Topoisomerases ;Structure function and mechanism.

Structural basis for gate-DNA recognition and bending by type IIA topoisomerases

Ken C. Dong1,2 & James M. Berger2

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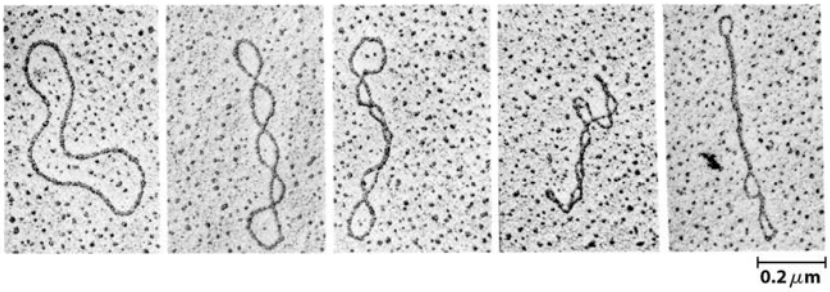
Topoisomerases

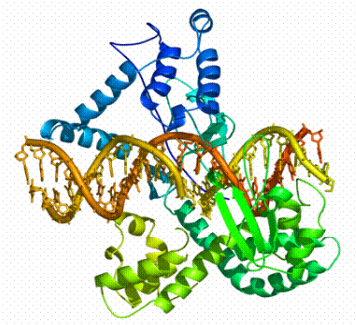
Enzymes that solves the topological problems associated with DNA

- Replication transcription and recombination
- Chromatin remodeling
- Relax negative and positive supercoils
- Decatenation and cantenation
- Knotting and unknotting

Supercoiling of Cellular DNA



- Type I topoisomerases:
Cleaves one DNA strands
- Type II topoisomersases
Cleaves both DNA strands (double strand break)
- Both types of enzyme form covalent intermediates with the DNA
- **Tyrosine is considered be in the active site of the enzyme**



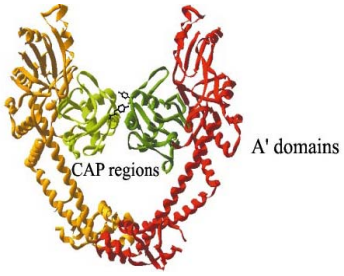


Table 1 | **Subfamilies of DNA topoisomerases**

Subfamily	Representative members
IA	Bacterial DNA topoisomerases I and III Yeast DNA topoisomerase III *Drosophila melanogaster* DNA topoisomerases IIIα and IIIβ Mammalian DNA topoisomerases IIIα and IIIβ
IB	Eukaryotic DNA topoisomerase I Mammalian mitochondrial DNA topoisomerase I Pox virus topoisomerase
IIA	Bacterial gyrase, DNA topoisomerase IV Phage T4 DNA topoisomerase Yeast DNA topoisomerase II *Drosophila* DNA topoisomerase II Mammalian DNA topoisomerases IIα and IIβ
IIB	*Sulfolobus shibatae* DNA topoisomerase VI (subunit A homologous to yeast Spo11)

Nature Reviews Molecular Cell Biology 3, 430-440 (June 2002)

Topoisomerase I Action

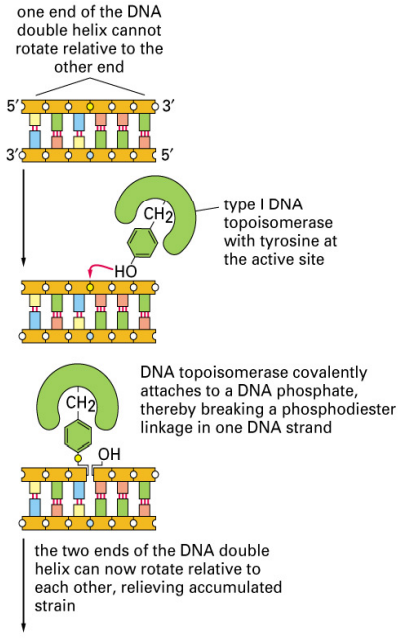


Figure 5–25 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Topoisomerase I Action

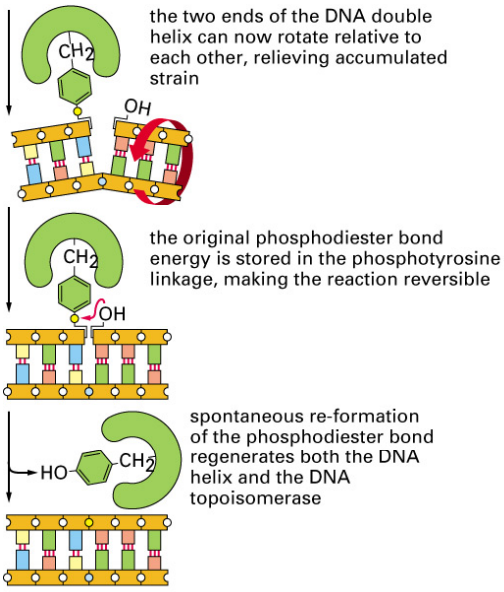
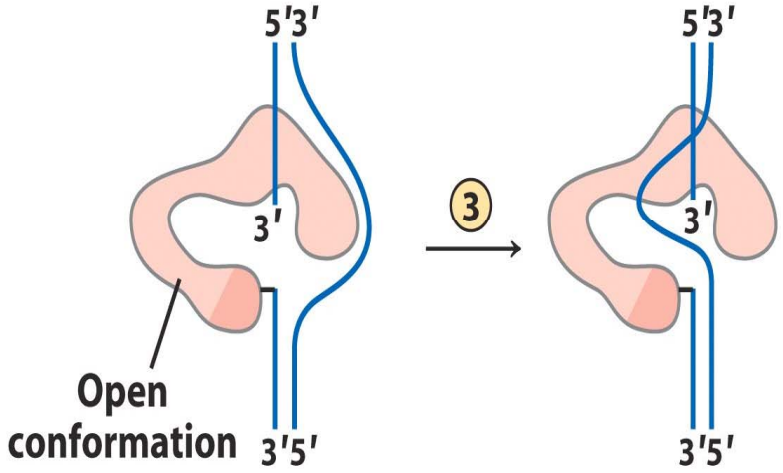


Figure 5–25 part 2 of 2. Molecular Biology of the Cell, 4th Edition.



Topoisomerase II Action

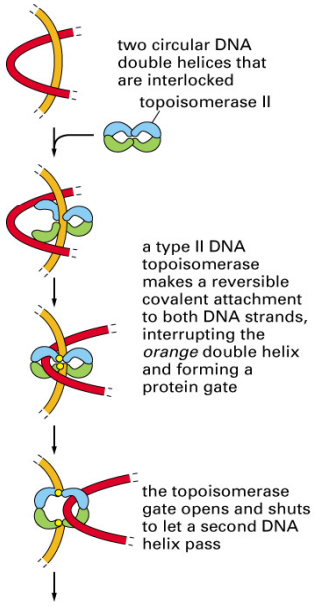


Figure 5–27 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Topoisomerase II Action

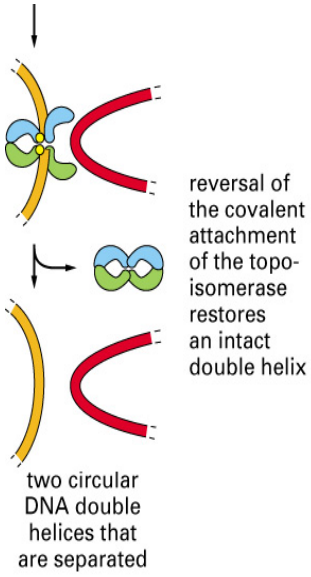
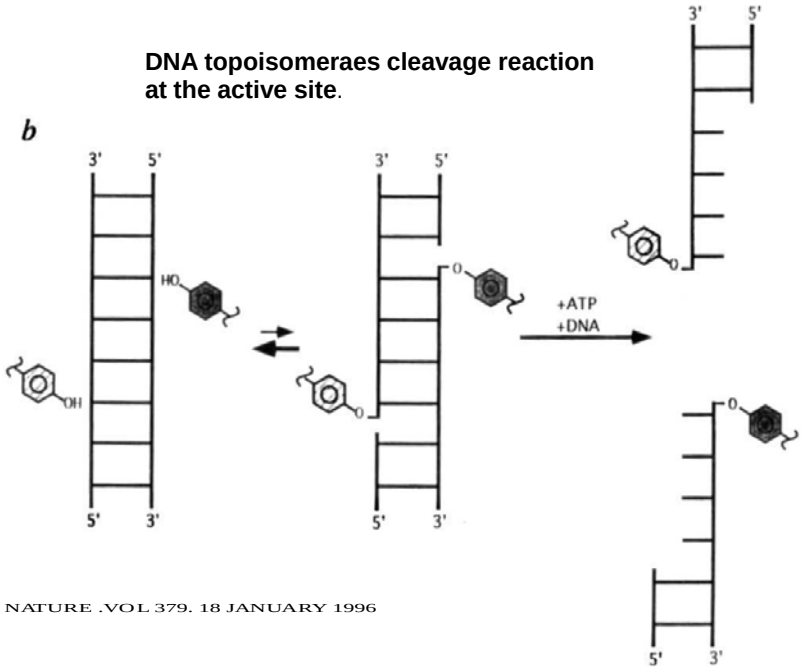


Figure 5–27 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

DNA topoisomeraes cleavage reaction at the active site.



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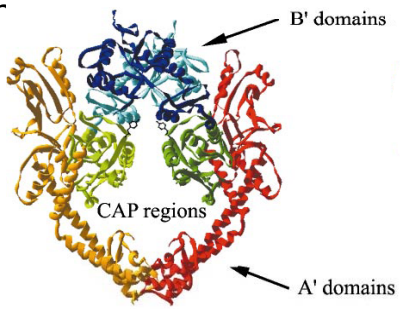
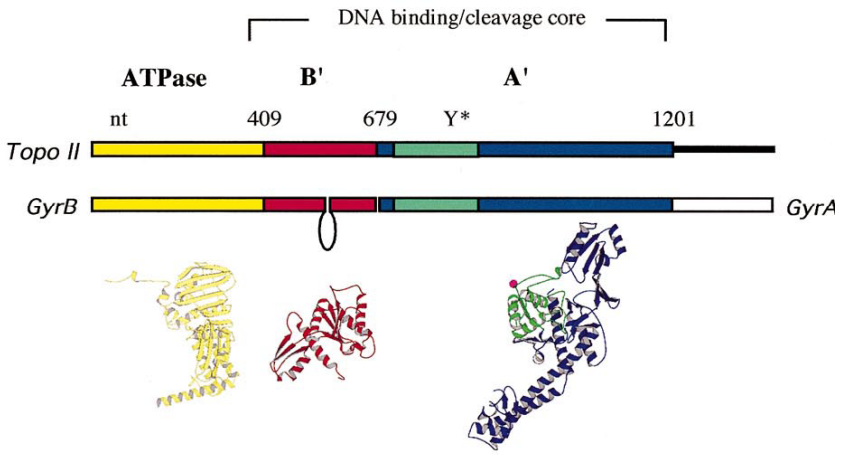
Type II Topoisomerases

- Divided into IIA and IIB subclasses
- Prokaryotic Type II topoisomerase are also known as DNA gyrase.
- They are ATP dependent.
- They catalse stepwise negative supercoiling of
- DNA with hydrolysis of ATP to ADP+Pi.

Structure and mechanism of Type II topoisomerses.

- Heart shaped dimeric proteir with large central hole.
- catalyze the passage of one DNA double helix through another
- ATP modulated clamp with two sets of jaws at the opposite end
- The mechanism depends on ATP binding and hydrolysis.





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S.cerevisiase Topo II complex has been used for this study

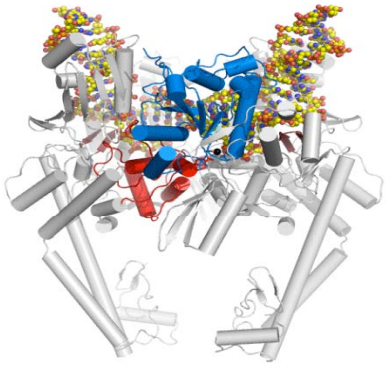
Two ATPASE domain
- N-gate

TOPRIM Domain
- Metal binding domain
- Found interior of the enzyme

Helps in formation of covalent protein.DNA intermediate by coordinating metal ion (Mg+).

WHD -Two winged helix Domains (DNA Gate)
- Also in interior of the enzyme
- Contains Tyr responsible for DNA cleavage and sepration.

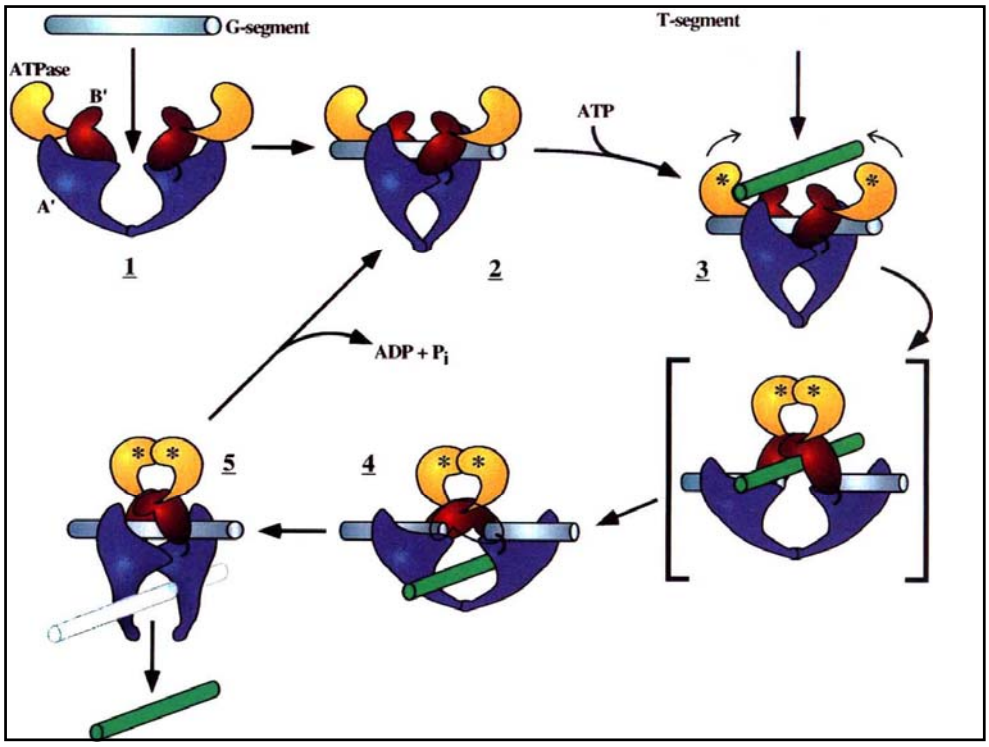
C-Gate
- Carboxy terminal dimerization

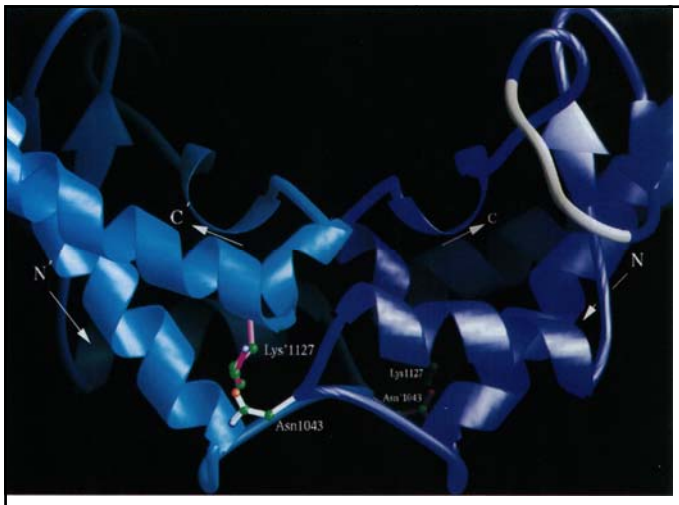


Two gate Model

The mechanism is outlined as follows:

- One DNA duplex, the G-segment, associates with the DNA binding and cleavage core.
- ATP binding promotes the capture of a second DNA duplex, the T-segment, and dimerization of the ATPase domains.
- Closure of the ATPase domains (the N-gate), stimulates cleavage and opening of the G-segment, and transport of the T-segment through the break.
- The G-segment is religated and the T segment expelled through the dimerization domains (the C-gate)

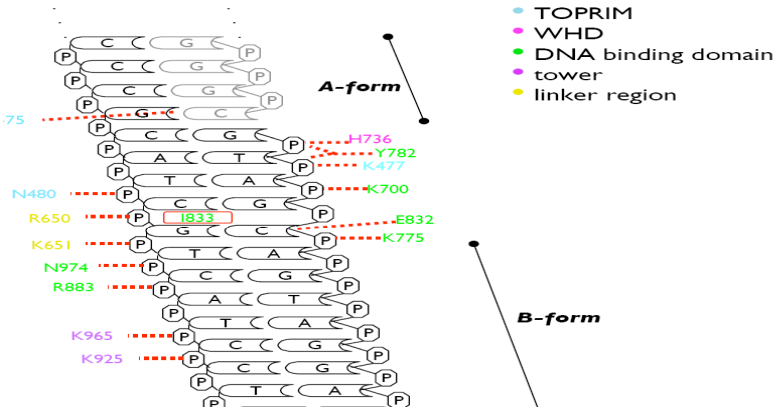




Evidence that DNA enters through N-gate

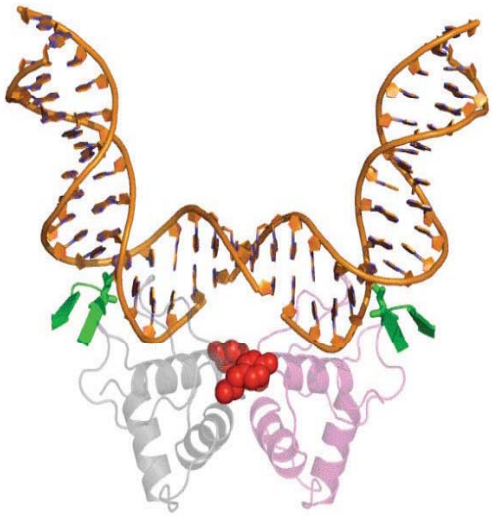
PNAS
Vol. 93, pp. 4057-4062,
April 1996
Biochemistry

- Mutant has been designed Asn and Lys were replaced by Cys in c-terminus.
- A pair of disulfide cross links formed
- The G-segment binds to the super coiled DNA
- C-gate is locked in the cross linked enzyme
- G-segment must have entered the enzyme through the N-gate.

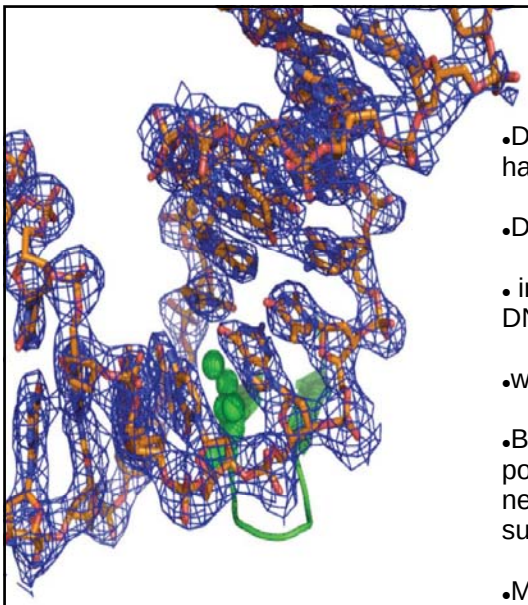


- 15 bp of DNA bind to single TOPO II promoter.
- Binding orientation of DNA allows base pairing to occur between four bp of 5'end in central position of the DNA binding site.
- 34 bp are involved in binding to single TOPO II enzyme
- Hence total 64 bp of DNA are involve in binding to the enzyme.

Reshaping of DNA

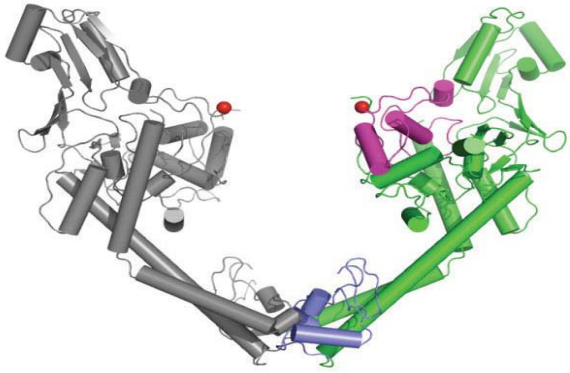


- 150 degree bend in the bound DNA segment
- DNA is present in A form in the catalytic site(verified by hydroxyl radical footprinting)
- B form in the outermost edges.
- No direct side chain base contacts present (verified by footprinting)
- 26 bp of the helix are within van der Waals distance of the protein

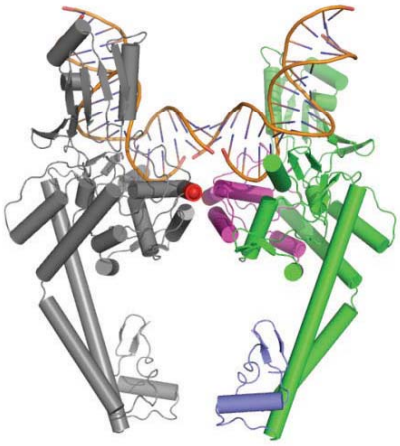


- DNA binding groove is beta-hairpin
- DNA bending is induced by Ileu
- intercalation in bound bp of DNA
- widening the minor grove.
- Bent form is also stabilized by positively charged groove formed near WHD (His736,Lys700 and sugar phosphate backbone)
- Mutation of Lys to Ala inactivates Topo II

Reshaping of Topo II



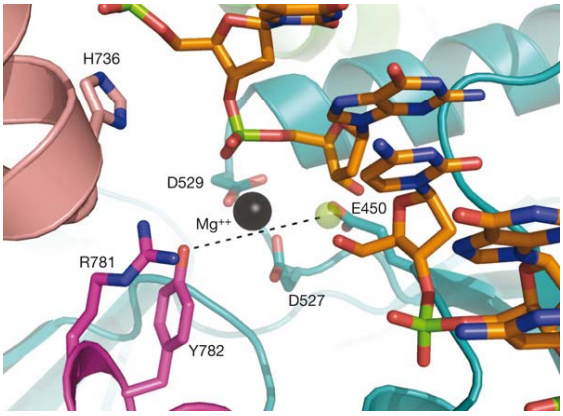
- Tyr move towards one another.
- TOPRIM domain is rotated by 80 degree.
- DNA can be modeled into the central cavity without steric clashes



Change in C-terminal

- linked to the main body by alpha helix
- Provides flexible movement
- Results in 17A gap between the subunits.

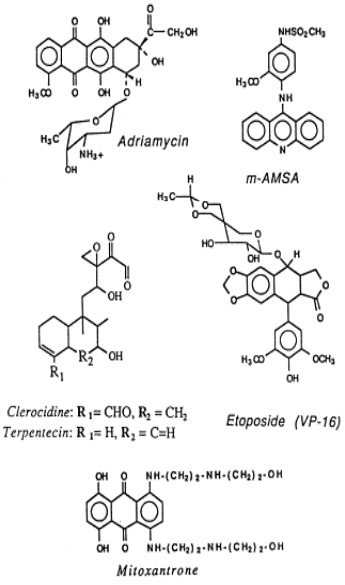
Changes in active site



Leads to the formation of bipartite active site in Topo II sububits
The triad in TOPRIM metal binding domain gets close to catalytic Tyr 728

Topoisomerase as drug targets.

- used as chemotherapeutic agents.
- inhibit various genetic processes involving the enzyme, dna replication and chromosome dynamics
- They act by stablilzing DNA-Topoisomerase complex.
- Camptothecin -- Topo I inhibitor
- Doxorubicin -- Topo II inhibitor



Topoisomerases poisions

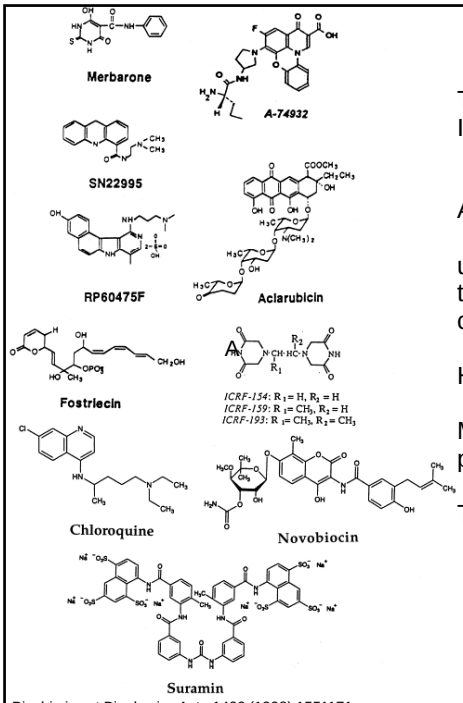
Topoisomerase II is a specifc target for several clinically important antitumor drugs

They act by stabilizing covalent `cleavable complex'

OR "poison" the DNA- enzyme complex.

Poisoned Topoisomerase-DNA-inhibitor complexes are unable to execute a complete enzymatic cycle

Biochimica et Biophysica Acta 1400 (1998) 155^171



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TOPOISOMERASE II CATALYTIC INHIBITORS

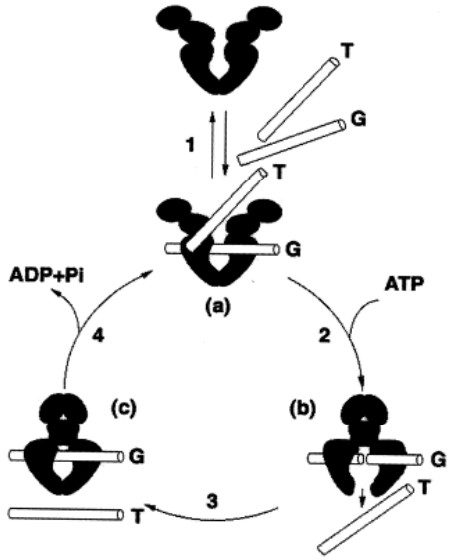
Also inhibit topoisomerase II activity

unlike the classical topoisomerase II poisons they lack the ability to stabilize the cleavable complex.

Hence are called ‘catalytic inhibitors’.

May act as antagonists to topoisomerase II poisons.

Their mechanism is less clearly understood.



- Topoisomerase II poisons stabilize the cleavable complex(3b)
- inhibit reaction 3 to proceed
- Results in DNA damage
- catalytic inhibitors inhibit reaction 1, 2 or 4, depending on the type of drug

T. Andoh, R. Ishida / Biochimica et Biophysica Acta 1400 (1998) 155^171

Perspectives as drug targets.

- Conversion of an essential enzyme for cell proliferation into a lethal poison is a unique mechanism for killing tumor cells.
- Because of the specificity of these topoisomerases drugs, DNA damage can be more conveniently studied.
- These drugs can also be conveniently used to probe the resistance mechanisms of tumor cells.
- These studies may also lead to a better understanding of the mechanism of induced cellular differentiation by anticancer drugs.