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

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Abstract | Topoisomerases are complex molecular machines that modulate DNA topology to maintain chromosome superstructure and integrity. Although capable of stand-alone activity *in vitro*, topoisomerases are frequently 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.

Supercoil
The result of duplex DNA twisting on itself in three-dimensional space.

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doi:10.1038/nrm3228

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 (supercoil) nucleic acid segments (FIG. 1a), interfering with appropriate gene expression. Furthermore, DNA repair and replication 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 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 that direct topoisomerase action. It also has become clear that a rich and growing variety of natural and synthetic agents blocks topoisomerase function for the purposes of evolutionary competition or therapeutic gain.

In this Review, we touch on a few of the many recent findings that have broadened our understanding of the parts played by topoisomerases in supporting chromosome biology and cell viability. Our goal is not to provide a comprehensive overview for those studying these enzymes, but rather to convey a sense of the vitality and richness of the topoisomerase field to the general biological community, particularly in relation to 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 owing 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 can also be assigned to 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 after their name

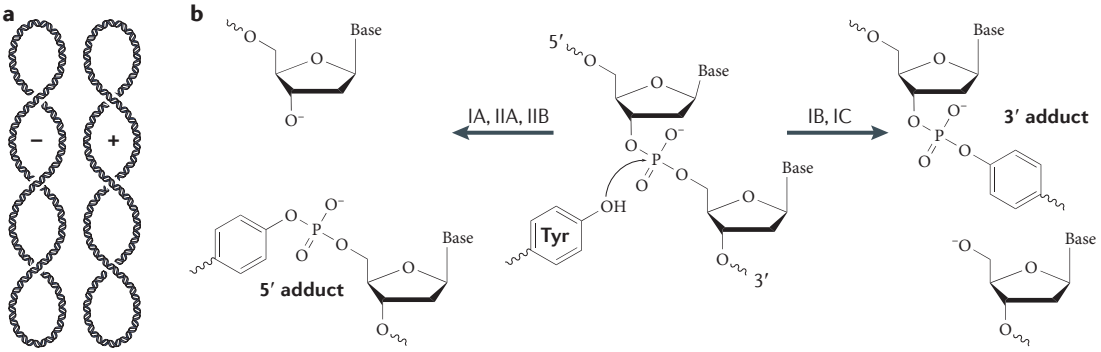

Figure 1 | **DNA cleavage. a** | Negative (–) and positive (+) plectonemic DNA supercoiling. The two forms can be distinguished by their right- and left-handed superhelical wrapping, respectively. **b** | The DNA cleavage reaction. Topoisomerases catalyse strand scission by forming a reversible, covalent enzyme–DNA adduct through their active-site tyrosine. Type IB and IC topoisomerases become attached to 3′ DNA ends, and type IA, IIA and IIB topoisomerases attach to 5′ DNA ends.

(for example, topo I or topo V) fall into the type I class, whereas those with an even Roman numeral after their name are type II. Topoisomerase subtypes — A, B or C — are then used to distinguish between enzyme families that have distinct amino acid sequences and/or global structures. Some topoisomerase subtypes further encompass multiple paralogues that have diverged from one another in terms of their specific activities. A brief overview of these groupings follows to provide a context for the subsequent discussion. For more extensive coverage of topoisomerase evolution, structure and mechanism, the reader is referred to REFS 1,2.

Type IA: single-strand-DNA '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 strand is navigated through the gap; following passage of the second DNA segment, the broken strand is resealed (FIG. 2a). Type IA topoisomerases comprise three distinct subfamilies that are found throughout all three cellular domains of life. These subfamilies are bacterial topo I (hereafter called topo IA), bacterial and eukaryotic topo III, and bacterial and archaeal reverse gyrase[1]. The primary activity of topo IA is to relax negatively supercoiled DNA[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, which is found exclusively in thermophilic bacteria and archaea, positively supercoils DNA and renatures melted DNA strands through the action of an ATP-dependent superfamily 2 helicase (SF2 helicase) 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 duplex 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. 2b). Type IB proteins preferentially bind positively or negatively supercoiled substrates rather than relaxed substrates[17,18], using an interaction surface outside of the 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 homologues 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 homologues in this group[15].

Type IC: a second class of swivelase. Thus far, type IC topoisomerases 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]. However, the active site of type IC enzymes 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 in DNA[26,27]. Only one topoisomerase variant, topo V, is presently known to constitute the type IC family[25].

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

Paralogues
Homologous genes separated by a duplication event that have evolved new functions.

Domains of life
The three major evolutionary branches (Bacteria, Archaea and Eukarya) of modern-day cellular lineages.

Negatively supercoiled DNA
DNA that is under-twisted (wound to <10.5 base pairs per turn). Negative supercoiling destabilizes the DNA, allowing complementary strands to be more easily denatured. By contrast, DNA becomes more resistant to denaturation when positively supercoiled (wound to >10.5 base pairs per turn).

Superfamily 2 helicase
A member of 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.

Abasic lesions
Regions of DNA damage in which the nucleobase has been excised from the sugar backbone.

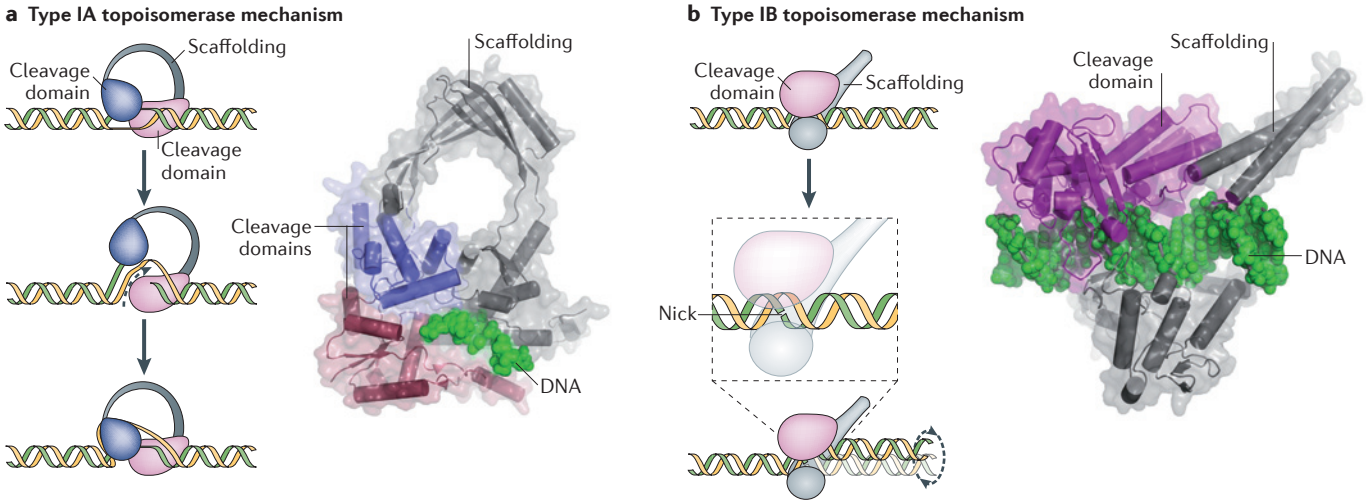

Figure 2 | **Type I topoisomerase mechanisms. a** | Type IA topoisomerases pass a single-stranded DNA segment (yellow) through a transient break in a second single DNA strand (green). The structure of *Escherichia coli* topo III (a type IA topoisomerase) bound to single-stranded DNA is shown on the right[210]. **b** | Type IB topoisomerases nick one DNA strand (green), allowing one duplex end to rotate with respect to the other around the remaining phosphodiester bond (yellow). The structure of human topo IB bound to duplex DNA is shown on the right[20].

a second intact duplex through the transient break[30–32] (FIG. 3a), 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 to disentangle long intertwined chromosomes and DNA catenanes[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 homologous subfamilies — eukaryotic topo II, bacterial topo IV, and bacterial and archaeal 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], whereas topo IV enzymes (and certain topo II isoforms) are robust decatenases that preferentially relax positively supercoiled substrates over those that are negatively supercoiled[38,39] (BOX 1). Interestingly, many type IIA topoisomerases show some ability to distinguish, and preferentially act on, regions of high DNA curvature or DNA crossovers owing to their ability to sharply bend DNA segments[37,40–43]. By contrast, *Saccharomyces cerevisiae* and *Drosophila melanogaster* topo II enzymes do not show any preference for one type of supercoiled DNA over another[44,45].

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. 3b) 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 arrangements of these elements in the primary sequence and their overall structural organizations differ substantially[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-strand DNA breaks that initiate meiotic recombination[46,52]. Thus far, only one family member, topo VI, is known to constitute the type IIB topoisomerase clade[46].

Topoisomerase families — why so many? Given the 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 whether different accessory factors and/or modifications that alter topoisomerase localization and activity are in use (see Supplementary information S1 (table)). Topoisomerases use a combination of these regulatory strategies in the majority of the nucleic acid transactions in which they participate. A discussion of the roles 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 alone has been estimated to condense DNA by two to three orders of magnitude[54] (FIG. 1a).

Catenanes
Topologically interlinked duplex DNA rings.

Plectonemic supercoiling
The natural tendency of supercoiled DNA to wrap back on itself, forming intramolecularly wound structures known as plectonemes. 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).

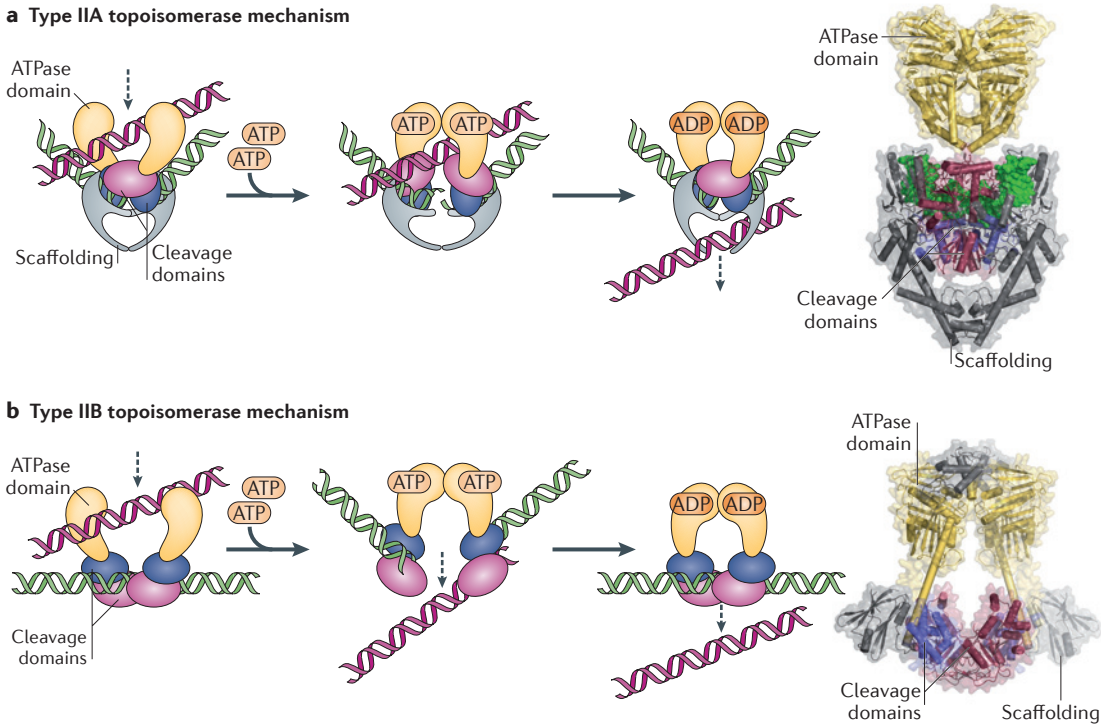

Figure 3 | **Type II topoisomerase mechanisms. a** | Type IIA topoisomerases cleave both strands of a duplex DNA (green) and pass another duplex DNA (pink) through the transient break in a reaction that is coupled to ATP turnover. The cleaved strands are religated, and the products of the reaction are released from the enzyme. The DNA cleavage domains are homologous to those of type IA topoisomerases. The structure of the *Saccharomyces cerevisiae* topo II ATPase and DNA cleavage domains are shown on the right[187,211]. **b** | Type IIB topoisomerases use a duplex strand passage mechanism similar to that of type IIA enzymes and have the same ATPase and cleavage domains but differ in overall tertiary structure. The structure of the *Methanosarcina mazei* topo VI holoenzyme is shown on the right[51].

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 domain[53,55]. More recent studies, however, suggest that there are additional layers of complexity to this view. For example, two closely related bacterial organisms, *Escherichia coli* and *Salmonella enterica* (specifically, *S. enterica* subsp. *enterica* serovar Typhimurium), have about 90% sequence identity across homologous genes[56] but have highly dissimilar superhelical densities, with *E. coli* DNA being substantially more underwound than that of *S. enterica*[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 amino-terminal domains, but is 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 bacteria and archaea has not been measured directly, multiple findings suggest that many species, particularly those that are adapted to growth at high temperatures, have 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 'set point' of the chromosome, is not fully understood.

Topoisomerases play another part in chromosome compaction by working with large condensation machineries (see Supplementary information S1 (table)) — principally a group of ATP-binding-cassette ATPases (ABC ATPases) known as structural maintenance of chromosomes (SMC) proteins[63–67]. SMCs, and their paralogous counterparts RAD50 (in eukaryotes) and SbcC and RecF (in bacteria), are conserved factors involved in multiple aspects of chromosome cohesion and condensation, and DNA repair[68]. SMCs and type IIA topoisomerases indirectly colocalize as part of the protein network that helps stabilize long-range contacts between chromosomal segments[67,69]. SMCs, or their affiliated accessory factors, have also been reported to directly associate with both topo II and topo IV. For example, the *D. melanogaster* subunit Barren (an orthologue of condensin H (also known as condensin complex subunit 2)) co-immunoprecipiates with topo II *in vitro*[66], and a dimerization region of the *E. coli* SMC homologue, MukB, interacts with the carboxy-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

Superhelical densities
Measurements of the over- or under-twistedness of DNA, generally expressed as the ratio by which the twist of the supercoiled state differs from that of the relaxed state.

ATP-binding-cassette ATPases
(ABC ATPases). A family of proteins that include membrane-bound transporters, DNA repair factors and structural maintenance of chromosomes (SMC) proteins. ABC ATPases possess a conserved ATPase site that is often formed at dimer interfaces. ATP binding results in conformational changes (often dimerization) that affect the associated partner proteins and substrates.

Box 1 | **Selectivity and function of type IIA DNA topoisomerase paralogues**

Vertebrates possess two topoisomerase IIA paralogues, topo IIα and topo IIβ, which have almost 77% sequence homology[198]. However, the carboxy-terminal regions of the two enzymes differ. In particular, the C terminus of topo IIα shifts the activity of the enzyme towards 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], whereas topo IIβ tends to be associated with DNA repair, transcription and development[82,83,134].

Bacteria also often use two type IIA topoisomerase paralogues (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 DNA-binding domain of bacterial type IIA topoisomerases is not conserved in eukaryotic topo II, indicating that the improved action of topo IV and topo IIα on positively supercoiled DNA arose independently during their evolution.

associated topoisomerase, although how stimulation is achieved is unresolved. At present, little is known about the effect 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 case of developmental strategies, the 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 DNA topoisomerase, TOP1mt, which is a paralogue of the nuclear type IB DNA topoisomerase, TOP1 (REFS 76,77). Mitochondrial DNA replication requires TOP1mt to form its regulatory displacement loop[77], which in turn is thought to control 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, as recent studies have uncovered 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, *oriC*; this supercoiling is regulated by the opposing activities of topo IA 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 colocalize 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, and inhibition of topo IB interferes directly with origin firing[80] (see Supplementary information S1 (table)). The formation of replication initiation complexes at the human *ORS8* origin, (a late-firing origin), as well as at the lamin B2 origin, has also 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–KU80 (also known as XRCC6–XRCC5) and poly(ADP-ribose) polymerase 1 (PARP1)[84]. Why topoisomerases are needed during these early stages of eukaryotic replication is not well understood, but may involve 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 the positive supercoiling that arises from DNA unwinding mediated by replicative helicases (FIG. 4a). 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 relaxation of positive supercoils, 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 are under evolutionary pressure to acquire particularly robust relaxases that can accommodate rapidly moving replication forks.

A second consequence of a progressing fork is the formation of intertwined daughter duplexes (termed precatenanes in circular chromosomes) (FIG. 4a). Such structures are produced as natural by-products of replication and, if left unchecked, give rise to tangled or catenated DNAs that can lead to abnormal DNA segregation on entry into cell division[92–96]. The duplex strand passage activity of type II topoisomerases plays a key part in resolving these topological linkages.

As two forks near one another, the unreplicated region between them represents a topological linkage, known as a hemicatenane, which must be resolved before chromosome segregation can occur (FIG. 4b). If the two strands of the hemicatenane can be fully replicated before resolution, the resulting duplex DNA segments become natural substrates for type II topoisomerases. Consistent with a need for these enzymes in resolving interchromosome crossovers, the removal or inactivation of topo II or topo IV can lead to hypercatenated DNAs and the production of broken chromosomes during cell division[96–101].

Origin
A chromosomal site for replisome assembly.

Origin recognition complex
A multisubunit protein complex that localizes to origins to initiate DNA replication in eukaryotes.

Poly(ADP-ribose) polymerase 1
An enzyme that adds chains of ADP-ribose to proteins as a response to DNA damage and cell death.

Precatenanes
Entangled daughter duplexes that are formed behind a replication fork during strand synthesis.

Hemicatenane
A junction between two double-stranded DNA molecules, in which one strand of one DNA molecule forms a duplex with the complementary strand on the other DNA molecule.

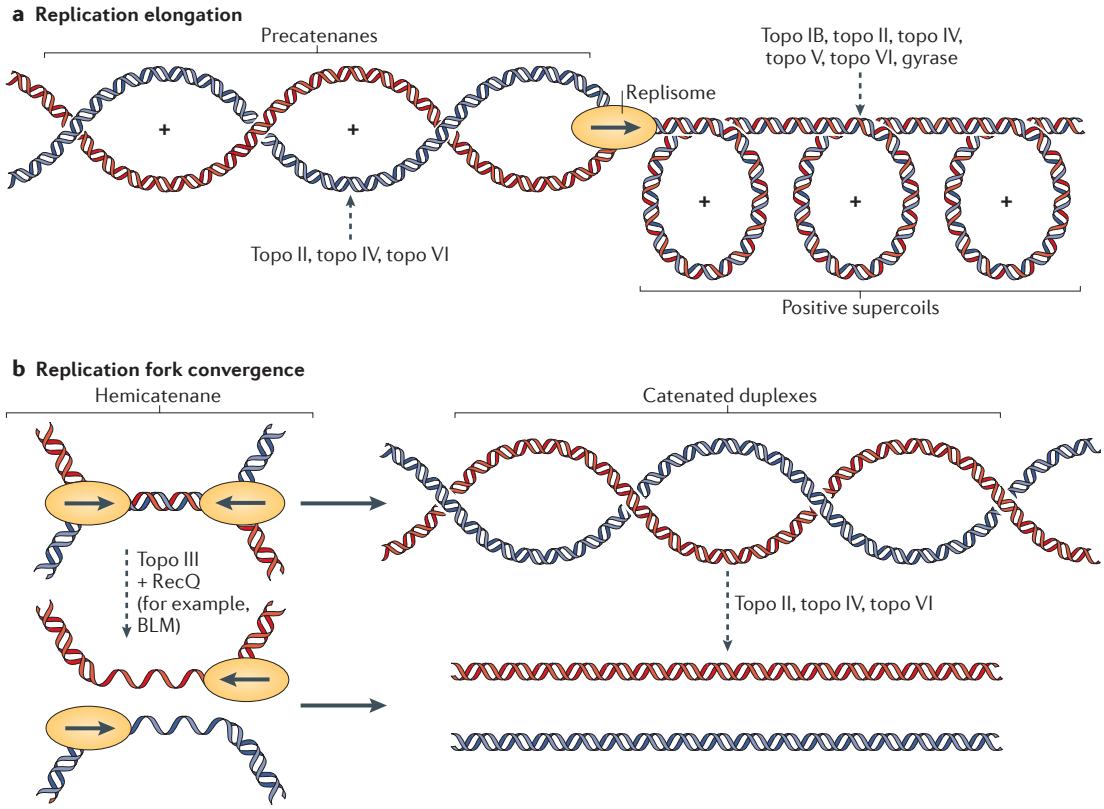

Figure 4 | **Topoisomerase functions during DNA replication.** Topological problems that arise during DNA replication. The names of the topoisomerases that resolve these superstructures are listed. Topoisomerase action is indicated by a dashed arrow. **a** | Replication elongation. As a replisome progresses, positive supercoils form ahead of the fork, and newly replicated precatenanes form behind it. If unresolved, precatenanes can give rise to tangled or catenated DNAs that lead to abnormal DNA segregation on entry into cell division. Unresolved positive supercoils can impede fork progression and terminate DNA replication prematurely. **b** | Replication fork convergence. Hemicatenanes are formed as two forks converge, and must be resolved before chromosome segregation can occur. The unreplicated parental duplex can be unlinked by topo III, together with a RecQ-family helicase (for example, Bloom's syndrome protein (BLM) in eukaryotes), after which the single-stranded gaps are filled in. Alternatively, the unreplicated parental duplex can be replicated to form a catenane with duplex linkages, which are then removed by a type II topoisomerase.

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, temperature-sensitive topo IV-encoding 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 DNA helicases to disentangle hemicatenated structures (see Supplementary information S1 (table)); in this partnership, the helicase may help generate single-stranded DNA to aid topo III function. The functions of these two enzyme classes are entwined, as evidenced by the fact that they frequently form stable or colocalized complexes along with auxiliary single-stranded-DNA-binding proteins such as Ssb (in bacteria) or one or more replication protein A (RPA)-like factors (that is, RecQ-mediated genome instability protein 1 (RMI1), present in all eukaryotes, and RMI2, a second protein present in metazoans)[9,103–105]. Consistent with the collaboration between these proteins being part of a functional 'resolvosome', ablation of the RecQ family partner of topo III in eukaryotes, Bloom's syndrome protein (BLM), leads to enhanced formation of ultrafine DNA tangles, termed microbridges, between condensed chromosomes[106]. The natural fusion of a RecQ-like SF2 helicase domain with a type IA topoisomerase module from reverse gyrase — which likewise can resolve hemicatenanes[13,107,108] — further highlights the frequent need for pairing RecQ helicase and type IA topoisomerase activities in the cell.

Topoisomerases in replication termination. With respect to replication termination, topoisomerases have been found to play a part in at least two instances. In bacteria, the completion of DNA synthesis can be assisted by chromosomally encoded termination regions (*Ter* sites), 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, terminus site-binding protein (Tus), to block progression of the replicative helicase, DnaB[109]. This effect may arise as a consequence of the increased levels of negative supercoiling resulting from the absence of topo IA: increased supercoiling can stimulate DNA unwinding by DnaB and

may also serve to reduce the duration of the Tus–DnaB interaction. In *S. cerevisiae*, topo II appears to facilitate fork progression at termination elements by localizing to such sites and working with the helicase Rrm3 to resolve the torsional stress arising from converging forks[110]. This controlled pausing at termination elements ensures complete replication and counteracts abnormal genome rearrangements or breaks arising from fork convergence at replication termination sites. Whether other, more specific connections exist between topoisomerases and replication termination remains to be determined.

Topoisomerases in chromosome segregation. On completion of DNA replication, 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 help unlink tangled DNA regions[112,113] (see Supplementary information S1 (table)). A physical association between *E. coli* topo IV and the actin-like protein MreB has also 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 kinases Aurora kinase B and Polo-like kinase 1 (PLK1)[115,116] (see Supplementary information S1 (table)). The biological rationale for these interactions is not fully understood; however, the colocalization of topo II with Aurora kinase B and PLK1 may be important for centromere resolution between sister chromatids. Additional links between topoisomerases and chromosome segregation are 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, precatenanes and supercoils), but also by topoisomerase-associated partner proteins. Other intriguing interactions have also been implicated in topoisomerase function during replication. For example, topo IV can associate both with the polymerase processivity clamp loader assembly, which may localize the enzyme to *oriC* to decatenate newly synthesized chromosomes[117], and with the factor SeqA, a protein that prevents replication re-initiation (by sequestering hemimethylated DNA within *oriC*) and stimulates the decatenation and supercoil relaxation activity of topo IV[118] (see Supplementary information S1 (table)). These connections further highlight cellular needs for particular topoisomerase activities during elongation and initiation, respectively.

Transcription and gene regulation

As with DNA 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. 5a). Left unchecked, these supercoiling alterations can lead to considerable 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 resultant suppression of R-loop formation[123,125]. Indeed, the C terminus of the *E. coli* topo IA interacts directly with RNA polymerase, an interaction that 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 crucial part in eukaryotic transcription, and topo IV and gyrase handling these substrates in bacteria[37,55,127–129].

Topoisomerases have also been linked to specific events during transcription, such as the activation or repression of particular promoters, and nucleosome remodelling. 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 *Schizosaccharomyces 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]. In addition, 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 and/or arginine-rich) proteins[131], thus regulating the localization of these splicing factors and enhancing their activity[132,133]; in turn, topo IB-mediated phosphorylation of SR proteins can be negatively regulated by the presence of poly(ADP-ribose)[133] (see 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 PARP1, KU70–KU80 and DNA repair proteins such as DNA-PK (DNA-dependent protein kinase)[81,83]. DNA cleavage and the subsequent recruitment of PARP1 and associated repair proteins appear to be necessary to exchange histone H1 for high mobility group (HMG) factor HMGB1 or HMGB2 and to stimulate transcription[83]. Similarly, in human acute promyelocytic leukaemia cell lines, topo IIβ associates with the promoter regions of genes that are regulated by retinoic acid receptor elements, initially stimulating their transcription; sustained transcriptional activation of these genes then leads to decreased transcript levels and the inhibition of granulocytic differentiation in a topo IIβ-dependent manner[134].

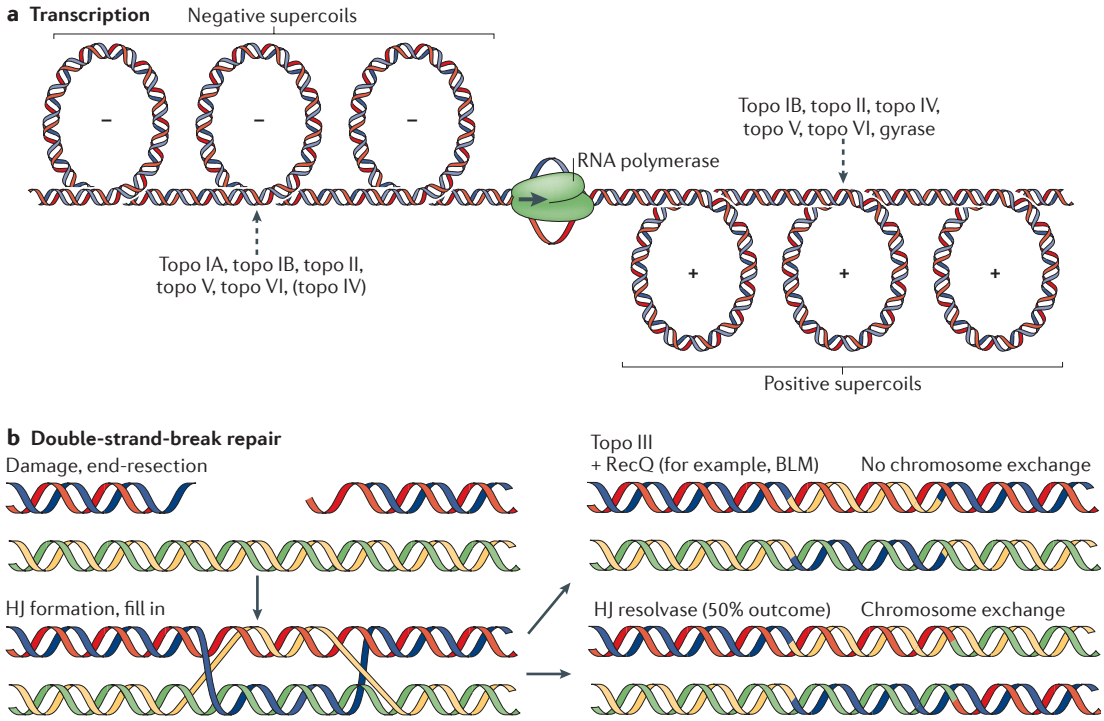

Figure 5 | **Topoisomerase functions during transcription and DNA repair. a** | Topological problems that arise during transcription. As RNA polymerase progresses, positive and negative supercoils form ahead of and behind it, respectively. The names of the topoisomerases that act on these superstructures are listed. Topo IV can remove negative supercoils; however, 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 Bloom's syndrome protein (BLM) in eukaryotes) can resolve these junctions, generating disentangled chromosomes that have no crossovers between DNA ends. By contrast, the resolution of Holliday junctions (HJs) by branch endonucleases (such as HJ resolvase) has a 50% chance of giving rise to chromosomes for which the arms have been reciprocally exchanged.

Some of these findings, such as those suggesting that topo IB directly phosphorylates target proteins or that cells rely on a potentially mutagenic topo IIβ-meditated DNA cleavage event to control gene expression, raise as many questions as they answer. Nonetheless, although 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

Another area in which topoisomerases have a crucial role is in forming and managing double-strand DNA breaks. As noted before, DNA cleavage by at least one topoisomerase, topo IIβ, has been implicated in the control of promoter activity[81,83,134]. However, the formation of double-strand breaks through a variety of topoisomerases and topoisomerase-like proteins also affects processes such as meiotic recombination, cell cycle checkpoint activation and DNA repair. For instance, during meiotic recombination, the type IIB (topo VI) topoisomerase A subunit homologue 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 is 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]. Other eukaryotes do not appear to possess any clear homologues of the ATP-binding B subunit plant of topo VI, and how SPO11 is activated is unknown at present[136].

The creation of a nick or a double-strand break is potentially a deleterious event 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 MRE11–RAD50–NBS1 (MRN) complex[136,137], RAD51 and DMC1-like factors[138,139], and the nuclease Sae2 (in yeast; also known as CtIP and RBBP8 in other eukaryotes)[140]. Meiotic recombination elicits a p53 damage response, which appears to be independent of the kinases ATM (ataxia telangiectasia mutated) and ATR

(ATM Rad3-related) 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 complexes. However, in these cases additional systems that induce cell cycle arrest and DNA repair can be activated (for example, the ATM and ATR pathways, and the BRCA1 or BRCA2 proteins[142,143]). When topoisomerases become inadvertently trapped in a covalent complex with DNA, they can be targeted for destruction by sumoylation- and ubiquitylation-mediated mechanisms, and the covalent tyrosine–DNA adducts can be repaired by enzymes such as tyrosyl-DNA phosphodiesterase 1 (TDP1) and TDP2 (REFS 143–152) (see Supplementary information S1 (table)). Some of these repair events may be promoted by collision encounters with proteins such as RNA polymerase[153–155].

Other evidence also links 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 the 2–3-nucleotide-long deletions that are 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 (REFS 157,158). Alternatively, topo IB may cleave at the scissile phosphate of a misincorporated ribonucleotide within or adjacent to 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 caused by DNA misalignments during repair. In this regard, it is interesting to note that many 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[162-164]. Thus, multiple classes of enzyme (types IB and IIB) may contribute to a steady-state background of damage events with mutagenic potential.

Topoisomerases further play an important part during and after the repair of damaged DNA strands. This is particularly true during homologous recombination, which generates interlinked intermediates that bear some similarities to those occurring during replication fork convergence (FIG. 5b). Recent work has found ample evidence for the participation of topo III in homologous recombination, during which, as in hemicatenane resolution during replication, it breaks up double Holliday junctions in concert with both a RecQ-type helicase (Sgs1 in *S. cerevisiae*[165] and BLM in metazoans[166,167]) and single-stranded-DNA-binding proteins such as Ssb[168], RMI1 (REF. 104) and RMI2 (REFS 105,169). Notably, the cooperative activity of this resolvosome allows homologous recombination to proceed without the generation of crossover products that exchange large chromosomal segments (as can occur when branch endonucleases resolve double Holliday junctions), and may therefore be the dominant means of resolving Holliday junctions in mitotic cells[104,166,167]. Topo III also may help recruit other homologous recombination proteins to the ends of broken DNA. For example, Sgs1, Dna2 and Rpa (also known as Rpf) derived from budding yeast can resect double-strand breaks 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 the ends of broken DNA[170,171]. How the disparate activities of topoisomerases, helicases and single-stranded-DNA-binding proteins collaborate to disentangle hemicatenated substrates is not known; studies of reverse gyrase may provide some mechanistic insights into this problem, as reverse gyrase is composed of an Sgs1-like SF2 helicase domain fused to a type IA topoisomerase element[107,108].

PTMs 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 structure on which topoisomerases preferentially act. Thus far, four types of topoisomerase PTM have been observed: phosphorylation[172], acetylation[173], sumoylation[145,151] and ubiquitylation[146].

For poisoned topoisomerases that are trapped on DNA, modification by sumoylation and ubiquitylation 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 is 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, RANBP2 and protein inhibitor of activated STATγ (PIASγ). In *Xenopus laevis*, pias4 (the homologue of PIASγ) has been linked to the sumoylation of topo IIα, a modification that appears to reduce the ability of topo IIα 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 to 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]. Some of these differences are almost certainly due to which particular topoisomerase site is being modified. In general, the regulation of eukaryotic topoisomerases through PTMs is a highly interesting, but still murky, area of research that undoubtedly holds significant surprises.

Scissile phosphate
The phosphate at which a nucleic acid backbone is broken by nucleophilic attack.

E3 ligases
Enzymes that attach ubiquitin or ubiquitin-like proteins to target proteins.

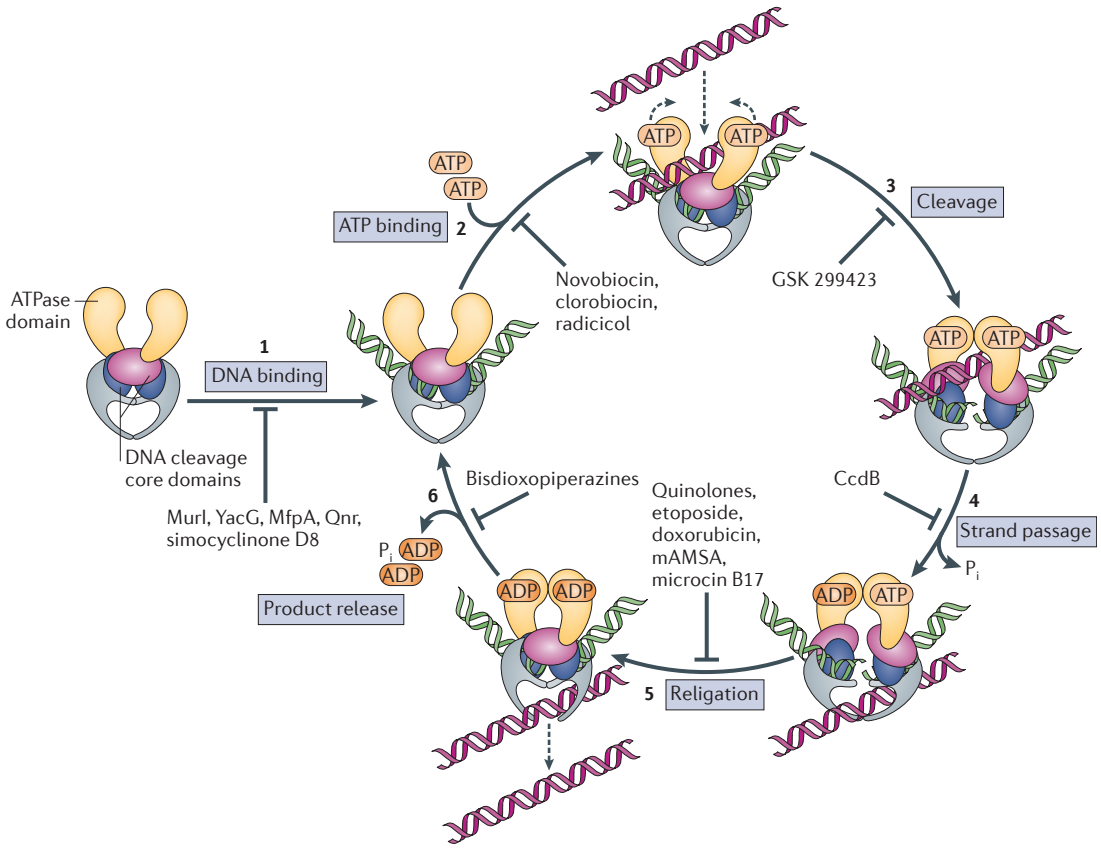

Figure 6 | **Inhibition or poisoning points for type II topoisomerases.** The type II topoisomerase reaction cycle and all of the points at which exogenous agents can disrupt function. During catalysis, the topoisomerase initially binds one duplex DNA segment (step 1). Following binding, the topoisomerase can then associate with a second duplex DNA segment. ATP binding (step 2) stimulates cleavage and opening of the first DNA (step 3), and passage of the second through the opening (step 4). The broken strands are then religated (step 5), and the product is released (step 6). Agents have been identified that interfere with each step, as indicated, but only a partial list of inhibitors and poisons is given here. mAMSA, amsacrine; MurI, glutamate racemase.

Topoisomerase inhibition

Although topoisomerases are indispensible for cell viability, it is clear that these enzymes 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 that lead to cell death. The evolution of general repair systems, as well as more specific ones such as the TDP system[147–150], help cells to deal with these problems. However, nature and humans have also been able to exploit topoisomerase inactivity and stalling through diverse means in order 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 (see 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. When considering protein-based antitopoisomerase 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 potential ease with which topoisomerase activity can be subverted or exploited and strongly suggests that more antitopoisomerase systems remain to be discovered.

On the biomedical side, topoisomerase inhibition has proved to be a highly versatile and useful approach for therapeutic intervention. Small-molecule agents targeting topoisomerases fall into two broad classes on the basis of their mode of action: inhibitors, which attenuate enzyme activity, and poisons, which stabilize DNA cleavage complexes (see FIG. 6 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 camptothecin) have been used for decades, it is only relatively recently that their mechanisms of action have 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 active site of the enzyme[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′ ends of a broken DNA strand to prevent resealing and release[42]. Other compounds act in a different manner, by blocking ATPase activity (for example, novobiocin and clorobiocin[185,186], bisdioxopiperazines[187] and radicicol[188]), DNA binding (simocyclinone D8)[189] or DNA cleavage (GSK 299423)[190]. These compounds have been imaged in the presence of their respective targets; together with biochemical studies, these findings have not only highlighted the molecular determinants that enable drug binding and explained the inhibitory effects of these agents, but also demonstrated that there is a rich abundance of molecular scaffolds capable of inhibiting topoisomerases (see Supplementary information S2 (figure)). At present, no small-molecule agent is known to target type IA topoisomerases, although recent analyses have suggested that such compounds would probably be cytotoxic[191]. A hopeful, but presently unrealized, goal for these combined efforts is to point the way towards the design of new inhibitors that have 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 recently discovered 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 some antitopoisomerase agents can kill by a second pathway[194]. In eukaryotes, treatment of cells with poisons leads to the ubiquitylation 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 proteins have shown some promise in promoting tumour cell killing when administered with topoisomerase poisons. Initially, the PARP1 inhibitor NU1025 was reported to potentiate damage mediated by the topo IB 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 PARP1 are more sensitive to the topo II-inhibitor C-1305 than wild-type cells[196], and that the PARP inhibitor ANI (4-amino-1,8-naphthalimide) enhances the activity of the topo II inhibitor doxorubicin against liver cells[197]. Fully mapping the pathways and mechanisms by which cells contend with antitopoisomerase 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 topoisomerases were first discovered more than 40 years ago, the study of these enzymes 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 are many significant gaps in our knowledge of 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 are similarly poorly understood. At the same time, although studies are at long last explaining how certain classes of antitopoisomerase 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 that 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 generate new surprises and insights into the action and use of these essential and complex molecular machines.

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Acknowledgements
The authors thank K. Drlica for thoughtful discussions and editing. This work was supported by a US National Science Foundation pre-doctoral fellowship (to S.M.V.) and the US National Cancer Institute (grant CA077373 to J.M.B.).

Competing interests statement
The authors declare no competing financial interests.

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