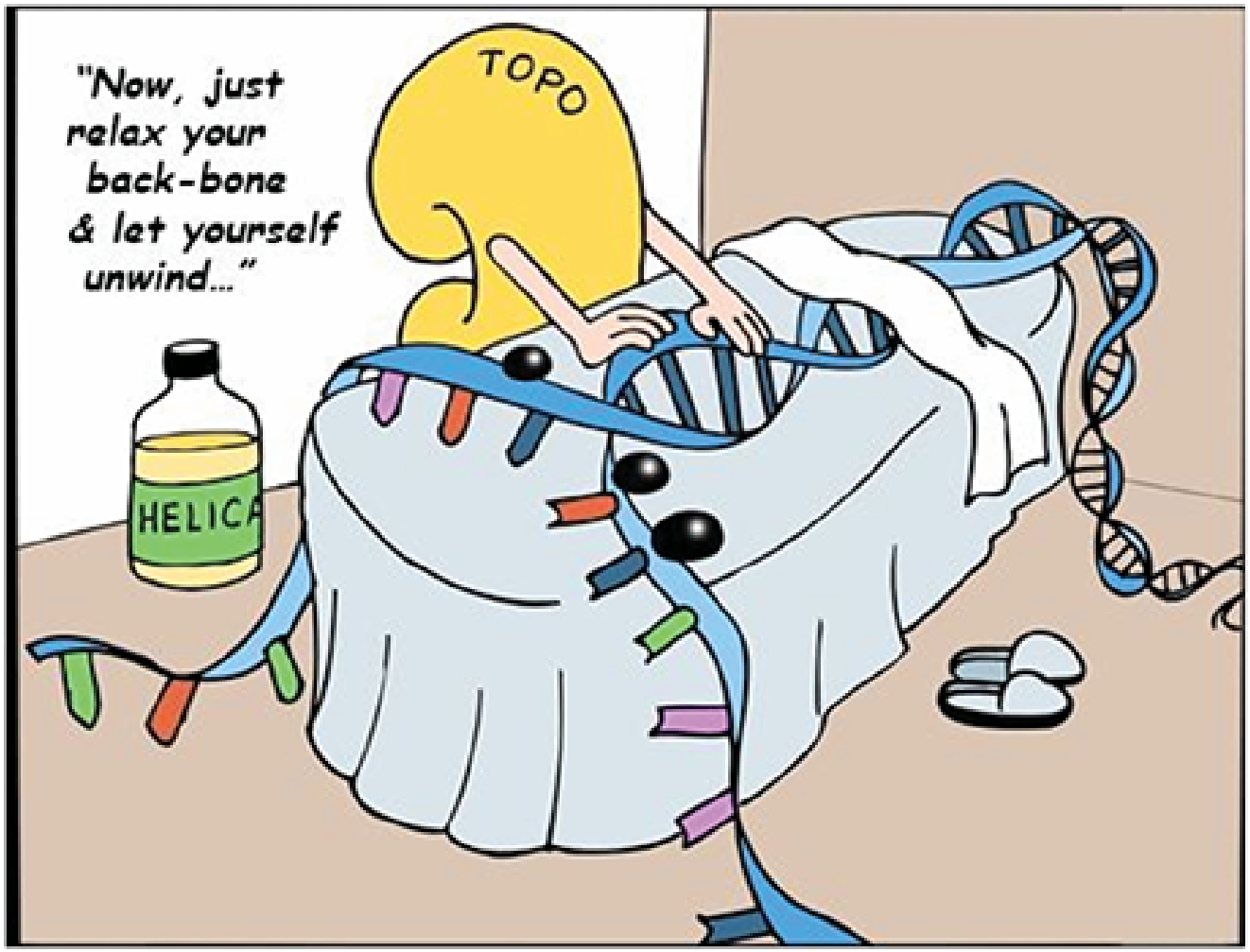
# Topoisomerase



# DNA Topoisomerases

Chemistry & Biology
Review

17, May 28, 2010

Cell PRESS

## DNA Topoisomerases and Their Poisoning by Anticancer and Antibacterial Drugs

Yves Pommier,[1,*] Elisabetta Leo,[1] HongLiang Zhang,[1] and Christophe Marchand[1]
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## Drugging Topoisomerases: Lessons and Challenges

Yves Pommier*

Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, United States

# Review

REVIEWS

NATURE REVIEWS | MOLECULAR CELL BIOLOGY

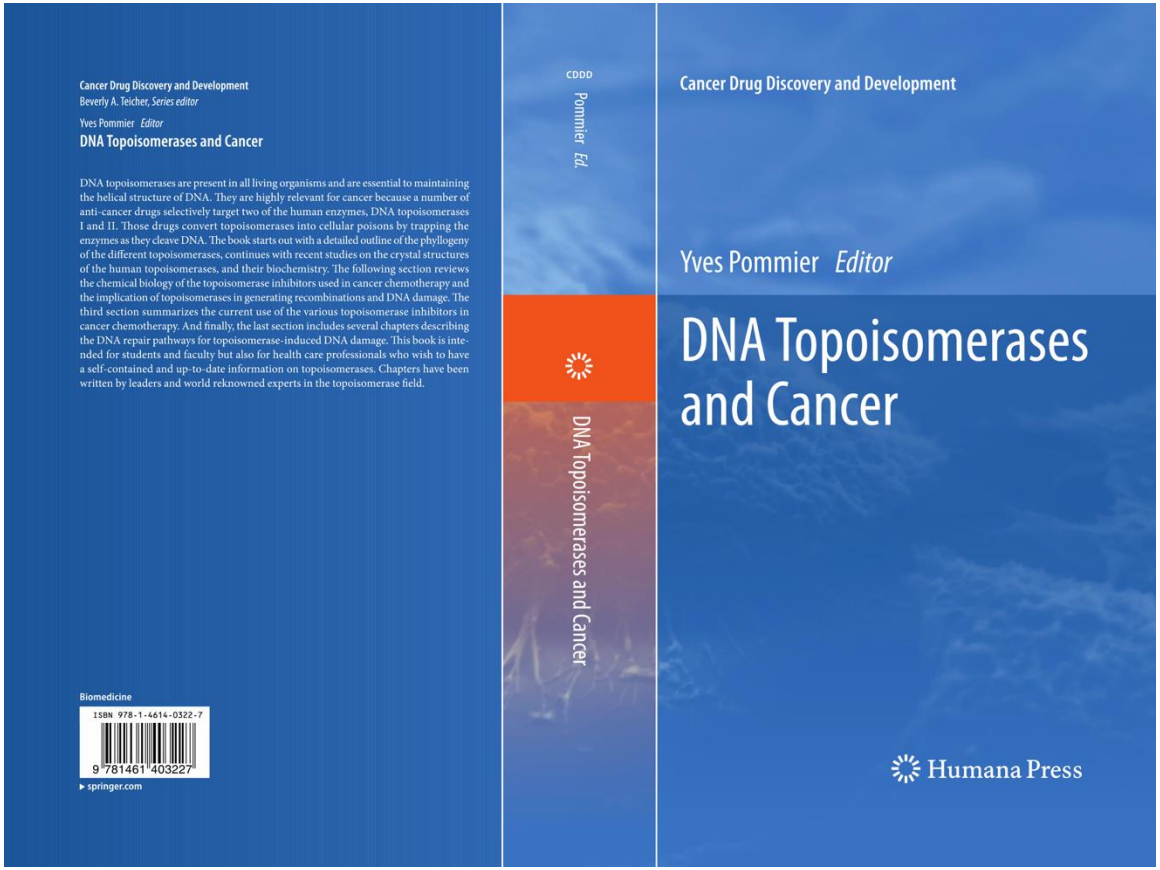
ADVANCE ONLINE PUBLICATION | October 2016

## Roles of eukaryotic topoisomerases in transcription, replication and genomic stability

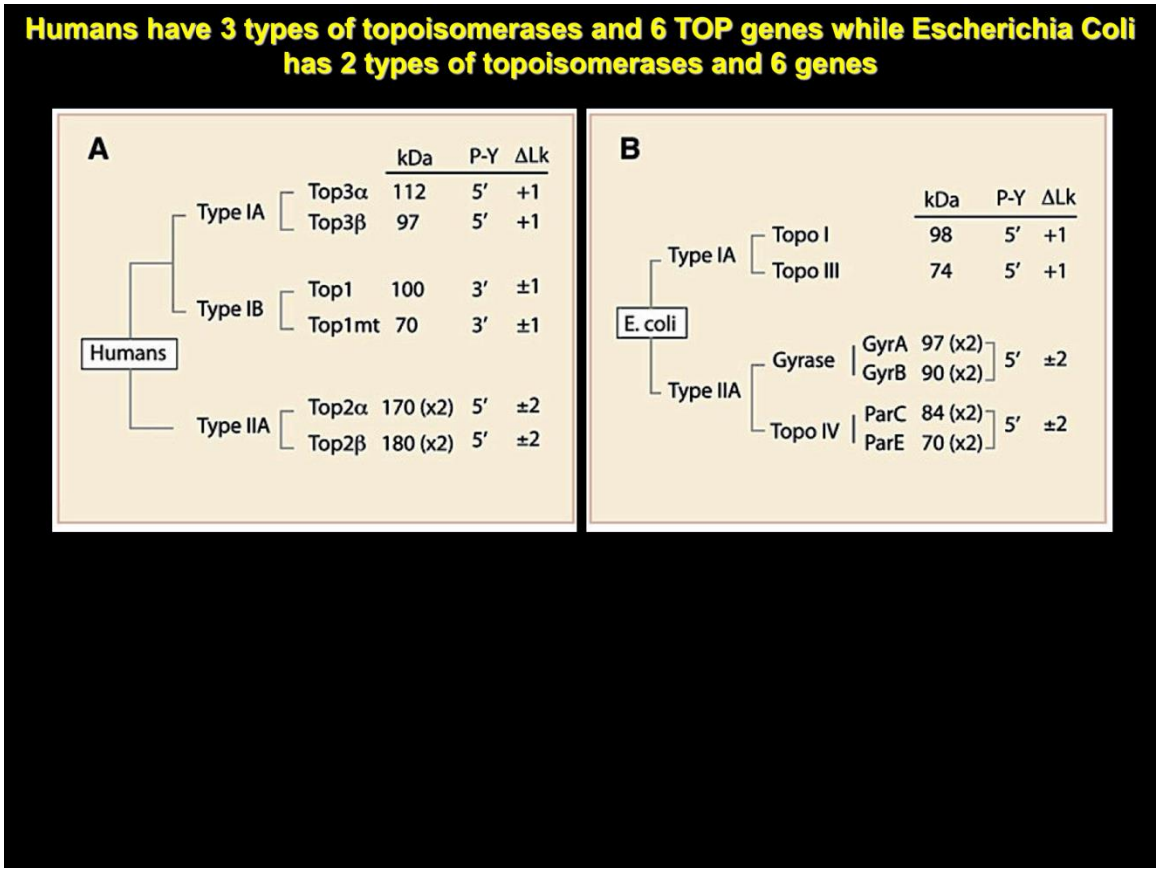
*Yves Pommier[1], Yilun Sun[2], Shar-yin N. Huang[1] and John L. Nitiss[2]*

Abstract | Topoisomerases introduce transient DNA breaks to relax supercoiled DNA, remove catenanes and enable chromosome segregation. Human cells encode six topoisomerases (TOP1, TOP1mt, TOP2α, TOP2β, TOP3α and TOP3β), which act on a broad range of DNA and RNA substrates at the nuclear and mitochondrial genomes. Their catalytic intermediates, the topoisomerase cleavage complexes (TOPcc), are therapeutic targets of various anticancer drugs. TOPcc can also form on damaged DNA during replication and transcription, and engage specific repair pathways, such as those mediated by tyrosyl-DNA phosphodiesterase 1 (TDP1) and TDP2 and by endonucleases (MRE11, XPF–ERCC1 and MUS81). Here, we review the roles of topoisomerases in mediating chromatin dynamics, transcription, replication, DNA damage repair and genomic stability, and discuss how deregulation of topoisomerases can cause neurodegenerative diseases, immune disorders and cancer.

# DNA Topoisomerases And Cancer

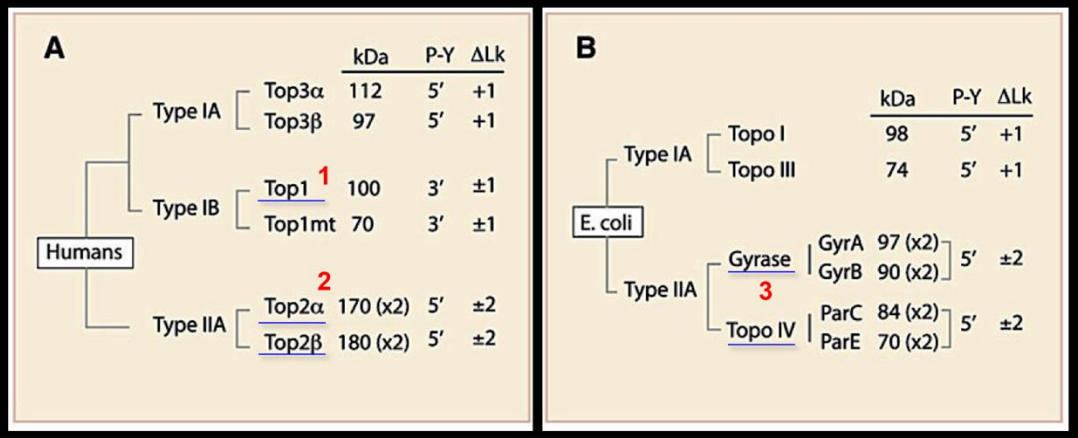


# Humans vs. Escherichia Coli



# Humans vs. Escherichia Coli

**Humans have 3 types of topoisomerases and 6 TOP genes while Escherichia Coli has 2 types of topoisomerases and 6 genes**



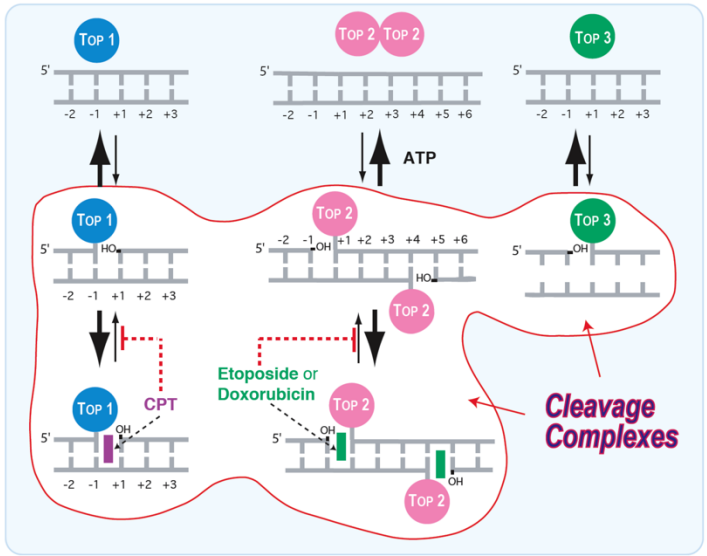

[1] Top1 is the anticancer target of camptothecins and indenoisoquinolines

[2] Top2α and β are the anticancer targets of etoposide, doxorubicin, mitoxantrone…

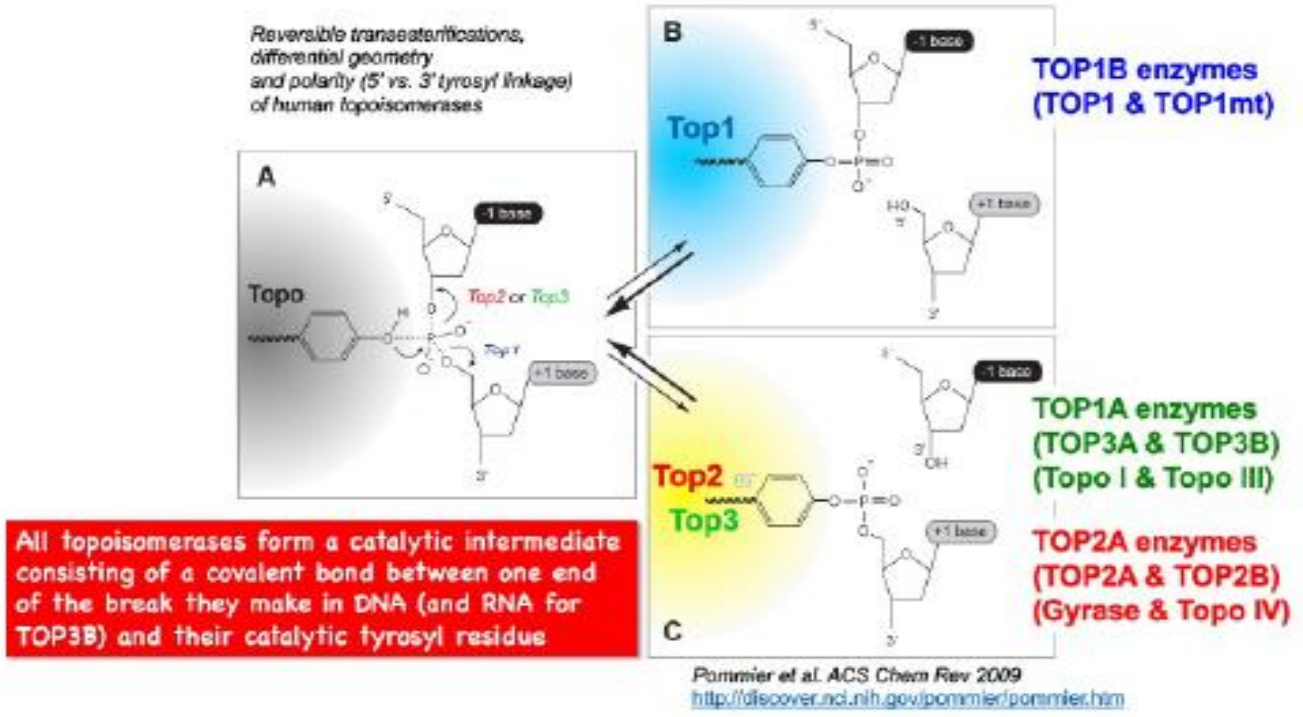
[3] Gyrase and Topo IV are the antibacterial targets of quinolones

# Topoisomerases and TOP Genes in Humans

**Not counting SPO11, there are 3 types of topoisomerases and 6 TOP genes in humans**

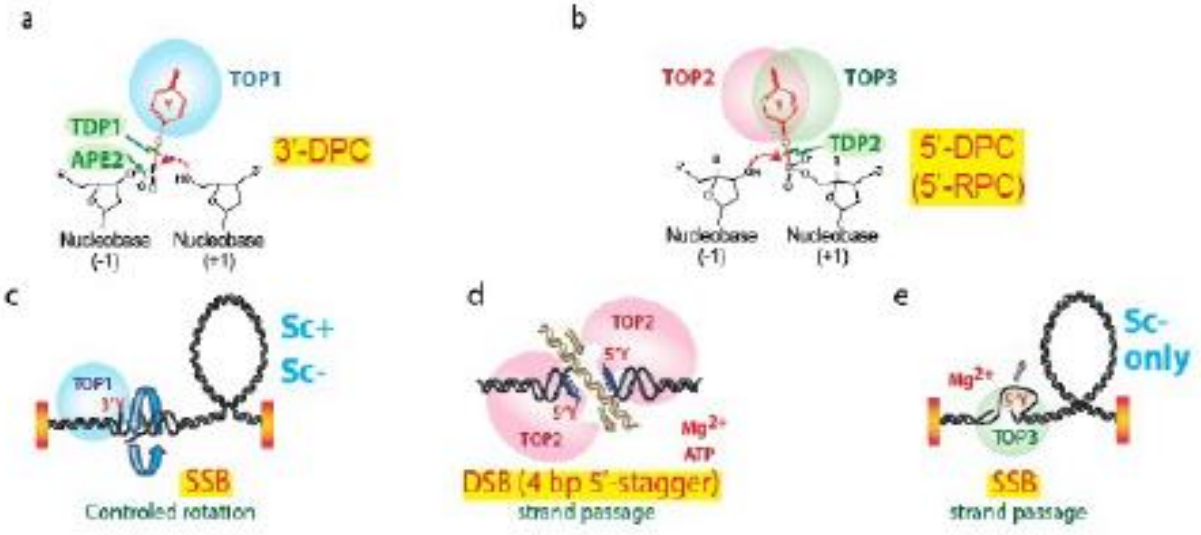


# Enzyme intermediates

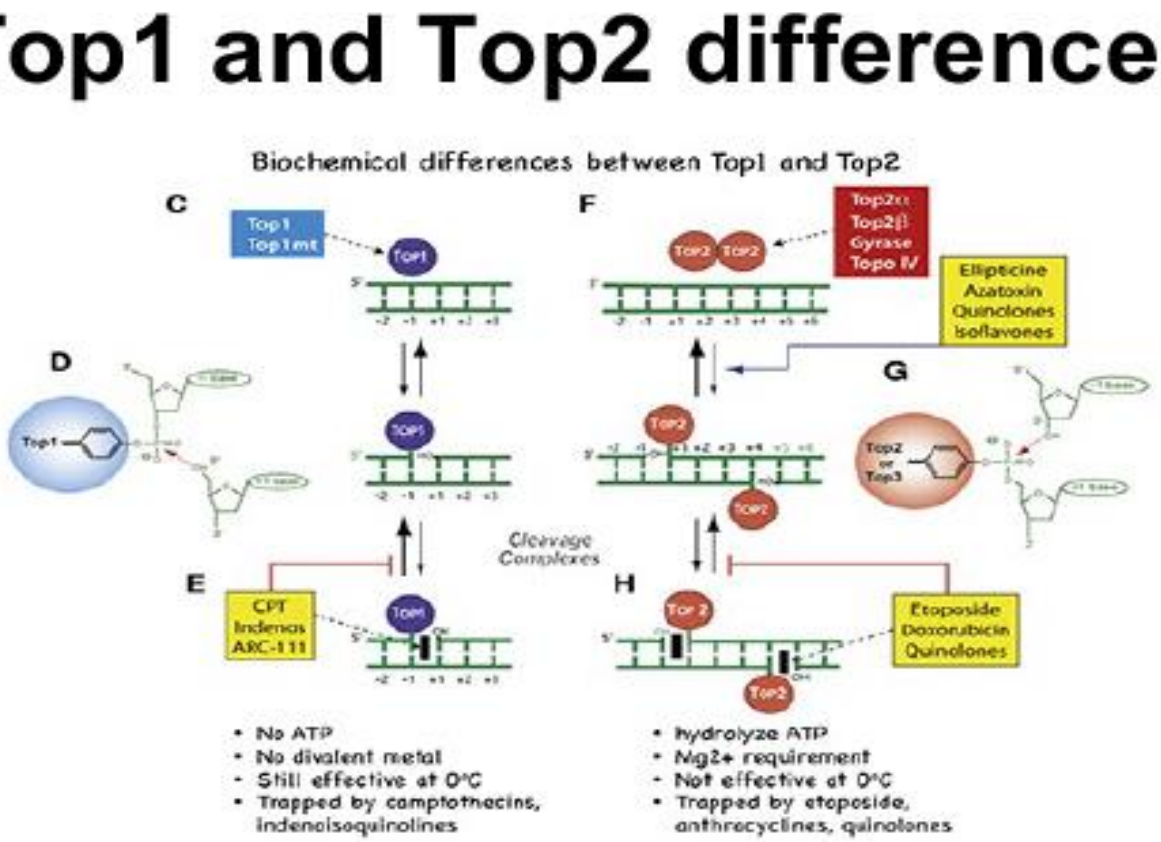


# Enzyme mechanism

Enzyme mechanism



# Top1 and Top2 differences



# Comparisons

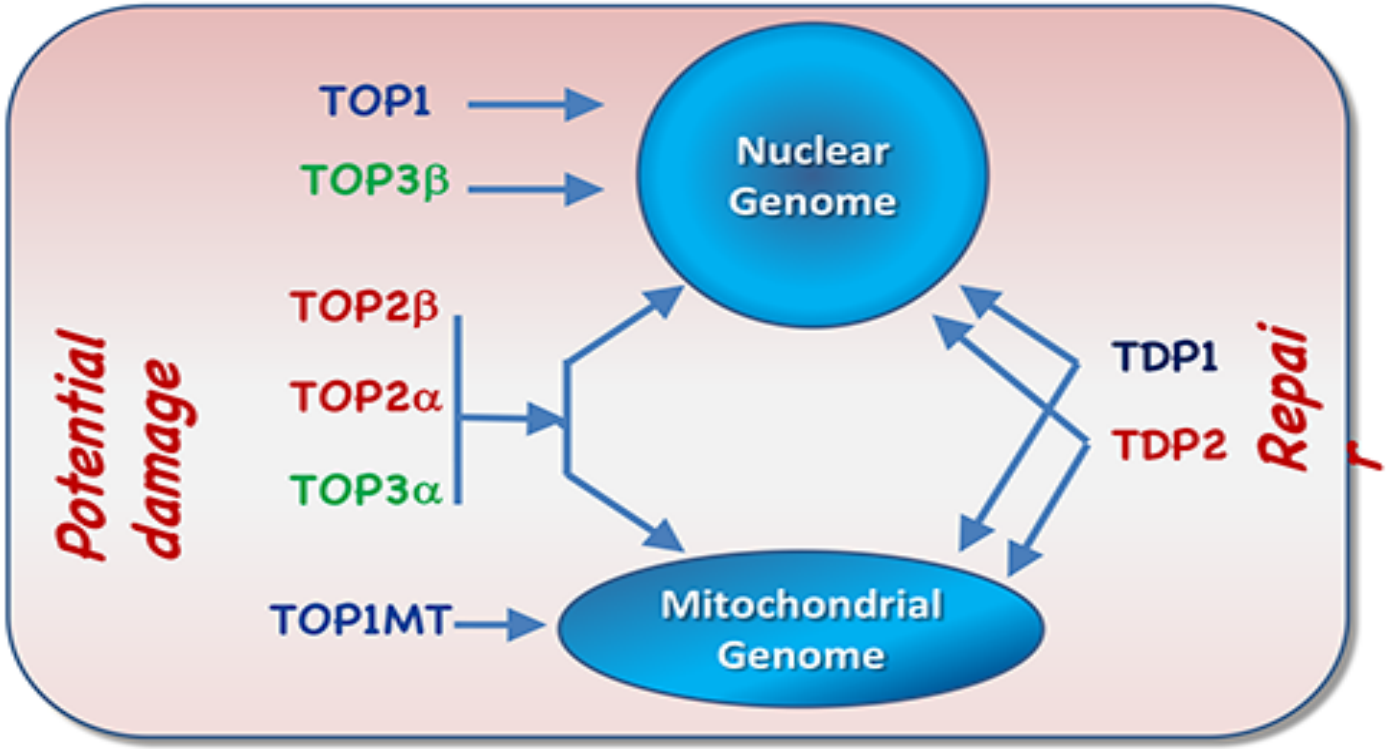
Comparison of the 6 human topoisomerases

| Genes | Chromosome | Proteins | Localization | Drugs | Mechanism | Polarity* | Main functions |
|---|---|---|---|---|---|---|---|
| *TOP1* | 20q12-q13.1 | **Top1** 100 kDa monomer | Nucleus | Camptothecins Indenos (LMPS) | Swivelling controlled rotation dsDNA | 3'-PY | Nuclear supercoiling relaxation |
| *TOP1MT* | 8q24.3 | **Top1mt** 100 kDa monomer | Mitochondria | none | | | mitochondrial supercoiling relaxation |
| *TOP2A* | 17q21-q22 | **Top2α** 170 kDa dimer | Nucleus Mitochondria | Anthracyclines, (doxorubicin) Etoposide mitoxantrone | Strand passage dsDNA ATPase | 5'-PY | Decatenation/replication |
| *TOP2B* | 3p24 | **Top2β** 180 kDa dimer | Nucleus Mitochondria | | | | Transcription; Unknotting |
| *TOP3A* | 17p12-p11.2 | **Top3α** 100 kDa monomer | Nucleus Mitochondria | none | Strand passage within single strands | 5'-PY | DNA Replication with BLM** |
| *TOP3B* | 22q11.22 | **Top3β** 100 kDa monomer | Nucleus cytoplasm | | | | RNA topoisomerase with TDRD3 |

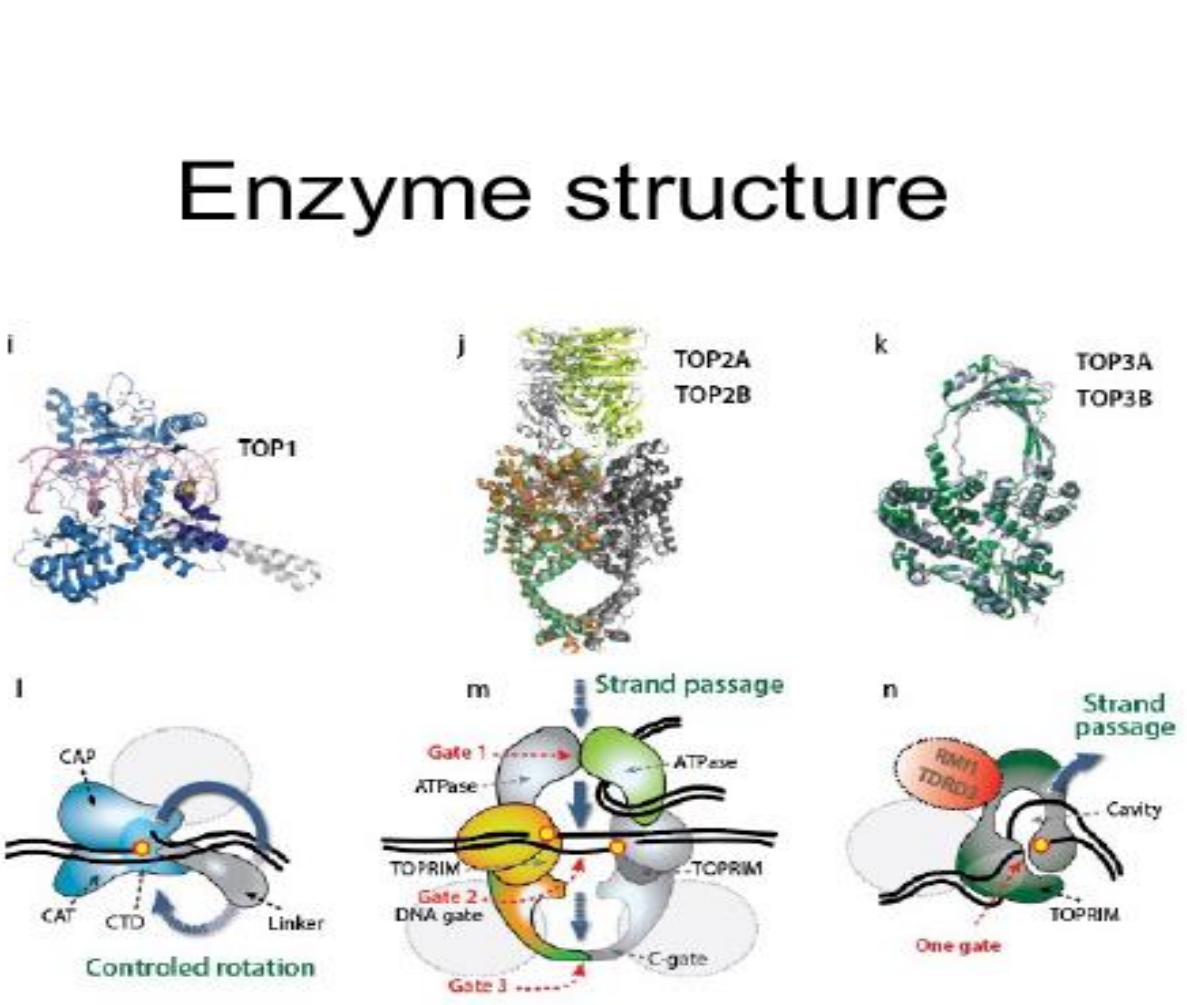
*: Covalent linkage between the catalytic tyrosine and the end of the broken DNA
**: Bloom syndrome, RecQ helicase

# Topoisomerase and genomes

Topoisomerases and tyrosyl DNA phosphodiesterases (TDPs) handle both the nuclear and mitochondrial genomes and their imbalance is source of genomic instability

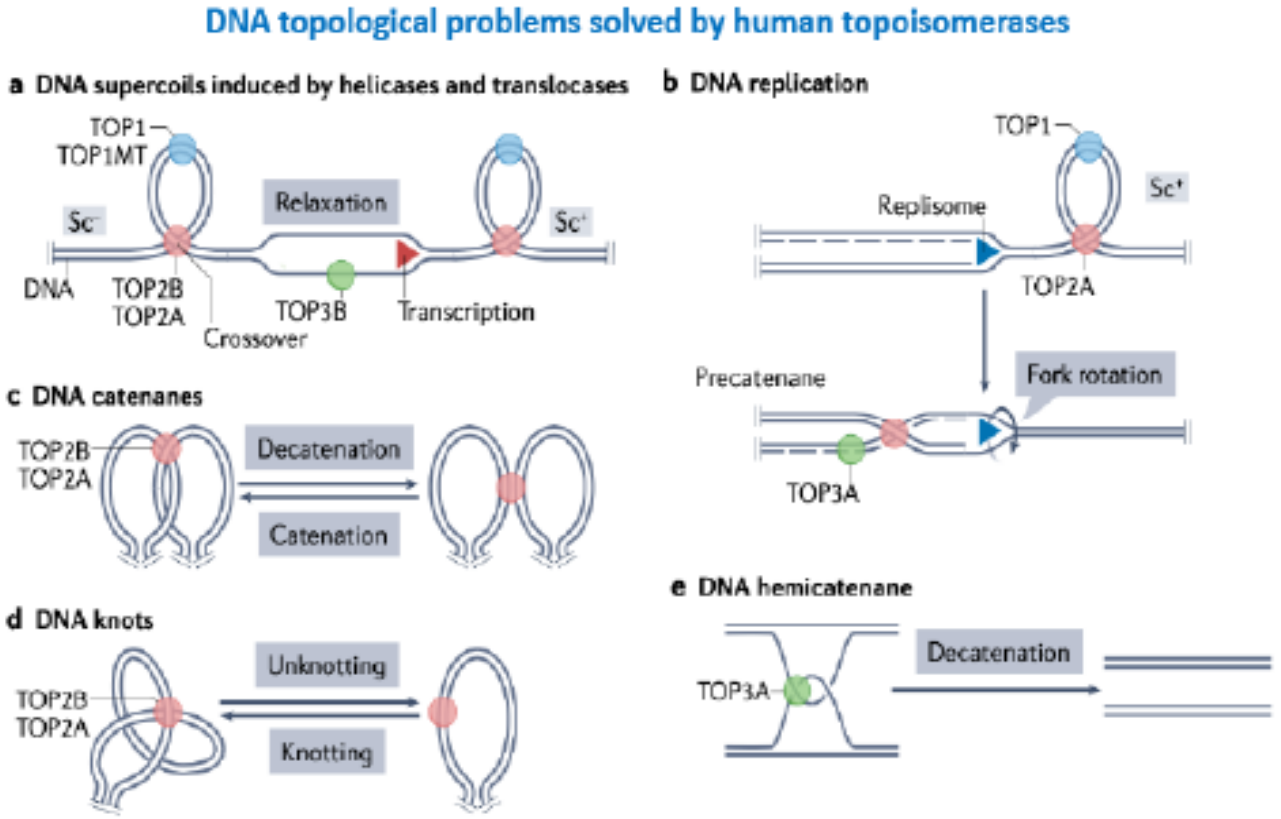


# Enzyme structure



# DNA topologicaI proble

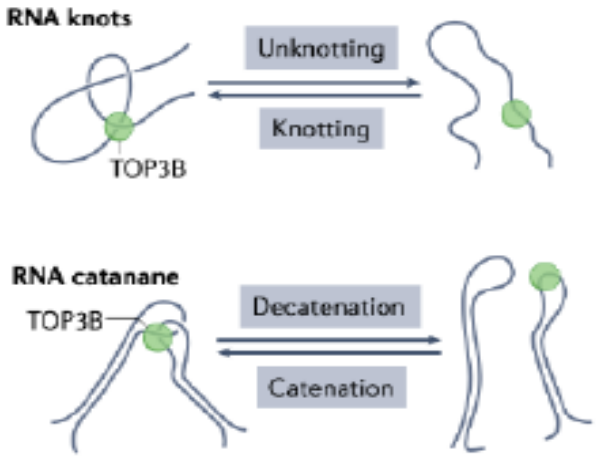
DNA topological problem



# TOP38

TOP38

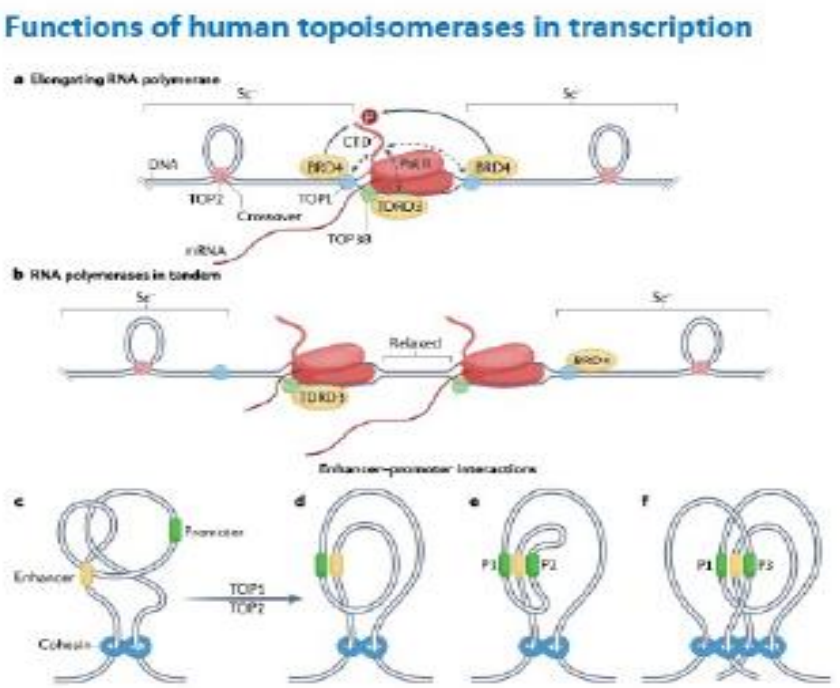
RNA topological problems solved by human topoisomerase III beta (TOP3B)




Hypernegative RNA supercoil relaxation?

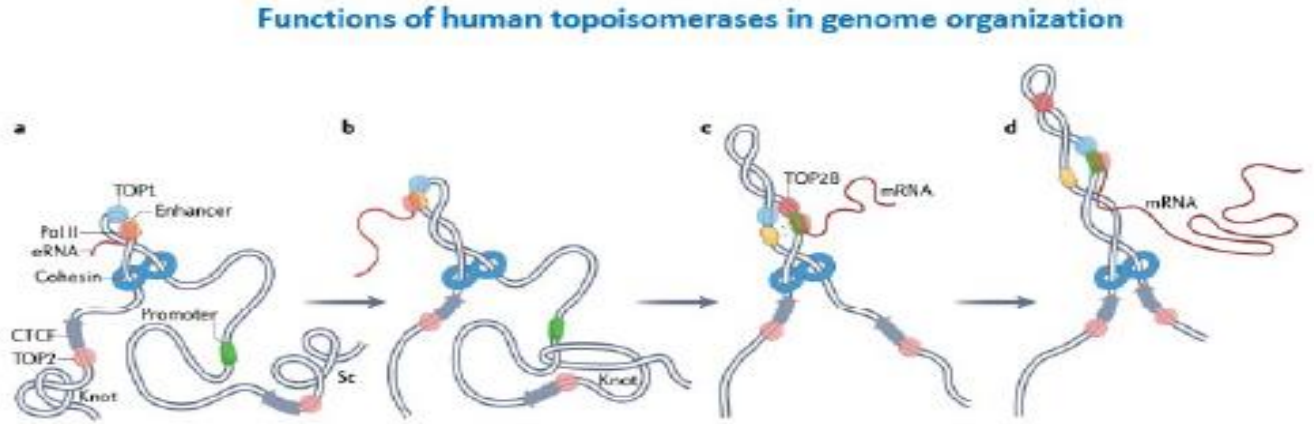
# TOP transcription

## TOP in transcription



# Genome organization

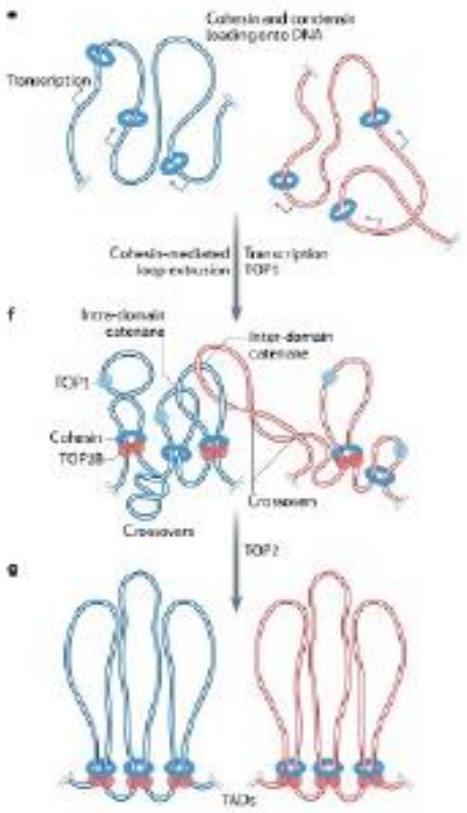
Genome organization



# TOP function

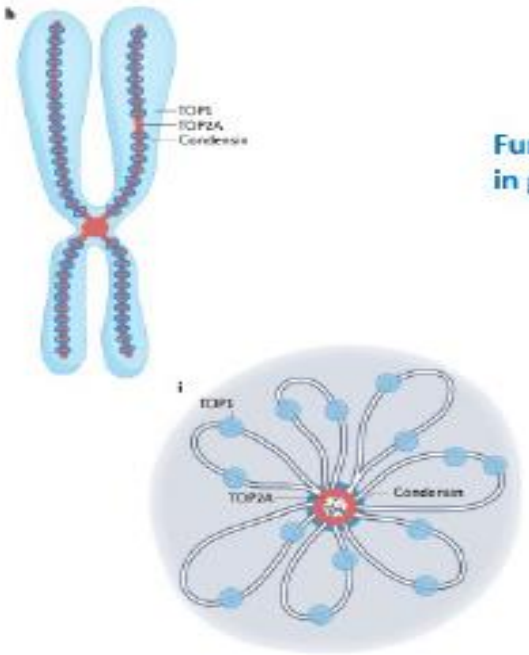
TOP function

**Functions of human topoisomerases in genome organization**



# TOP in mitosis

TOP in mitosis



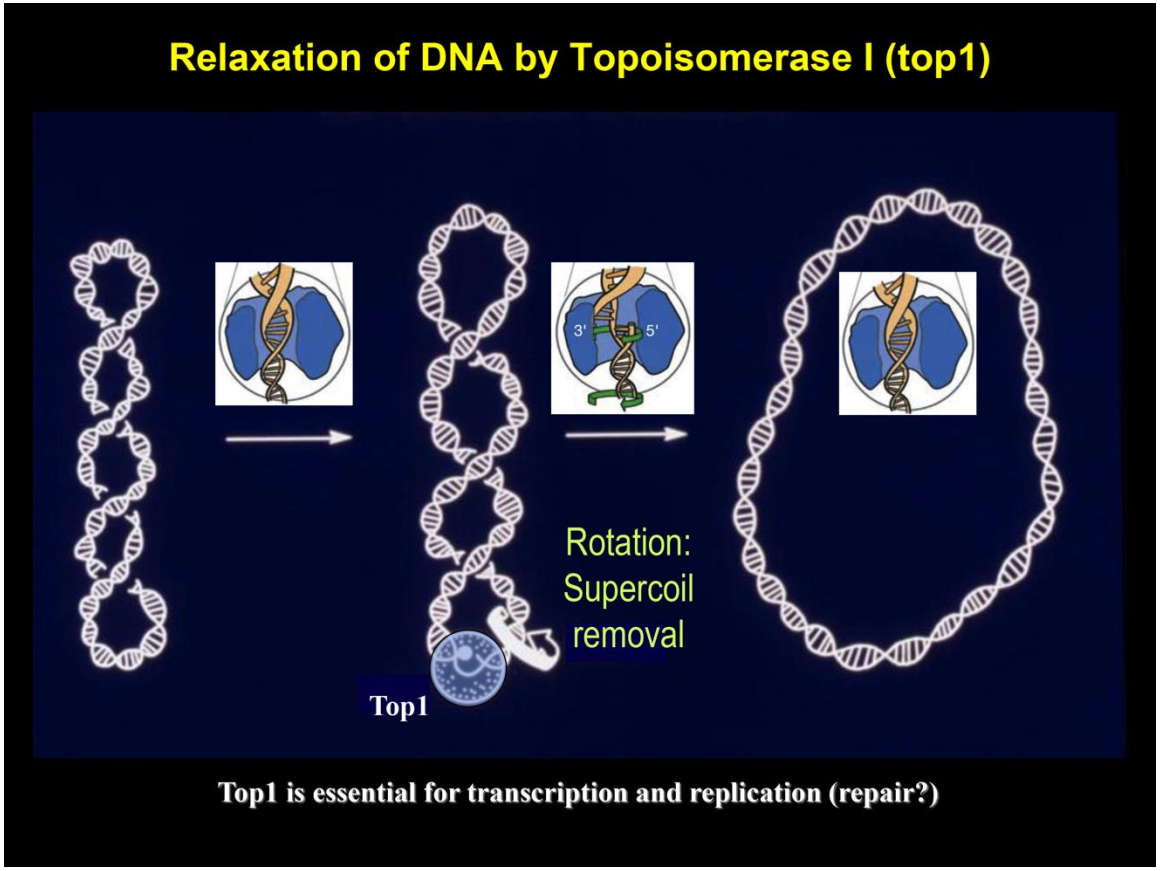

**Functions of human topoisomerases in genome organization in mitosis**

# Top1 and Top2 differences

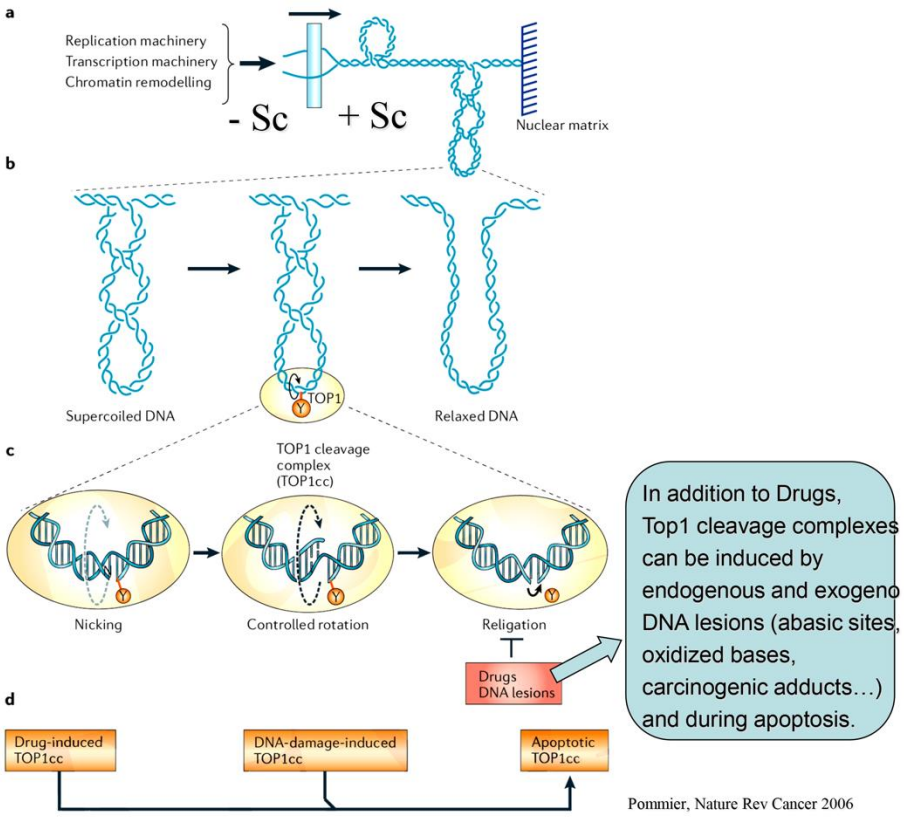
## Top1

TOP1 (nuclear Top1)
TOP1MT (mitochondrial Top1)

# Relaxation of DNA



# Top1




In addition to Drugs, Top1 cleavage complexes can be induced by endogenous and exogeno DNA lesions (abasic sites, oxidized bases, carcinogenic adducts…) and during apoptosis.

Pommier, Nature Rev Cancer 2006

# DNA supercoiling

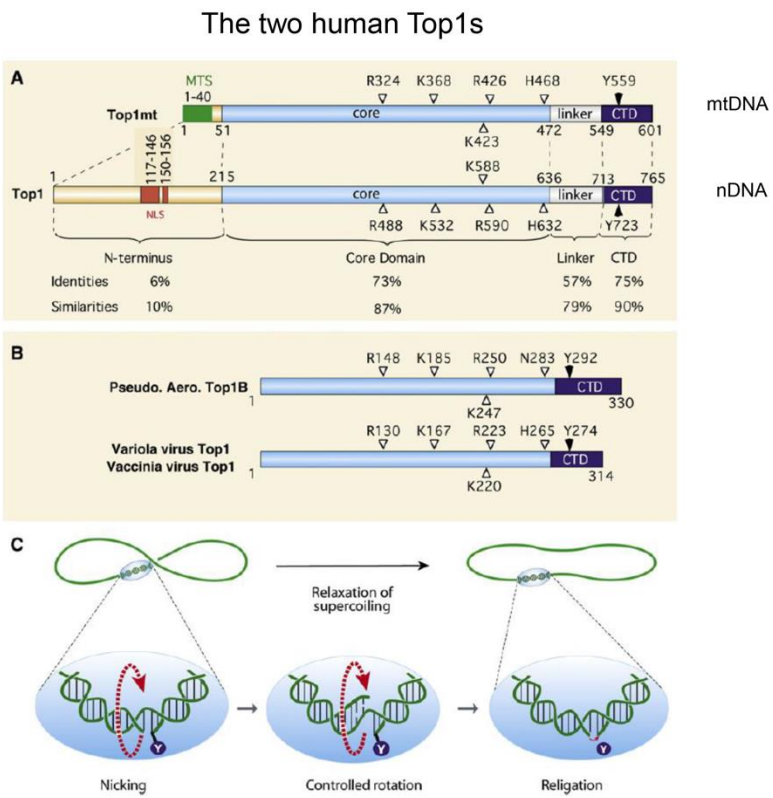
**DNA supercoiling**

In the context of chromatin, where the rotation of DNA is constrained, DNA supercoiling (over- and under-twisting and writhe) is readily generated. TOP1 and TOP1mt remove supercoiling by DNA untwisting, acting as "swivelases", whereas TOP2a and TOP2b remove writhe, acting as "writhases" at DNA crossovers (see TOP2 section).

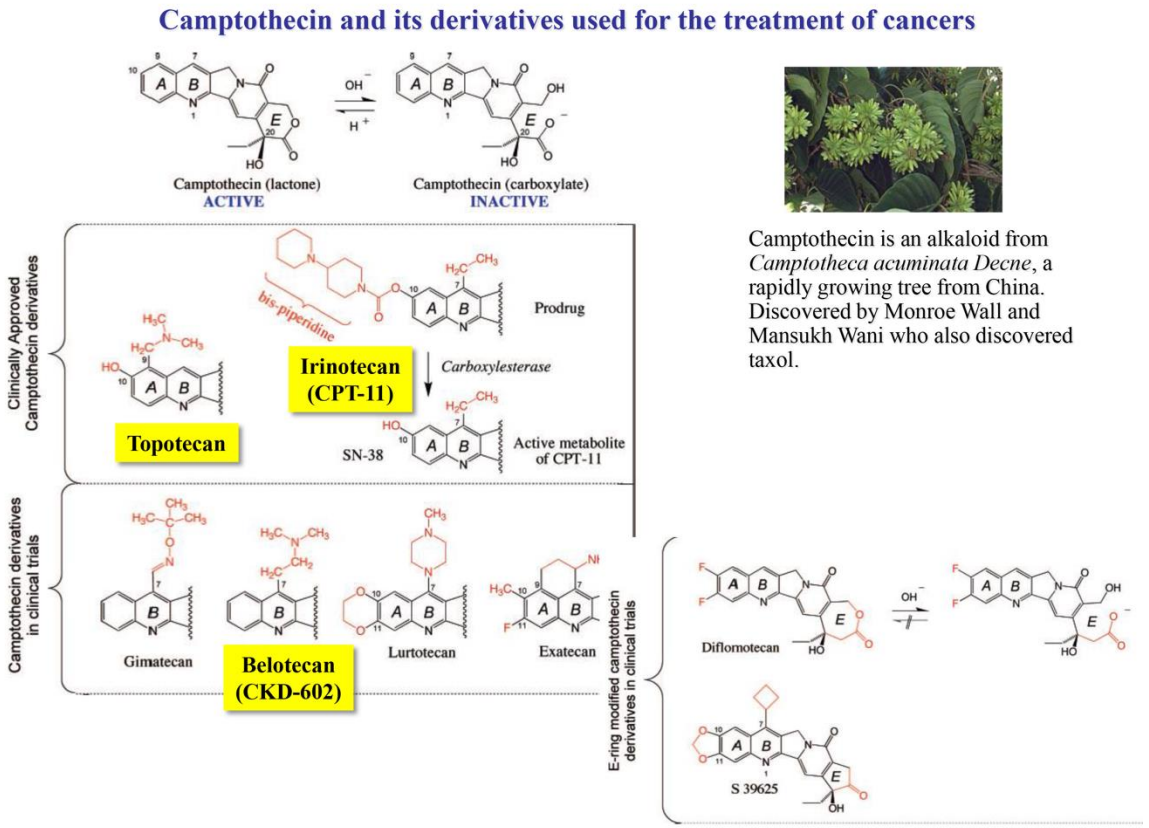
Here are some basic facts concerning DNA supercoiling that are relevant to topoisomerase activity:

- Positive supercoiling (Sc+) tightens the DNA helix whereas negative supercoiling (Sc-) facilitates the opening of the duplex and the generation of single-stranded segments.
- Nucleosome formation and disassembly absorbs and releases Sc-, respectively.
- Polymerases generate Sc+ ahead and Sc- behind their tracks.
- Excess of Sc+ arrests DNA tracking enzymes (helicases and polymerases), suppresses transcription elongation and initiation, and destabilizes nucleosomes.
- Sc- facilitates DNA melting during the initiation of replication and transcription, D-loop formation and homologous recombination and nucleosome formation.
- Excess of Sc- favors the formation of alternative DNA structures (R-loops, guanine quadruplexes, right-handed DNA (Z-DNA), plectonemic structures), which then absorb Sc- upon their formation and attract regulatory proteins.

# The Two Human Top 1s



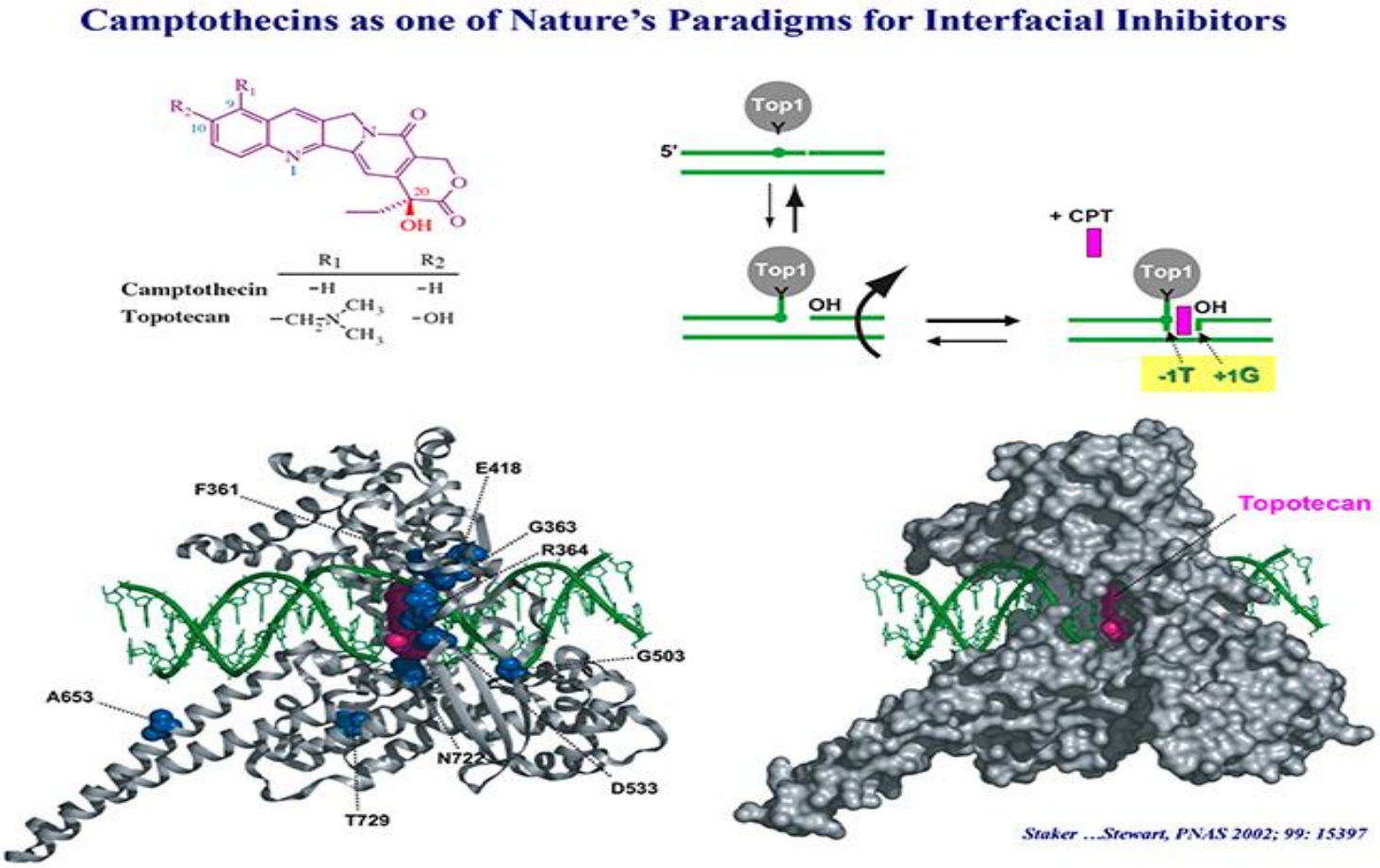
# Camptothecin






Camptothecin is an alkaloid from *Camptotheca acuminata Decne*, a rapidly growing tree from China. Discovered by Monroe Wall and Mansukh Wani who also discovered taxol.

# Interfacial inhibitor



# CPT analogs

758 Eng *et al.* 1988, Mol. Pharmacol

TABLE 1

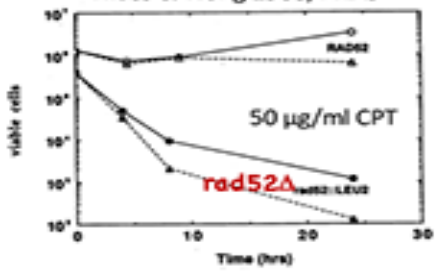
**Activity of Cpt analogs in repair-deficient and/or topoisomerase I-deficient yeast mutants**

$IC_{10}$ is the concentration required to produce a zone of inhibition of 12 mm diameter. Values are mean ± standard error of $IC_{10}$ values determined in multiple independent concentration-response studies. Values without standard errors represent results of single concentration-response studies.

| Drug | $IC_{10}$ | | | rad52Δ | |
|---|---|---|---|---|---|
| | Repair-proficient | | | Repair-deficient (rad52) | |
| | TOP1* | top1 | TOP1** | Top1* | top1 |
| | μg/ml | | | | |
| Cpt | >800 | >800 | 25 ± 3 | 4.5 ± 0.4 | >800 |
| 9-$CH_2O$-Cpt | >800 | >800 | 21 | 5.1 ± 2.1 | >800 |
| 9-Nitro-Cpt | >800 | >800 | 180 ± 38 | 39 ± 5 | >800 |
| 10-$CH_2O$-Cpt | >800 | >800 | 29 ± 3 | 5.0 ± 1.4 | >800 |
| 10-$CH_2O$-7-ethyl-Cpt | >800 | >800 | >800 | 9.4 ± 3.6 | >800 |
| 7-Methyl-Cpt | >800 | >800 | 91 ± 6 | 16 ± 0.2 | >800 |
| 10-HO-Cpt | >800 | >800 | >800 | 210 ± 60 | >800 |
| 10-HO-7-ethyl-Cpt | >800 | >800 | >800 | >800 | >800 |

* TOP1** refers to strain RS190 bearing the topoisomerase I-overproducing plasmid pWE3 GAL-TOP1 under induced conditions.

Nitiss & Wang 1988, PNAS




Homologous recombination is a key pathway for survival to camptothecins

=> Camptothecins were the 1st drugs showing synthetic lethality in homologous recombination deficient (HRD) cells

# Why New Top1 Inhibitors?

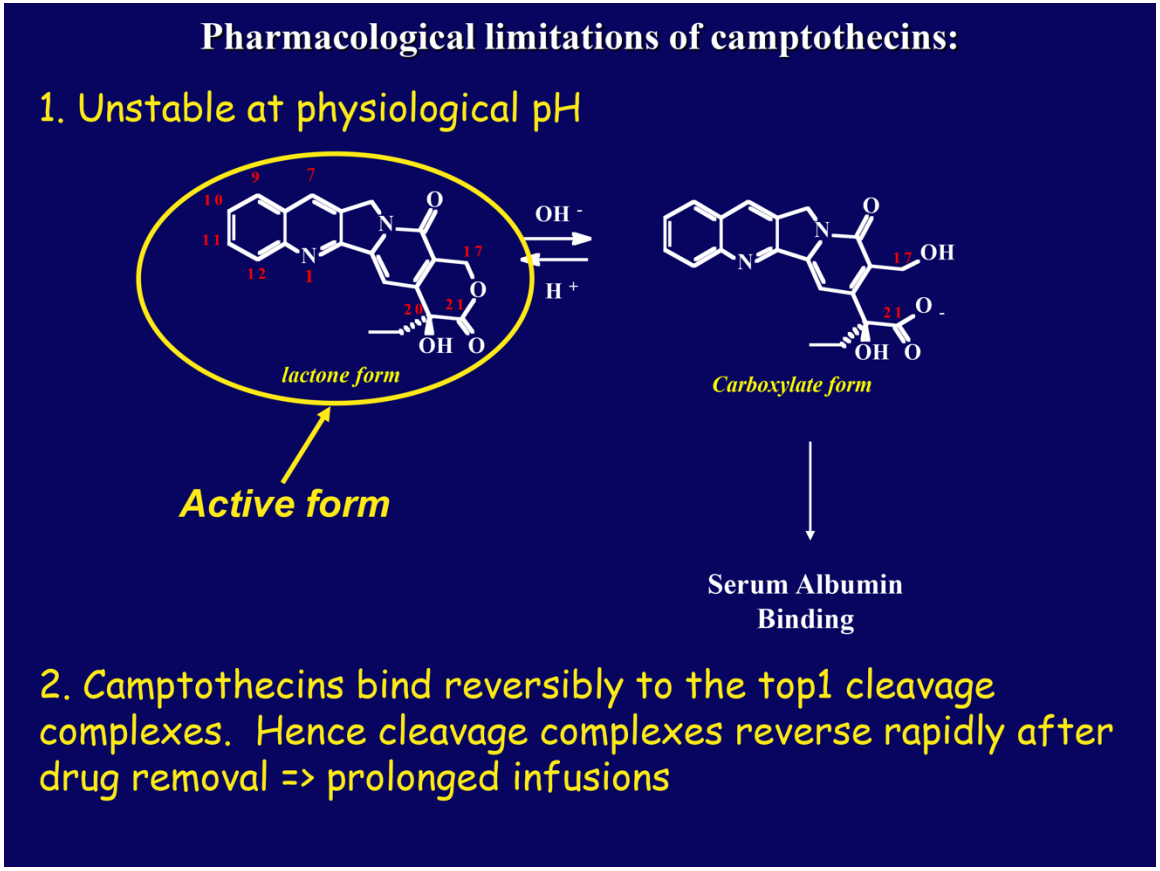
**Why New Top1 Inhibitors?**

1. Because camptothecins are effective anticancer drugs.
   Hence, Top1 is a validated target for cancer treatment.

2. Because agent with a common target have different pharmacology, toxicology and exhibit different anticancer activity (for instance top2 poisons or tubulin inhibitors [colchicine <-> vinblastine]).

3. Because camptothecins have limitations:
   - Bone marrow and intestinal toxicity (adults).
   - Drug efflux substrates (ABCG2).
   - Chemically unstable: E-ring opening.

# Pharmacological Limitations of Camptothecins:



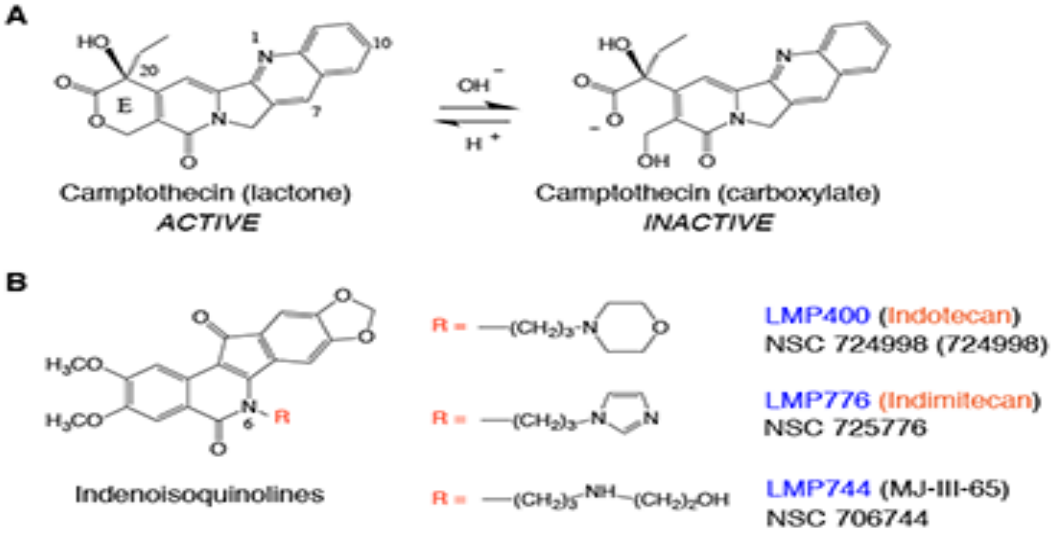
# TOP1 inhibitors

## Rationale for the development of non-camptothecin TOP1 inhibitors

- Camptothecin derivatives (Irinotecan and Topotecan) are potent anticancer agents and highly selective TOP1 inhibitors
- Camptothecins are selective for HR (BRCA) deficient tumors
- Camptothecins are the only chemical class of TOP1 inhibitors (many tubulin, TOP2...)
- Camptothecins have well-established limitations
  - ✓ Chemically unstable (inactivated within minutes in plasma)
  - ✓ Reversibly block TOP1-DNA complexes (long exposure required to maximize effect)
  - ✓ Eliminated from cancer cells by ABC drug efflux transporters (ABCG2 – ABCB1)
  - ✓ Short plasma half-life (2-3 hours due to rapid clearance)
  - ✓ Dose-limiting bone marrow toxicity
  - ✓ Severe diarrhea (Irinotecan)

# Indenoisoquinolines and LMPs

**Non-camptothecin TOP1 inhibitors developed by the NCI-Purdue: the Indenoisoquinolines: the "LMPs"**

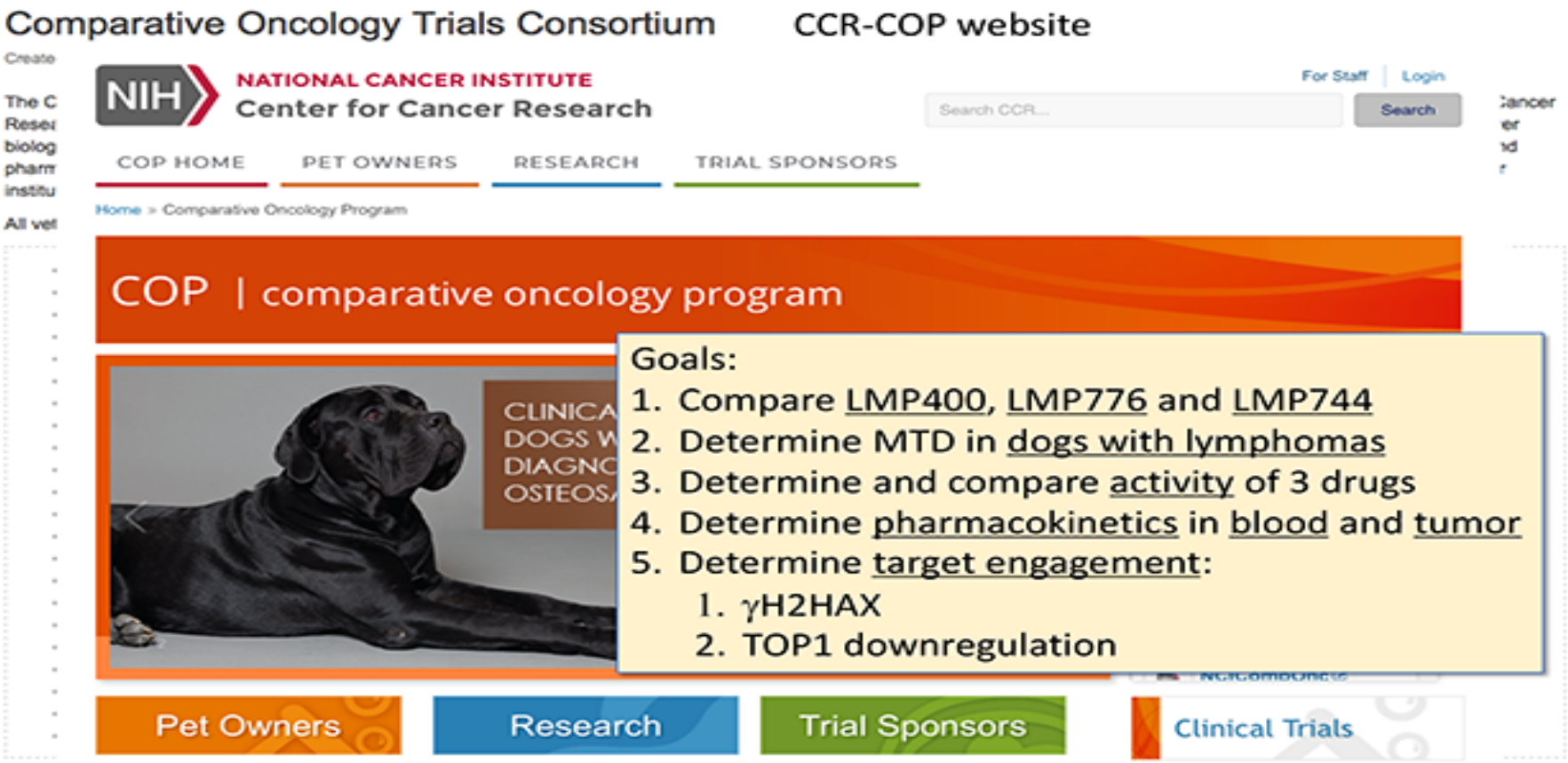



LMP400 (Indotecan) and LMP776 (Imidotecan) completed Phase 1
LMP744 is in phase 1

Joint NCI-Purdue University patent, licensed to Linus Oncology

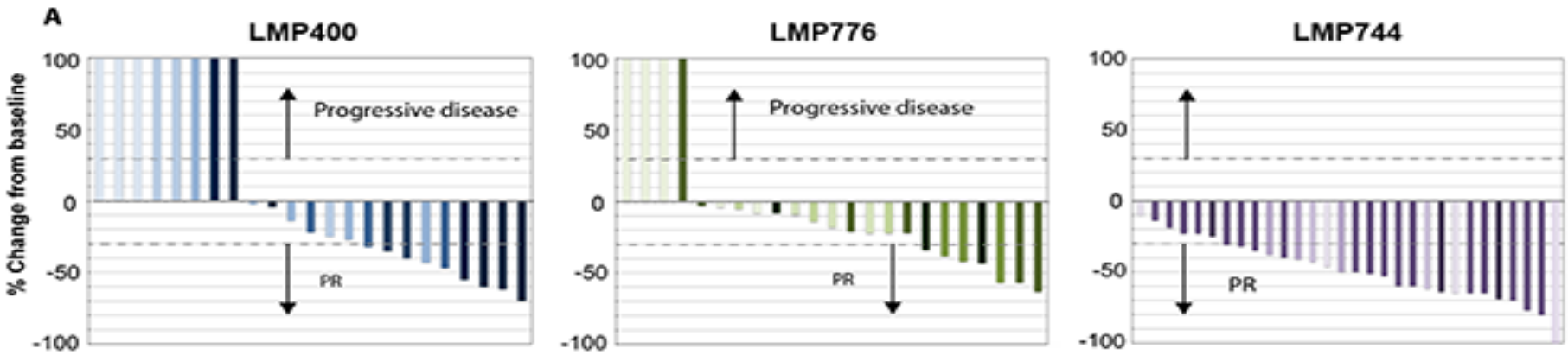
Antony, S,,,Kiselev,,,Pommier,,,Cushman

# Comparative oncology trials



# Dog lymphoma

All drugs exhibit antitumor activity in primary dog lymphoma




Amy LeBlanc
CCR COP

James Doroshow
DCTD - CCR

# Indotecan and imidotecan trials

Summary of the clinical oncology trial:

- The two clinical indenoisoquinolines, LMP400 (indotecan) and LMP776 (imidotecan) exhibit antitumor activity in dog lymphoma.
- The 3rd indenoisoquinoline, LMP744 shows even greater antitumor activity.
- The dose limiting toxicity of the indenoisoquinolines (MTD = 17.5 mg/m$^2$ for LMP776; MTD > 65 mg/m$^2$ for LMP400; MTD = 100 mg/m$^2$ for LMP744) is bone marrow suppression. No diarrhea.
- The PK of the LMPs shows long half-lives: LMP744: 17 h; LMP400: 11 h; LMP776: 6 h.
- LMP744 shows remarkable tumor retention and accumulation
- γH2AX response demonstrates target engagement for all drugs

# Precision therapeutics

Precision therapeutics can be defined as the ability to:

1. prescribing effective therapies only to those patients who will respond effectively (cure) ⇔ Tumor molecular signature: SLFN11 + HRD…
2. while limiting toxicity to normal tissues and minimizing side effects ⇔ Targeted delivery

# Camptothecins

## Second Generation Camptothecins with Targeted Delivery

| Name | Company | Active Derivative (Payload) | Formulation (Conjugate; Target) |
|---|---|---|---|
| Onivyde™ = MM398* | Merrimack | Irinotecan (CPT11) | Liposome |
| CRLX101 | Cerulean Pharma Inc. | Camptothecin | PEG |
| NKTR-102 | Nektar Therapeutics | Etirinotecan (20 position) | PEG (Pegol) |
| PLX038 | ProLynx | SN-38 | PEG |
| IMMU-132 = Sacituzumab govitecan ** | Immunomedics (Seattle Genetics) | SN-38 (20 position) | ADC - TROP2 (TACSD2) |
| IMMU-130 = Labetuzumab govitecan | Immunomedics | SN-38 | ADC-CEACAM5 |
| DS-8201a *** | Daichi Sankyo | DXd (Exatecan) | ADC – HER2 |
| PEN-866 | Tarveda Therapeutics | SN-38 (10 position) | HDC - Conjugate Hsp90 |
| NK012 | Nippon Kayaku | SN-38 | Polymeric micelles (PEG-polyglutamate) |
| ALOS4-CPT | Ariel University | Camptothecin | HDC – ALOS-4 |
| SN38-TOA | CHOP Philadelphia | SN-38 | Tocopherol oxyacetate nanoparticles |

Camptothecins as warheads

Tumor-specific delivery

* FDA Approved, October 2015

** FDA Breakthrough, February 2016

*** FDA Breakthrough, August 2017 (Breast)

# Top2

Top2

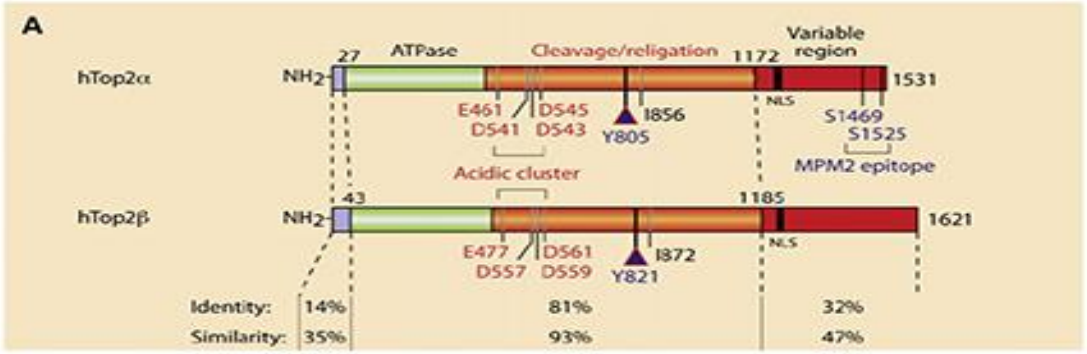
Top2α – TOP2A: Replication

Highly expressed in replicating and cancer cells

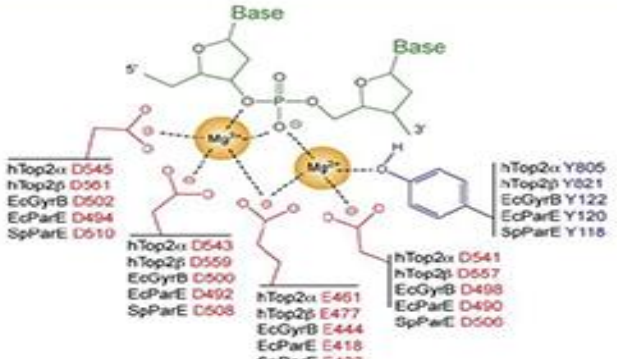
Top2β – TOP2B: Transcription

Expressed both in replicating and differentiated cells

# Two Top2 enzymes



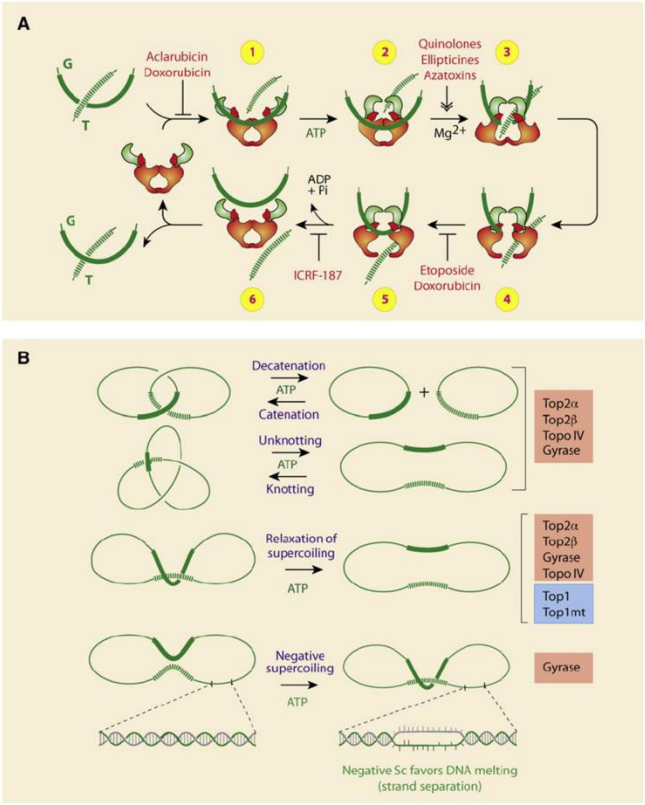





Humans have two Top2 enzymes

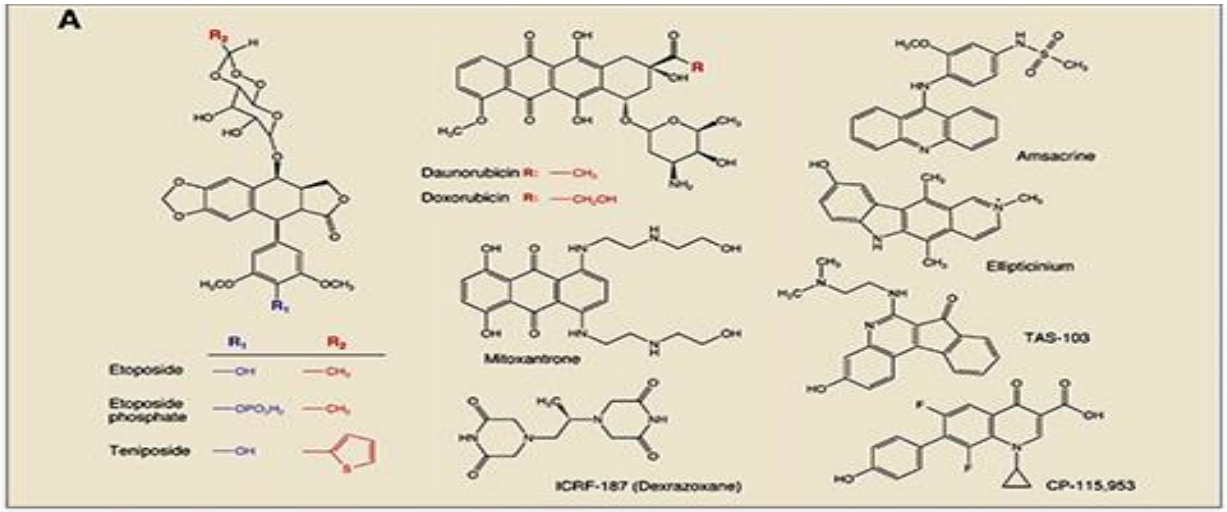
# Top2DNA replication

Top2 catalyze a broad range of reactions

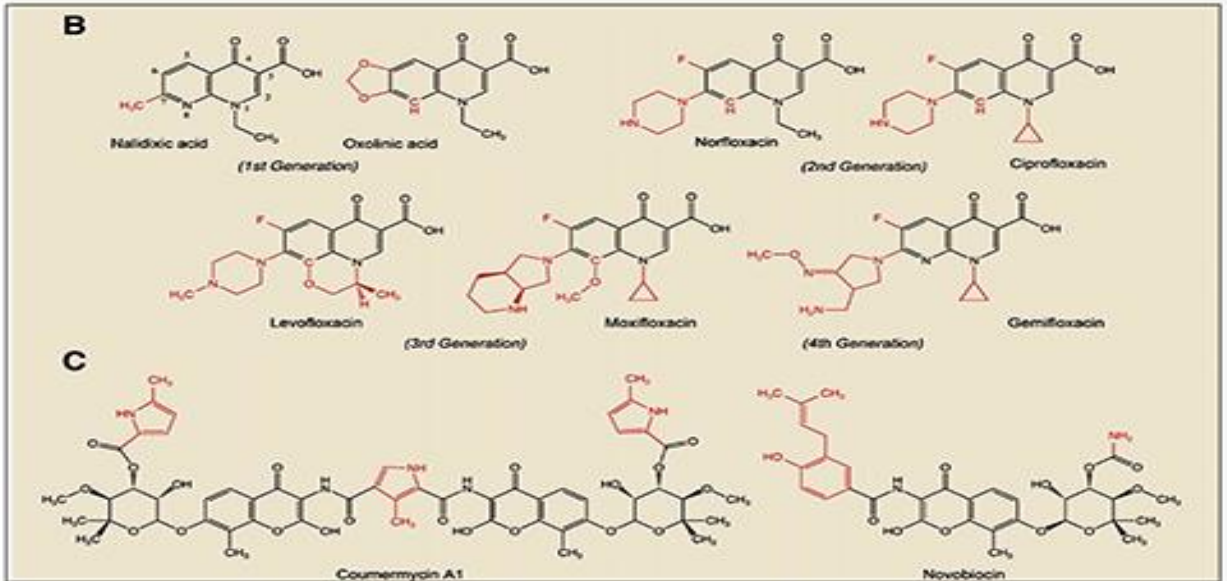


# Top2 drugs

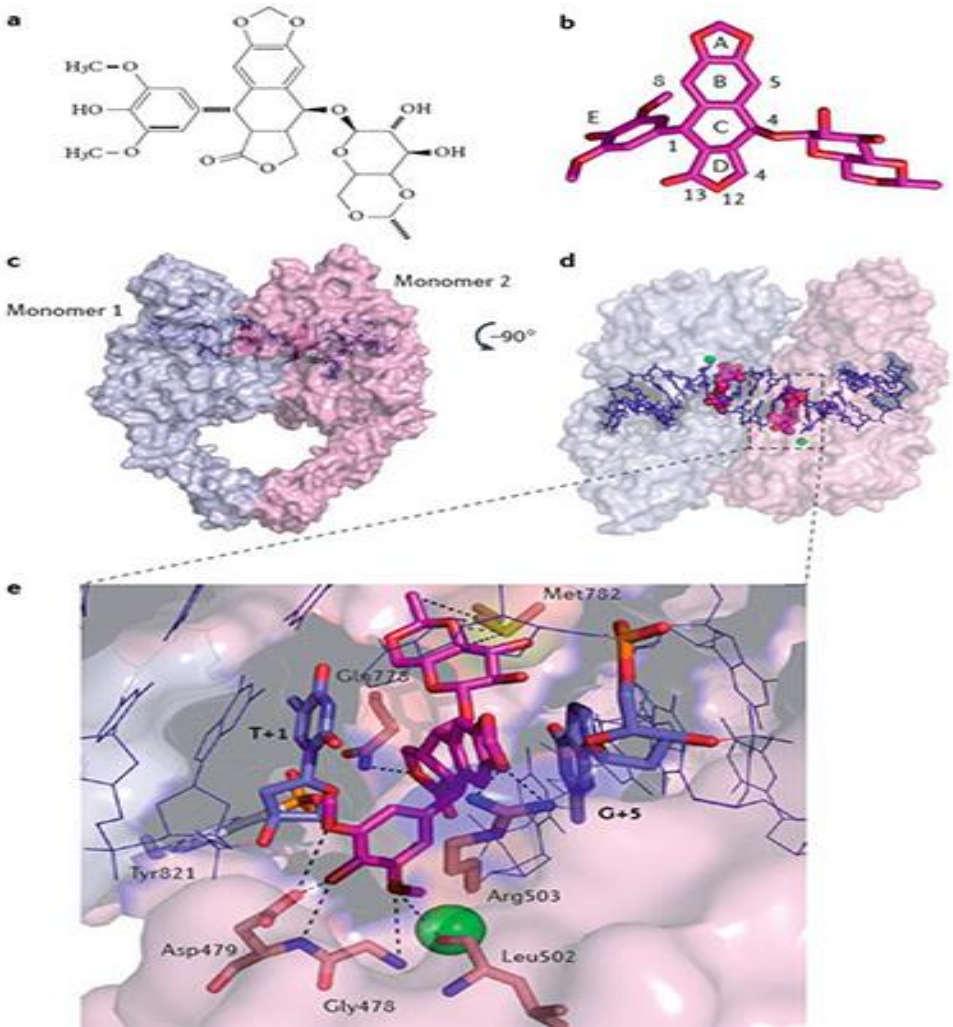
**Anticancer**
Top2-targeted drugs




**Antibiotics**
Top2-targeted drugs



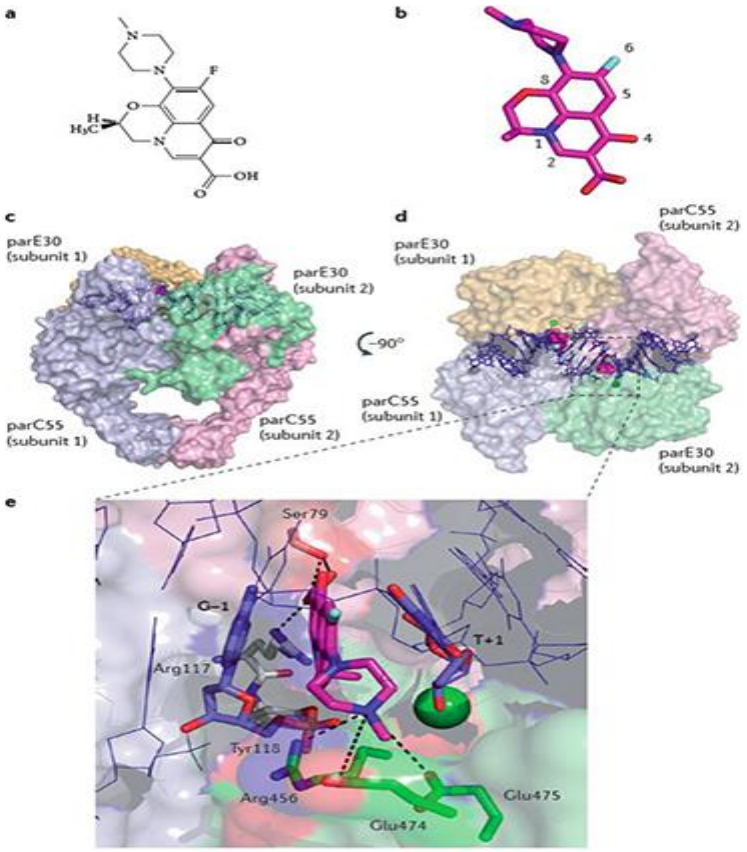
# Etoposide




Structure of a topoisomerase II cleavage complex (Top2cc) trapped by etoposide (VP-16)

# Levofloxacin

Antibacterials




Structure of a topoisomerase IV cleavage complex (Topo IVcc) trapped by the quinolone, levofloxacin

# Interfacial inhibition

*TRENDS in Pharmacological Sciences* Vol.26 No.3 March 2005

Review *TRENDS in Pharmacological Sciences* Vol.26 No.3 March 2005



## Interfacial inhibition of macromolecular interactions: nature's paradigm for drug discovery

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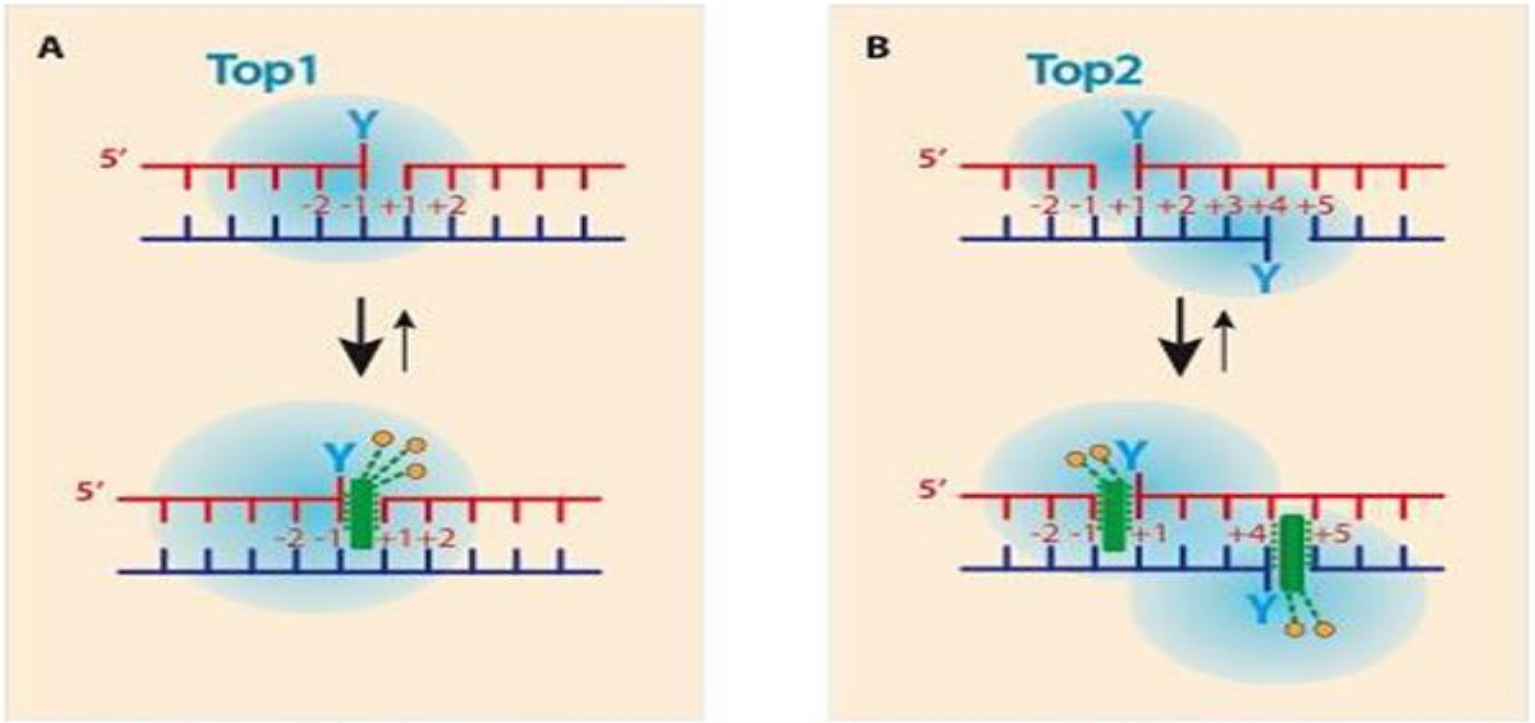
NATURE REVIEWS | DRUG DISCOVERY VOLUME 11 | JANUARY 2012

## Interfacial inhibitors: targeting macromolecular complexes

*Yves Pommier[1] and Christophe Marchand[1]*

Abstract | Interfacial inhibitors belong to a broad class of natural products and synthetic drugs that are commonly used to treat cancers as well as bacterial and HIV infections. They bind selectively to interfaces as macromolecular machines assemble and are set in motion. The bound drugs transiently arrest the targeted molecular machines, which can initiate allosteric effects, or desynchronize macromolecular machines that normally function in concert. Here, we review five archetypical examples of interfacial inhibitors: the camptothecins, etoposide, the quinolone antibiotics, the vinca alkaloids and the novel anti-HIV inhibitor raltegravir. We discuss the common and diverging elements between interfacial and allosteric inhibitors and give a perspective for the rationale and methods used to discover novel interfacial inhibitors.

# Topoisomerase drugs




Camptothecins
Indenoisoquinolines

Etoposide
Doxorubicin

# Top 3

## Top3

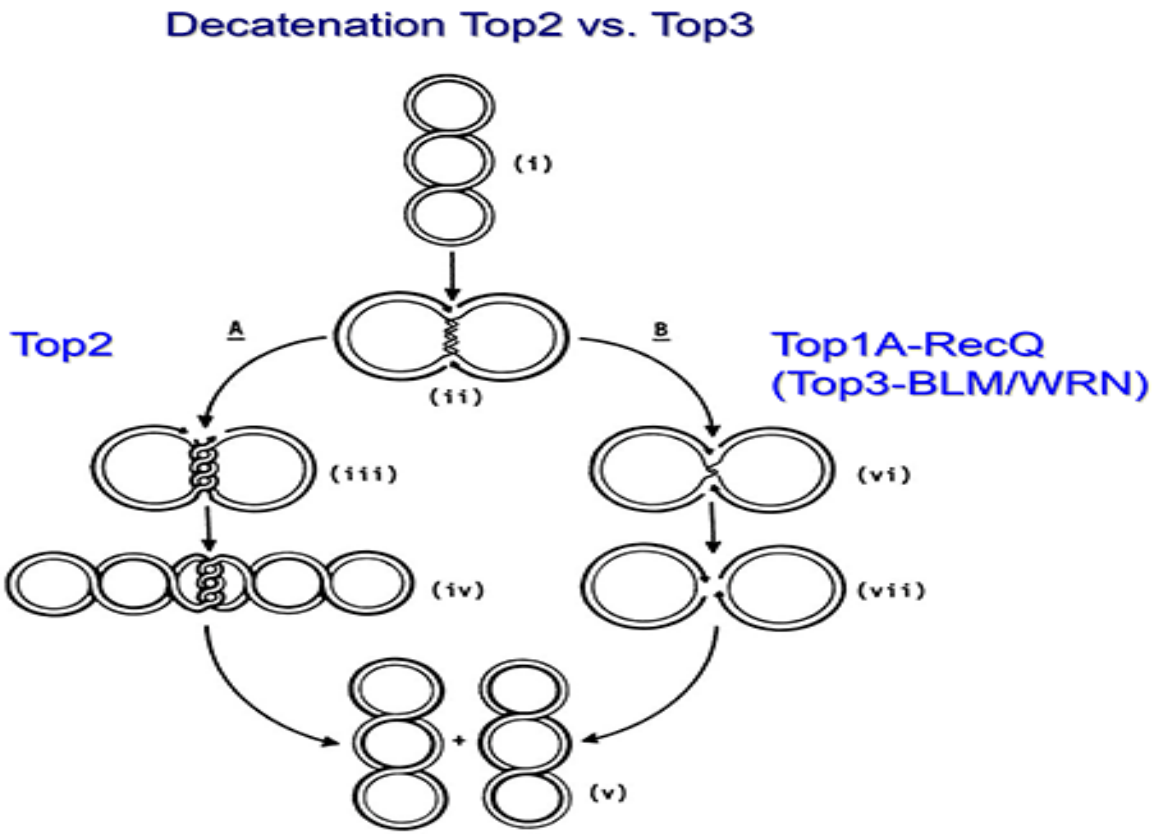
**Top3α – TOP3A**: Replication

DNA topoisomerase (single-strands);
resolves hemicatenanes and
prevents recombinations

**Top3β – TOP3B**: Transcription

DNA topoisomerase (R-loops);
RNA topoisomerase

# Decatenation



# Top-3beta

Nature NeuroScience 2013

## Deletion of TOP3β, a component of FMRP-containing mRNPs, contributes to neurodevelopmental disorders

Georg Stoll[1,32], Olli P H Pietiläinen[2–4,32], Bastian Linder[1,32], Jaana Suvisaari[5], Cornelia Brosi[1], William Hennah[3,5], Virpi Leppä[3], Minna Torniainen[5], Samuli Ripatti[2,3], Sirpa Ala-Mello[6], Oliver Plöttner[7], Karola Rehnström[2], Annamari Tuulio-Henriksson[5], Teppo Varilo[3,4], Jonna Tallila[2], Kati Kristiansson[3], Matti Isohanni[8], Jaakko Kaprio[3,5,9], Johan G Eriksson[10–14], Olli T Raitakari[15,16], Terho Lehtimäki[17], Marjo-Riitta Jarvelin[18–21], Veikko Salomaa[22], Matthew Hurles[2], Hreinn Stefansson[23], Leena Peltonen[2–4,24,25], Patrick F Sullivan[26,27], Tiina Paunio[3,4,28], Jouko Lönnqvist[5,6], Mark J Daly[29,30], Utz Fischer[1], Nelson B Freimer[31] & Aarno Palotie[2,3,30]

Implicating particular genes in the generation of complex brain and behavior phenotypes requires multiple lines of evidence. The rarity of most high-impact genetic variants typically precludes the possibility of accruing statistical evidence that they are associated with a given trait. We found that the enrichment of a rare chromosome 22q11.22 deletion in a recently expanded Northern Finnish sub-isolate enabled the detection of association between *TOP3B* and both schizophrenia and cognitive impairment. Biochemical analysis of TOP3β revealed that this topoisomerase was a component of cytosolic messenger ribonucleoproteins (mRNPs) and was catalytically active on RNA. The recruitment of TOP3β to mRNPs was independent of RNA *cis*-elements and was coupled to the co-recruitment of FMRP, the disease gene product in fragile X mental retardation syndrome. Our results indicate a previously unknown role for TOP3β in mRNA metabolism and suggest that it is involved in neurodevelopmental disorders.

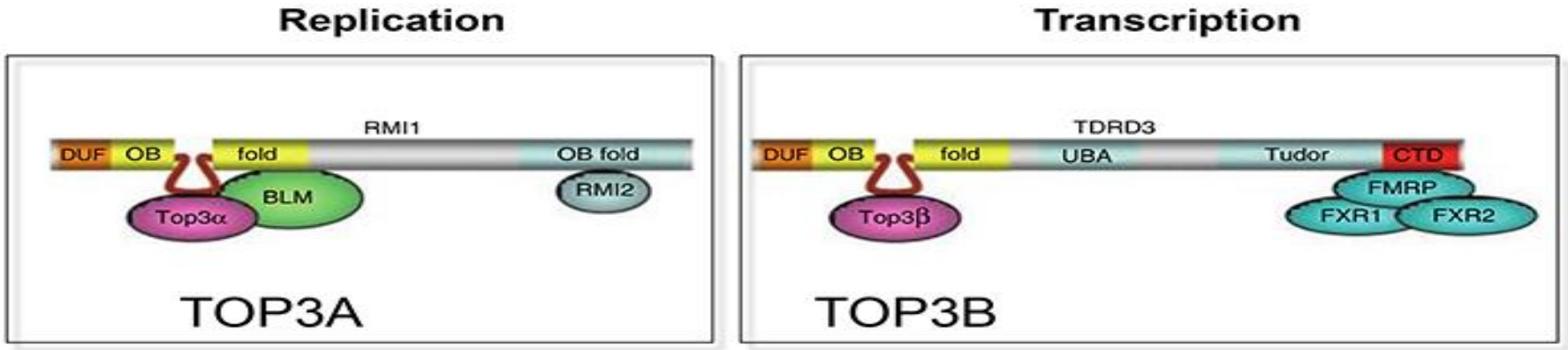
## Top3β is an RNA topoisomerase that works with fragile X syndrome protein to promote synapse formation

Dongyi Xu[1,2,10], Weiping Shen[1,10], Rong Guo[1], Yutong Xue[1], Wei Peng[1], Jian Sima[3], Jay Yang[4], Alexei Sharov[5], Subramanya Srikantan[6], Jiandong Yang[1], David Fox III[1], Yong Qian[5], Jennifer L Martindale[6], Yulan Piao[5], James Machamer[7], Samit R Joshi[8], Subhasis Mohanty[8], Albert C Shaw[8], Thomas E Lloyd[7], Grant W Brown[4], Minoru S H Ko[5], Myriam Gorospe[6], Sige Zou[9] & Weidong Wang[1]

Topoisomerases are crucial for solving DNA topological problems, but they have not been linked to RNA metabolism. Here we show that human topoisomerase 3β (Top3β) is an RNA topoisomerase that biochemically and genetically interacts with FMRP, a protein that is deficient in fragile X syndrome and is known to regulate the translation of mRNAs that are important for neuronal function, abnormalities of which are linked to autism. Notably, the FMRP-Top3β interaction is abolished by a disease-associated mutation of FMRP, suggesting that Top3β may contribute to the pathogenesis of mental disorders. Top3β binds multiple mRNAs encoded by genes with neuronal functions linked to schizophrenia and autism. Expression of one such gene, that encoding protein tyrosine kinase 2 (ptk2, also known as focal adhesion kinase or FAK), is reduced in the neuromuscular junctions of *Top3β* mutant flies. Synapse formation is defective in Top3β mutant flies and mice, as well as in FMRP mutant flies and mice. Our findings suggest that Top3β acts as an RNA topoisomerase and works with FMRP to promote the expression of mRNAs that are crucial for neurodevelopment and mental health.

# Top3A and Top3B

TOP3 alpha and beta function in different protein complexes and biological processes



# Topoisomerases

Topoisomerases
Genomic Integrity
and
Human diseases

# DNA damage

Topoisomerase-induced DNA damage

**A**


* **Conversion of TOP1cc into DSB by replication "run-off"**
  **=> TOP1 needs to be removed by TDP1**
  **and/or 3'-flap endonucleases (XPF-ERCC1)**
  **=> DSB repaired by homologous recombination**
* **TOP1cc also form DSB when on opposite strands or opposite to a preexisting single-strand break**

**B**


* **TOP2cc readily form DSB when concerted cleavage on both strands and disjonction of the homodimer**

**C**


* **Collisions of polymerases and helicases (green ellipse) with trapped Top cleavage complexes (Stop sign)**
  **=> Protein-DNA complexes blocking DNA metabolism**

**D**


* **Topological defects resulting from enzyme sequestration in the cleavage complexes: accumulation of**
  **=> supercoils (Top1 and Top2)** *(1)*
  **=> knots (Top2)** *(2)*
  **=> catenanes (Top2)** *(3)*

# Topoisomerases and disease

Table 1 | **Drugs, DNA alterations and physiological processes that lead to the formation of persistent TOPcc**

| Causes | Consequences for TOP1 enzymes | Consequences for TOP2 enzymes |
|---|---|---|
| Anticancer drugs acting as interfacial inhibitors[155] | Trapping of TOP1cc by irinotecan, topotecan, indenoisoquinolines* and tumour-targeting camptothecin derivatives[3,154,155] | Trapping of TOP2cc by etoposide, teniposide, doxorubicin, epirubicin, idarubicin and mitoxantrone[4] |
| Oxidative DNA lesions (8-oxoguanine, 8-oxoadenosine and 5-hydroxycytosine) | Induction and trapping of TOP1cc[218,219] | Induction and trapping of TOP2cc[220] |
| Abasic sites and DNA mismatches | Formation of irreversible TOP1cc[221] | Formation of irreversible TOP2cc[220,222–225] |
| Carcinogenic base adducts (methylated bases, exocyclic adducts, benzo[*a*]pyrene adducts and crotonaldehyde adducts) | Induction and trapping of TOP1cc[226–232] | Induction and trapping of TOP2cc[220,233–235] |
| Nicks and DNA strand breaks | Formation of irreversible TOP1cc, double-stranded breaks, genomic deletions and recombination[18,167,168,236,237] | Formation of irreversible TOP2cc[235] |
| UV lesions (pyrimidine dimers and 6.4-photoproducts) | Induction of TOP1cc[238,239] | Enzymatic inhibition[240] |
| Ribonucleotide incorporation into DNA | Formation of TOP1cc that generate nicks with 2′,3′-cyclic phosphate ends and short deletions in repeat sequences[166–168] | Stabilization of TOP2cc with asymmetrical cleavage[20,169,241] |
| Natural and food products | Unknown | Stabilization of TOP2cc by flavones, tea and wine products[205] |
| Genetic defects | Unrepaired TOP1cc due to TDP1 defects[177,206,210] in cooperation with ATM defects[179] | Unrepaired TOP2cc due to TDP2 defects[69] |
| Transcription activation | Stabilization of TOP1cc at enhancers[42] | Stabilization of TOP2cc at promoters[62,65,242,243] |

ATM, ataxia telangiectasia mutated; TDP, tyrosyl-DNA phosphodiesterase; TOPcc, topoisomerase cleavage complex.
*Indenoisoquinoline derivatives are in clinical trials.

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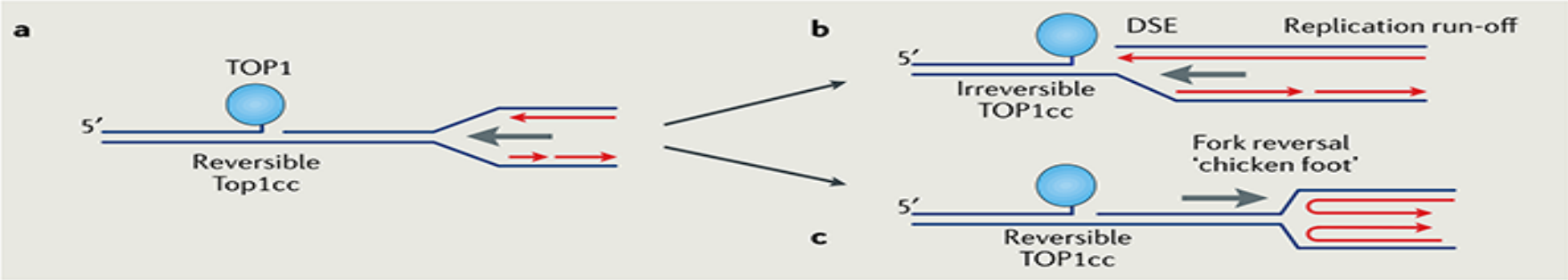
2016 Nature Rev Mol Cell Biol

# Replicative DNA damage

Replicative DNA damage induced by TOP1cc (Topoisomerase I cleavage complexes)

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# Human diseases

**Human Diseases linked with topoisomerases**

TOP1: Neurological diseases due to lack of removal of TOP1cc (in conjuction with TDP1 and ATM deficiencies)

TOP2B: Chromosomose translocations at TOP2Bcc (leukemia, prostate cancers…)

TOP3B: Neurodevelopmental disorders (schizophrenia and cognitive impairment)

TDP1: SCAN1 (Spinocerebellar Ataxia and peripheral Neuropathy)

TDP2: Intellectual disability, seizures and ataxia

# DNA repair

Box 1 | **DNA–protein crosslink repair pathways and human health**

It is intriguing that germline mutations in almost all identified genes that encode components of the three main DNA–protein crosslink (DPC) repair pathways result in human syndromes that are characterized by genome instability, cancer predisposition, premature ageing and/or neurological pathologies. Whether all of these phenotypes are directly related to a defect in DPC repair or to other cellular functions of these proteins, is not entirely clear in all cases. The MRN complex, for example, has crucial functions during repair of DSBs, which are clearly related to the radiosensitivity and immunodeficiency that are observed in patients with mutations in genes that encode MRN subunits. Below, we briefly discuss the main diseases that are associated with mutations in DPC repair proteins.

**Repair by tyrosyl-DNA phosphodiesterases**

Spinocerebellar ataxia, autosomal recessive, with axonal neuropathy (SCAN1; OMIM: 607250) was first identified in a large Saudi Arabian family (nine affected individuals) that had homozygous mutations in the tyrosyl-DNA phosphodiesterase 1 (*TDP1*) gene, which map to chromosome 14q31–14q32 (REF. 91). Clinical features of SCAN1 include spinocerebellar ataxia (with late onset and slow progression) and areflexia, followed by signs of peripheral neuropathy, with the absence of non-neurological symptoms that are otherwise common in ataxia telangiectasia (telangiectasias, immunodeficiency, and cancer predisposition). Interestingly, the TDP1-H493R variant, which causes SCAN1, is not only catalytically compromised but also becomes covalently trapped in the process of repairing Top1 adducts[92]. However, despite this pathological gain-of-function of the TDP1-H493R variant, this form of SCAN1 is a recessive disorder, as wild-type TDP1 is able to repair the TDP1-H493R adducts in heterozygous individuals.

Spinocerebellar ataxia, autosomal recessive 23 (SCAR23; OMIM: 616949) has been identified in three Irish brothers who were born to consanguineous parents, and in an unrelated Egyptian case. SCAR23 has been associated with a homozygous mutation in the *TDP2* gene on chromosome 6p2 (REF. 40). Clinical features include progressive spinocerebellar ataxia, epilepsy and intellectual disabilities.

**Repair by the MRN complex**

Clinical features of ataxia telangiectasia-like disorder 1 (ATLD1; OMIM: 604391) include slowly progressive cerebellar degeneration that results in ataxia and oculomotor apraxia, and dysarthria, but without telangiectasia or major defects in immunoglobulin production, and without major cancer predisposition but with radiosensitivity. ATLD1 is caused by homozygous or compound heterozygous mutations in the *MRE11* gene on chromosome 11q21 (REFS 93,94).

Nijmegen breakage syndrome (NBS) ataxia telangiectasia variant V1 (OMIM: 251260) is caused by homozygous or compound heterozygous mutations in the *NBS1* gene on chromosome 8q21. More than 90% of patients are homozygous for a five base pair deletion (657del5), which leads to a frameshift and truncation of the NBS1 protein[95–98]. There are no reliable estimates of worldwide prevalence, but it is likely to approximate to 1 in 100,000 live births (most common in the Slavic populations of Eastern Europe)[99]. Clinical features of this syndrome include microcephaly, growth retardation, immunodeficiency, predisposition to cancer (mainly non-Hodgkin lymphoma), and radiosensitivity; neither ataxia nor telangiectasia are present. Compound heterozygous mutations in the *RAD50* gene (on chromosome 5q31.1) that give rise to low levels of RAD50 cause Nijmegen breakage syndrome-like disorder (NBSLD; OMIM 613078)[100]. Clinical features of NBSLD include microcephaly, growth retardation, chromosome instability, radioresistant DNA synthesis, radiation hypersensitivity and slight, non-progressive ataxia; there are no signs of telangiectasia or immunodeficiency and no evidence of cancer predisposition[100,101].

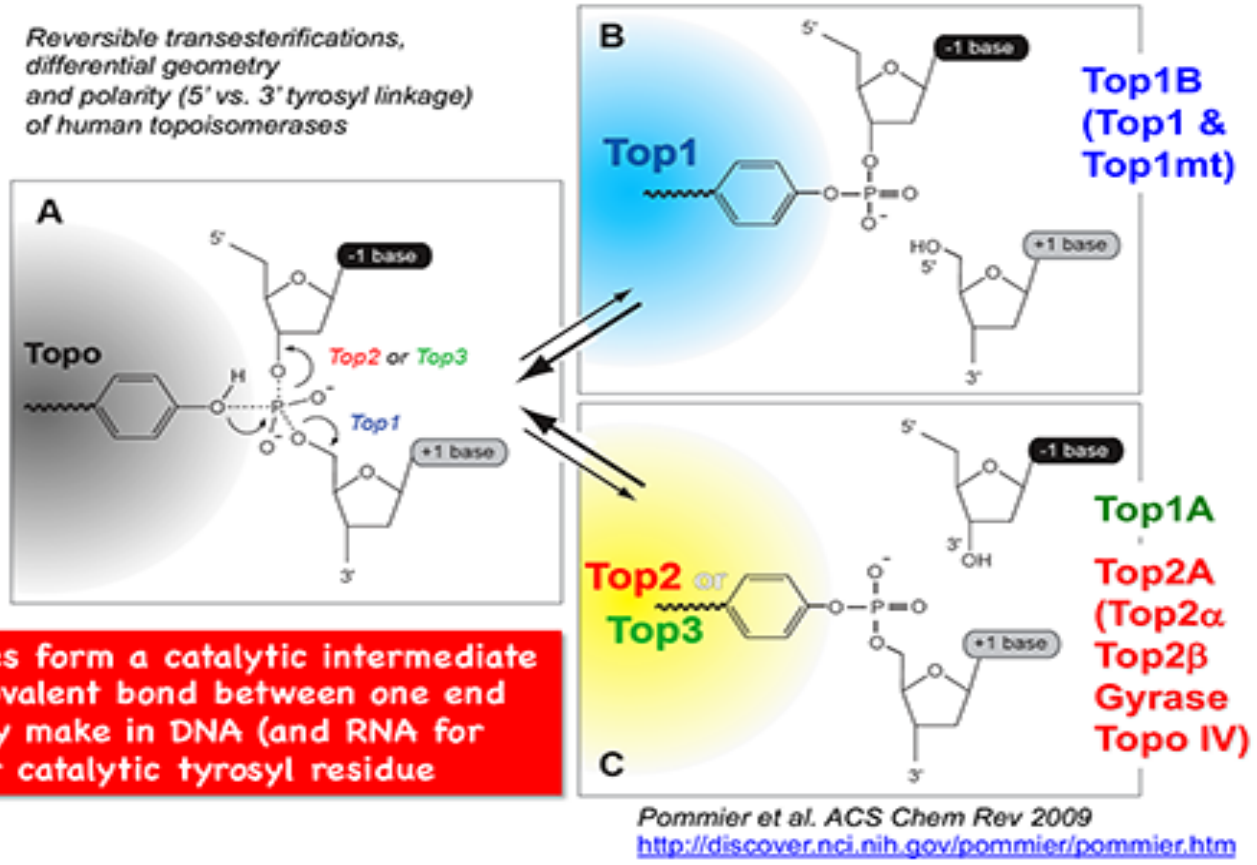
**Repair by DPC proteases**

Homozygous or compound heterozygous mutations in the *SPRTN* gene (on chromosome 1q42) cause Ruijs–Aalfs syndrome (RJALS; OMIM: 616200). Clinical features of RJALS include growth retardation, early-onset hepatocellular carcinomas, micrognathia, chromosomal instability and sensitivity to genotoxic agents[68,69].

# Covalent complexes

Repair of Topoisomerase covalent complexes

# Catalytic intermediate



# Topoisomerase

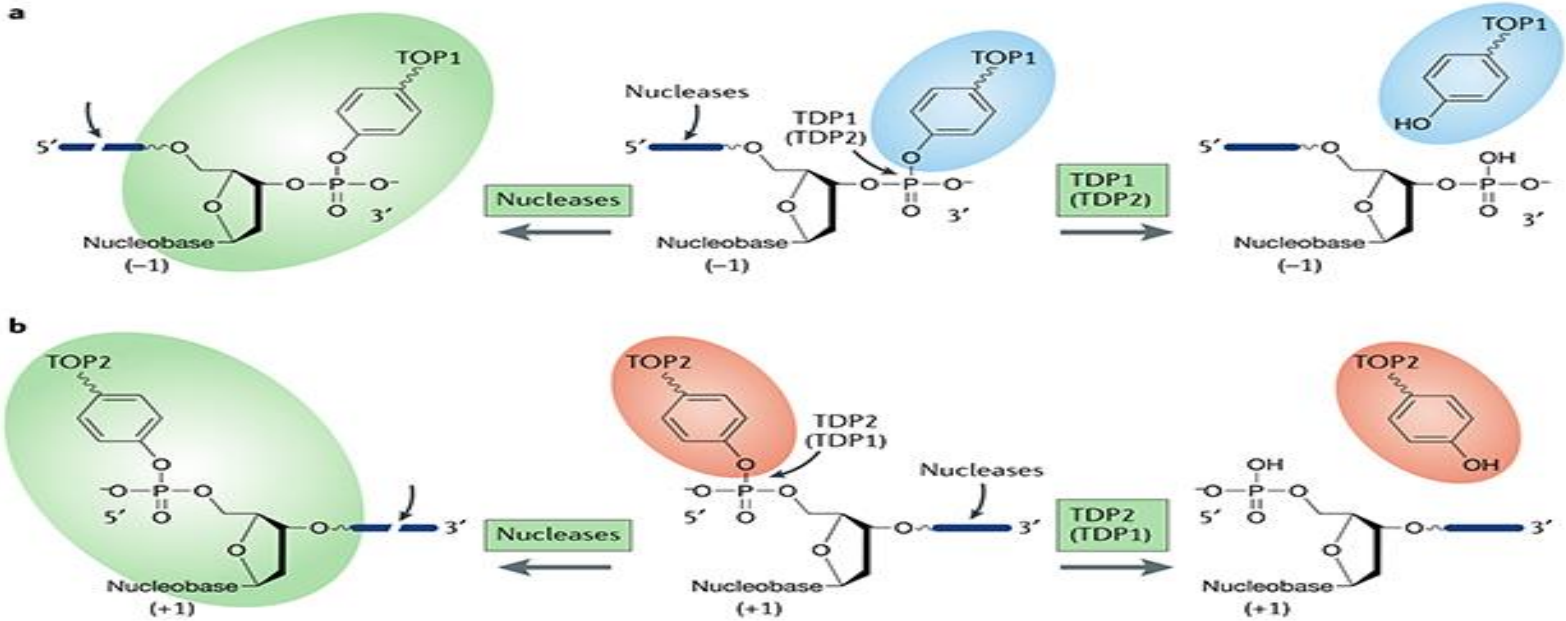



Figure 5 | **TOPcc repair. a** | Tyrosyl-DNA phosphodiesterase 1 (TDP1) and TDP2 (although much less efficiently and therefore shown in parentheses) cleave the TOP1 tyrosyl–DNA covalent bond (middle), releasing TOP1 and leaving a 3′-phosphate end (right) that needs to be further processed by polynucleotide kinase phosphatase (not shown). **b** | TOP2 cleavage complexes (TOP2cc) are preferentially repaired by TDP2 and much less efficiently by TDP1 (middle) in vertebrates, releasing TOP2 and leaving a 5′-phosphate (right), which can be readily ligated. Yeast, which do not encode a TDP2 orthologue, use Tdp1 to excise both Top1cc and Top2cc. In the endonuclease pathways (left), topoisomerases are released with the segment of DNA to which they are attached by the action of endonucleases; the polarity is opposite for TOP1cc (part **a**) and TOP2cc (part **b**).

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# Repair pathways

**Parallel repair pathways for abortive topoisomerase cleavage complexes:**

- **Excision by two dissimilar tyrosyl DNA phosphodiesterases: TDP1 and TDP2**
- **Endonucleases (Mre11; NER…)**


TDP1 has a broad range of DNA repair functions beyond TOP1cc repair:

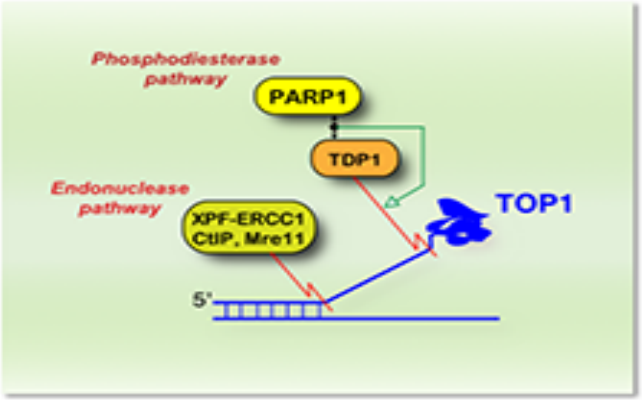
- 3'-end cleansing activity: 3'-phosphoglycolates ($H_2O_2$, bleomycin, IR)
- 3'-dRP (MMS, alkylating agents) (JBC)*
- Excises chain terminator nucleosides (AraC, AZT, abacavir, sapacitabine) (JBC; NAR)*; 3'-nucleosidase
- Both in the nucleus and mitochondria (EMBO J)
- Role in genomic stability in the nervous system (PNAS)*
- Coupled with PARP1 (JBC; DNAR)*
- Also excises TOP2cc (JBC)* (no TDP2 in yeast)

TDP2 also has DNA repair functions beyond TOP2cc:

- 5'-end tyrosyl-DNA phosphodiesterase: VpG unlinkase (poliovirus replication) (HPV replication)
- Crystal structures (NSMB; JBC)*: similarity with APE1 ($Mg^{2+}$; 5 fingers) but different from TDP1
- Recruitment to TOP2cc by Ub (JBC)*
- Activity on TOP2cc requires denaturation/ proteolysis (JBC)*

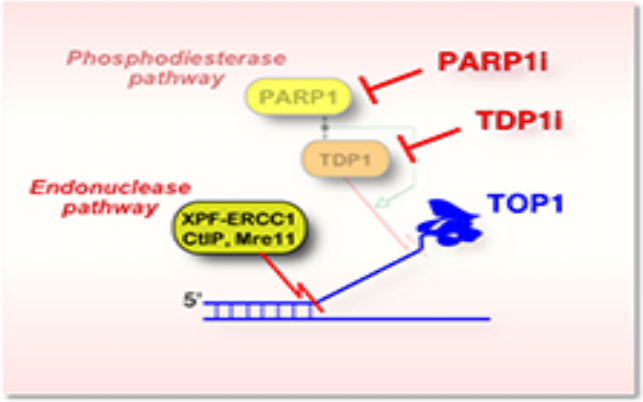
# Parallel repair pathways

Normal cells have parallel repair pathways for abortive TOP1cc




TDP1 is coupled with PARP1

*Discovered this review cycle (NAR; DNAR; JBC)*




PARP1 inhibitors synergize with TOP1 inhibitors

*(Sci Transl Med 2016; JPET 2014)*

=> Synthetic lethality

in Mre11- or XPF-ERCC1-deficient cancers?

# Acknowledgements


## Acknowledgements for Indenoisoquinolines

- **Mark Cushman** (Purdue University)
- William Bonner (LMP-CCR)
- Smitha Antony (LMP-CCR)
- George Vande Woude (DBS)
- Susan Bates (COB-CCR)
- Robert Wiltrout (CCR)
- Patricia Steeg (LMP-CCR)
- Lyuba Varticovski (LHC-CCR)
- **James Doroshow** (DCTD-LMP-NCI)
- Joseph Tomaszewski (DCTD-NCI)
- Jerry Collins (DTP-DCTD-NCI)
- Barbara Mroczkowski (DCTD)
- Ralph Parchment (SAIC-FCRDC)
- Bob Kinders (SAIC-FCRDC)
- **Kenneth Paull** (DTP)
- Melinda Hollingshead (DTP)
- Jiuping Ji (SAIC-FCRDC)
- Joe Covey (DTP)
- Liz Glaze (DTP)
- Prabhakar Risbood (DTP)
- Jim Cradock (DTP)
- Rao Vishnuvajjala (DTP)
- Sima Hayavi (DTP)
- Tiziano DiPaolo (DCTP)
- Vali Sevastita (DTP)
- Gina Uhlenbrauck (DTP)
- Shivaani Kummar (COB-CCR)
- Giuseppe Giaconne (COB-CCR)