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All tangled up: how cells direct, manage and exploit topoisomerase function

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Preface

Topoisomerases are complex molecular machines that modulate DNA topology to maintain chromosome superstructure and integrity. Although capable of stand-alone activity *in vitro*, topoisomerases frequently are linked to larger pathways and systems that resolve specific DNA superstructures and intermediates arising from cellular processes such as DNA repair, transcription, replication, and chromosome compaction. Topoisomerase activity is indispensible to cells, but requires the transient breakage of DNA strands. This property has been exploited, often for significant clinical benefit, by various exogenous agents that interfere with cell proliferation. Despite decades of study, surprising findings involving topoisomerases continue to emerge with respect to their cellular function, regulation, and utility as therapeutic targets.

Keywords

Topoisomerase; DNA replication; DNA repair; transcription; chromosome structure; topology; drug discovery

Introduction

The extended, double-helical structure of DNA poses a unique challenge to living organisms. Chromosomes must be folded and compacted in an orderly manner to fit within the confines of the cell. Natural nucleic-acid transactions such as transcription, replication, and repair – all of which require access to nucleotide sequence information – necessitate duplex melting events that overwind and underwind (or **supercoil**•) (Fig. 1A) nucleic acid segments, interfering with appropriate gene expression. DNA repair and replication further generate entanglements between chromosomal regions that, if left unresolved, can lead to potentially mutagenic or cytotoxic DNA strand breaks.

Enzymes known as topoisomerases manage these transactions. Endowed with an ability to cut, shuffle, and religate DNA strands, topoisomerases can add or remove DNA supercoils, and disentangle snarled DNA segments. How topoisomerases carry out such complex

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•**Glossary Term: Supercoil.** The result of duplex DNA twisting upon itself in 3-dimensional space.

functions has long been a significant question of both biochemical and biophysical interest. However, in recent years it has become evident that topoisomerases do not work at random; they can instead preferentially distinguish between different types of DNA juxtapositions and collaborate with disparate factors direct their action. It also has become clear that a rich and growing variety of natural and synthetic agents block topoisomerase function for the purposes of evolutionary competition or therapeutic gain.

In this Review, we touch upon a few of the many recent findings that have broadened our understanding of the roles played by topoisomerases in supporting chromosome biology and cell viability. Our goal is not to provide a comprehensive overview for those studying these complex enzymes, but rather to convey a sense of the vitality and richness of the topoisomerase field to the general biological community, particularly insofar as the evolutionary and functional diversity of these complex machines, and their roles in the flow of genetic information, genome maintenance, and human health. Given these limitations, we apologize to those colleagues whose work is only topically discussed, or excluded due to space limitations.

Topoisomerase families

At first glance, a bewildering number of different topoisomerases exist. However, nature's topoisomerase collection is not as complicated as it first appears. For example, all topoisomerases contain a nucleophilic tyrosine, which they use to promote strand scission. This feature links DNA cleavage to the formation of a transient, covalent enzyme–DNA adduct, which in turn prevents the inadvertent release of nicked or broken DNA duplexes that might otherwise damage the chromosome (Fig. 1B). All topoisomerases also can be assigned into one of two primal classes, type I and type II, depending on whether they cleave one or two strands of DNA, respectively. Enzymes with an odd Roman numeral (for example, "I" or "V") after their name fall into the type I class, whereas those with an even-numbered Roman numeral after their name are type II. Topoisomerase subtypes – "A," "B," or "C" – are then used to distinguish between enzyme families that have significantly different amino acid sequence relationships and/or structures. Some topoisomerase subtypes further encompass multiple **paralogs**[1] that have diverged from one another in terms of their specific activities. A brief overview of these groupings follows to provide a context for subsequent discussion. For more extensive coverage of topoisomerase evolution, structure, and mechanism, the reader is invited to see [1,2].

Type IA: single-stranded 'strand-passage' enzymes

Type IA topoisomerases have a toroidal protein structure that bears a superficial resemblance to a padlock [3]. These enzymes effect topological changes in DNA through a "strand-passage" mechanism [4,5], in which a single DNA strand is cleaved and physically opened, and a second DNA is navigated through the gap; following passage of the second DNA, the broken DNA is resealed (Fig. 1C). Type IA topoisomerases comprise three distinct subfamilies that are found throughout all three cellular **domains of life**[2]. These

[1]**Glossary term: Paralogs.** Homologous genes separated by a gene duplication event, which can evolve new functions.

subfamilies are bacterial topo I (hereafter called topo IA), bacterial and eukaryotic topo III, and reverse gyrase [1]. The primary activity of topo IA is to relax **negatively-supercoiled DNA**[3]6,7. By contrast, topo III preferentially acts to resolve single-stranded DNA entanglements that can arise during DNA replication and repair [7–11]. Reverse gyrase, found exclusively in thermophilic bacteria and archaea, positively supercoils DNA and renatures melted DNA strands through the action of an ATP-dependent **superfamily-2 (SF-2) helicase**[4] domain fused to its type IA topoisomerase element[12, 13].

Type IB: DNA "swivelases"

Type IB topoisomerases differ fundamentally in structure and mechanism from type IA enzymes [14]. Rather than relying on strand passage, type IB topoisomerases effect supercoil relaxation by nicking a single strand of duplex DNA, and allowing one DNA end to rotate with respect to the other around the intact phosphodiester bond on the opposing strand [15]. Rotation is controlled by friction between the DNA and the enzyme, which aids in aligning the broken ends for resealing [15,16] (Fig. 1D). Type IB proteins preferentially bind positively- or negatively- supercoiled substrates rather than relaxed substrates [17,18] using an interaction surface outside of its primary active site to bridge distal DNA segments [19]. Some variants show a proclivity for positively-supercoiled DNA, which they can relax at a faster rate [18].

The active site architecture of type IB topoisomerases is evolutionarily related to that of tyrosine recombinases and integrases [20,21]. Type IB enzymes are ubiquitous among eukaryotes, with some scattered homologs extant in certain viruses and bacteria [22]. Type IB topoisomerases appear to be represented by a single family member (topo IB), although architectural differences that influence the rate and mechanism of supercoil relaxation are evident between various homologs in this group [15].

Type IC: a second class of swivelase

Type IC topoisomerases thus far have been found only in the archaeal genus *Methanopyrus* [23]. Like topo IB, type IC topoisomerases relax positively- and negatively-supercoiled DNA through a nicking and rotation mechanism [24,25] (Fig 1D). However, the type IC active site shows little structural similarity to that of type IB enzymes, and appears to have a different evolutionary lineage [23,26]. Type IC enzymes also retain functional elements that exhibit apurinic site lysase activity, which they can use for repairing **abasic lesions**[5] in DNA [26,27]. Only one topoisomerase variant, topo V, is presently known to comprise the type IC family [25].

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[2]**Glossary term: Domains of life**. Refers to the three major evolutionary branches – bacteria, archaea, and eukaryotes – of modern day cellular lineages.

[3]**Glossary Term: Negatively-supercoiled DNA.** DNA becomes negatively supercoiled when undertwisted (that is, when wound to < 10.5 bp/turn). This destabilizes DNA, allowing complementary strands to be more easily denatured. In contrast, DNA becomes positively supercoiled and more stabilized when over-twisted (that is, wound to > 10.5 bp/turn).

[4]**Glossary Term: Superfamily-2 (SF-2) helicase.** A diverse group of ATP-dependent nucleic-acid helicases and translocases that share a conserved domain architecture. Many members of this family can locally melt or unwind short duplex regions.

[5]**Glossary term: Abasic lesions.** A form of DNA damage in which the nucleobase has been excised from the sugar backbone.

Type IIA: duplex DNA 'strand-passage' enzymes

As with their type IA counterparts, type IIA topoisomerases employ an active strand-passage mechanism for effecting topological changes in DNA. Type IA and IIA topoisomerases also share certain catalytic domains used for DNA cleavage [28,29]. However, type IIA enzymes differ in that they cleave both strands of a DNA duplex and pass a second intact duplex through the transient break [30–32] (Fig 2A), and they use ATP to power strand passage [31,33,34]. These activities allow type IIA topoisomerases to resolve both positive and negative DNA supercoils, as well as disentangle long intertwined chromosomes and DNA **catenanes**[6]32,35. Type IIA topoisomerases are found throughout all cellular organisms, as well as in some viruses and organelles, and can be partitioned into three paralogous subfamilies – eukaryotic topo II, bacterial topo IV, and prokaryotic gyrase – that exhibit distinct functional properties [1,2]. For instance, gyrase actively adds negative supercoils to DNA and is only weakly able to unlink catenanes [33,36,37], while topo IV (and certain topo II isoforms) is a robust decatenase that preferentially relaxes positively-supercoiled substrates over negatively-supercoiled ones [38,39] (Box 1). Interestingly, type IIA topoisomerases show some ability to discriminate between, and preferentially act on, regions of high DNA curvature or DNA crossovers due to their ability to sharply bend DNA segments[37,40–43]. By contrast, yeast and *Drosophila melanogaster* topo II do not show any preference for one type of supercoiled DNA over another [44,45].

Box 1

Selectivity and function of type IIA DNA topoisomerase paralogs

Vertebrates possess two topoisomerase II paralogs, topo IIα and topo IIβ, which share almost 77% sequence homology [198]. However, the C-terminal regions of the two enzymes differ. In particular, the C-terminus of topo IIα shifts the activity of the enzyme toward the preferential relaxation of positive supercoils; by contrast, the equivalent region of topo IIβ does not appear to impart any supercoil preference [39]. These differences may be linked to particular cellular functions [199], as topo IIα is essential in proliferating cells and assists in chromosome condensation, segregation and replication [200–202], while topo IIβ tends to be associated with DNA repair, transcription, and development [82,83,134].

Bacteria also often utilize two type IIA topoisomerase paralogs (gyrase and topo IV) with different activity profiles. Gyrase relaxes positive supercoils and actively adds negative supercoils to DNA, whereas topo IV relaxes both positive and negative supercoils and shows a heightened activity on positively-supercoiled and catenated substrates [33,38,203,204]. An auxiliary domain appended to the primary strand-passage machinery of gyrase and topo IV appears to be responsible for controlling their functional distinctions [205,206], with sequence- and structure-based differences in this element permitting the wrapping of DNA by gyrase, but only DNA-binding by topo IV [206–209]. The auxiliary DNA wrapping and binding domain of bacterial type IIA topoisomerases is not conserved in eukaryotic topo II, indicating that the improved action

[6]**Glossary Term: Catenanes**. Topologically interlinked duplex DNA rings.

of topo IV and topo IIα on positively supercoiled DNA arose independently during their evolution.

Type IIB: a second class of duplex DNA strand-passage enzyme

Type IIB topoisomerases are found in archaea, plants, and a few bacterial, protist, and algal lineages [46,47]. As with their type IIA cousins, type IIB topoisomerases unlink tangled DNA duplexes by strand passage (Fig 2B) and relax both negative and positive supercoils [48]. Type IIB topoisomerases also possess ATPase and DNA-cleavage domains similar to those found in type IIA enzymes, although the relative arrangement of these elements in primary sequence space and their overall structural organization differs significantly [46,48–51]. The principal DNA binding subunit of type IIB topoisomerases is noteworthy in that it is evolutionarily related to Spo11, the factor responsible for creating double-stranded DNA breaks that initiate meiotic recombination [46,52]. Thus far, only one family member, topo VI, is known to comprise the type IIB topoisomerase clade [46].

Topoisomerase families – why so many?

Given this diversity of topoisomerases, and the seeming overlap in their basic function, how do cells decide which one to use for a particular purpose? This question has been difficult to address, in part because different organisms often possess markedly different topoisomerase repertoires [1,2]. Moreover, the regulatory strategies for a given topoisomerase can vary widely among species. At present, there appears to be no single answer. Rather, the molecular logic behind topoisomerase choice appears to derive from a confluence of factors, including: the type of topological problem that needs to be addressed, which topoisomerase genes happen to be present in the resident genome, whether specific architectural elements have been acquired to modulate topoisomerase function, and if different accessory factors and/or modifications that alter topoisomerase localization and activity are in use (Table 1 and Supplementary information S1 (table)). Topoisomerases utilize a combination of these regulatory strategies in the majority of nucleic-acid transactions in which they participate. A discussion of the role of specific topoisomerases in different cellular processes follows.

DNA packaging

The length of chromosomal DNA far exceeds that of the cell in which it resides. Organisms thus employ a variety of mechanisms to assist with DNA compaction, including supercoiling and the use of chromosome organizing factors such as histones or nucleoid associated proteins [53]. **Plectonemic supercoiling**[7] alone has been estimated to condense DNA by two to three orders of magnitude (Figure 1A)[54].

In bacteria, a need for compaction has been invoked as one of the principal reasons why chromosomal DNA is negatively supercoiled, and why gyrase, which adds those supercoils to DNA, is so ubiquitous throughout the bacterial kingdom [53,55]. More recent studies,

[7]**Glossary term: Plectonemic supercoiling.** The natural tendency of supercoiled DNA is to wrap back on itself, forming intra-molecularly wound structures known as plectonemes (Figure 1A). Because DNA is itself a chiral molecule, negatively- and positively-supercoiled states cause the DNA to coil in opposite directions (in the form of right- and left-handed supertwists, respectively).

however, suggest that there are additional layers of complexity to this view. For example, two very closely related bacterial species, *E. coli* and *S. typhimurium*, share about 90% sequence identity across homologous genes [56], but have highly-dissimilar **superhelical densities**[8], with *E. coli* DNA being significantly more underwound [57]. Gyrase itself is not always necessary for DNA packaging, as the lone type IIA topoisomerase present in the hyperthermophilic bacterium *Aquifex aeolicus* is not a gyrase, as might be predicted on the basis of its sequence similarity with other gyrase N-terminal domains, but rather a topo IV [58]. However, many thermophiles also possess reverse gyrase [59], which introduces positive supercoils that may aid compaction, as well as counteract thermal denaturation [60,61]. Although the steady-state superhelical density of DNA in most prokaryotes has not been measured directly, multiple findings suggest that many species, particularly those that are adapted to growth at high temperatures, may have relatively relaxed chromosomes [62]. How such organisms are able to sufficiently compact their chromosomes in the absence of supercoiling remains an open question, but the mechanism almost certainly relies on proteinaceous factors. Overall, the role of supercoiling in DNA compaction, and how topoisomerases collaborate to define the superhelical "setpoint" of the chromosome, is not fully understood.

Topoisomerases play another role in chromosome compaction by working with large condensation machineries (Table 1), principally a group of **ATP binding cassette (ABC)-family ATPases**[9] known as Structural Maintenance of Chromosomes (SMC) proteins [63–67]. SMCs, and their counterparts Rad50 (eukaryotes) and SbcC and RecF (*E. coli)*, are conserved factors involved in multiple aspects of chromosome cohesion, condensation, and DNA repair [68]. SMCs and type IIA topoisomerases indirectly co-localize as part of the protein network that helps stabilize long-range contacts between chromosomal segments [67,69]. SMCs, or their affiliated accessory factors, also have been reported to directly associate with both topo II and topo IV. For example, the *Drosophila melanogaster* Barren subunit (an ortholog of the condensin H protein) co-immunoprecipiates with topo II in vitro [66], while a dimerization region of the E. coli SMC homolog MukB interacts with the C-terminal domain of the ParC subunit of topo IV [64,65]. Interestingly, both interactions appear to potentiate the relaxation of negatively-supercoiled DNA by the associated topoisomerase, although how stimulation is achieved is unresolved. At present, little is known about the effect and role of these interactions on global DNA superstructure or about their utility to the cell, although the MukB–ParC interaction is important for cell viability [65]. Whether these associations are preserved in other species is not clear, and constitutes a clear avenue for additional investigation.

Replication and chromosome segregation

Topoisomerases are required both for the successful management of DNA replication, and for specific developmental strategies that depend on replication. In the latter instance, the

[8]**Glossary Term: Superhelical density.** A measurement of the over- or under-twistedness of DNA, which is generally expressed as the ratio to which the twist of the supercoiled state differs from that of the relaxed state.

[9]**Glossary Term: ATP Binding Cassette (ABC) ATPases.** A family of proteins that include membrane bound transporters, repair proteins, and SMCs. ABC ATPases possess a conserved ATP binding domain that is often formed at dimer interfaces. Binding of ATP results in conformational changes, often dimerization, that affect associated binding partners and substrates.

type IIB topoisomerase found in plants (topo VI) is required for a particular form of replication known as endoreduplication, a process by which chromosomes are copied multiple times in the absence of cell division [70–73]. Endoreduplication gives rise to polyploid nuclei, which in turn can be used to regulate cell size [74]. Why topo VI is needed specifically for endoreduplication, and why topo II cannot rescue a topo VI deficiency, is not clear [75]. Similarly, replication of vertebrate mitochondrial DNA requires a special mitochondrial type IB topoisomerase, top1mt, a paralog of the nuclear type IB topoisomerase, top1 [76,77]. Mitochondrial DNA replication requires top1mt to form its regulatory displacement (D)-loop[77], which in turn is thought to regulate DNA replication initiation and transcription [77]. Surprisingly, when nuclear top1 is localized to the mitochondria, it cannot complement top1mt function and arrests the cell cycle [76]. At present, it remains to be seen whether similar topoisomerase-dependent roles exist in other organisms. The role of topoisomerases in conventional DNA replication is better understood, with recent studies uncovering specific roles for topoisomerases in each of the three major replicative phases: initiation, fork progression, and termination.

Topoisomerases in replication initiation

In *E. coli*, replication initiation is dependent on local supercoiling at a lone **origin**[10], *oriC*, that is regulated by the opposing activities of topo I and gyrase [78,79]. During DNA replication initiation in eukaryotes, topoisomerases have been seen to associate directly with certain origins to aid in activation. For example, human topo IB and topo IIα co-localize with both the lamin B2 origin (an early firing, widely studied replication start site found on human chromosome 19) and the Orc2 subunit of the **origin recognition complex**[11], while inhibition of topo IB interferes directly with origin firing [80] (Table 1). Formation of replication initiation complexes at the human Ors8 origin, (a late firing origin), as well as the lamin B2 origin, also have been reported to require DNA cleavage by topo IIβ – a topoisomerase associated with repair and development [81–83] – and the association of DNA repair proteins such as Ku70/80 and poly(adenosine diphosphate-ribose) polymerase-1 (PARP1) [84]. Why topoisomerases are needed during these early stages of eukaryotic replication is not well understood, but they may be involved in the control of torsional stress or DNA deformations that could be used either to help clear DNA regions for replisome assembly, or to promote DNA unwinding in the early stages of replisome assembly.

Topoisomerases in replication fork progression

During strand synthesis, topoisomerases are required to relieve positive supercoiling that arises from DNA unwinding by replicative helicases (Fig 3A). In bacteria, this role is typically fulfilled by DNA gyrase and/or topo IV [37,85–87]. By contrast, eukaryotes rely primarily on topo IB for positive supercoil relaxation, although topo II can assist or even substitute for topo IB in this capacity [88–91]. Some type II topoisomerases (for example, topo IV and human topo IIα – see Box 1) are markedly more adept at removing positive supercoils than negative ones [38–40], suggesting that cells may be under evolutionary pressure

[10]**Glossary Term: Origin.** A chromosomal site for replisome assembly.
[11]**Glossary Term: Origin recognition complex**. A multi-subunit protein complex that localizes to specific DNA origins to initiate DNA replication in eukaryotes.

to acquire particularly robust relaxases that can accommodate rapidly moving replication forks.

A second consequence of a progressing fork is the formation of daughter duplex intertwinings (termed **precatenanes**[12] in circular chromosomes) behind it (Fig. 3A). Such structures are produced as a natural byproduct of replicating the double helix of the mother chromosome and, if left unchecked, give rise to tangled or catenated DNAs that can lead to abnormal DNA segregation upon entry into cell division [92–96]. The duplex strand-passage activity of type II topoisomerases plays a key role in resolving these topological linkages.

As two forks near one another, the unreplicated region between them represents a topological linkage, known as a **hemicatenane**[13], which must be resolved before chromosome segregation can occur (Fig. 3B). If the two strands of the hemicatenane can be fully replicated prior to resolution, the resulting duplex DNA segments become a natural substrate for type II topoisomerases. Consistent with a need for these enzymes in resolving inter-chromosome crossovers, the removal or inactivation of topo II or topo IV can lead to hyper-catenated DNAs and the production of broken chromosomes during cell division [96–101].

However, there also is mounting evidence that type IA topoisomerases, particularly topo III, can participate directly in hemicatenane resolution before forks converge. For example, in bacteria, topo IV temperature-sensitive alleles can be rescued by overexpression of topo III [102]. Topo III, which decatenates single-stranded DNA *in vitro*, has been shown to collaborate with RecQ-family helicases in disentangling hemicatenated structures (Table 1); in this partnership, the helicase may help generate single-stranded DNA to aid topo III function. The connection between these two enzyme classes is sufficiently entwined that they frequently form stable or co-localized complexes, along with auxiliary single-stranded DNA binding proteins such as SSB (in bacteria), or replication protein A (RPA)-like factors (Rmi1 in eukaryotes and Rmi2 in metazoans) [9,103–105]. Consistent with the collaboration between these proteins as part of a functional “resolvosome”, ablation of the RecQ-partner of topo III in eukaryotes, the BLM helicase, leads to the enhanced formation of ultrafine DNA tangles, termed “microbridges,” between condensed chromosomes [106]. The natural fusion of a RecQ-like (SF-2) helicase domain with a type IA topoisomerase module in reverse gyrase – which likewise can resolve hemicatenanes [13,107,108] – further highlights the frequent need for paired, RecQ-family helicase–topoisomerase activities in the cell.

Topoisomerases in replication termination

With respect to replication termination, topoisomerases have been found to play a role in at least two instances. In bacteria, the completion of DNA synthesis can be assisted by chromosomally-encoded termination regions (*Ter*s), which bind dedicated factors that help arrest replicative helicases. In *E. coli*, the absence of topo IA diminishes the ability of the bacterium’s cognate termination factor (Tus) to block progression of the replicative DnaB

[12]**Glossary term: Precatenanes.** Entangled daughter duplexes formed behind a replication fork during strand synthesis.
[13]**Glossary Term: Hemicatenane.** A junction between two double-stranded DNA molecules where one strand of one DNA forms a duplex with a complementary strand on the other duplex.

helicase [109]. This effect may arise as a consequence of the increased levels of negative supercoiling left by topo IA's absence, which can stimulate DNA unwinding by the replicative helicase, DnaB, and may also serve to reduce the duration of the Tus–DnaB interaction. In yeast, topo II appears to facilitate fork progression at termination elements by localizing to such sites and working with the Rrm3 helicase to resolve the torsional stress arising from converging forks [110]. This controlled pausing at termination elements ensures complete replication and counteracts abnormal genome rearrangements and breaks arising from fork convergence at replication termination (Table 1). Whether there exist other, more specific connections between topoisomerases and replication termination remains to be determined.

Topoisomerases in chromosome segregation

Once replication is complete, daughter chromosomes must be pulled apart and separated from each other. Topoisomerases promote these events by facilitating DNA compaction and disentangling (as described above) and by working directly with chromosome partitioning machineries and/or cytoskeletal elements. For instance, many bacteria use a dedicated motor protein, FtsK or SpoIIIE, to move newly replicated chromosomes into different cellular locales to aid segregation [111]. In *E. coli*, topo IV physically associates with, and is stimulated by, FtsK, possibly as a means to both counteract the supercoils created by the translocating motor and to help unlink tangled DNA regions [112,113] (Table 1). A physical association between *E. coli* topo IV and the actin-like protein MreB also has been observed, and this pairing stimulates DNA decatenation as a possible additional force promoting chromosome segregation [114]. Although no eukaryotic topoisomerase has been reported to bind to cytoskeletal elements, topo II does associate with factors responsible for centromere formation and chromosome segregation, including the aurora B kinase and the polo-like kinase (Plk)-1 [115,116] (Table 1). The biological rationale for these interactions is not fully understood; however, the colocalization of topo II with aurora B and Plk-1 may be important for centromere resolution between sister chromatids. Additional links between topoisomerases and chromosome segregation appear likely to exist, but have yet to be discovered.

What attracts a given topoisomerase to a particular point in the replicative process? As the aforementioned examples highlight, this control can be achieved not only by the types of DNA intermediates that derive from a particular process (for example, hemicatenanes, pre-catenanes and supercoils), but also by topoisomerase-associated partner proteins. Other intriguing interactions have been implicated in topoisomerase function during replication. For example, topo IV can associate with both the polymerase processivity clamp-loader assembly, which may localize the enzyme to *oriC* to decatenate newly synthesized chromosomes [117], and the SeqA factor, a protein that prevents replication reinitiation by sequestering hemimethylated DNA within *oriC,* and that stimulates topo IV decatenation and supercoil relaxation activity [118] (Table 1). These connections further highlight cellular needs for particular topoisomerase activities during elongation and initiation, respectively.

Transcription and gene regulation

As with replication, transcription induces changes in DNA topology. A moving RNA polymerase produces localized positive supercoiling ahead of the transcription bubble and negative supercoiling in its wake [119,120] (Fig. 4A). Left unchecked, these supercoiling alterations can lead to significant changes in gene expression [121]. Underwound DNA also has a higher propensity to form stable RNA–DNA hybrids (R-loops) than relaxed duplexes [122]. Such structures can block cell growth and may contribute to genomic instability by stalling replication forks and promoting DNA breaks at highly transcribed genes [123,124].

Both type IA and type IB topoisomerases have been implicated in the removal of negative supercoils and the resulting suppression of R-loop formation [123,125]. Indeed, the *E. coli* topo IA C-terminus interacts directly with RNA polymerase, and this interaction may help recruit the topoisomerase to negative supercoils [55,126]. Type IB and type II topoisomerases further help relax positive supercoils in front of transcribing polymerases, with topo IB playing a more significant role in eukaryotic transcription and topo IV and gyrase handling these substrates in bacteria [37,55,127–129].

Topoisomerases have been linked to specific transcriptionally-related events, such as the activation or repression of particular promoters or nucleosome remodeling. For example, inactivation of topo IB in *S. cerevisiae* leads to histone-specific acetylation and methylation events that increase the transcription of telomere-proximal genes [130]. In *S. pombe*, there is evidence that the presence or absence of topo IB activity can influence nucleosome disassembly and assembly, respectively, at certain promoter regions [129]. Eukaryotic topo IB has been implicated in the control of gene expression through an associated kinase activity, which is reported to phosphorylate splicing factors such as SR (serine/arginine-rich) proteins[131], regulating splicing factor localization and enhancing their activity[132,133]; topo IB-mediated phosphorylation of SR proteins in turn can be negatively regulated by the presence of poly(ADP-ribose) [133] (Table 1 and Supplementary information S1 (table)).

Remarkably, the transient, site-specific cleavage of DNA by human topo IIβ has been reported to be required to activate the transcription of genes regulated by certain nuclear receptors, a process that necessitates the activity of **PARP**[14] and Ku80/Ku70, as well as DNA repair proteins such as DNA-PK (DNA-dependent protein kinase) [81,83]. DNA cleavage and the subsequent recruitment of PARP and associated repair proteins appears necessary to exchange histone-1 for the high mobility group (HMG)B-1/2 factor, and to stimulate transcription [83]. Similarly, topo IIβ associates with the promoter region of genes in human acute promyelocytic leukemia cell lines that are regulated by retinoic acid receptor elements, initially stimulating transcription; sustained transcriptional activation then leads to decreased transcript levels and the inhibition of granulocytic differentiation in a topo IIβ-dependent manner [134].

[14]**Glossary Term: PARP.** Poly (ADP ribose) polymerase, an enzyme that adds chains of ADP to proteins as part of DNA damage and cell death responses.

Some of these findings, such as those suggesting that topo IB might directly phosphorylate target proteins, or that cells might rely on a potentially mutagenic topo IIβ-meditated DNA-cleavage event to control gene expression, raise as many questions as they answer. Nonetheless, even though it can be difficult to definitively determine whether the effects of topoisomerases on promoter function and structure are direct or indirect, it seems that these enzymes can influence transcriptional events in a manner that may not depend solely on their ability to control supercoiling. This complex area of investigation is likely to yield many more surprises.

Recombination and repair

Yet another area where topoisomerases play a critical role is in forming and managing double-stranded DNA (dsDNA) breaks. As noted earlier, DNA cleavage by at least one topoisomerase, topo IIβ, has been implicated in the control of promoter activity [81,83,134]. However, double-stranded DNA-break formation through a variety of topoisomerases and topoisomerase-like proteins also impacts processes such as meiotic recombination, cell cycle checkpoint activation, and DNA repair. For instance, during meiotic recombination, the type IIB topoisomerase A-subunit homolog, Spo11, creates double-strand breaks that allow chromosomes to exchange segments through homologous recombination [46,52,135]. Although there is no evidence presently linking Spo11 to a *bona fide* topoisomerase activity (in the sense that it would be capable of DNA strand passage) [136], its DNA-cleavage activity during meiosis is clearly controlled, as only a fraction of the total Spo11 pool bound to chromosomal loci is used to form DNA breaks and recombinogenic sites [137]. Eukaryotes (with the exception of plants) do not appear to possess any clear homologs of the ATP-binding B-subunit of topo VI, and how Spo11 is activated is unknown at present [136].

The creation of nicks and double-strand DNA breaks are potentially deleterious events for the cell. Hence, DNA cleavage by topoisomerases or topoisomerase-like factors has the capacity to elicit DNA damage responses. For example, following DNA-breakage by Spo11, the protein remains covalently attached to the DNA and must be removed [137]. The removal of Spo11 and downstream DNA-end processing events appear to be carried out by double-strand break repair proteins such as the Rad50–Mre11–Nbs1 (MRN) complex [136,137], Rad51 and Dmc1-like factors [138,139], and the nuclease Sae2/CtIP [140]. Meiotic recombination elicits a p53 damage response, which appears to be independent of the ATM and ATR (ataxia telangiectasia mutated and ATM Rad3-related) kinases and their associated repair pathways [141]. Similar responses are seen when topoisomerases become inactivated [96], or are aberrantly linked to DNA through the action of DNA lesions or small-molecule inhibitors that promote the formation of topoisomerase-DNA cleavage complexes. However, additional systems also can be activated in these instances (for example, the ATM and ATR pathways, and BRCA1 or BRCA2 proteins [142,143]) that induce cell cycle arrest and DNA repair. When topoisomerases become inadvertently trapped in a covalent complex with DNA, they further can be targeted for destruction by SUMOylation- and ubiquitinylation-mediated mechanisms, and the covalent tyrosine-DNA adducts can be repaired by enzymes such as Tdp1 and Tdp2 [143–152] (Supplementary information S1 (table)). Some of these latter events may be promoted by collision encounters with proteins such as RNA polymerase[153–155].

Other evidence also exists linking topoisomerase activity to mutagenesis. For instance, highly transcribed genes in eukaryotes are sometimes associated with enhanced levels of genetic instability and spontaneous mutation [156]. Several recent studies have found that 2–3 nucleotide deletions commonly observed in these regions are the result of topo IB activity [157,158]. The mechanism behind these alterations has not been fully elucidated; topo IB may generate lesions by binding and cleaving within and adjacent to tandem dinucleotide repeats, forming a stable covalent DNA link that is then processed in part by endonucleases such as Mus81 and Rad1 [157,158]. Alternatively, topo IB may cleave at the scissile phosphate [15] of a misincorporated ribonucleotide within or adjacent to the tandem dinucleotide repeats. The misincorporated ribonucleotide would then undergo an internal 2′-3′ cyclization event to release the enzyme and leave a small nick or gap in the DNA [159], giving rise to microdeletions from DNA misalignments during repair. In this regard, it is interesting to note that type II and type IB topoisomerases form stable adducts at sites of ribonucleotide incorporation in DNA [160], as well as at DNA lesions such as nicks [161] and abasic sites [162163,164]. Thus, both classes of enzymes may contribute to a steady-state background of damage events with mutagenic potential.

Topoisomerases further play an important role during and after the repair of damaged DNA strands. This is particularly true during homologous recombination (HR), which generates interlinked intermediates that bear some similarities to those occurring during replication fork convergence (Fig. 4B). Recent work has found ample evidence for the participation of topo III in HR where, as with hemicatenane resolution during replication, it breaks up double-Holliday junctions in concert with both a RecQ-type helicase (Sgs1 in yeast [165], BLM in metazoans [166,167]) and single-stranded DNA binding proteins such as SSB[168], Rmi1 [104] and Rmi2 [105,169] (Table 1). Notably, the cooperative activity of this resolvosome allows HR to proceed without the generation of crossover products that exchange large chromosomal segments, which occurs when branch-endonucleases resolve double-Holliday junctions, and may therefore constitute the dominant means for resolving Holliday junctions in mitotic cells [104,166,167]. Topo III also may help recruit other HR proteins to broken DNA ends. For example, Sgs1, Dna2, and RPA derived from budding yeast can resect DSBs in a topo III-independent manner *in vitro*; however, topo III stimulates Sgs1 helicase activity and is probably required at physiological concentrations to recruit Sgs1 to broken DNA ends [170,171]. How disparate topoisomerase, helicase, and SSB protein activities collaborate to disentangle hemicatenated substrates is not known; studies of reverse gyrase may provide some mechanistic insights into this problem, since reverse gyrase is composed of an Sgs1-like (SF-2) helicase domain fused to a type IA topoisomerase element [107,108].

PTM of eukaryotic topoisomerases

Eukaryotic topoisomerases are subject to a number of post-translational modifications (PTMs) that can alter their localization and activities at various stages in the cell cycle. These modifications may also influence the types of DNA structures on which topoisomerases preferentially act. Thus far, four types of topoisomerase PTM have been

[15]**Glossary Term : Scissile phosphate**. The phosphate at which a nucleic acid backbone is broken by nucleophilic attack.

observed: phosphorylation [172], acetylation [173], SUMOylation [145,151], and ubiquitinylation[146].

The modification of poisoned topoisomerases trapped on DNA by SUMOylation and ubiquitinylation, in response to DNA damage, appears to target these enzymes for processing and/or degradation [145,146,151–155]. However, apart from this function, the physiological role of topoisomerase PTMs has been somewhat controversial. Indeed, it is not uncommon to find studies that link the same type of modification to the regulation of different protein–protein partnering events, the activation or repression of specific topoisomerase activities, and/or proper and improper topoisomerase localization, sometimes in a mutually-exclusive manner. An example of this complexity is the role of SUMOylation in the temporal activation and localization of topo IIα through two **E3 ligases**[16], RanBP2 and protein-inhibitor-of-activated-STAT-1 γ (PIASγ). In *Xenopus laevis*, PIASγ has been linked to the SUMOylation of topo IIα, a modification that appears to reduce topo IIα's ability to decatenate DNA *in vitro* [174]. By contrast, PIASγ is reported to have no effect on topo IIα in mouse embryonic fibroblasts; instead, these cells appear to SUMOylate topo IIα through RanBP2, which enhances topo IIα localization and activation at centromeres during sister chromosome resolution in anaphase [175,176]. The role of phosphorylation has been similarly difficult to tease apart, with studies variously reporting that this modification can potentiate, depress, or exert no effect on topoisomerase function [116,177–179]. Nonetheless, some of these differences are almost certainly due to the particular site on the topoisomerase that is being modified. The regulation of eukaryotic topoisomerases through PTMs is a highly-interesting, but still relatively murky, area of research that undoubtedly holds significant surprises.

Topoisomerase inhibition

Although indispensible for cell viability, it is clear that topoisomerases also present a fundamental peril: they can be inadvertently inactivated, or their DNA cleavage activity can be corrupted, resulting in cytotoxic or mutagenic DNA breaks, which leads to cell death. The evolution of general repair systems, as well as more specific ones such as the tyrosyl-DNA phosphodiesterases (Tdps) [147–150], help cells to deal with these problems. However, nature and humans also have been able to exploit topoisomerase inactivity and stalling through diverse means to deliberately kill cells, at times for tangible therapeutic benefit.

A number of proteins and protein-like factors have been found that specifically inhibit topoisomerase activity, particularly in bacteria (Table 1 and Supplementary information S1 (table)). These proteins range from toxins that control plasmid stability (CcdB) [180,181] to agents that are involved in microbial competition (microcin B17) [182] and antibiotic resistance (Qnr and MfpA) [183]. Such factors have varied modes of action that include attenuating the DNA binding activity of topoisomerases, or promoting their ability to cleave DNA. In considering protein-based anti-topoisomerase factors, it is interesting to note that their identification has come not through directed screens, but from basic research and serendipity. The continual discovery of new proteinaceous inhibitors points to the utility –

[16]**Glossary term: E3 ligase.** An enzyme that attaches ubiquitin or ubiquitin like proteins to a target protein.

and potentially relative ease – by which topoisomerase activity can be subverted, and strongly suggests that more anti-topoisomerase systems remain to be discovered.

On the biomedical side, topoisomerase inhibition has proven to be a highly versatile and useful approach for therapeutic intervention. Small-molecule agents targeting topoisomerases fall into two broad classes based on their mode of action: “inhibitors,” which attenuate enzyme activity, and “poisons”, which stabilize DNA-cleavage complexes (see Fig. 5 for a partial list of type II topoisomerase antagonists and their modes of action). Although many of the most significant therapeutics (for example, epipodophyllotoxins, quinolones, and camptothecins) have been used for decades, only relatively recently have their mechanisms of action been understood in molecular detail. For instance, camptothecin, and its derivatives such as Topotecan, poison topo IB by intercalating between cleaved DNA ends in the enzyme active site [184], impeding DNA religation and the relaxation of supercoils. Fluoroquinolones, which are used primarily as antibiotics that target gyrase and topo IV, as well as topo II poisons such as etoposide, act in a similar manner, binding between the 5′ and 3′ end of a broken DNA strand to prevent resealing and release [42]. Other compounds that act in a very different manner, blocking either ATPase activity (for example, novo- and cholorobiocins [185,186], bisdioxopiperazines [187], and radicicol [188]), DNA binding (simocyclinone D8) [189], or DNA cleavage (NTBI GSK299423)[190]. These compounds have been imaged in the presence of their respective targets, yielding insights into their function. Together, these findings have not only highlighted the molecular determinants that enable drug binding and explained their inhibitory effects, but further demonstrated that there is a rich abundance of molecular scaffolds capable of inhibiting topoisomerases (Supplementary information S2 (figure)). At present, no small-molecule agent is known to target type IA topoisomerases, although biochemical studies have suggested that such compounds likely would be cytotoxic [191]. A hopeful, but presently unrealized, goal for these combined efforts is to point the way toward design of new inhibitors that show improved patient tolerance and that can circumvent emerging problems with drug-resistance.

How cells respond to topoisomerase poisons has been difficult to tease out. In bacteria, it was discovered relatively recently that fluoroquinolones help promote cell death by inducing the formation of reactive oxygen species (ROS) that further damage DNA [192,193]. Interestingly, however, some fluoroquinolones (moxifloxacin and PD161144) also kill cells even when the ROS cascade is blocked (or when oxygen is absent), suggesting that there exists a second pathway for killing by some anti-topoisomerase agents [194]. In eukaryotes, treatment of cells with poisons leads to the ubiquitinylation and SUMOylation of both topo I and topo II [145,146,151–155]. These modifications are necessary in part for initiating the repair responses described earlier. However, the need for active repair mechanisms also sensitizes cells to agents that block such pathways. In this regard, inhibitors of PARP have shown some promise when administered with topoisomerase poisons in promoting tumor cell killing. Initially, the PARP-1 inhibitor NU1025 was reported to potentiate damage mediated by the topo I inhibitor camptothecin in mouse lymphocytic cells, but not to promote further damage in conjunction with the topo II-targeting agent, etoposide [195]. However, more recent work has shown that mouse fibroblasts lacking PARP-1 are more sensitive to the topo II inhibitor C-1305 [196], and that the PARP inhibitor ANI enhances the activity of the topo II

inhibitor doxorubicin against liver cells [197]. Fully mapping the pathways and mechanisms by which cells contend with anti-topoisomerase agents, and looking for synergy between such compounds and other drugs, promises to be a rich vein of research, with powerful implications for the treatment of bacterial infections and cancer.

Concluding Remarks

Although first discovered over 40 years ago, the study of topoisomerases remains highly vigorous. Our knowledge of the role of cellular topoisomerases and their regulation by external factors continues to expand, but many pressing questions remain. For instance, there remain many significant gaps in describing the molecular operating principles that define where, when, and how a particular topoisomerase will act in the cell. The role of higher-order topoisomerase complexes (many of which are transient), the control of topoisomerases by post-translational modifications, and the mechanisms by which topoisomerases are directly involved in processes such as gene expression or the formation of DNA damage, similarly are poorly understood. At the same time, while studies are at long last explaining how certain classes of anti-topoisomerase agents function – particularly for small-molecule compounds – many of these insights have yet to be translated into the development of more efficacious drugs. Moreover, the available evidence suggests novel inhibitors and poisons remain to be identified, indicating that the therapeutic potential of topoisomerases is far from exhausted. Future cellular, biochemical, and structural efforts in this area will no doubt continue to offer up new surprises and insights into the action and use of these essential and complex molecular machines.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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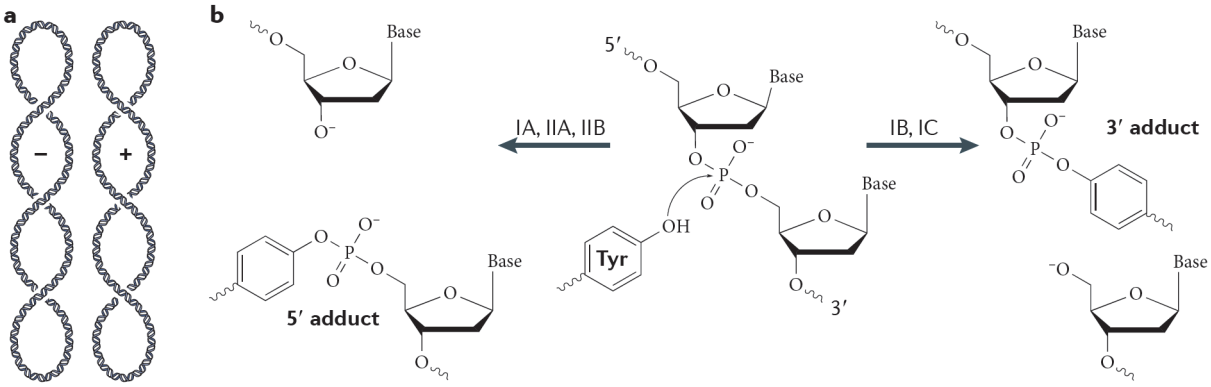

Fig 1. DNA cleavage and type I topoisomerase mechanisms

(A) Schematic of negative (−) and positive (+) plectonemic DNA supercoiling. The two forms can be distinguished by their right- and left-handed superhelical wrapping, respectively. (B) Schematic of the DNA cleavage reaction. Topoisomerases catalyze strand scission by forming a reversible, covalent enzyme–DNA adduct through their active site tyrosine. Type IB and IC topoisomerases become attached to 3′ DNA ends, and type IA and II topoisomerases attach to 5′ DNA ends. (C) Type IA topoisomerases pass a single-stranded DNA segment (yellow) through a transient break in a second, single DNA strand (green). The structure of *E. coli* topo III (a type IA topoisomerase) bound to single-stranded DNA is shown [210]. (D) Type IB topoisomerases nick one DNA strand (yellow), allowing one duplex end (green strand) to rotate with respect to the other around the remaining phosphodiester bond. The structure of human topo IB bound to duplex DNA is shown [20].

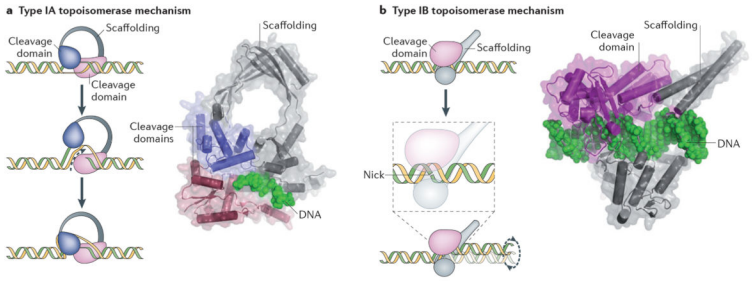

Fig 2. Type II topoisomerase mechanism

(A) Type IIA topoisomerases cleave both strands of a duplex DNA (green) and pass another duplex DNA (purple) through the transient break in a reaction coupled to ATP turnover. The cleaved strands are religated, and the products of the reaction are released from the enzyme. The movement of the purple DNA duplex is indicated by a dashed arrow. The DNA cleavage domains are homologous to those of type IA topoisomerases. The structure of the yeast topo II ATPase and DNA cleavage domains are shown [187,211]. (B) Type IIB topoisomerases use a duplex strand passage mechanism similar to that of type IIA enzymes, and share the same ATPase and cleavage domains but differ in overall tertiary structure. The structure of the topo VI holoenzyme is shown [51].

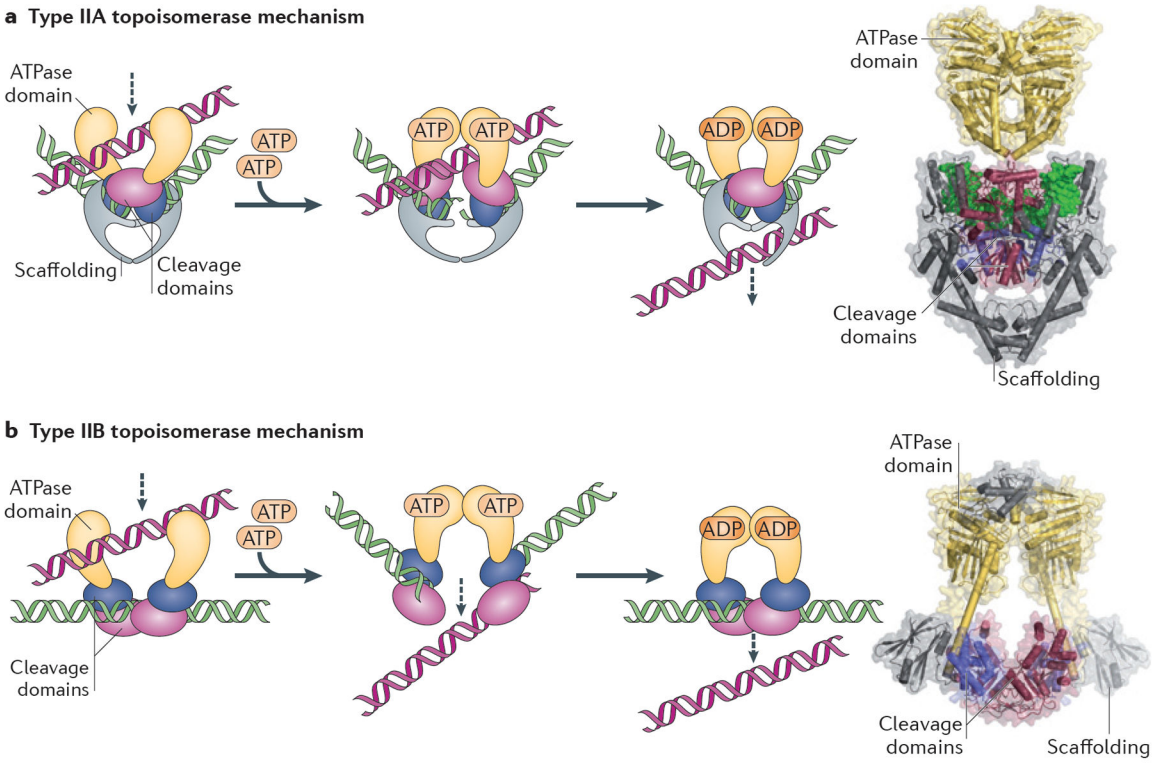

Fig 3. Topoisomerase functions during DNA replication

Schematic of topological problems that arise during DNA replication. The names of the topoisomerases that resolve these superstructures are listed. Topoisomerase action is indicated by a dashed arrow. (A) Replication elongation. As a replisome progresses, positive supercoils form ahead of the fork, and newly-replicated precatenanes behind it. If unresolved, precatenanes can give rise to tangled or catenated DNAs that can lead to abnormal DNA segregation upon entry into cell division. Unresolved positive supercoils can impede fork progression and terminate DNA replication prematurely. (B) Replication termination. Hemicatenanes are formed as two forks converge, and these must be resolved before chromosome segregation can occur. The unreplicated mother duplex can be resolved by topo III, together with a RecQ-family helicase (for example, the BLM helicase in eukaryotes), after which the single-stranded gaps are filled in (1). Alternatively, the unreplicated mother duplex can be replicated to form duplex linkages, which are then removed by a type II topoisomerase (2).

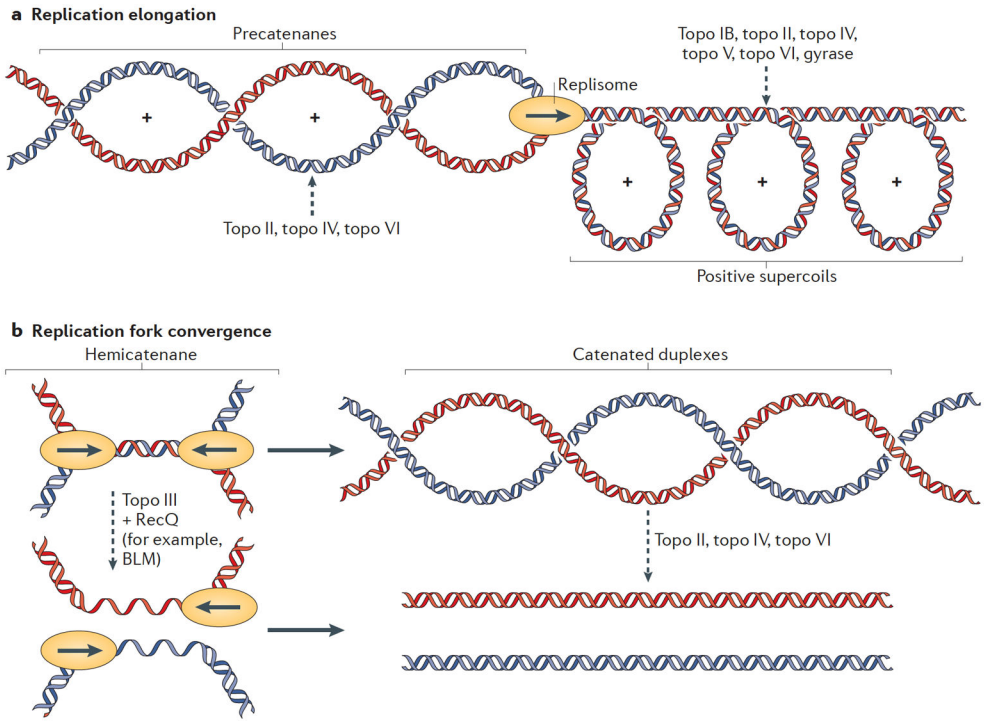

Fig 4. Topoisomerase functions during transcription and DNA repair

(A) Schematic of topological problems that arise during transcription. As RNA polymerase progresses, positive and negative supercoils form ahead and behind it, respectively. The names of the topoisomerases that act on these superstructures are listed; topoisomerase action is indicated by a dashed arrow. Topo IV can remove negative supercoils; however, it is listed in brackets because it is not the primary enzyme used by cells to resolve these superstructures. (B) Double-strand break repair through homologous recombination. The repair of broken DNA ends can proceed through a pathway that leads to the formation of a double-Holliday junction (HJ). Topo III, together with a RecQ-type helicase (such as BLM in eukaryotes) can resolve these junctions, generating disentangled chromosomes that have no crossovers between DNA ends (1). By contrast, the resolution of Holliday junctions by branch-endonucleases have a 50% chance of giving rise to repair chromosomes, the arms of which are now swapped (2).

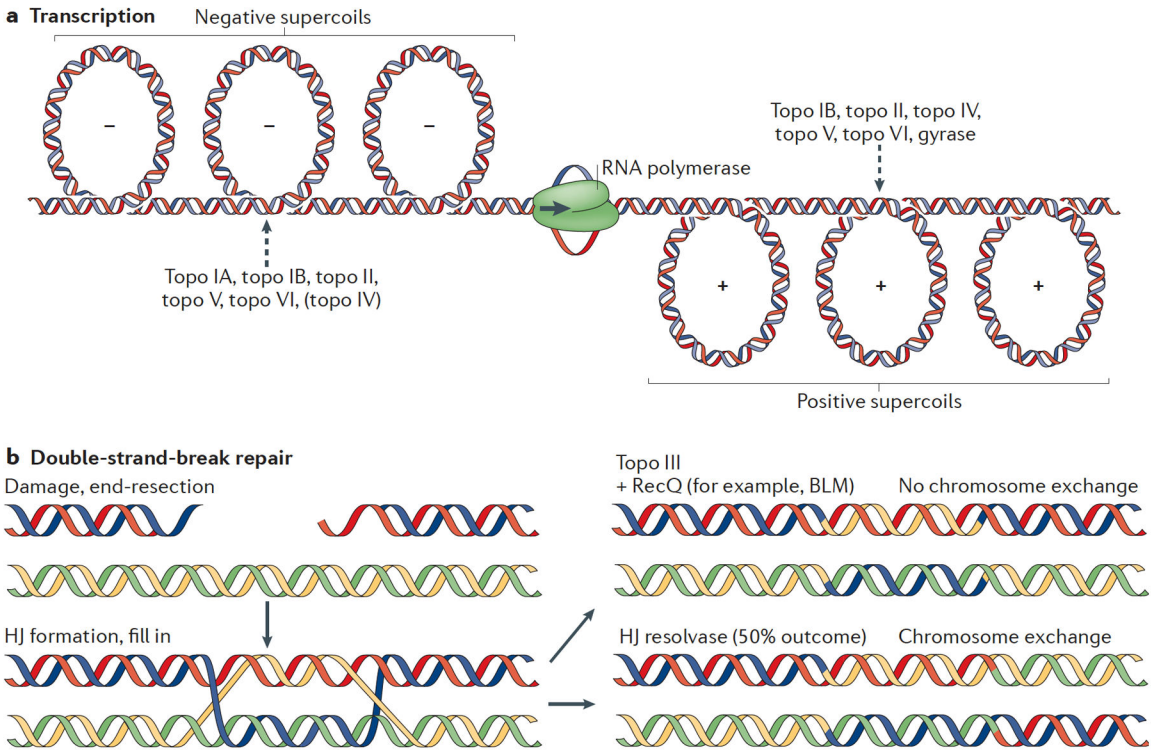

Fig 5. Inhibition or poisoning points for type II topoisomerases

Detailed schematic of the type II topoisomerase reaction cycle, showing all of the points where exogenous agents can disrupt function. During its reaction cycle, the topoisomerase initially binds one duplex DNA segment. Following binding, the topoisomerase can then associate with a second duplex DNA segment. ATP binding stimulates cleavage and opening of the first DNA, and passage of the second through the opening. Agents have been identified that interfere with: (1) DNA binding, (2) ATP binding, (3) DNA cleavage, (4) strand passage, (5) religation, and (6) product release. Only a partial list of all inhibitors and poisons is shown.