoils or catenanes. The major role of topoisomerases is to prevent DNA tangling.

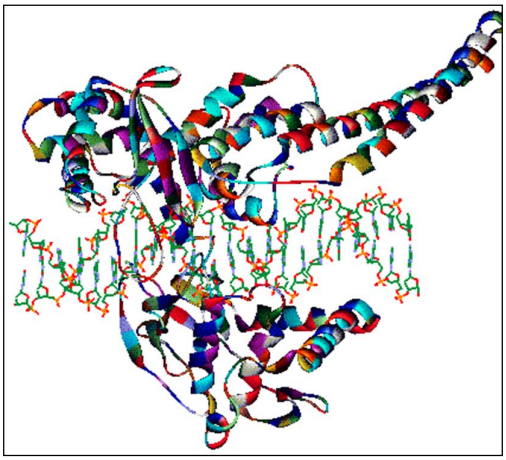

Figure 1: Structure of the Topo I/DNA complex. PDB ID = 1A36.

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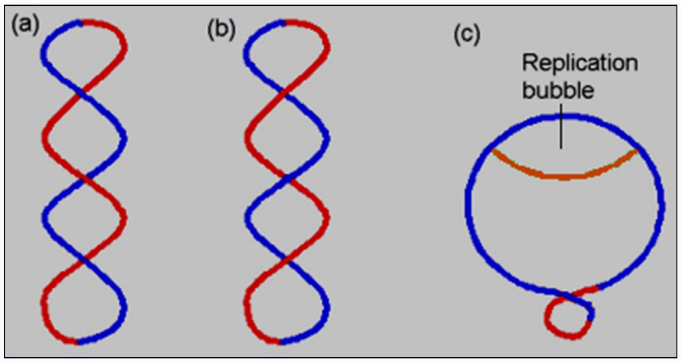

Figure 2: The structure of supercoils. (a) Positive supercoils - the front segment of a DNA molecule cross over the back segment from left to right. (b) Negative supercoils. (c) The positive supercoil in bacteria during DNA replication.

There are two types of topoisomerases: type I produces transient single-strand breaks in DNA and types II produces transient double-strand breaks. As a result, the type I enzyme removes supercoils from DNA one at a time, whereas the type II enzyme removes supercoils two at a time. Although the type II topoisomerase is more efficient in removing supercoils, this enzyme requires the energy from ATP hydrolysis, but the type I topoisomerase does not. The topo I of both prokaryotes and eukaryotes are the type I topoisomerase. The eukaryotic topo II, bacterial gyrase and bacterial topo IV belong to the type II. In eukaryotes, the topo I and topo II can remove both positive and negative supercoils. In bacteria, the topo I can remove only negative supercoils. The bacterial topo II is also called the gyrase, which has two functions: (a) to remove the positive supercoils during DNA replication, (2) to introduce negative supercoils (one supercoil for 15-20 turns of the DNA helix) so that the DNA molecule can be packed into the cell. During replication, these negative supercoils are removed by topo I. The bacterial topo IV belongs to the type II topoisomerase. This enzyme is involved in decatenation.

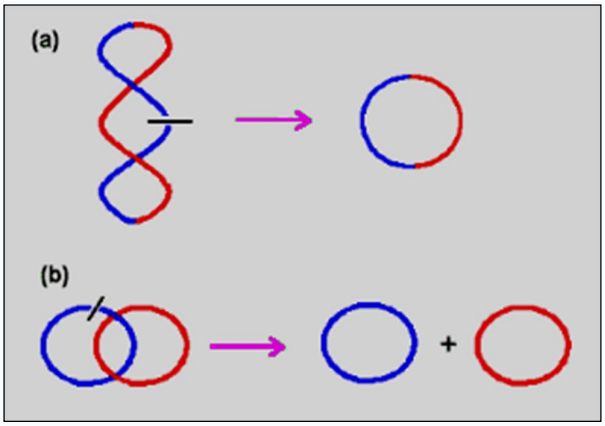

Figure 3: The function of topo II: (a) To remove supercoils. This involves a double-strand break (indicated by a short line), allowing the tangled segment to pass through. The break is then resealed. (b) To remove catenanes. The topo II makes a double-strand break in one DNA molecule (the blue one), allowing the other molecule to pass through. The break is then resealed.

Without topoisomerases, the DNA cannot replicate normally. Therefore, the inhibitors of topoisomerases have been used as anti-cancer drugs to stop the proliferation of malignant cells. However, these inhibitors may also stop the division of normal cells. Some cells (e.g., hair cells) which need to continuously divide will be most affected. This explains a noticeable side effect: the hair loss.

V. TYPES I TOPOISOMERASES

5.1 Type IA Topoisomerses

A. Introduction

Type IA topoisomerases, historically said to be found in prokaryotes, creates a single break in DNA and passes a second

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strand or duplex through the break. This strand passage mechanism shares several features with type IIA topoisomerases. They both form a 5' phosphotyrosine intermediate and require a divalent metal ion to perform its work. Unlike type II topoisomerases, type IA topoisomerases do not use energy to do its work.

B. Structure

Type IA topoisomerases have several domains, often number Domain 1-4. Domain I contains a Toprim domain (a Rossman fold known to coordinate Magnesium ions), domain IV and domain III each consist of a helix-turn-helix (HTH) domain; the catalytic tyrosine resides on the HTH of domain III. Domain II is a flexible bridge between domains III and IV. The structure of type IA topoisomerase resembles a lock, with Domains I, III and IV lie on the bottom of the structure The structure of topo III (see below) bound to single-stranded DNA (pdb id = 1I7D) shows how the HTH and Toprim domain are coordinated about the DNA.

Figure 4: Structure of topo III bound to single stranded DNA (pdb ID 1I7D). Note that the DNA resembles B-form DNA

C. Type IA Topo Variants

There are several variants of Type IA topoisomerases, differing by appendages attached to the main core (sometimes referred to as the "topo-fold"). Members of this subclass include topo I, topo III (which contain additional Zinc-binding motifs) and reverse gyrase. Reverse gyrase is particularly interesting because a ATPase domain, that resembles the helicase-like domain of the Rho transcription factor, is attached The enzyme uses the hydrolysis of ATP to introduce positive supercoils and overwinds DNA, a feature attractive in hyperthermophiles, in which reverse gyrase is known to exist. Rodriguez and Stock have done further work to identify a "latch" that is involved in communicating the hydroylsis of ATP to the introduction of positive supercoils. The topo III variant is likewise very interesting because it has zinc-binding motifs that is thought to bind single-stranded DNA. Topo III has been identified to be associated with the BLM (for Bloom Syndrome) helicase during recombination.

D. Mechanism

Type IA topoisomerases operate through a strand-passage mechanism, using a single gate (in contrast with type II topoisomerases). First, the single-stranded DNA binds domain III and I. The catalytic tyrosine cleaves the DNA backbone, creating a transient 5' phosphotyrosine intermediate. The break is then separated, using domain II as a hinge and a second duplex or strand of DNA is passed through. Domain III and I close and the DNA is re-annealed.

5.2 Type IB Topoisomerases

A. Introduction

In contrast to type IA topoisomerases, type 1B Topoisomerase solves the problem of overwound and underwound (also referred to as positively or negatively supercoiled) DNA through a hindered rotary mechanism. Crystal structures, biochemistry and single molecule experiments have contributed to a general mechanism. The enzyme first wraps around DNA and creates a single, 3' phosphotyrosine intermediate. The 5' end is then free to rotate, twisting it about the other strand, to relax DNA until the topoisomerase religates the broken strands.

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B. Structure

The structure of topo IB bound to DNA has been solved (pdb id = 1A36). Topo IB is composed of a NTD, a capping lobe, a catalytic lobe and a C-terminal domain. The capping lobe and catalytic lobe wrap around the DNA.

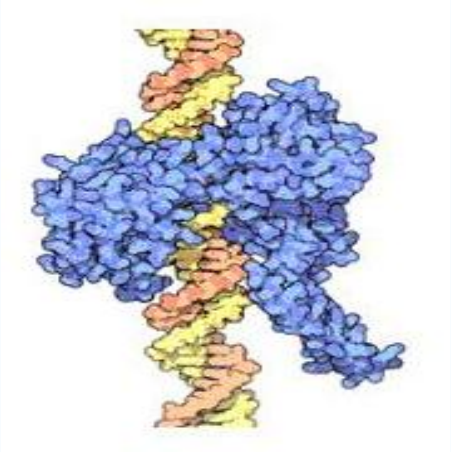

Figure 5: Structure of DNA bound to eukaryotic topo I (PDB ID = 1A36)

C. Mechanism

Relaxation is not an active process in the sense that energy in the form of ATP is not spent during the nicking or ligation steps as the reaction between the tyrosine residue at the active site of the enzyme with the phosphodiester DNA backbone simply replaces one phosphomonoester bond with another. The topoisomerase also does not use ATP during uncoiling of the DNA; rather, the torque present in the DNA drives the uncoiling and proceeds on average energetically downhill. Recent single molecule experiments have confirmed what bulk-plasmid relaxation experiments have proposed earlier, which is that uncoiling of the DNA is torque-driven and proceeds until religation occurs. No data suggest that Topo IB "controls" the swiveling insofar as that it has a mechanism in place that triggers religation after a specific number of supercoils removed. On the contrary, single-molecule experiments suggest that religation is a random process and has some probability of occurring each time the swiveling 5'-OH end comes in close proximity with the attachment site of the enzyme-linked 3'-end.Type IB topoisomerases were originally identified in eukaryotes and in viruses. Viral topo I is unique because it binds DNA in a sequence-specific manner.

5.3 Type IC Topoisomerases

Recently, a new type of topoisomerase was identified, topo V. Topo V is the founding member and so far the only member, of the type IC topoisomerase. The crystal structure of topo V was solved. Type IC topoisomerases work through a controlled rotary mechanism, much like type IB topoisomerases (pdb ID = 2CSB and 2CSD), but the fold is unique.

A. Intermediates

All topoisomerases form a phosphotyrosine intermediate between the catalytic tyrosine of the enzyme and the scissile phosphoryl of the DNA backbone.

- Type IA topoisomerases form a covalent linkage between the catalytic tyrosine and the 5'-phosphoryl.
- Type IB enzymes form a covalent 3'-phosphotyrosine intermediate.
- Type 1C topoisomerases form a covalent 3'-phosphotyrosine intermediate.

This intermediate is isoenergetic, meaning that the forward cleavage reaction and the backward religation reaction are both energetically equal. As such, no outside energy source is necessary to conduct this reaction.

D. Inhibition

As topoisomerases generate breaks in DNA, they are targets of small-molecule inhibitors that inhibit the enzyme. Type 1 topoisomerase is inhibited by irinotecan, topotecan and camptothecin.

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5.4 Type II Topoisomerase

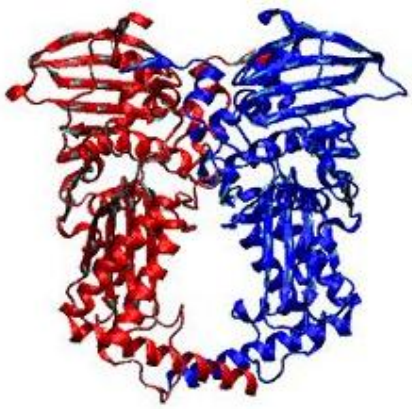

Figure 6: Structure of the 42 KDa fragment of the N-terminal ATPase of DNA gyrase homologous to all other type IIA topoisomerases.

A. Function

Once cut, the ends of the DNA are separated and a second DNA duplex is passed through the break. Following passage, the cut DNA is religated. This reaction allows type II topoisomerases to increase or decrease the linking number of a DNA loop by 2 units and promotes chromosome disentanglement. Reactions involving the increase in supercoiling require two molecules of ATP. Janet Lindsley has done much work to examine how the hydrolysis of ATP translate to topo function. For example, DNA gyrase, a type II topoisomerase observed in *E. coli* and most other prokaryotes, introduces negative supercoils and decreases the linking number by 2. Gyrase also is able to remove knots from the bacterial chromosome. Along with gyrase, most prokaryotes also contain a second type IIA topoisomerase, termed topo IV. Gyrase and topo IV differ by their C-terminal domains which is believed to dictate substrate specificity and functionality for these two enzymes. Footprinting indicates that gyrase, which forms a 140 base-pair footprint, wraps DNA allowing it to introduce negative supercoils, while topo IV, which forms a 28 base-pair footprint, does not wrap DNA.Eukaryotic type II topoisomerase cannot introduce supercoils; it can only relax them. This is thought to be unnecessary because the binding of DNA by histones increases potential superhelicity.The role of type IIB topoisomerases is less understood. Unlike type II topoisomerases, it cannot simplify DNA topology but it shares several structural features with type IIA topoisomerases.

B. Topology Simplification

Type IIA topoisomerases are essential in the separation of daughter strands at the end of replication. This function is performed by topo II in eukaryotes and by topo IV in prokaryotes. Failure to separate these strands leads to cell death. Type IIA topoisomerases have the special ability to relax DNA to a state below that of thermodynamic equilibrium, a feature unlike type IA, IB and IIB topoisomerases. This ability, known as topology simplification, was first identified. The hydrolysis of ATP drives this simplification, but a clear molecular mechanism for this simplification is still lacking. Several models to explain this phenomenon have been proposed, including two models that rely on the ability of type IIA topoisomerases to recognize bent DNA duplexes. Biochemistry, electron microscopy and the recent structure of topo II bound to DNA reveals that type IIA topoisomerases bind at the apices of DNA, supporting this model.

C. Classification

There are two subclasses of type II topoisomerases, type IIA and IIB.

- Type IIA topoisomerases include the enzymes DNA gyrase, eukaryotic topoisomerase II (topo II) and bacterial topoisomerase IV (topo IV). These enzymes span all domains of life and are essential for function.
- Type IIB topoisomerases are structurally and biochemically distinct and comprise a single family member, topoisomerase VI (topo VI). Type IIB topoisomerases are found in archaea and some higher plants.

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In cancers, the topoisomerase IIalpha is highly expressed in highly proliferating cells. In certain cancers, such as peripheral nerve sheath tumors, high expression of its encoded protein is also associated to poor patient survival.The two classes of topoisomerases share a similar strand passage mechanism and domain structure (see below), however they also have several important differences. Type IIA topoisomerases form double-stranded breaks with four-base pair overhangs, while type IIB topoisomerases form double-stranded breaks with two base overhangs. In addition, type IIA topoisomerases are able to simplify DNA topology.

D. Structure of Type IIA Topoisomerases

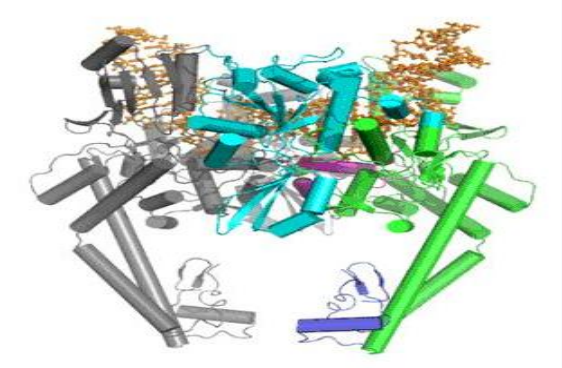

Figure 7: Structure of yeast topoisomerase II bound to a doubly-nicked 34-mer duplex DNA (PDB ID =2RGR). The Toprim fold is colored cyan, the DNA is colored orange, the HTH is colored magenta and the C-gate is colored purple. Notice that the DNA is bent by ~160 degrees through an invariant isoleucine (Ile833 in yeast).

Type IIA topoisomerases consist of several key motifs: an N-terminal GHKL ATPase domain (for Gyrase, Hsp, Kinase and MutL), a Toprim domain (sometimes called a Rossman fold) which exists both in Type II topoisomerases, type IA topoisomerases and bacterial primase (DnaG), a central DNA-binding core (which structurally forms a heart shaped structure) and a variable C-terminal domain. Eukaryotic type II topoisomerases are homodimers (A_2), while prokaryotic type IIs are heterodimers (A_2B_2). Prokaryotes have the ATPase domain and the Toprim fold on one polypeptide, while the DNA cleavage core and the CTD lies on a second polypeptide. For gyrase, the first polypeptide is called GyrB and the second polypeptide is called GyrA. For topo IV, the first polypeptide is called ParE and the second polypeptide is called ParC.The structure of the N-terminal ATPase domain of gyrase and yeast topo II have been solved in complex with AMPPNP (an ATP analogue).

Showing that two ATPase domains dimerize to form a closed conformation. For gyrase, the structure has a substantial hole in the middle, presumably to capture the T-segment. Linking the ATPase domain to the Toprim fold is a helical element known as the transducer domain. This domain is thought to communicate the nucleotide state of the ATPase domain to the rest of the protein. Modifications to this domain effects topoisomerase activity and structural work done by the Verdine group shows that the ATP state effects the orientation of the transducer domain.

E. Structures of Type IIB Topoisomerases

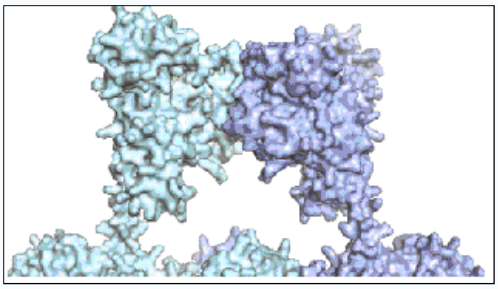

Figure 8: Structure of topo VI (PDB ID =2Q2E). Monomers are colored differently. The N-terminal ATPase domain lies on the top of the molecule, forming the DNA gate, while the DNA gate lies on the bottom.

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The organization of type IIB topoisomerases are similar to that of type IIAs, except that all type IIBs have two genes and form heterodimers. On one gene, termed topo VI-B (since it resembles gyrB), contains the ATPase domain, a H2TH domain and the transducer domain. The second gene, termed topo VI-A, contains the WHD and the Toprim domain.The ATPase domain of topo VI B was solved in multiple nucleotide states. It closely resembles that of the GHKL domain of topo II and MutL and shows that the nucleotide state (ADP versus ATP) effects the orientation of the transducer domain (pdb ID= 1MU5 and 1MX0).The structure of topo VI-A was solved, showing that the HTH and Toprim fold had a novel conformation compared with that of topo IIA.A recent structure of the topo VI A/B complex was solved, showing an open and closed conformation, two states that are predicted in the two-gate mechanism (see below). These structures, of which one is a X-ray crystal structure and the other is a Small Angle X-ray Scattering (SAXS) reconstruction shows that the ATPase domain can either be open or closed.

F. Mechanism of Type II Topoisomerases

Type IIA topoisomerase operates through a "two-gate" mechanism, a mechanism supported by biochemistry (Roca and Wang) as well as by structural work (Berger and Wang).A strand of DNA, called the polymorphic-delta segment, or PDS-segment is bound by a central DNA-binding gate (DNA-gate). A second strand of DNA, called the Transporter exchanger (sometimes called the transfer), or T-segment is captured by the oxidation of the N-terminal ATPase domain (the ATPase-gate) as two molecules of ATP are bound. Hydrolysis of ATP and release of a inorganic phosphate leads to the cleavage of the G-segment, as the catalytic tyrosines form a covalent phosphotyrosine bond with the 5' end of the DNA. This creates a four-base overhang and a double stranded break in the G-segment. As the DNA-binding gate separates, the T-segment is transferred through the G-segment. The G-segment is sealed, leading to the C-terminal gate (or C-gate) to open, allowing for the release of the T-segment. Release of product ADP leads to a reset of the system and allows a second T-segment to be captured.Type IIB topoisomerases operate through a similar fashion, except that the protein forms a two-base overhang in the G-segment and that the C-terminal gate is completely missing.

Catenation

Catenation is where two circular DNA strands are linked together like chain links. This occurs after DNA replication where two single strands are catenated can still replicate but cannot separate into the two daughter cells. As Type II topoisomerses break a double strand they can fix this state (Type I topoisomerases could only do this if there was already a single strand nick) and the correct chromosome number can remain in daughter cells. As linear DNA in eukaryotes is so long they can be thought of as being without ends and Type II topoisomerases are needed for the same reason.

Inhibition

Small molecules that target type II topoisomerase are divided into two classes: inhibitors and poisons.

- Inhibitors of type II topoisomerase include HU-331, ICRF-187, ICRF-193 and mitindomide. These molecules work by inhibiting the ATPase activity, specifically by acting as a un-competitive inhibitor of ATP. This has been shown through structural studies and biochemical studies performed by the Lindsley group.
- Poisons of type II topoisomerases include etoposide, Novobiocin and teniposide. These small molecules target the DNA-protein complex. Some of these molecules lead to increased cleavage, while others, such as etoposide, inhibit religation.

The experimental anti-tumor drug m-AMSA (4'-(9'-acridinylamino)methanesulfon-m-anisidide) also inhibits type 2 topoisomerase.

VI. TOPO CLONING

TOPO Cloning is a molecular biology technique in which DNA fragments amplified by either Taq or Pfu polymerases are cloned into specific vectors without the requirement for DNA ligases.

Principle

The technique utilises the inherent biological activity of DNA topoisomerase I. The biological role of topoisomerase is to cleave and rejoin supercoiled DNA ends to facilitate replication. Vaccinia virus topoisomerase I specifically recognises

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 2, March 2022
Impact Factor: 6.252

DNA sequence 5´-(C/T)CCTT-3'. During replication, the enzyme digests DNA specifically at this sequence, unwinds the DNA and re-ligates it again at the 3' phosphate group of the thymidine base.

Technique

TOPO vectors are designed in such a way that they carry this specific sequence 5´-(C/T)CCTT-3' at the two linear ends. The linear vector DNA already has the topoisomerase enzyme covalently attached to both of its strands' free 3' ends. This is then mixed with PCR products. When the free 5' ends of the PCR product strands attack the topoisomerase/3' end of each vector strand, the strands are covalently linked by the already bound topoisomerase. This reaction proceeds efficiently when this solution is incubated at room temperature with required salt. Different types of vectors are used for cloning fragments amplified by either Taq or Pfu polymerase as Taq polymerase (unlike Pfu) leaves an extra "T" nucleotide at the 3'end during amplification.TA TOPO Cloning technique relies on the ability of adenine (A) and thymine (T) (complementary basepairs) on different DNA fragments to hybridize and, in the presence of ligase, become ligated together. PCR products are usually amplified using Taq DNA polymerase which preferentially adds an adenine to the 3' end of the product. Such PCR amplified inserts are cloned into linearized vectors that have complementary 3' thymine overhangs. The insert is created by PCR using Taq DNA polymerase. This polymerase lacks 5' to 3' proofreading activity and with a high probability adds a single, 3'-adenine overhang to each end of the PCR product. It is best if the PCR primers have guanines at the 5' end as this maximizes probability of Taq DNA polymerase adding the terminal adenosine overhang. Thermostable polymerases containing extensive 3´ to 5´ exonuclease activity should not be used as they do not leave the 3´ adenine-overhangs. The target vector is linearized and cut with a blunt-end restriction enzyme. This vector is then tailed with dideoxythymidine triphosphate (ddTTP) using terminal transferase. It is important to use ddTTP to ensure the addition of only one T residue. This tailing leaves the vector with a single 3'-overhanging thymine residue on each blunt end. Manufacturers commonly sell TA Cloning "kits" with with a wide range of prepared vectors that have already been linearized and tagged with an overhanging thymine residue.

Clinical Significance

Many drugs operate through interference with the topoisomerases. The broad-spectrum fluoroquinolone antibiotics act by disrupting the function of bacterial type II topoisomerases.Some chemotherapy drugs work by interfering with topoisomerases in cancer cells:

- type 1 is inhibited by irinotecan and topotecan.
- type 2 is inhibited by etoposide(VP-16), teniposide and HU-331, a quinolone synthesized from cannabidiol.

Topoisomerase I is the antigen recognized by Anti Scl-70 antibodies in scleroderma.

These small molecule inhibitors act as efficient anti-bacterial and anti-cancer agents by hijacking the natural ability of topoisomerase to create breaks in chromosomal DNA. These breaks in DNA accumulate, ultimately leading to programmed cell death, or apoptosis.

VII. Conclusion

Topoisomerases are enzymes, found selectively in DNA of cells. They help for unwinding the twisted structure of DNA and hence for cell replication alsoss. Cancers like colorectal cancer or ovarian cancer can be very well treated with topoisomerase inhibitors that block the ligation step of the cell cycle, generating single and double stranded breaks that harm the integrity of the genome. The fluoroquinolones act by disrupting the function of bacterial type II topoisomerases and therefore act as broad spectrum antibacterials. There is tremendous scope for the research on topoisomerases as target for newer antineoplastic and antibacterial drugs.

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IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 2, March 2022
Impact Factor: 6.252

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