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DNA Topoisomerases and Their Poisoning by Anticancer and Antibacterial Drugs

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Drugging Topoisomerases: Lessons and Challenges

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REVIEWS

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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.

Cancer Drug Discovery and Development
Beverly A. Teicher, *Series editor*

Yves Pommier *Editor*

DNA Topoisomerases and Cancer

DNA topoisomerases are present in all living organisms and are essential to maintaining the helical structure of DNA. They are highly relevant for cancer because a number of anti-cancer drugs selectively target two of the human enzymes, DNA topoisomerases I and II. Those drugs convert topoisomerases into cellular poisons by trapping the enzymes as they cleave DNA. The book starts out with a detailed outline of the phyllogeny of the different topoisomerases, continues with recent studies on the crystal structures of the human topoisomerases, and their biochemistry. The following section reviews the chemical biology of the topoisomerase inhibitors used in cancer chemotherapy and the implication of topoisomerases in generating recombinations and DNA damage. The third section summarizes the current use of the various topoisomerase inhibitors in cancer chemotherapy. And finally, the last section includes several chapters describing the DNA repair pathways for topoisomerase-induced DNA damage. This book is intended for students and faculty but also for health care professionals who wish to have a self-contained and up-to-date information on topoisomerases. Chapters have been written by leaders and world reknowned experts in the topoisomerase field.

Biomedicine

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CDDD

Pommier *Ed.*

DNA Topoisomerases and Cancer

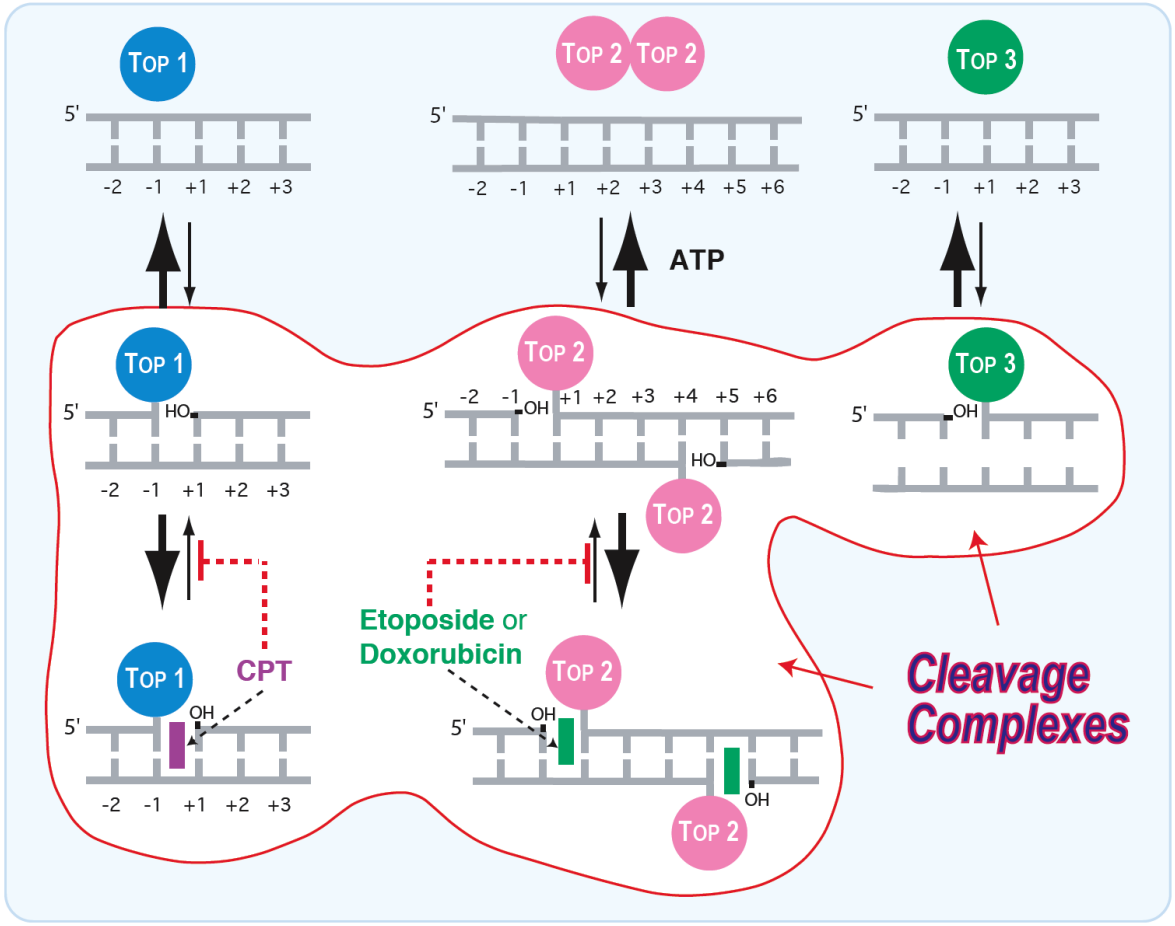
Cancer Drug Discovery and Development

Yves Pommier *Editor*

DNA Topoisomerases and Cancer

Humana Press

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



Top1 (Type IB)

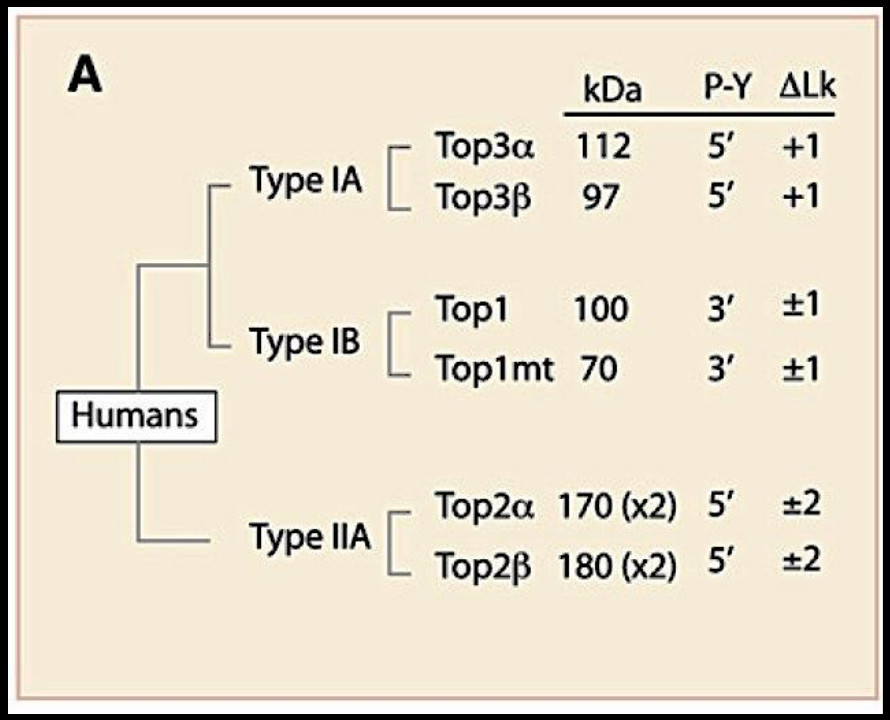
Top2 (Type IIA)

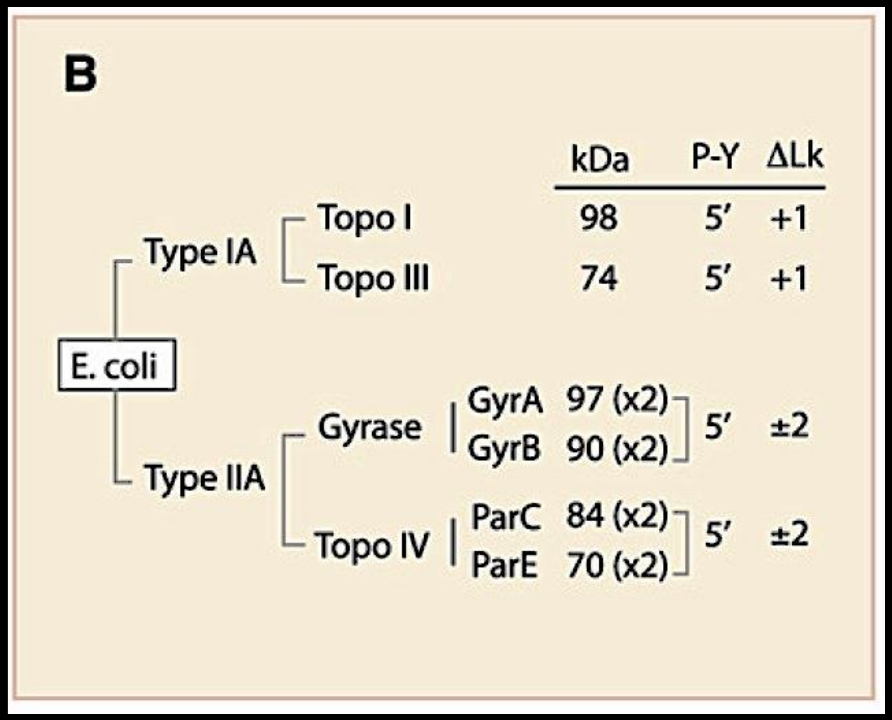
Top3 (Type IA)

6 genes:

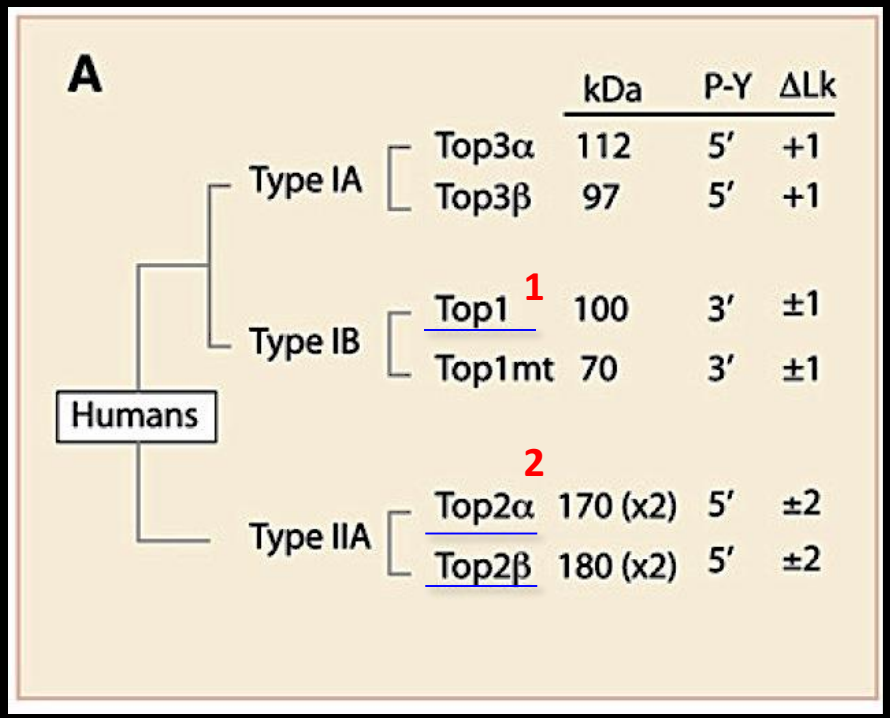
Top1	Top1mt	Top2α	Top2β	Top3α	Top3β
100 kDa	70 kDa	170x2 kDa	180x2 kDa	100 kDa	100 kDa

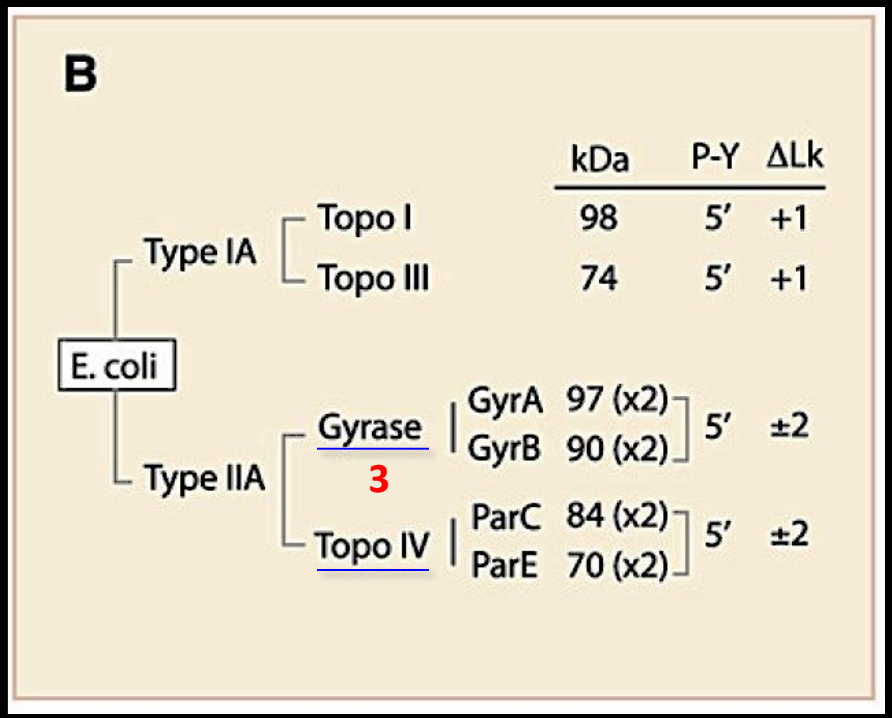
Humans have 3 types of topoisomerases and 6 TOP genes while Escherichia Coli has 2 types of topoisomerases and 6 genes





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



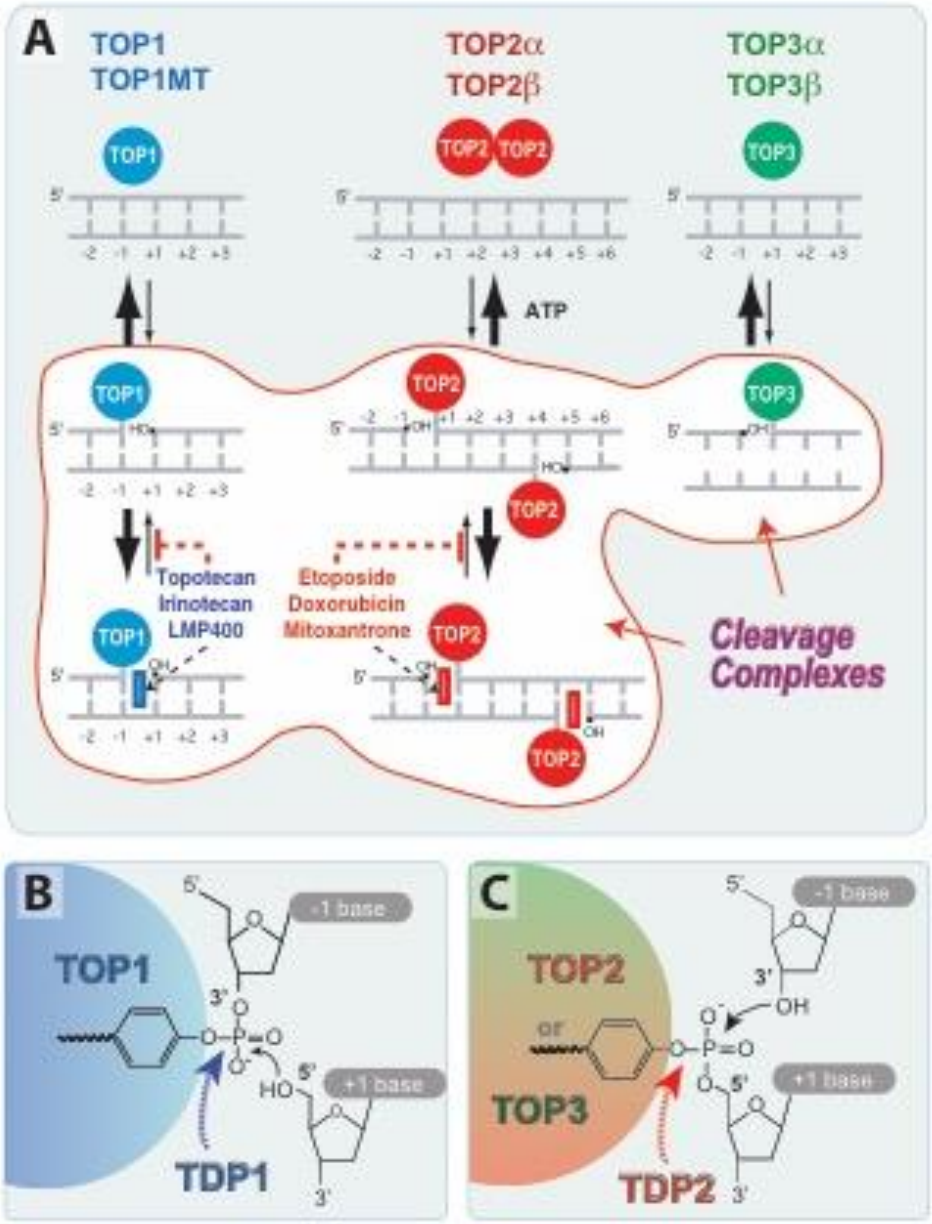


[1] Top1 is the anticancer target of camptothecins and indenoisoquinolines

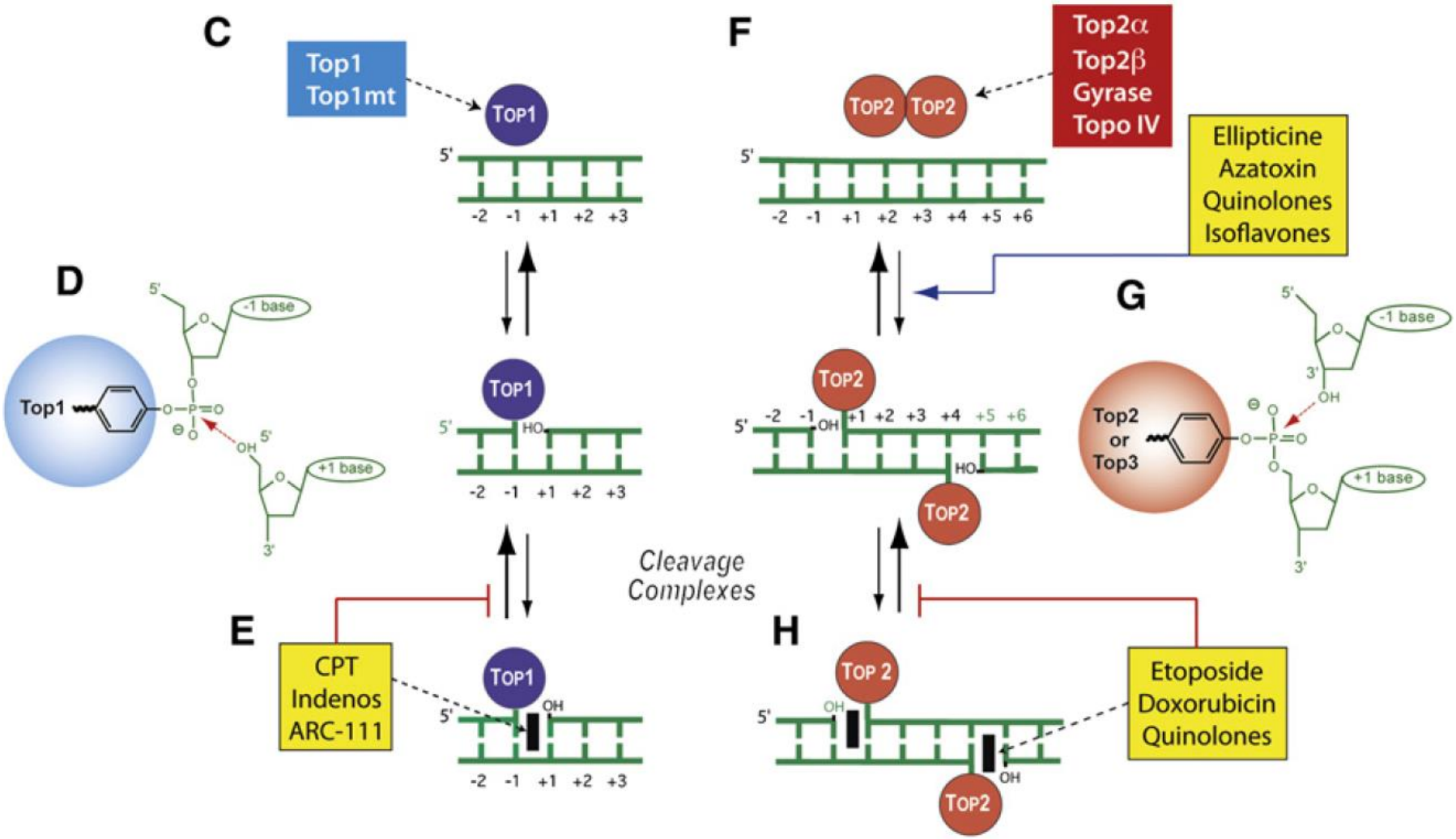
[2] Top2α and β are the anticancer targets of etoposide, doxorubicin, mitoxantrone…

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

The 3 families of human toposomerases



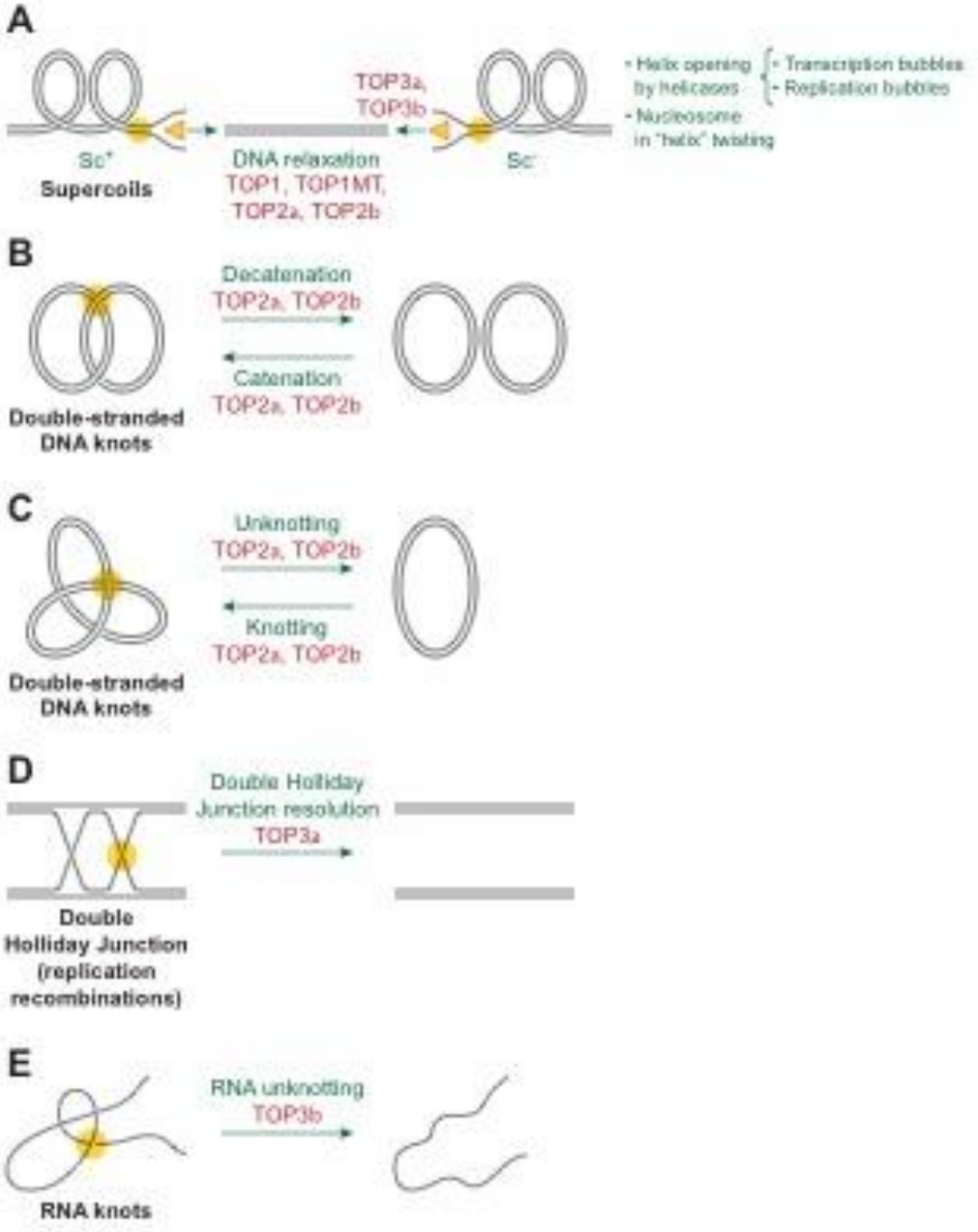
Biochemical differences between Top1 and Top2



- No ATP
- No divalent metal
- Still effective at 0°C
- Trapped by camptothecins, indenoisoquinolines

- hydrolyze ATP
- Mg2+ requirement
- Not effective at 0°C
- Trapped by etoposide, anthracyclines, quinolones

Topological Problems associated with nucleic acid metabolisms



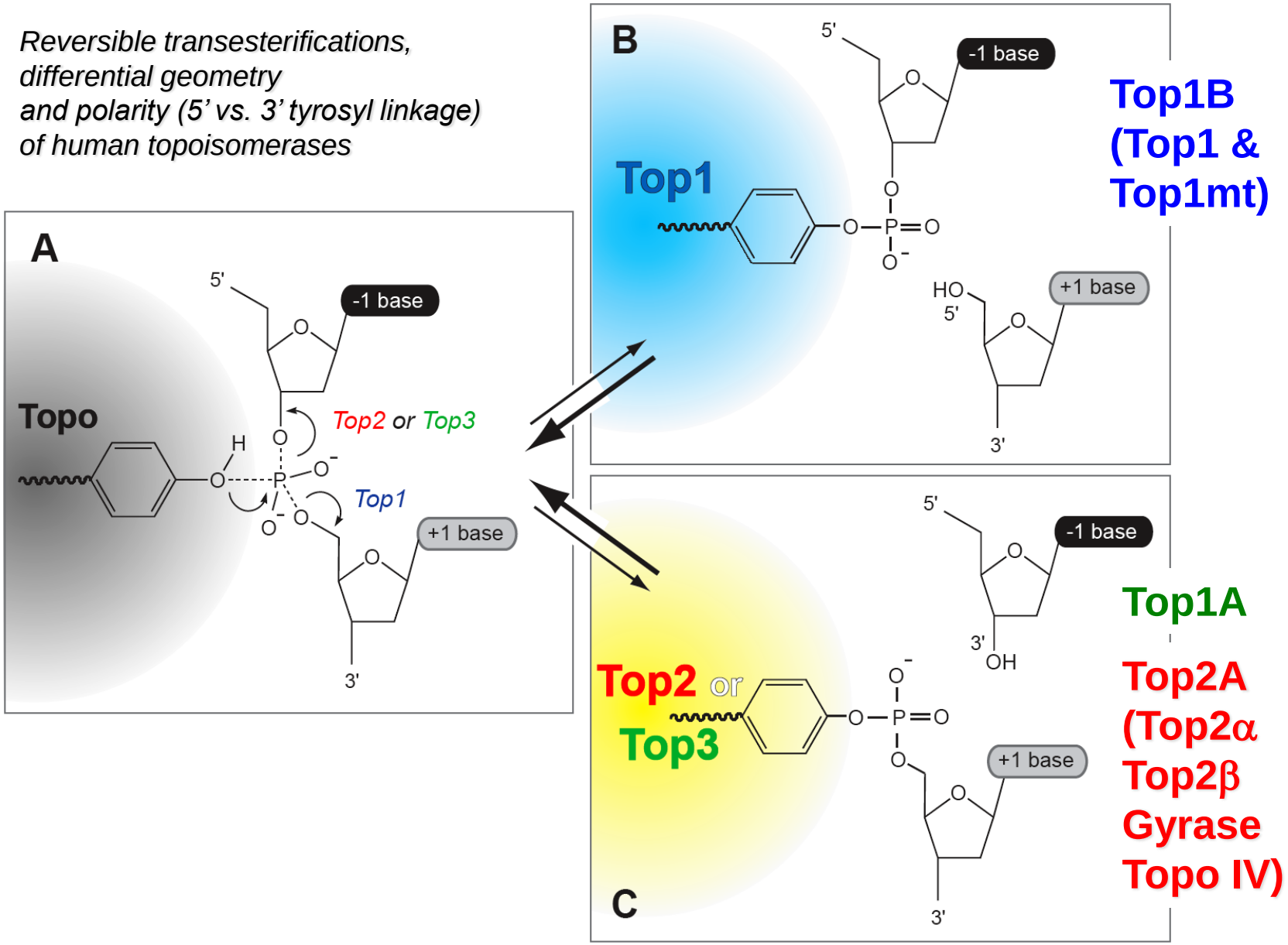
Comparison of the 6 human topoisomerases

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

Reversible transesterifications, differential geometry and polarity (5' vs. 3' tyrosyl linkage) of human topoisomerases



Top1B (Top1 & Top1mt)

Top1A

Top2A (Top2α Top2β Gyrase Topo IV)

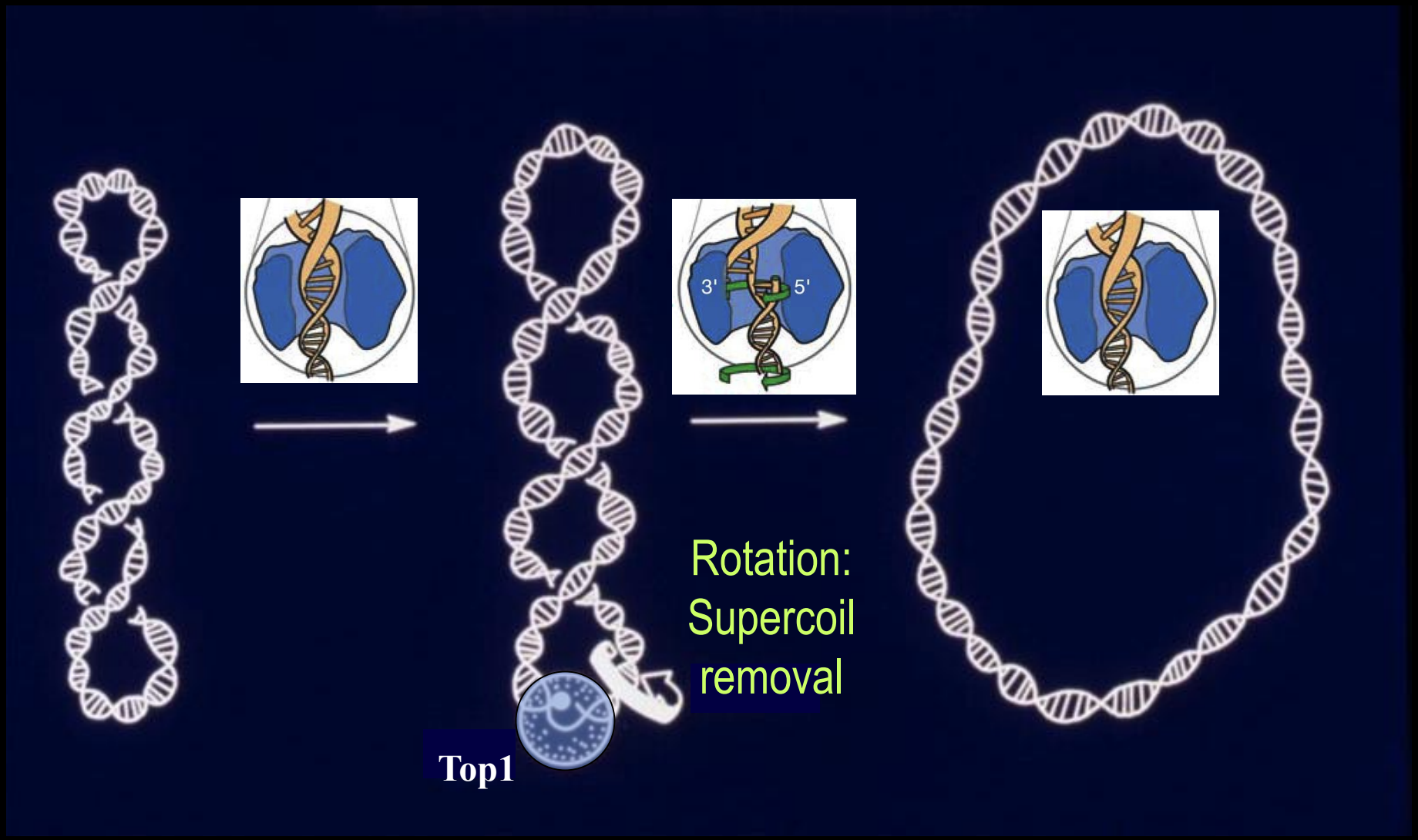
Pommier et al. ACS Chem Rev 2009
http://discover.nci.nih.gov/pommier/pommier.htm

Top1

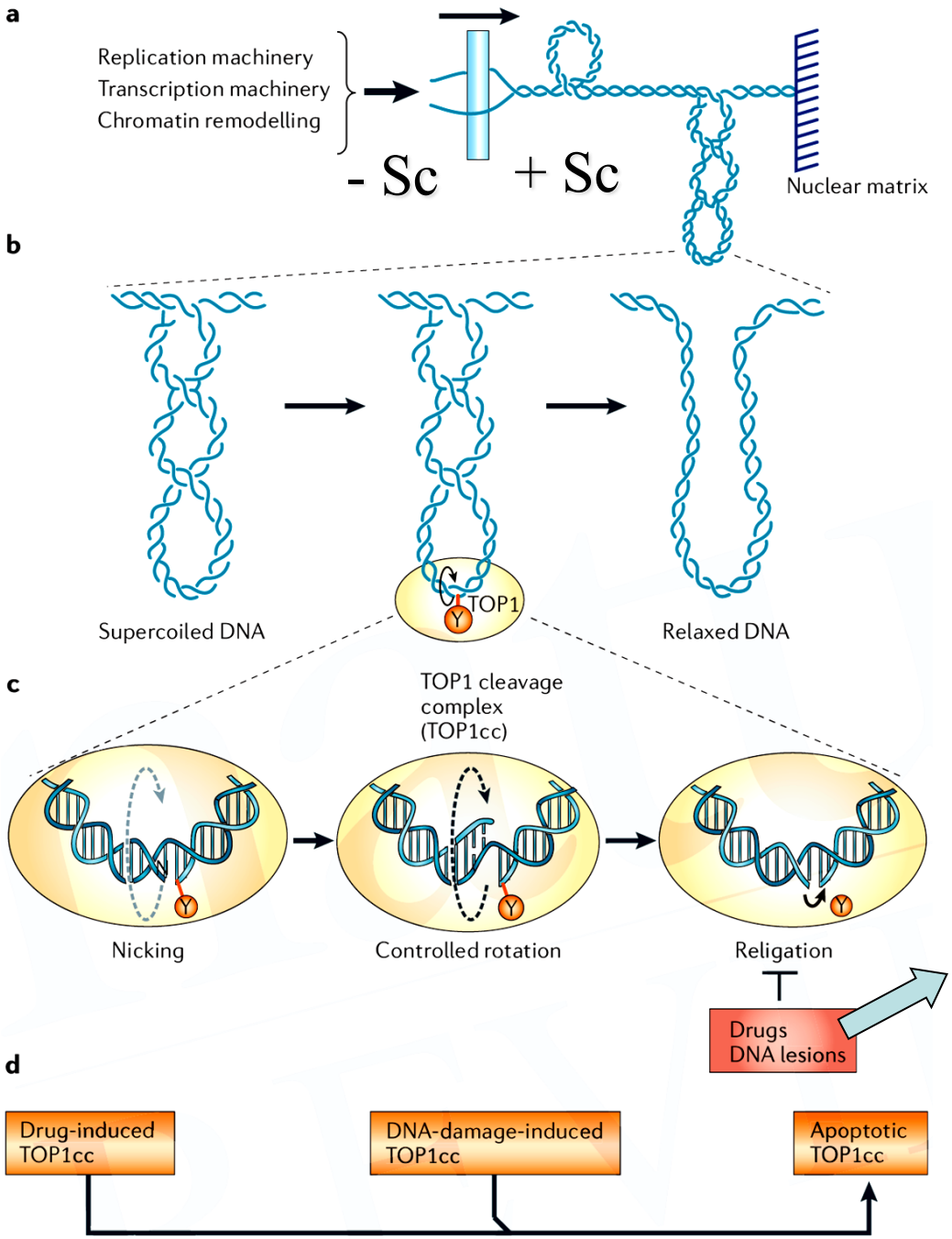
TOP1 (nuclear Top1)

TOP1MT (mitochondrial Top1)

Relaxation of DNA by Topoisomerase I (top1)



Top1 is essential for transcription and replication (repair?)



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

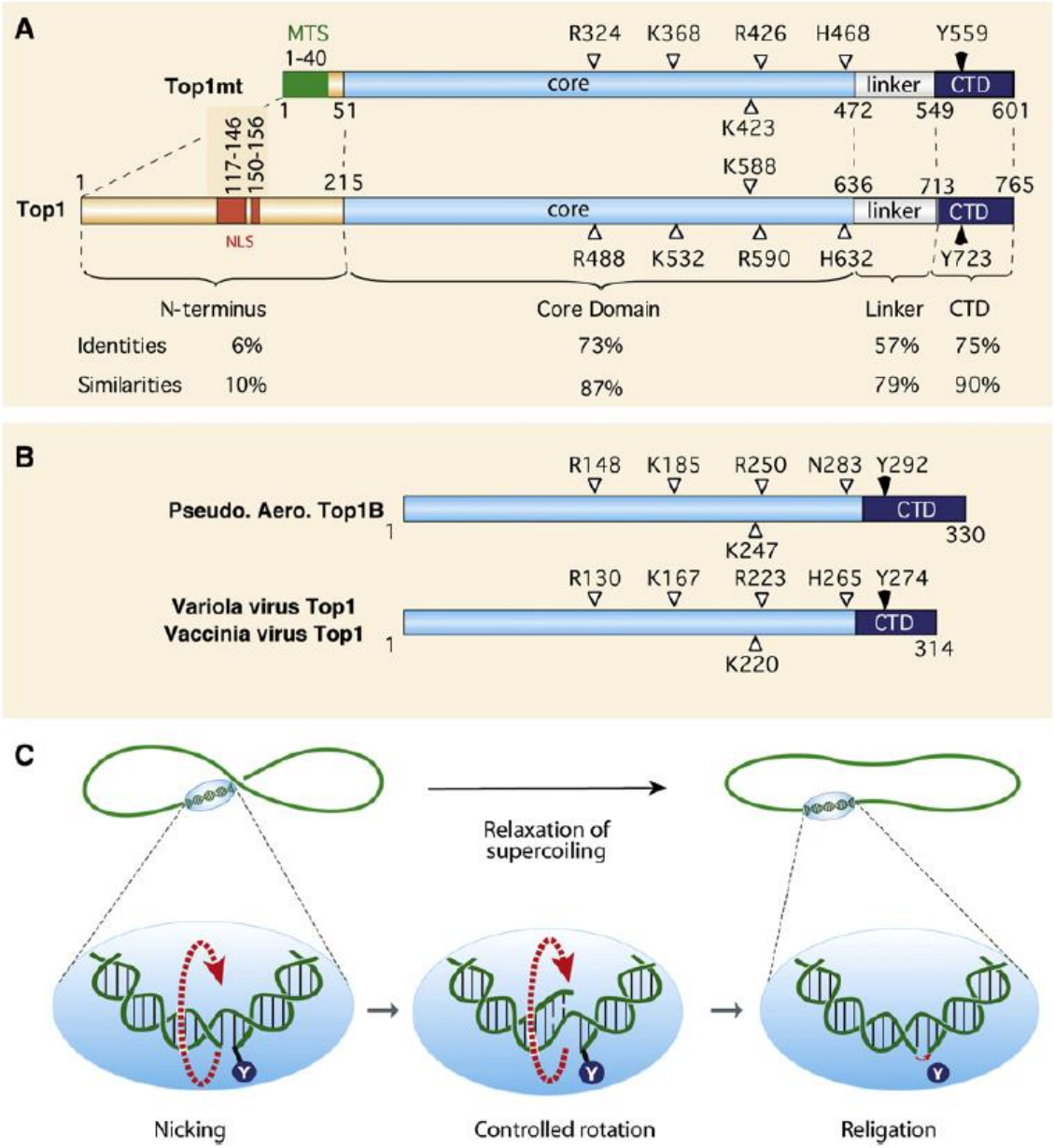
DNA supercoiling

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).

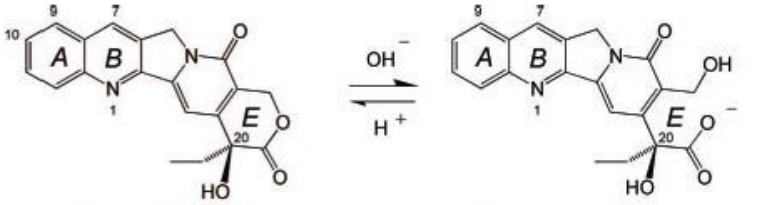
Here are some basic facts concerning DNA supercoiling that are relevant to topoisomerase activity:

- 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.

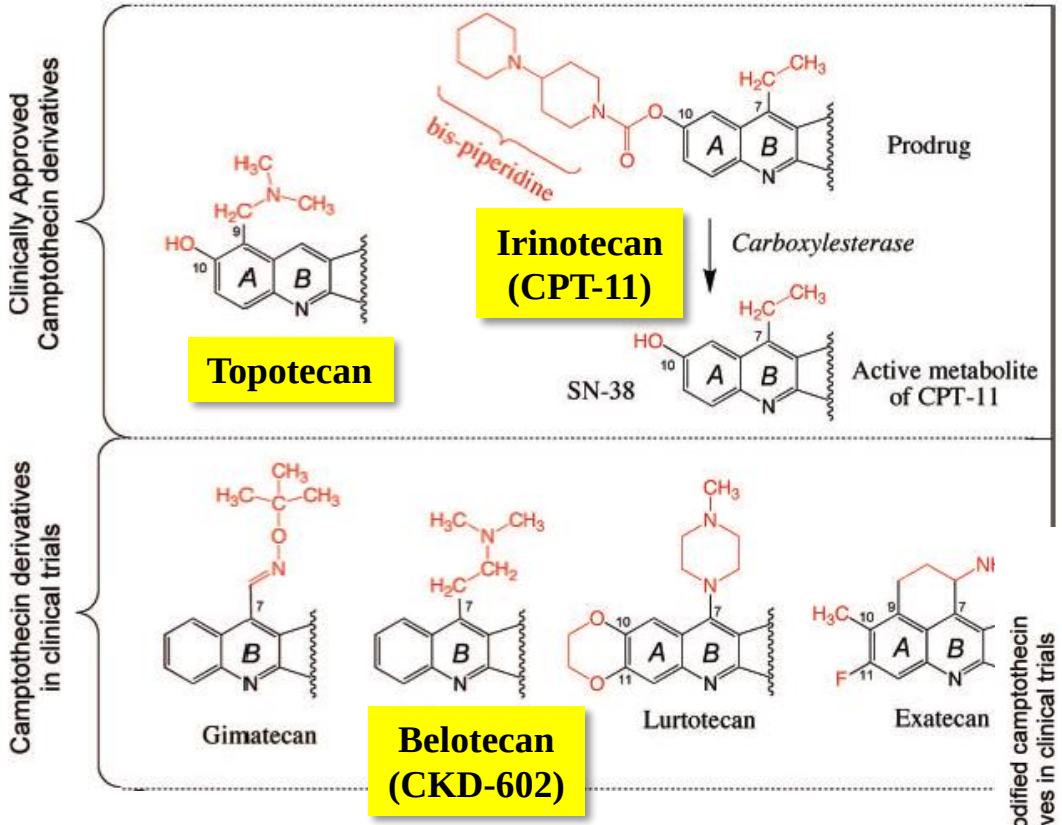
The two human Top1s

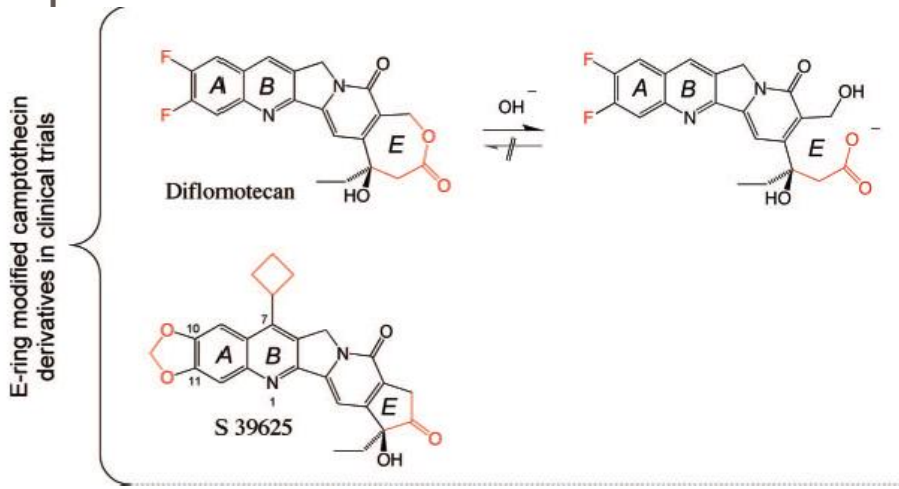


Camptothecin and its derivatives used for the treatment of cancers

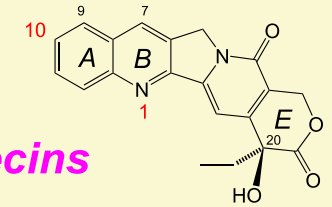


Camptothecin is an alkaloid from *Camptotheca acuminata Decne*, a rapidly growing tree from China. Discovered by Monroe Wall and Mansukh Wani who also discovered taxol.

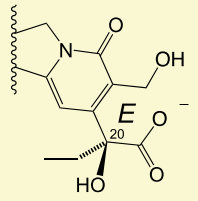




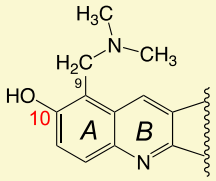
Camptothecins



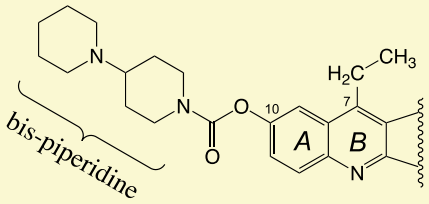
Camptothecin (lactone)
ACTIVE



Camptothecin (carboxylate)
INACTIVE

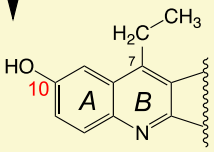


Topotecan

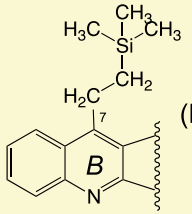


Irinotecan
(CPT-11)
Prodrug

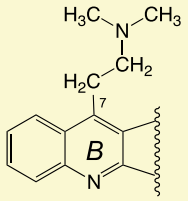
Carboxylesterase



SN-38
Active metabolite

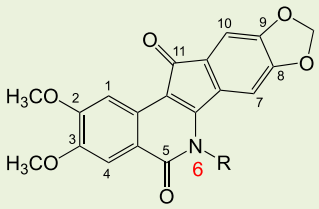


Cositecan
(Karenitecin)

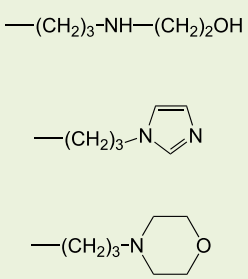


Belotecan
(CKD-602)

Indenoisoquinolines



R =

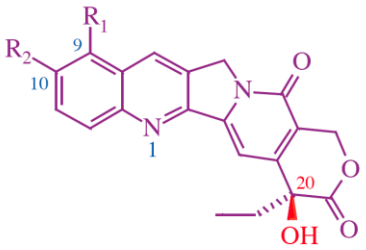


NSC 706744
MJ-III-65
= LMP-744

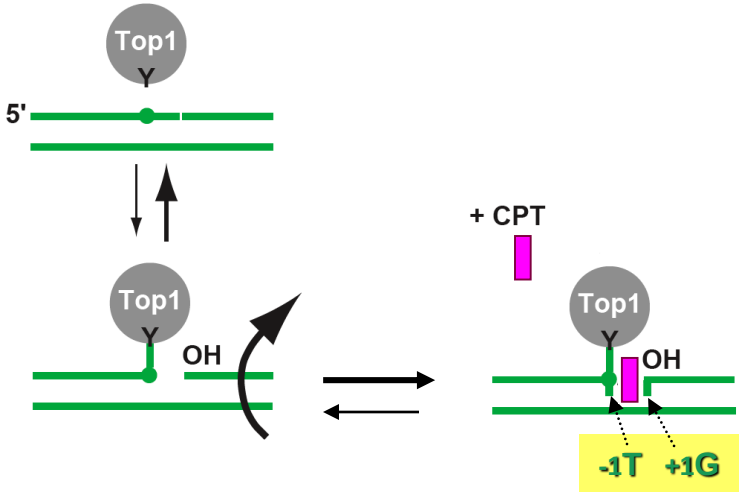
NSC 725776
= LMP-776
= Indimitecan

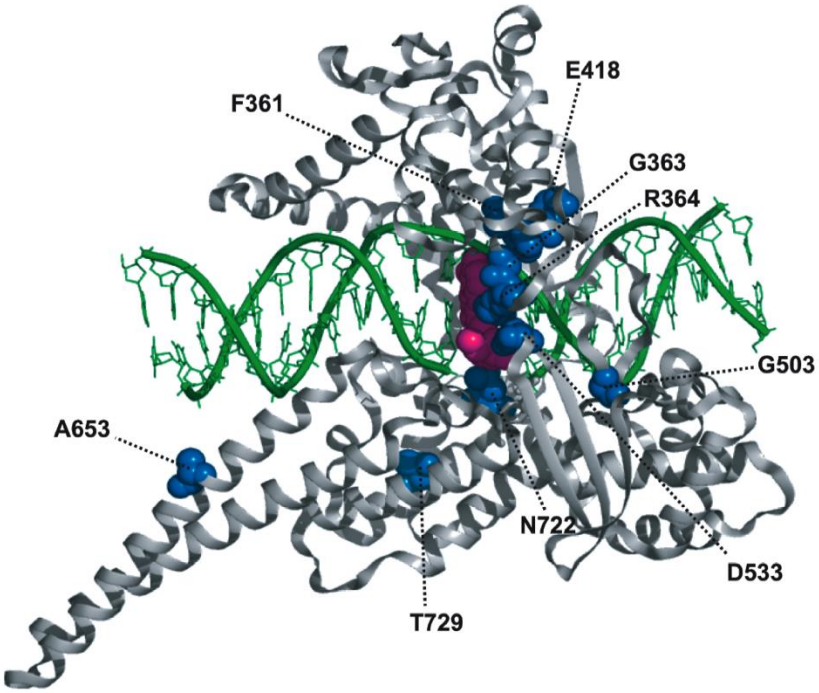
NSC 724998
= LMP-400
= Indotecan

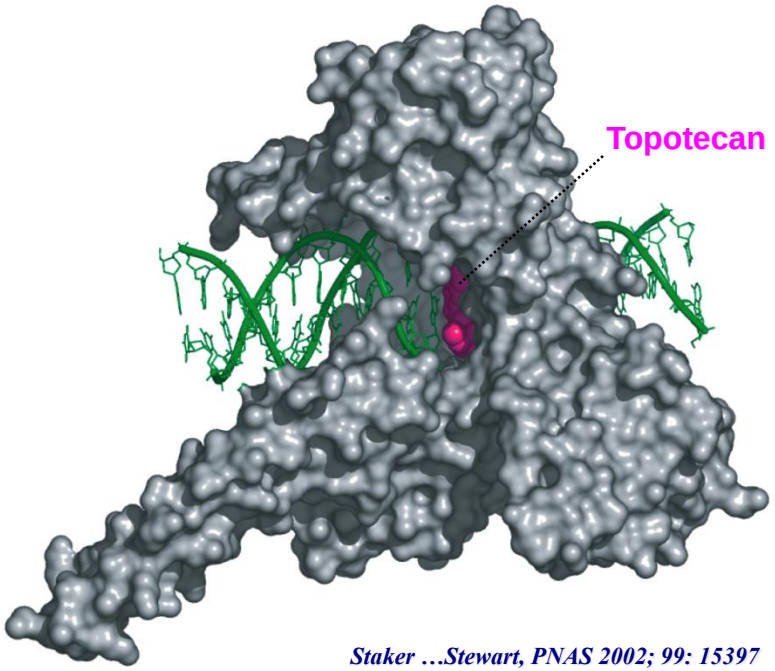
Camptothecins as one of Nature's Paradigms for Interfacial Inhibitors



	R_1	R_2
Camptothecin	-H	-H
Topotecan	$-CH_2-N(CH_3)_2$	-OH



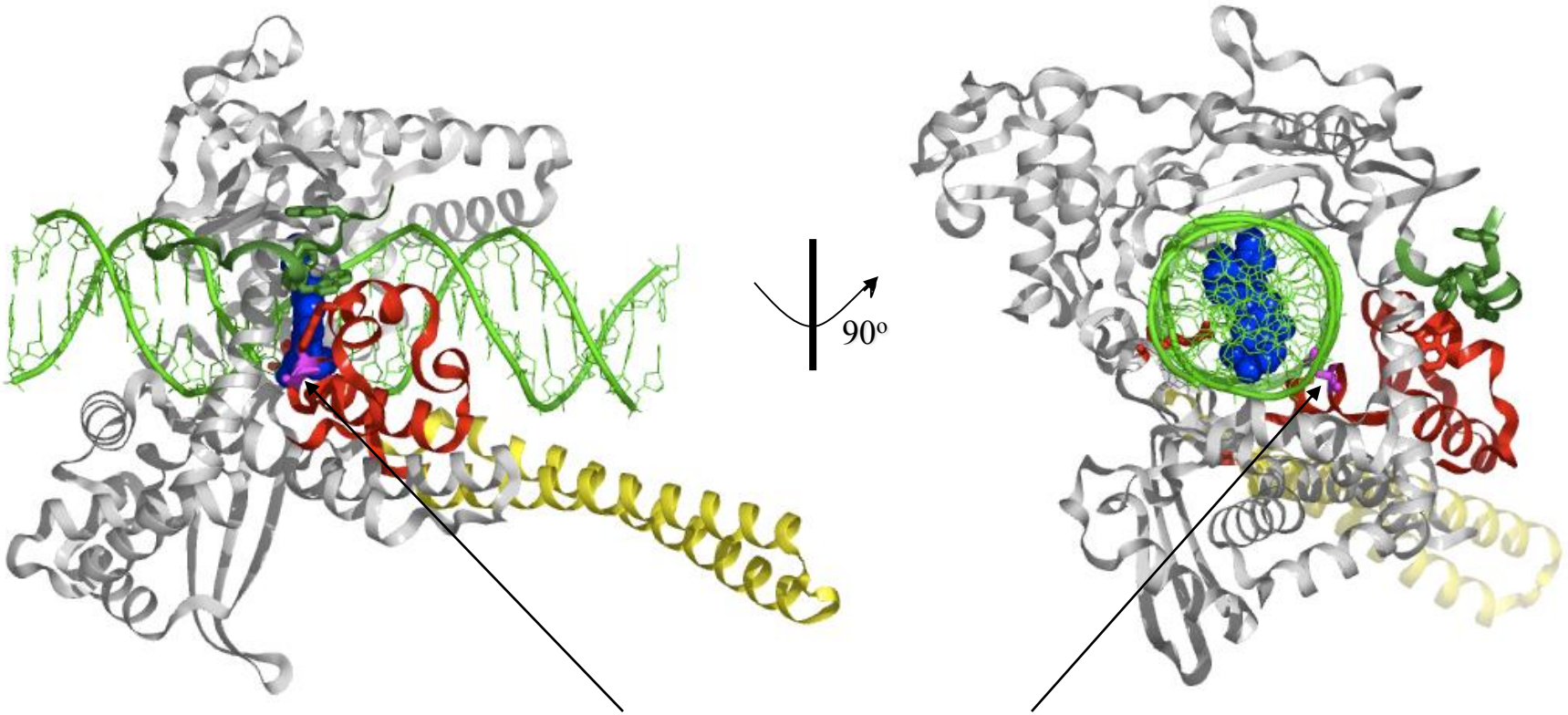




Staker …Stewart, PNAS 2002; 99: 15397

Top1 is the only cellular target for camptothecin
=> Camptothecins are highly Targeted Therapies

- Camptothecin-producing plants encode N722S mutation (Saito and coworkers, PNAS 2008)
- The N722S mutation was first found in human leukemia CEM cells selected for CPT resistance (Cancer Res 2001)



Why New Top1 Inhibitors?

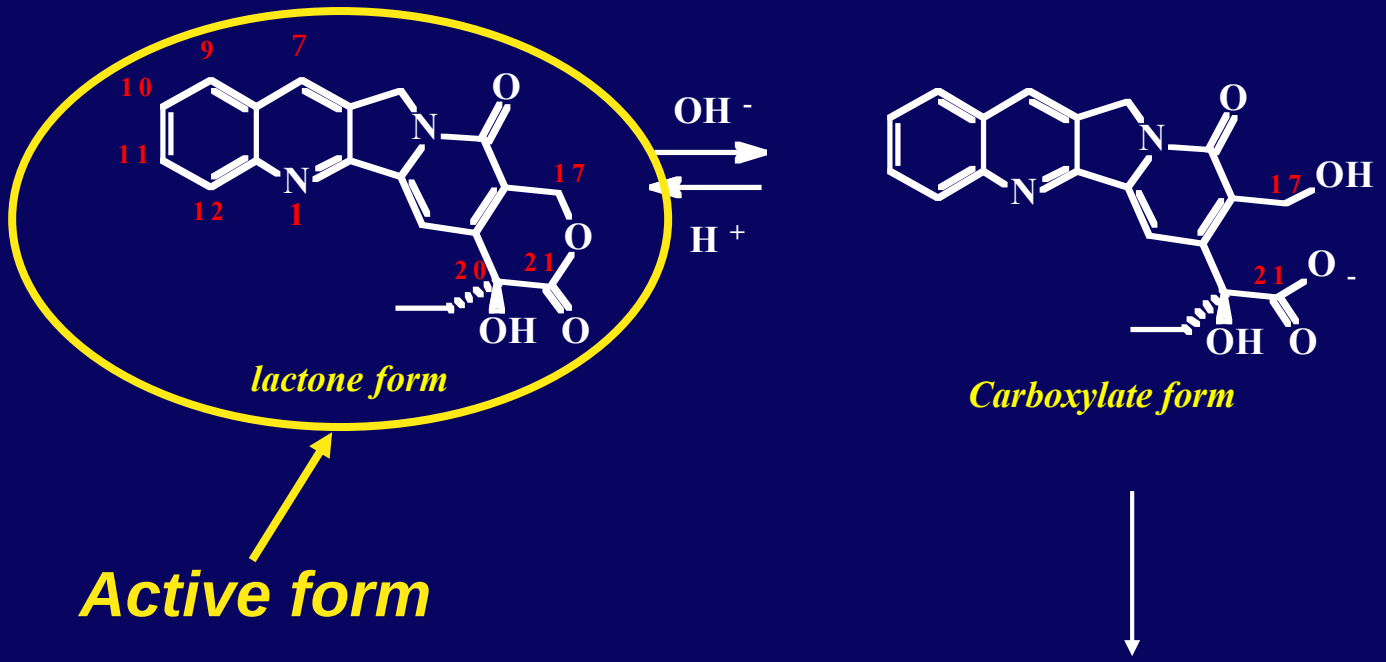
1. Because camptothecins are effective anticancer drugs.
   Hence, Top1 is a validated target for cancer treatment.

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:

1. Unstable at physiological pH

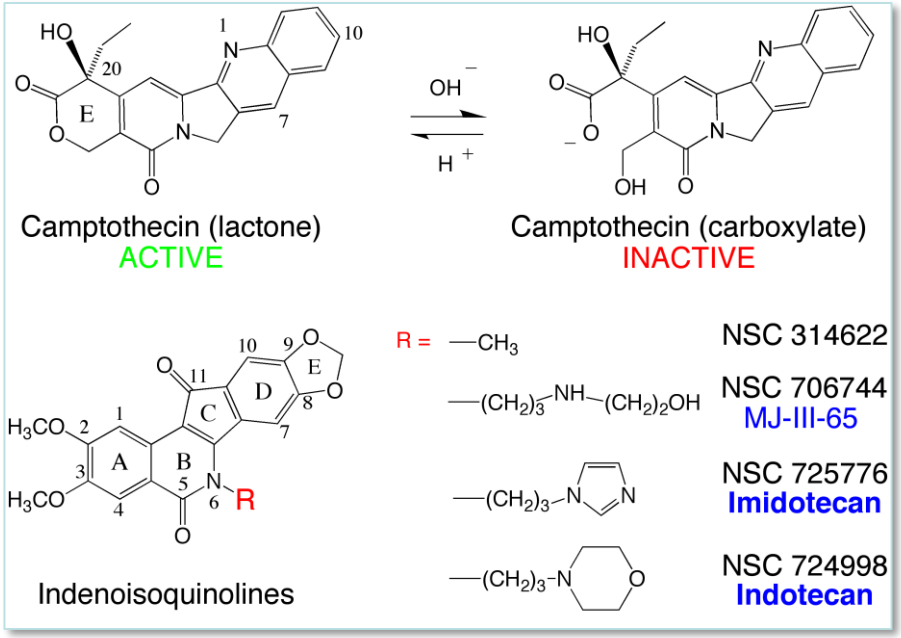


2. Camptothecins bind reversibly to the top1 cleavage complexes. Hence cleavage complexes reverse rapidly after drug removal => prolonged infusions

Clinical development of the indenoisoquinolines

Two compounds selected LMP-776 and LMP-400:

- **Potent and specific Top1cc-targeted drugs**
- **Overcoming limitations of camptothecins:**
  - **<u>Chemical stability</u> (no alpha-hydroxylactone)**
  - **Overcome resistance <u>drug efflux pumps</u> (collaboration with S. Bates & M. Gottesman)**
- **Trap Top1cc at different genomic sites compared to camptothecins => target ≠ regions of the genome.**



Second Generation Camptothecins with Targeted Delivery

Name	Company	Active Derivative (Payload)	Formulation (Conjugate; Target)
Onivyde™ = MM398*	Merrimack	Irinotecan	Liposome
CRLX101	Cerulean Pharma Inc.	Camptothecin	PEG
NKTR-102	Nektar Therapeutics	Etirinotecan (20-position)	PEG (Pegol)
IMMU-132	Immunomedics	SN-38 (20-position)	CDA - TROP2 (TACSD2)
STA-12-8666	Synta Pharmaceuticals	SN-38 (10-position)	HDC – Hsp90

* *FDA approved October 2015*

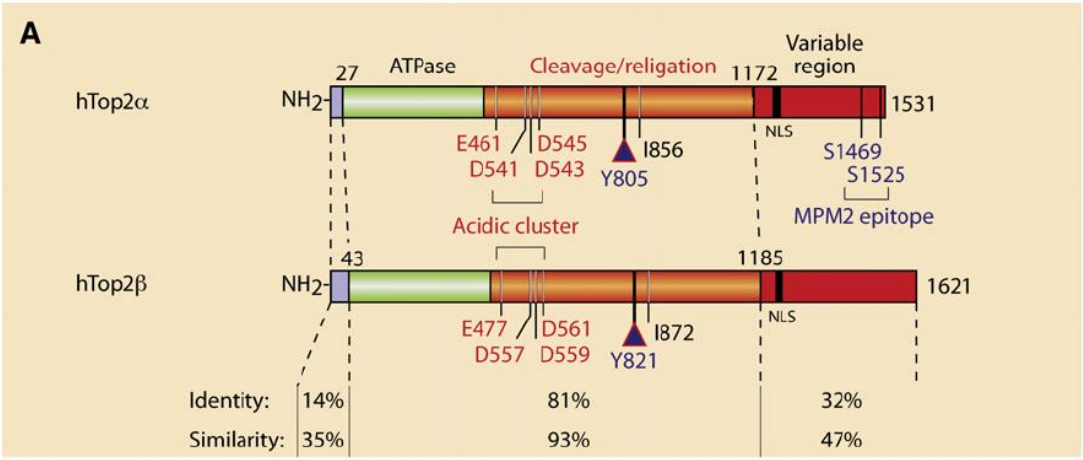
Top2

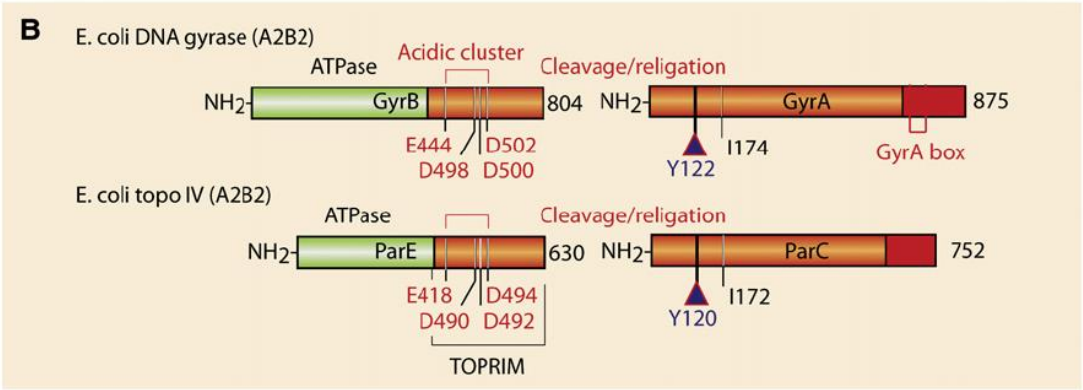
Top2α – TOP2A: Replication

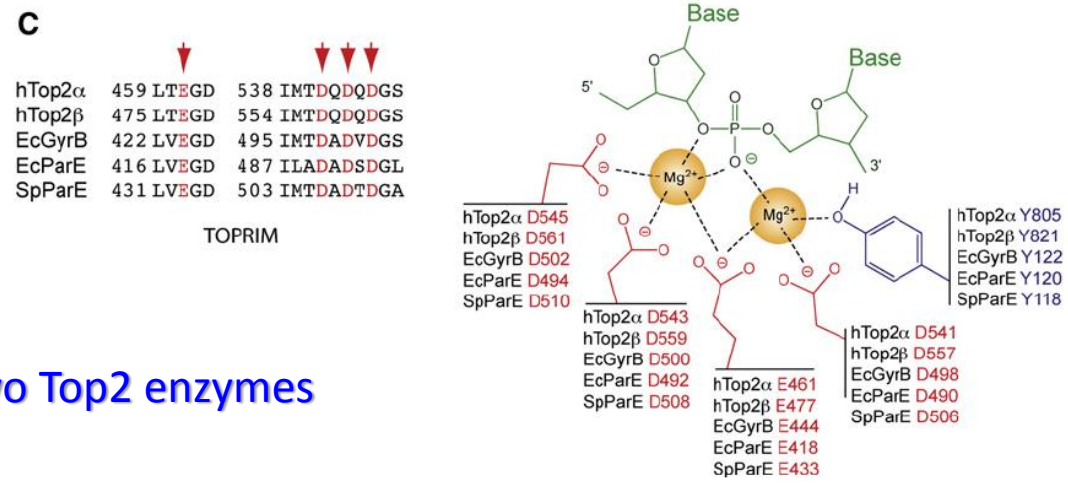
Highly expressed in replicating and cancer cells

Top2β – TOP2B: Transcription

Expressed both in replicating and differentiated cells

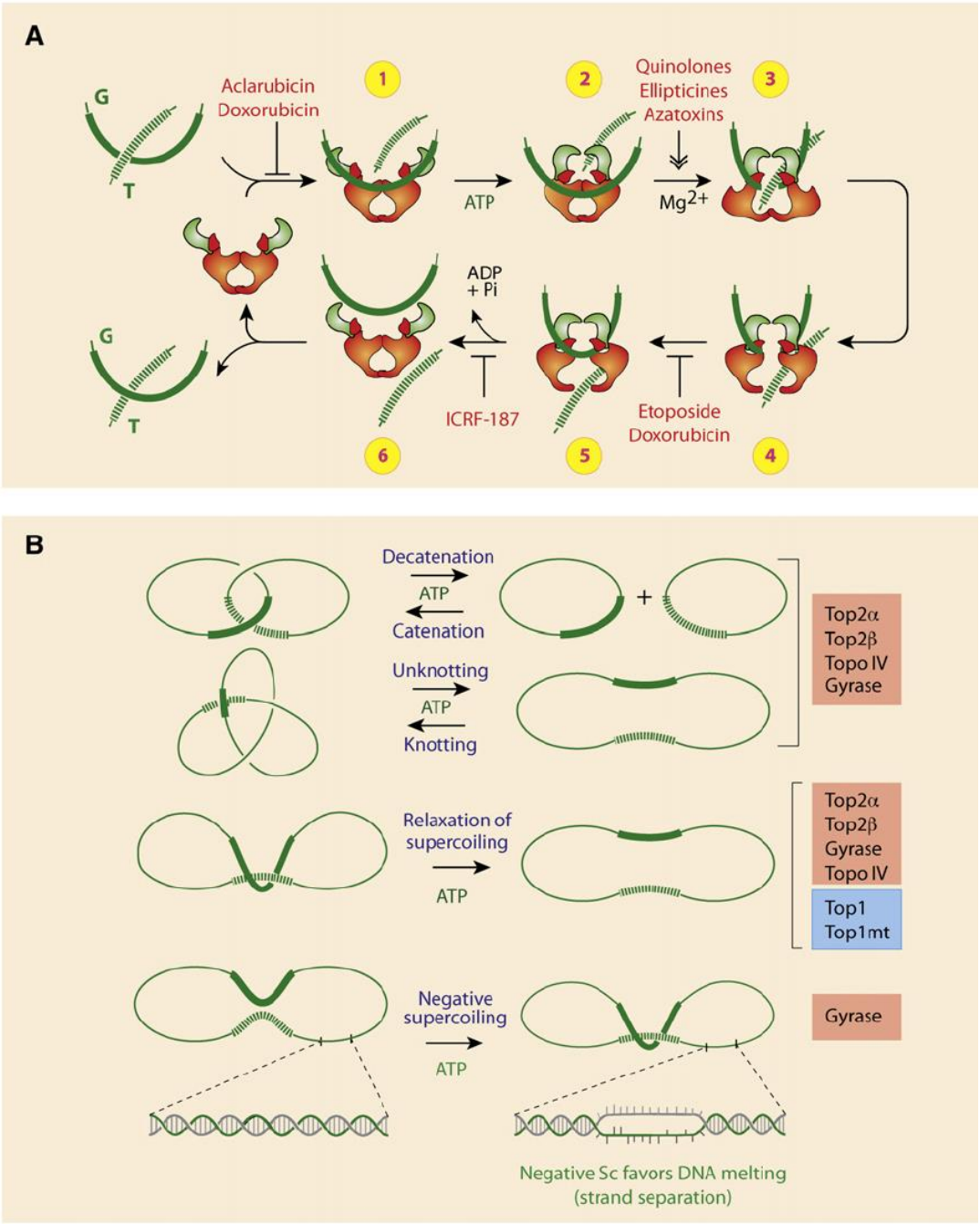






Humans have two Top2 enzymes

Top2 catalyze a broad range of reactions



Pommier, Y., Sun, Y., Huang, S. & Nitiss, J.L.
2016 Nature Rev Mol Cell Biol

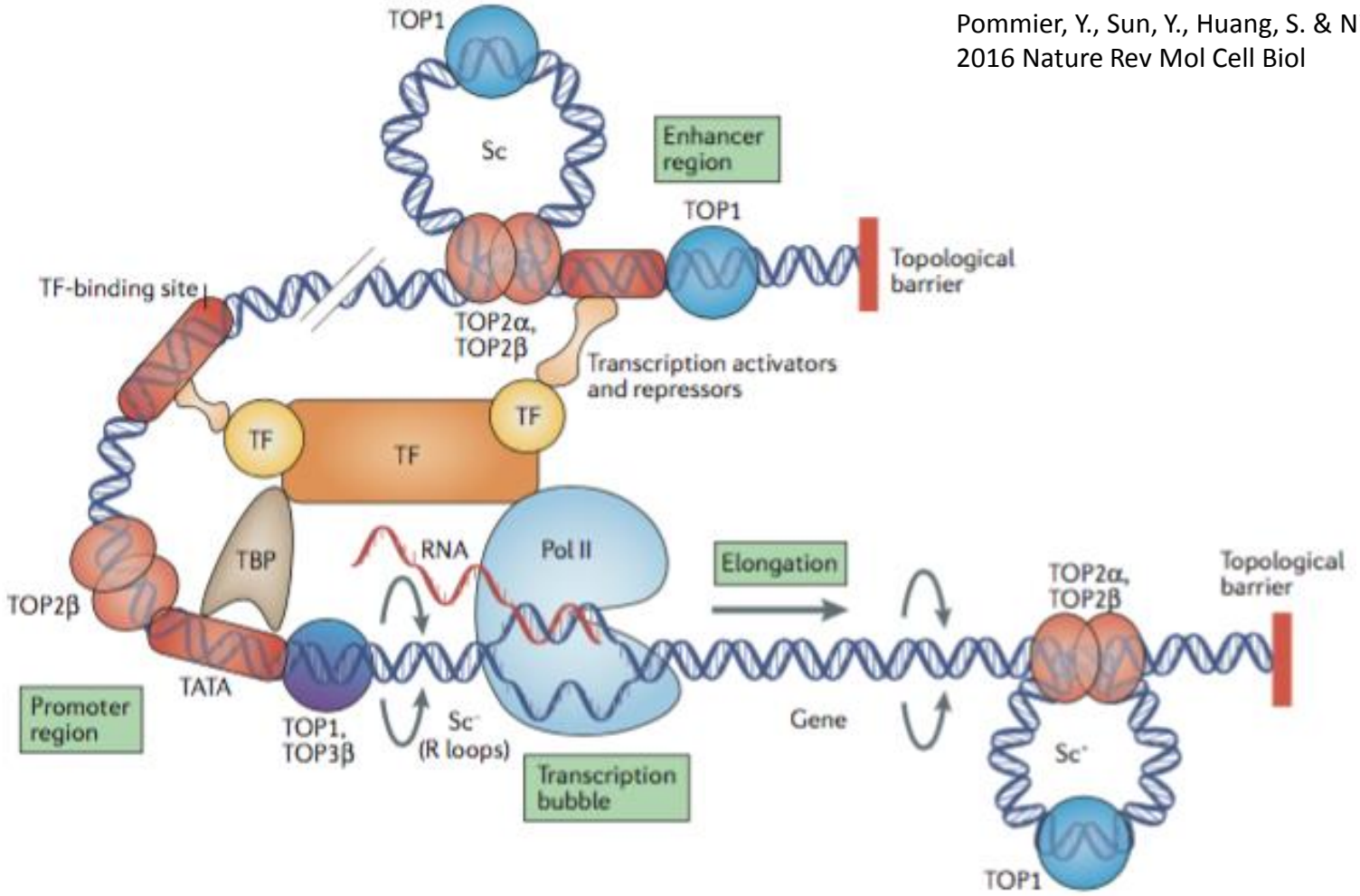
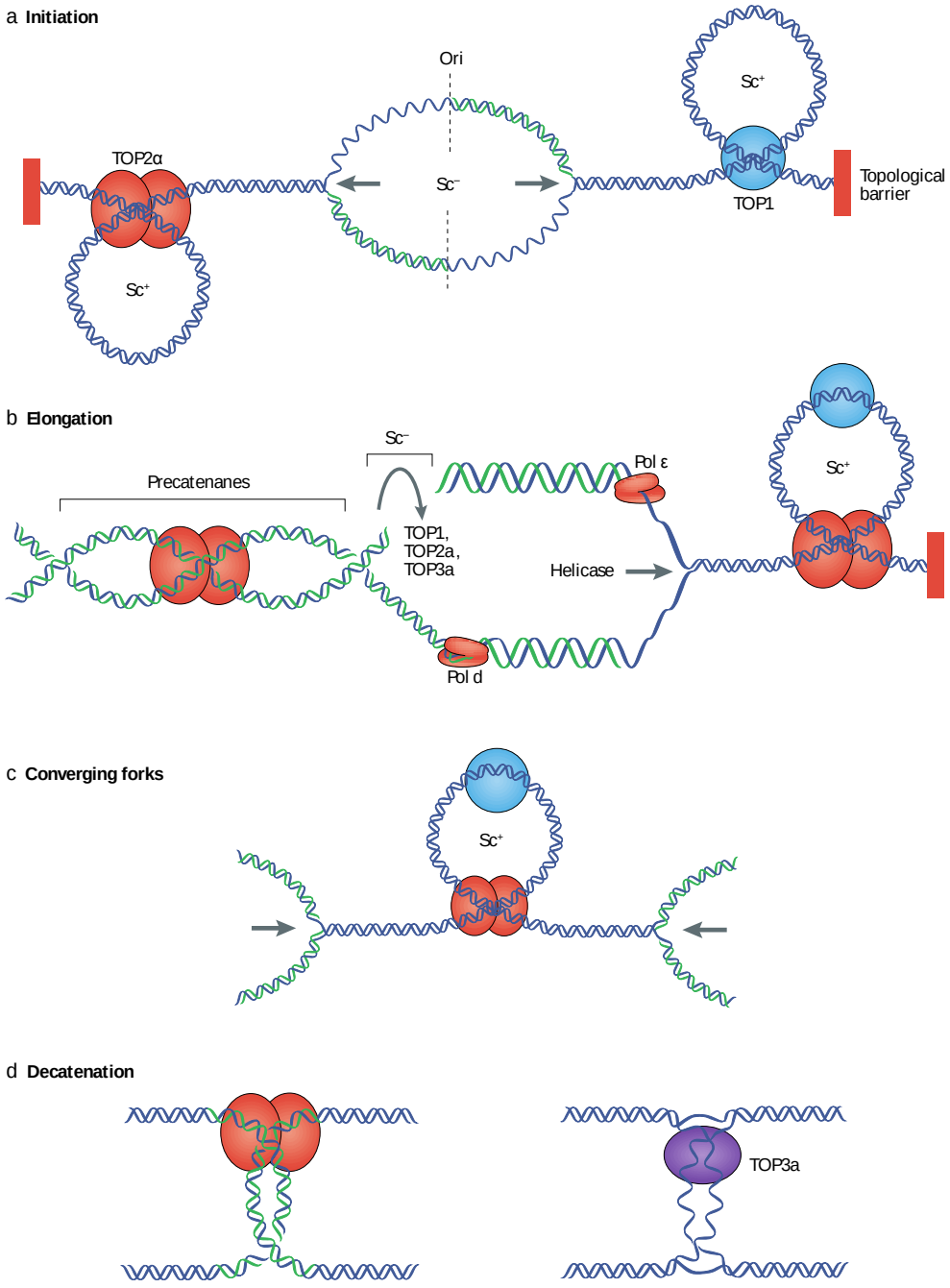


Figure 2 | **Topoisomerases and transcription.** Transcription incurs topological constraints that result from the progression of RNA polymerase II (Pol II). Positive supercoiling (Sc^+) of the DNA template takes place ahead of the transcription bubble, which in turn obstructs further Pol II movement, and negative supercoiling (Sc^-), which promotes the formation of RNA–DNA hybrids (R loops), accumulates behind it. TOP2 and especially TOP1 enzymes function ahead of Pol II to remove positive supercoils, whereas relaxation of negative supercoils behind the transcription apparatus relies on TOP1 and TOP3β. In addition, TOP1 regulates the activity of the transcription factor TATA-box-binding protein (TBP) at promoter TATA boxes independently of its catalytic activity. The formation of TOP2β-mediated transient DNA double-stranded breaks at promoter regions in certain genes is crucial for transcription activation. TOP1 is also recruited to certain enhancer regions to promote (ligand-dependent) enhancer activation by generating transient DNA single-stranded breaks. Topological barriers are genomic regions where the DNA is not free to rotate around its axis and require TOP1 and TOP2 to relax supercoils (Sc). TF, transcription factor.



Functions of topoisomerases in DNA replication. **a.** Initiation of DNA replication requires separation of the two parental strands, which generates negative supercoiling (Sc-) at the origin of replication and positive supercoiling in the flanking regions due to topological barriers, such as nuclear matrix attachment sites or insulators. Positive supercoiling is dissipated by TOP1 and TOP2α to allow replication fork progression (arrows). **b.** Replication elongation generates positive supercoiling ahead of the replication fork and negative supercoiling behind it. Positive supercoiling is removed by TOP1 and TOP2a, whereas negative supercoiling can be removed by TOP1, TOP2α or TOP3α. TOP2α can also remove precatenanes, which are formed when the fork rotates during elongation. **c.** Converging forks generate high positive supercoiling between them. **d.** Upon replication completion, catenanes are removed by TOP2α (left) and hemicatenanes by TOP3α (right). Topological barriers are genomic regions where the DNA is not free to rotate around its axis, for example owing to hindrance by macromolecular complexes.

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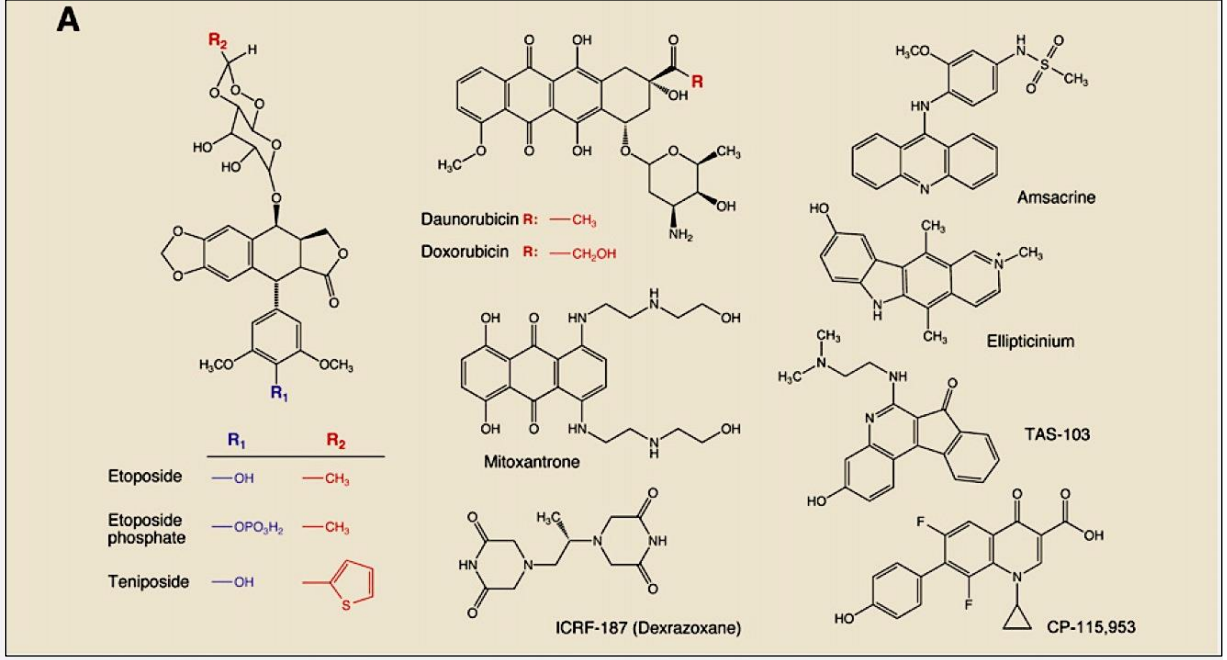
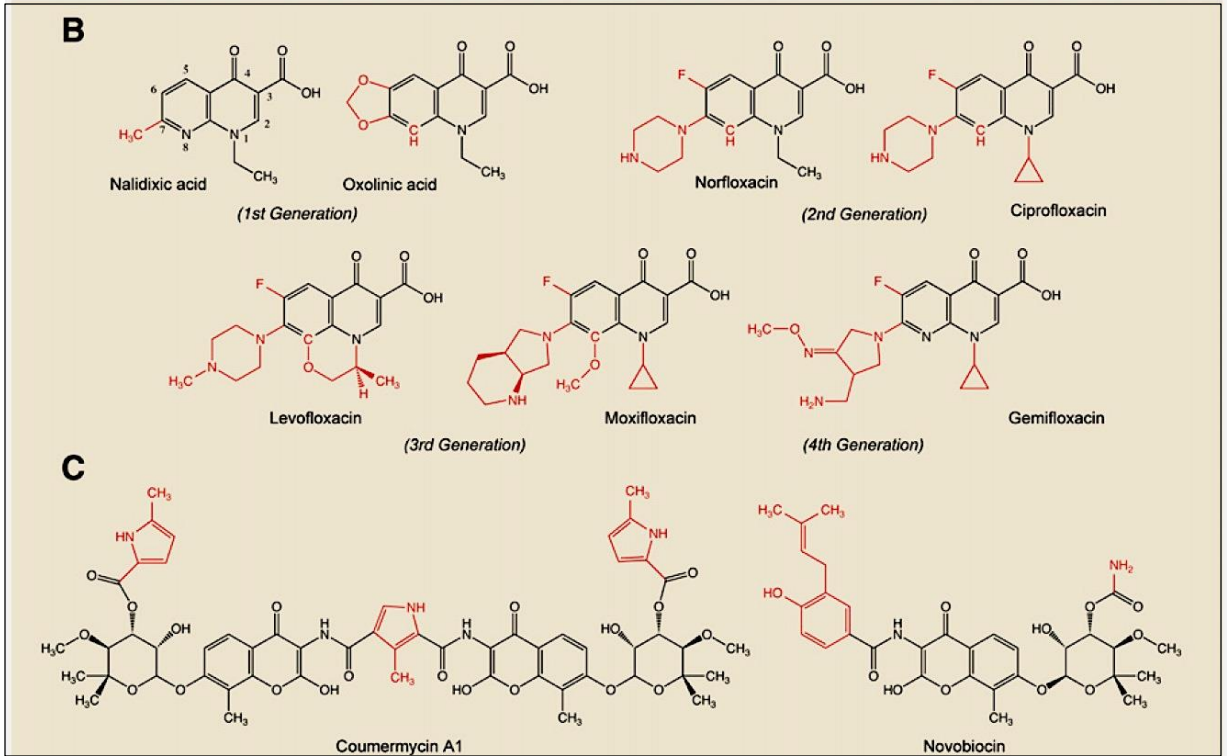
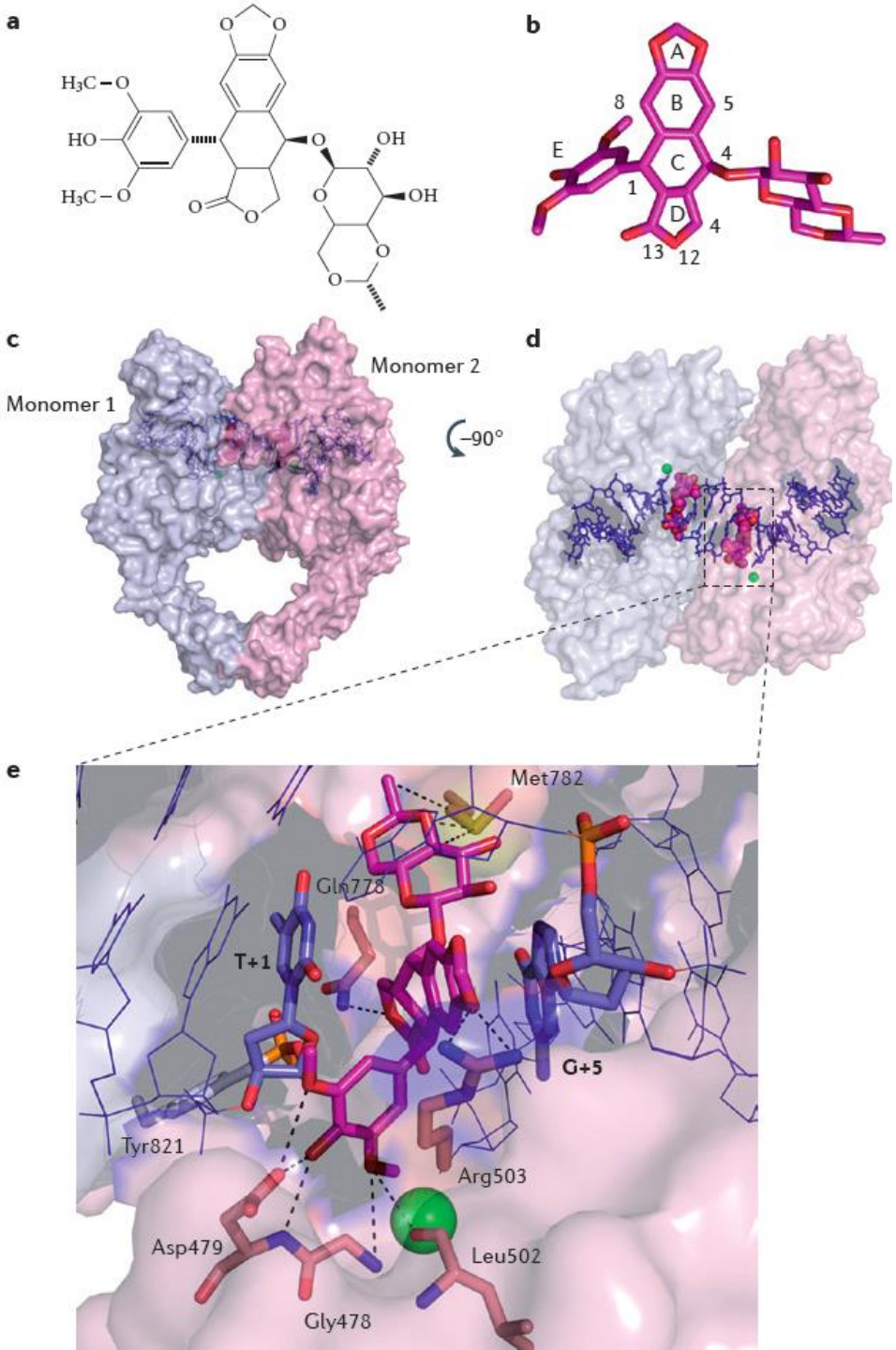
Anticancer
Top2-targeted drugs



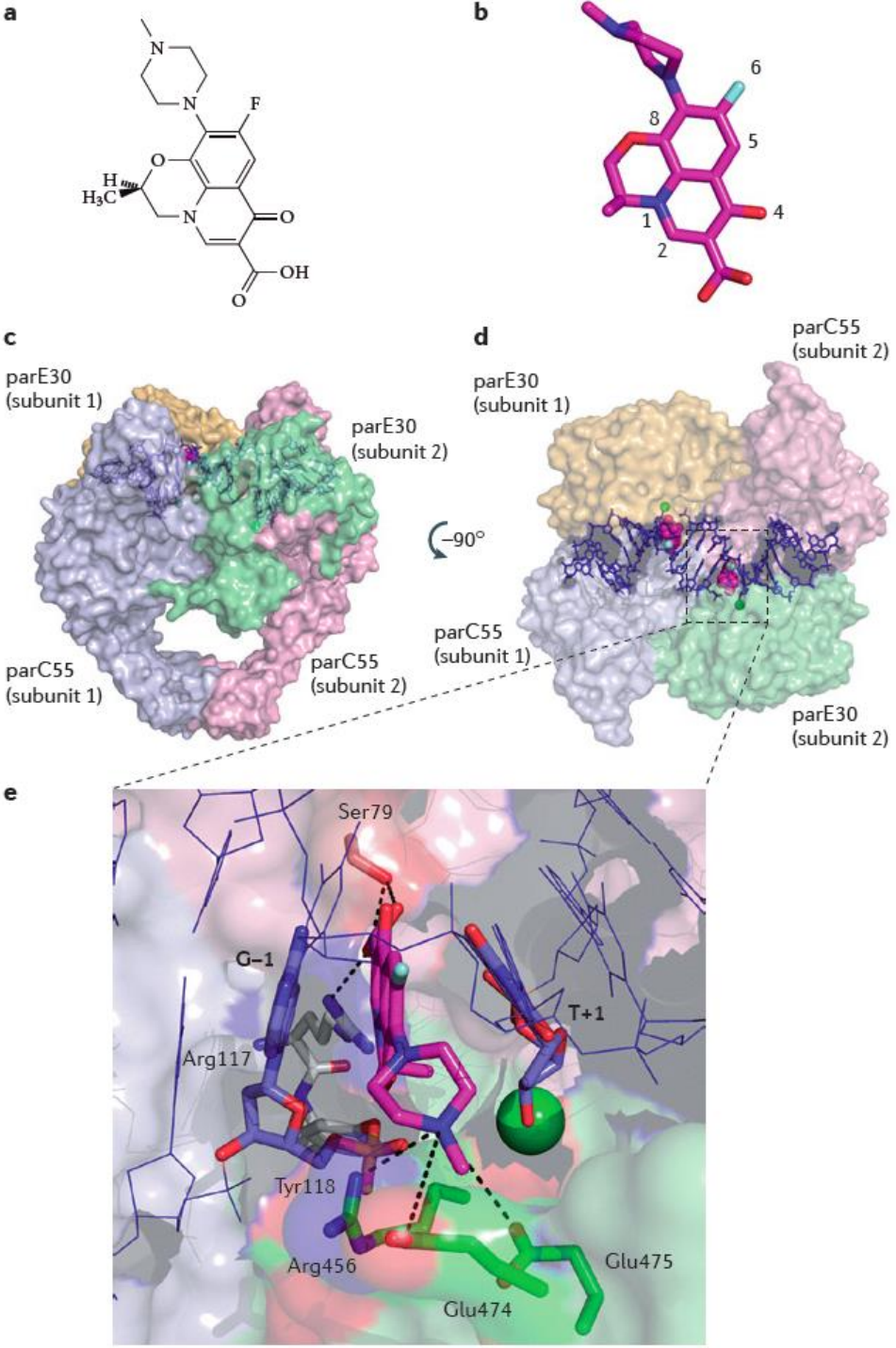
Antibiotics
Top2-targeted drugs





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

Antibacterials



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

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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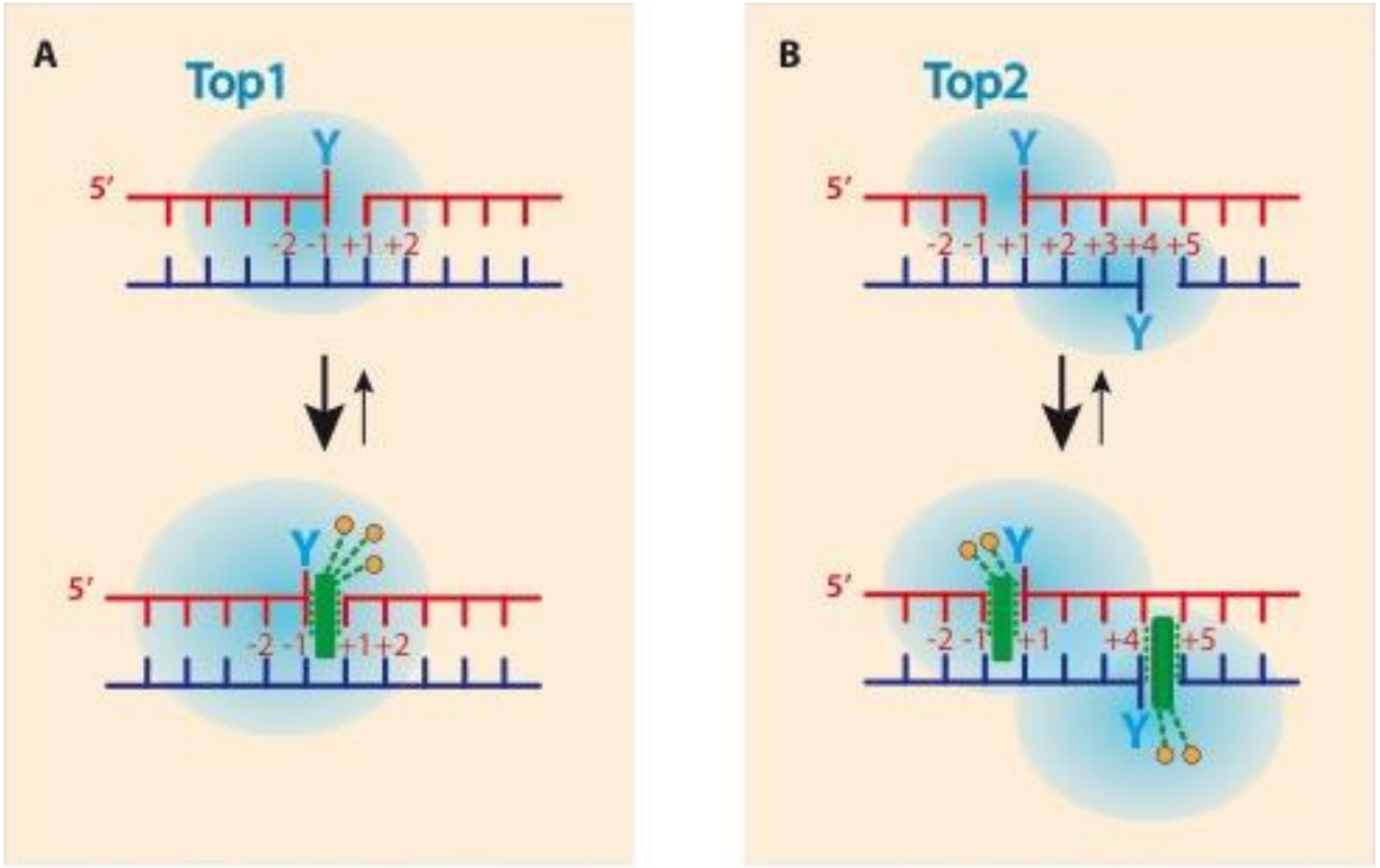
Interfacial inhibitors: targeting macromolecular complexes

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.



Camptothecins
Indenoisoquinolines

Etoposide
Doxorubicin

Top3

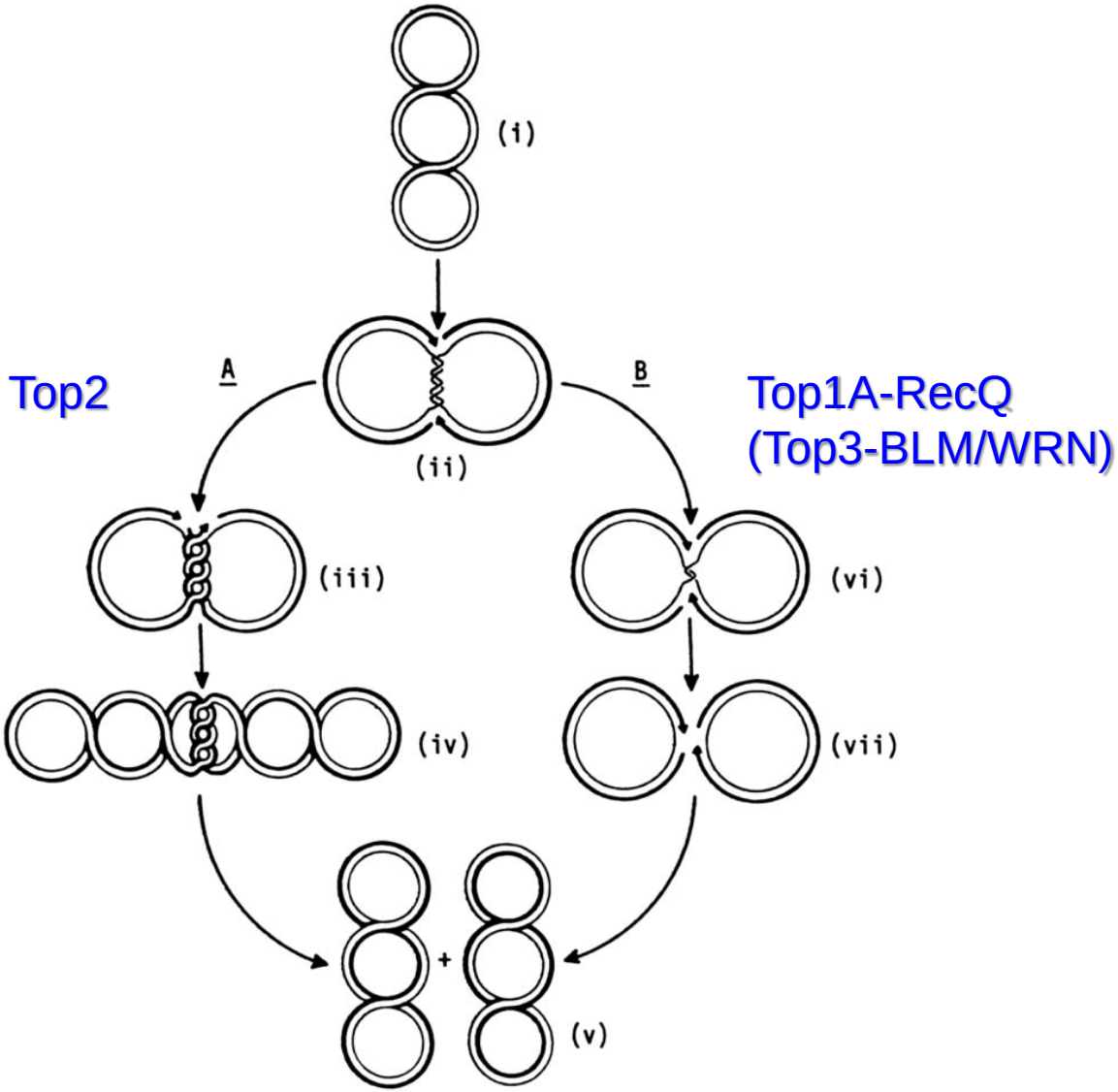
Top3α – TOP3A: Replication

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

Top3β – TOP3B: Transcription

DNA topoisomerase (R-loops);
RNA topoisomerase

Decatenation Top2 vs. Top3



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.

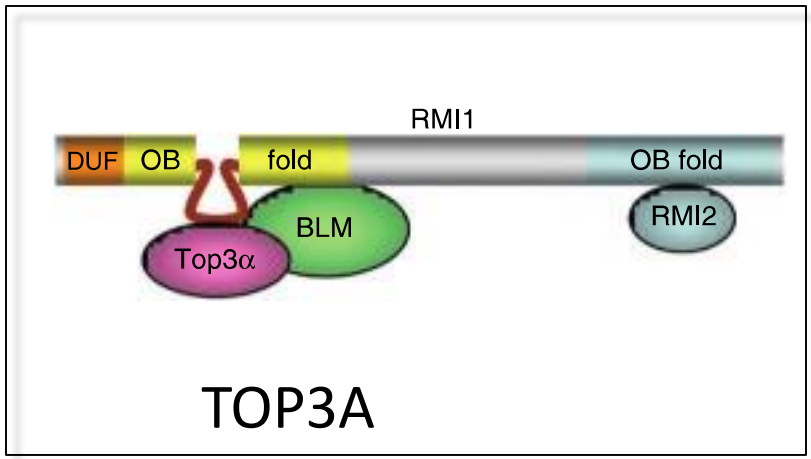
Top3β is an RNA topoisomerase that works with fragile X syndrome protein to promote synapse formation

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]

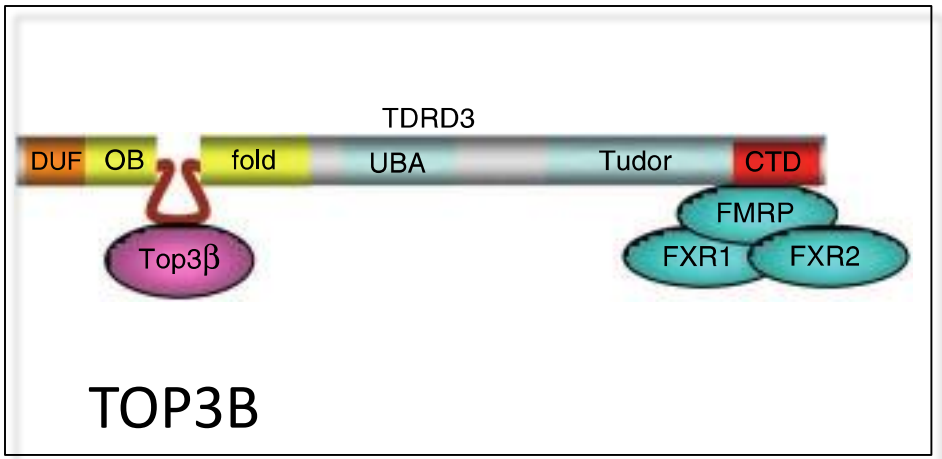
Topoisomerases are crucial for solving DNA topological problems, but they have not been linked to RNA metabolism. Here we show that human topoisomerase 3β (Top3β) is an RNA topoisomerase that biochemically and genetically interacts with FMRP, a protein that is deficient in fragile X syndrome and is known 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.

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

Replication



Transcription



Topoisomerases
Genomic Integrity
and
Human diseases

Topoisomerase-induced DNA damage

A

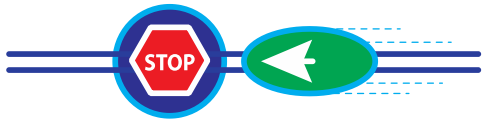
- **Conversion of TOP1cc into DSB by replication "run-off"**
  => TOP1 needs to be removed by TDP1
  and/or 3'-flap endonucleases (XPF-ERCC1)
  => DSB repaired by homologous recombination
- **TOP1cc also form DSB when on opposite strands or opposite to a preexisting single-strand break**

B

Top2cc proteolysis or mechanical disjoining

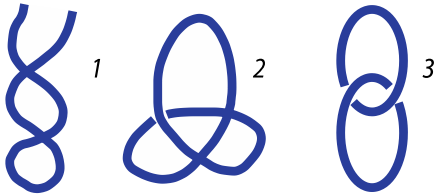
- **TOP2cc readily form DSB when concerted cleavage on both strands and disjonction of the homodimer**

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)*

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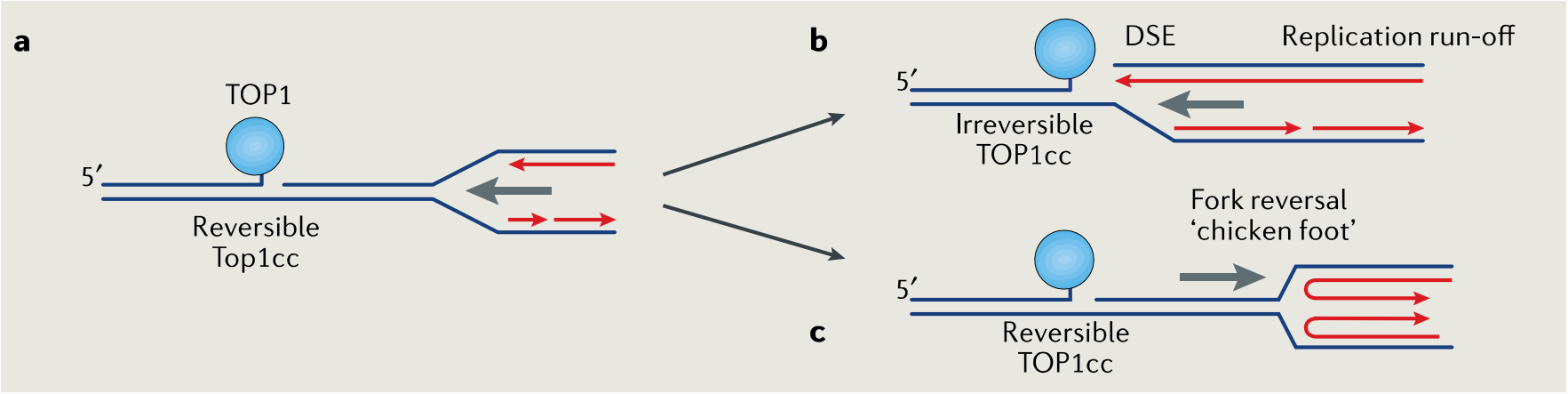
Replicative DNA damage induced by TOP1cc (Topoisomerase I cleavage complexes)



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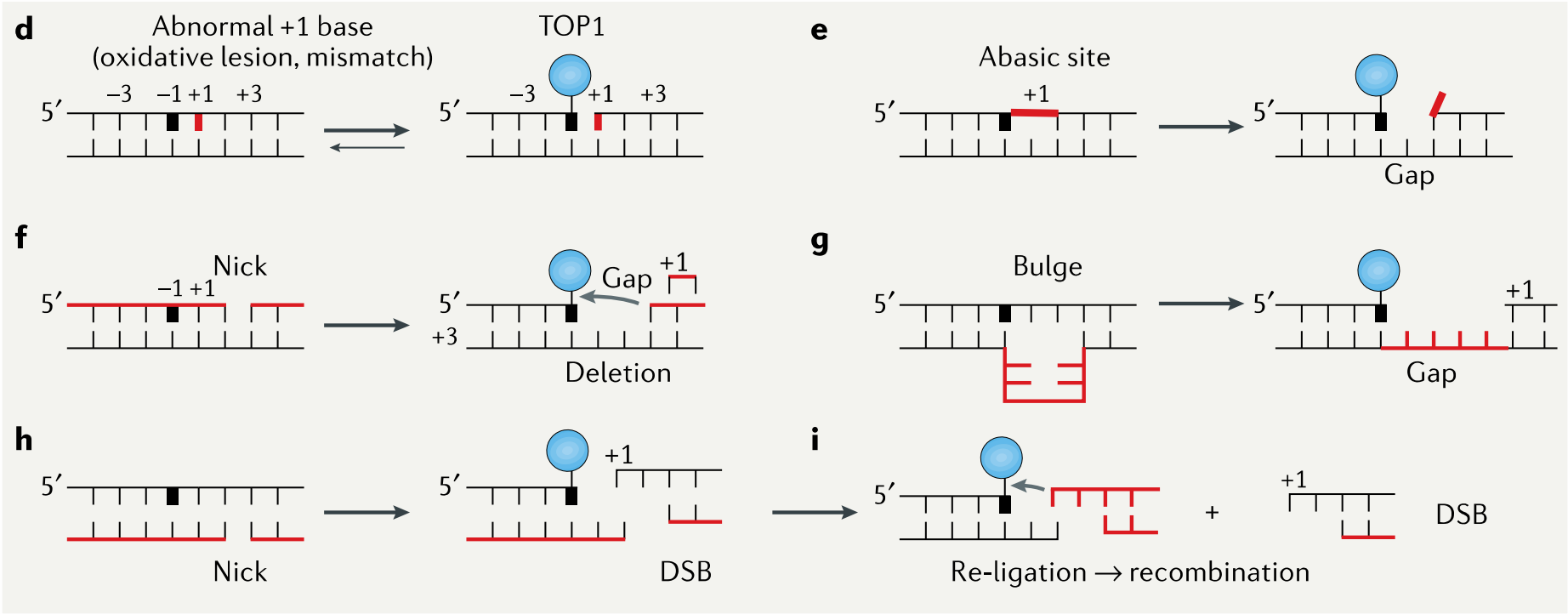
DNA damage induced by TOP1cc at preexisting DNA alterations



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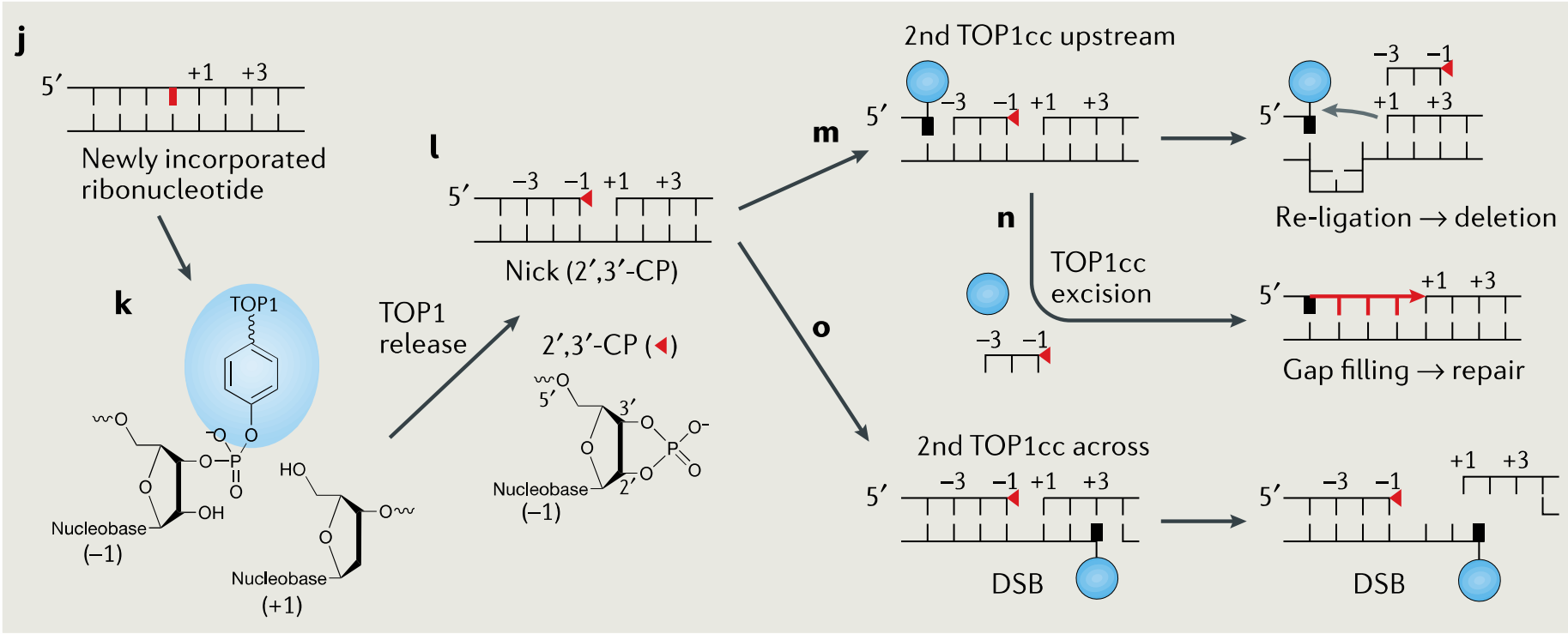
DNA damage induced by TOP1cc at incorporated ribonucleotides in the genome

Ribonucleotide incorporation by DNA polymerases is the most frequent non-canonical DNA ”alterations”



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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

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].

Stingele, J. Bellili, R. & Boulton, S.J. 2017 Nat Rev Mol Cell Biol

Repair of Topoisomerase covalent complexes

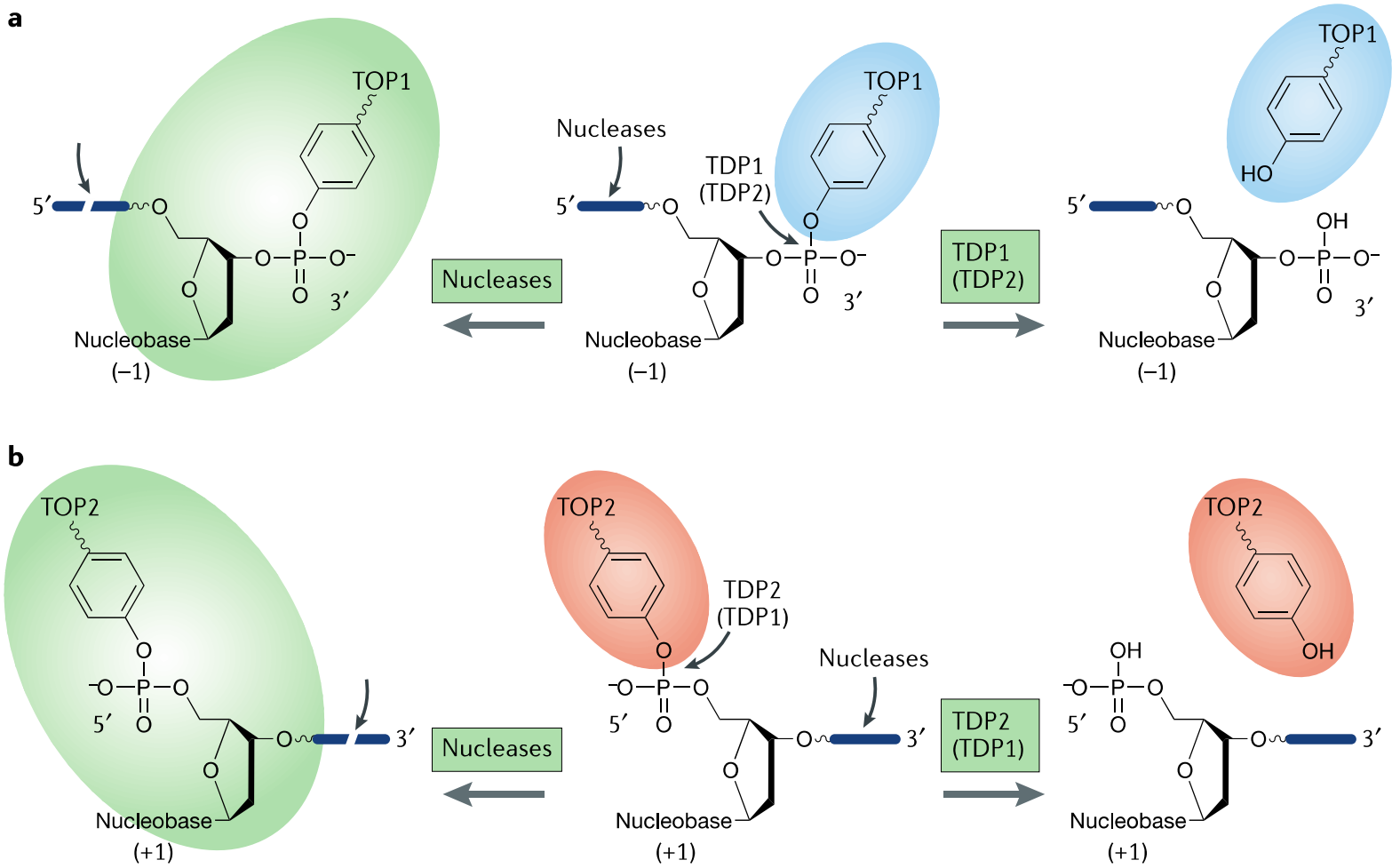

Figure 5 | **TOPcc repair. a** | Tyrosyl-DNA phosphodiesterase 1 (TDP1) and TDP2 (although much less efficiently and therefore shown in parentheses) cleave the TOP1 tyrosyl–DNA covalent bond (middle), releasing TOP1 and leaving a 3′-phosphate end (right) that needs to be further processed by polynucleotide 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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- Rao Vishnuvajjala (DTP)
- Sima Hayavi (DTP)
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