Topoisomerase

“Now, just relax your back-bone & let yourself unwind...”
DNA Topoisomerases

DNA Topoisomerases and Their Poisoning by Anticancer and Antibacterial Drugs

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Drugging Topoisomerases: Lessons and Challenges

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Roles of eukaryotic topoisomterases in transcription, replication and genomic stability

Yves Pommier¹, Yilun Sun², Shar-yin N. Huang¹ and John L. Nittis²

Abstract | Topoisomerase 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 topoisomerase in mediating chromatin dynamics, transcription, replication, DNA damage repair and genomic stability, and discuss how deregulation of topoisomeras can cause neurodegenerative diseases, immune disorders and cancer.
DNA Topoisomerases and Cancer
Unwinding DNA
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

A

<table>
<thead>
<tr>
<th>Type</th>
<th>kDa</th>
<th>P-Y</th>
<th>ΔLk</th>
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<td>5'</td>
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<tr>
<td>Top3β</td>
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<td>5'</td>
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<tr>
<td>Top1</td>
<td>100</td>
<td>3'</td>
<td>±1</td>
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<tr>
<td>Top1mt</td>
<td>70</td>
<td>3'</td>
<td>±1</td>
</tr>
<tr>
<td>Top2α</td>
<td>170 (x2)</td>
<td>5'</td>
<td>±2</td>
</tr>
<tr>
<td>Top2β</td>
<td>180 (x2)</td>
<td>5'</td>
<td>±2</td>
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B

<table>
<thead>
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<th>Type</th>
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<tr>
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<tr>
<td>Top III</td>
<td>74</td>
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<tr>
<td>GyrA</td>
<td>97 (x2)</td>
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<tr>
<td>GyrB</td>
<td>90 (x2)</td>
<td>5'</td>
<td>±2</td>
</tr>
<tr>
<td>ParC</td>
<td>84 (x2)</td>
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<td>±2</td>
</tr>
<tr>
<td>ParE</td>
<td>70 (x2)</td>
<td>5'</td>
<td>±2</td>
</tr>
</tbody>
</table>
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.

**A**
- Type IA
  - Top3α: 112 kDa, 5’ +1
  - Top3β: 97 kDa, 5’ +1

- Type IB
  - Top1: 100 kDa, 3’ ±1
  - Top1mt: 70 kDa, 3’ ±1

- Type IIA
  - Top2α: 170 (x2) kDa, 5’ ±2
  - Top2β: 180 (x2) kDa, 5’ ±2

**B**
- Type IA
  - Topo I: 98 kDa, 5’ +1
  - Topo III: 74 kDa, 5’ +1

- Type II A
  - Gyrase
    - GyrA: 97 (x2) kDa, 5’ ±2
    - GyrB: 90 (x2) kDa, 5’ ±2

  - Topo IV
    - ParC: 84 (x2) kDa, 5’ ±2
    - ParE: 70 (x2) kDa, 5’ ±2
Topoisomerases and TOP Genes in Humans

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:

<table>
<thead>
<tr>
<th></th>
<th>Top1</th>
<th>Top1mt</th>
<th>Top2α</th>
<th>Top2β</th>
<th>Top3α</th>
<th>Top3β</th>
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<tbody>
<tr>
<td>Weight</td>
<td>100 kDa</td>
<td>70 kDa</td>
<td>170×2 kDa</td>
<td>180×2 kDa</td>
<td>100 kDa</td>
<td>100 kDa</td>
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## Comparisons

### Comparison of the 6 human topoisomerases

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

*: 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.
Top 1

Top1

TOP1 (nuclear Top1)

TOP1MT (mitochondrial Top1)
Top1 and Top2 differences

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
Relaxation of DNA

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 exogenous DNA lesions (abasic sites, oxidized bases, carcinogenic adducts...) and during apoptosis.
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 TOP2 and TOP2 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

The two human Top1s

A

B

mtDNA

nDNA

Identities 6%

Core Domain 73%

Similarities 10%

Linker 79%

CTD 90%

C

Relaxation of supercoiling

Nicking

Controlled rotation

Religation
Camptothecin

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.
Interfacial inhibitor

Camptothecins as one of Nature’s Paradigms for Interfacial Inhibitors

Staker ...Stewart, PNAS 2002; 99: 15397
CPT analogs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cu (µg/ml)</th>
<th>TOP1**</th>
<th>TOP1***</th>
<th>rad52A**</th>
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<th>TOP1†</th>
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<tbody>
<tr>
<td>Cpt</td>
<td>&gt;800</td>
<td>&gt;800</td>
<td>25 ± 3</td>
<td>4.5 ± 0.4</td>
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<tr>
<td>9-CH0-CPT</td>
<td>&gt;800</td>
<td>&gt;800</td>
<td>21 ± 2</td>
<td>5.1 ± 2.1</td>
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<tr>
<td>9-Nitro-CPT</td>
<td>&gt;800</td>
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<td>180 ± 38</td>
<td>39 ± 5</td>
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<tr>
<td>10-CH0-CPT</td>
<td>&gt;800</td>
<td>&gt;800</td>
<td>29 ± 3</td>
<td>5.0 ± 1.4</td>
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<tr>
<td>10-CH0-7-ethyl-CPT</td>
<td>&gt;800</td>
<td>&gt;800</td>
<td>&gt;800</td>
<td>9.4 ± 3.6</td>
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<tr>
<td>7-Methyl-CPT</td>
<td>&gt;800</td>
<td>&gt;800</td>
<td>91 ± 6</td>
<td>16 ± 0.2</td>
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<tr>
<td>10-HO-CPT</td>
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<td>210 ± 60</td>
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<td>&gt;800</td>
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</tbody>
</table>

*TOP1*** refers to strain RS190 bearing the topoisomerase 1-overproducing plasmid pWE3 GAL-TOP1 under induced conditions.

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?

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

2. Because agents 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
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"

A
Camptothecin (lactone) \( \text{ACTIVE} \)
Camptothecin (carboxylate) \( \text{INACTIVE} \)

B
Indenoisoquinolines

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, A., Pommier, Y., Cushman, R.
Comparative oncology trials

**Goals:**
1. Compare LMP400, LMP776 and LMP744
2. Determine MTD in dogs with lymphomas
3. Determine and compare activity of 3 drugs
4. Determine pharmacokinetics in blood and tumor
5. Determine target engagement:
   1. γH2HAX
   2. TOP1 downregulation
Dog lymphoma

All drugs exhibit antitumor activity in primary dog lymphoma

A

LMP400

Progressive disease

PR

% Change from baseline

-100

0

50

100

LMP776

Progressive disease

PR

% Change from baseline

-100

0

50

100

LMP744

% Change from baseline

-100

0

50

100

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 3\textsuperscript{rd} indenoisoquinoline, LMP744 shows even greater antitumor activity.
- The dose limiting toxicity of the indenoisoquinolines (MTD = 17.5 mg/m\textsuperscript{2} for LMP776; MTD > 65 mg/m\textsuperscript{2} for LMP400; MTD = 100 mg/m\textsuperscript{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 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
### Second Generation Camptothecins with Targeted Delivery

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<th>Name</th>
<th>Company</th>
<th>Active Derivative (Payload)</th>
<th>Formulation (Conjugate; Target)</th>
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<td>Onivyde™ = MM398*</td>
<td>Merrimack</td>
<td>Irinotecan (CPT11)</td>
<td>Liposome</td>
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<td>CRLX101</td>
<td>Cerulean Pharma Inc.</td>
<td>Camptothecin</td>
<td>PEG</td>
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<td>NKTR-102</td>
<td>Nektar Therapeutics</td>
<td>Etirinotecan (20 position)</td>
<td>PEG (Pegol)</td>
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<td>PLX038</td>
<td>ProLynx</td>
<td>SN-38</td>
<td>PEG</td>
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<td>IMMU-132 = Sacituzumab govitecan</td>
<td>Immunomedics (Seattle Genetics)</td>
<td>SN-38 (20 position)</td>
<td>ADC - TROP2 (TACSD2)</td>
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<td>DXd (Exatecan)</td>
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<td>HDC - Conjugate Hsp90</td>
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<tr>
<td>NK012</td>
<td>Nippon Kayaku</td>
<td>SN-38</td>
<td>Polymeric micelles (PEG-polyglutamate)</td>
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<td>ALOS4-CPT</td>
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<td>Camptothecin</td>
<td>HDC – ALOS-4</td>
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<td>SN38-TOA</td>
<td>CHOP Philadelphia</td>
<td>SN-38</td>
<td>Tocopherol oxyacetate nanoparticles</td>
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* FDA Approved, October 2015
** FDA Breakthrough, February 2016
*** FDA Breakthrough, August 2017 (Breast)
Top2

Top2α – TOP2A: Replication
Highly expressed in replicating and cancer cells

Top2β – TOP2B: Transcription
Expressed both in replicating and differentiated cells
Two Top2 enzymes
Top2 catalyze a broad range of reactions

A

- Adanubicin
- Doxorubicin
- ATP
- Decatenation
- Catenation
- unknotting
- knotting
- Relaxation of supercoiling
- Negative supercoiling
- ATP

B

- Quinolones
- Etopoisines
- Anatoxins
- Mg^{2+}
- ICRF-187
- Top1
- Top2α
- Top2β
- Top3α
- Top3β
- Top3γ
- Top4α
- Gyrase
- Top1
- Top1α
- Negative sc favors DNA melting (strand separation)
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.
DNA replication

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 TOP2α, whereas negative supercoiling can be removed by TOP1, TOP2α or TOP3α. TOP2α can also remove precatenanes, which are formed when the fork separates 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.

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

Structure of a topoisomerase IV cleavage complex (Topo IVcc) trapped by the quinolone, levofloxacin.
Interfacial inhibition of macromolecular interactions: nature’s paradigm for drug discovery

Yves Pommier¹ and Jacqueline Cherfils²

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²Laboratoire d’Enzymologie et Biochimie Structurales, CNRS, Gif sur Yvette, France

Interfacial inhibitors: targeting macromolecular complexes

Yves Pommier¹ and Christophe Marchand¹

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

(A) Top1

Camptothecins
Indenoisoquinolines

(B) Top2

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

Decatenation Top2 vs. Top3

Top2

A

Top1A-RecQ
(Top3-BLM/WRN)

B

(vi)

(vi)
Deletion of TOP3β, a component of FMRP-containing mRNPs, contributes to neurodevelopmental disorders


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 TOP3β 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 Xu1,2,18, Weiping Shen1,10, Rong Guo1, Yutong Xue1, Wei Peng1, Jian Sima3, Jay Yang4, Alexei Sharov5, Subramanya Srikanthan6, Jiandong Yang1, David Fox III7, Yong Qian5, Jennifer L. Martindale8, Yulan Fiao9, James Machamer7, Samit R Joshi8, Subhasis Mohanty9, Albert C Shaw4, Thomas E Lloyd7, Grant W Brown4, Minoru S H Ko4, Myriam Gorospe6, Sige Zou9 & Weidong Yang1

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 genes with such nucleotides, that encoding protein tyrosine kinase 2 (p652, 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
Topoisomerase

Topoisomerase
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 disjoinction 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

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Replicative DNA damage

Replicative DNA damage induced by TOP1cc (Topoisomerase I cleavage complexes)
Human diseases
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 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. 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). 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 (NBSD1; OMIM 613078). Clinical features of NBSD1 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.

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.
Covalent complexes

Repair of Topoisomerase covalent complexes
Catalytic intermediate

All topoisomerases form a catalytic intermediate consisting of a covalent bond between one end of the break they make in DNA (and RNA for TOP3B) and their catalytic tyrosyl residue.

Pommier et al. ACS Chem Rev 2009
http://discover.nci.nih.gov/pommier/pommier.htm
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).
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 (H2O2, 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 (Mg2+; 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 2014; JPET 2014)

⇒ Synthetic lethality
in Mre11- or XPF-ERCC1-deficient cancers?
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