



Human topoisomerases and their roles in genome stability and organization

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Abstract | Human topoisomerases comprise a family of six enzymes: two type IB (TOP1 and mitochondrial TOP1 (TOP1MT), two type IIA (TOP2A and TOP2B) and two type IA (TOP3A and TOP3B) topoisomerases. In this Review, we discuss their biochemistry and their roles in transcription, DNA replication and chromatin remodelling, and highlight the recent progress made in understanding TOP3A and TOP3B. Because of recent advances in elucidating the high-order organization of the genome through chromatin loops and topologically associating domains (TADs), we integrate the functions of topoisomerases with genome organization. We also discuss the physiological and pathological formation of irreversible topoisomerase cleavage complexes (TOPccs) as they generate topoisomerase DNA–protein crosslinks (TOP-DPCs) coupled with DNA breaks. We discuss the expanding number of redundant pathways that repair TOP-DPCs, and the defects in those pathways, which are increasingly recognized as source of genomic damage leading to neurological diseases and cancer.

Tyrosyl-DNA phosphodiesterases (TDPs). Referring to TDP1 and TDP2, enzymes that excise topoisomerase–DNA crosslinks by hydrolysing 3'-phosphodiester and 5'-phosphodiester bonds, respectively. TDP2 also excises TOP3–RNA crosslinks.

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DNA topoisomerases are present in all domains of life to resolve a wide variety of topological problems arising from the length of the human double-helix DNA polymer (about 3×10^9 bp) as it is folded, bent and highly compacted into the cell nucleus while remaining accessible to RNA and DNA polymerases. In addition, each human cell contains 100–1,000 copies of circular, ~16,000 bp mitochondrial DNA (mtDNA), as well as long and folded RNAs that are even more abundant than DNA and present in all subcellular compartments.

Human topoisomerases and the associated repair enzymes tyrosyl-DNA phosphodiesterases (TDPs; TDP1 and TDP2) are located both in the nucleus and in mitochondria; in addition, cytoplasmic RNAs are handled by TOP3B and TDP2 (REFS^{1,2}) (Supplementary Table 1).

In this Review, we first provide basic information on the six human topoisomerases. We discuss their specific and overlapping roles as regulators of nucleic acid topology and metabolism, thereby complementing other recent reviews^{1,3–8}. We emphasize the need to further define the roles of topoisomerases in genome organization and stability, as well as the increasingly recognized implication of topoisomerases in generating deleterious genomic lesions associated with irreversible topoisomerase cleavage complexes (TOPccs). We describe the molecular mechanisms by which abortive TOPccs damage the genome and the multiple pathways that repair those cellular lesions and how they relate to genomic instability.

DNA and RNA topological problems

To manage the topology of the long, folded and intertwined DNA and RNA polymers that are attached to scaffolding structures and are metabolically and dynamically processed by large molecular machines (such as transcription, DNA replication, chromatin remodelling and DNA repair complexes), human cells use their six topoisomerases often redundantly, but also in specific ways depending on the topological problem, the surrounding cellular structures and the differentiation status of the cell. This section outlines topological problems and the molecular solutions provided by each of the topoisomerases. Additional details are provided for the TOP3 enzymes, which were not covered in depth in our previous Review¹.

DNA and RNA topological conversions by topoisomerases

One of the most common changes in DNA topology is the opening of the double helix by helicases during transcription and replication and by ATPase translocase 'motors' during chromatin remodelling (FIG. 1a,b). In addition, each nucleosome absorbs about two left-handed (negative) supercoils. Due to the length of the genome, its attachment to the nuclear scaffold and the viscosity of the nuclear milieu, DNA cannot freely rotate on its axis and the opening of the double helix generates DNA overtwisting (positive DNA supercoiling (Sc⁺)) in front (downstream) of the helicase or other motor proteins, and DNA under-twisting (negative

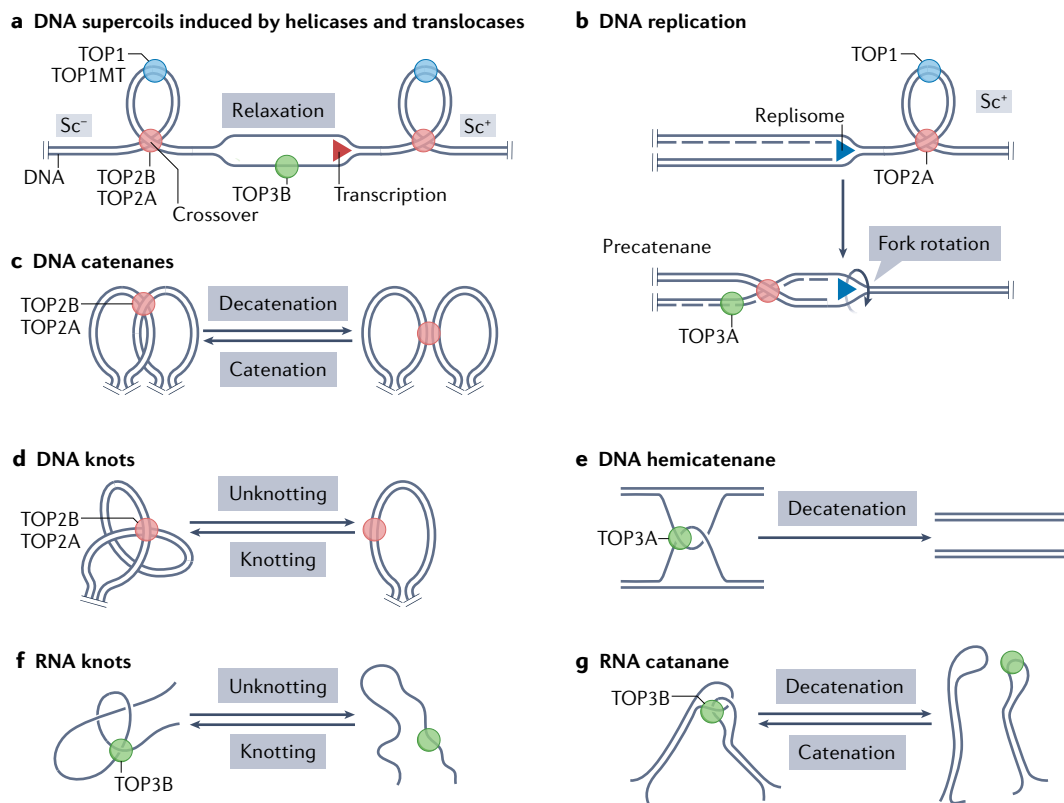


Fig. 1 | Topological problems solved by human topoisomerases. Sites of action of topoisomerases (red, blue and green circles), duplex nucleic acids (parallel lines) without their double-helix structure and DNA segments whose ends are not free to rotate on their helical axis due to steric and physical constraints (parallel pegs) representing topological domains. **a** | Twin supercoiled domain model¹⁴. Opening the DNA duplex by helicases and ATPase translocases within a topological domain during transcription and chromatin remodelling generates positive DNA supercoiling (Sc^+) ahead of the moving helicase (or translocase) and negative supercoiling (Sc^-) behind it. Excessive supercoiling generates writhe that brings together distant regions of DNA that form crossovers. Topoisomerase 1 (TOP1), mitochondrial TOP1 (TOP1MT), TOP2A and TOP2B remove both Sc^+ and Sc^- by incising double-stranded DNA; TOP3B (and TOP3A) relax hyper-negative supercoiling by nicking and closing single-stranded DNA segments. **b** | Replication forks generate Sc^+ in front of the translocating replisome, which is removed by TOP1 and TOP2A. If the replisome swivels due to the twisting force, Sc^+ diffuses behind the replisome and generates precatenanes, which are removed by TOP2A³⁷. TOP3A may also remove precatenanes if they include single-stranded DNA segments. **c** | TOP2A and TOP2B decatenate topological domains by passing one DNA molecule through the double-stranded DNA break made in the other DNA molecule (double-strand passage). **d** | TOP2A and TOP2B resolve DNA knots by double-strand passage. **e** | TOP3A in association with the Bloom syndrome protein (BLM)–TOP3A–RecQ-mediated genome instability proteins (RMI1/2) (BTR) dissolvosome complex (not shown) resolves DNA hemicatenanes arising during replication and recombination by passing a single strand of DNA through a break made in another DNA strand (single-strand passage)^{6,357}. **f** | TOP3B is the only RNA-only topoisomerase¹⁴; it resolves intramolecular RNA intertwinings (knots) by single-strand passage. **g** | TOP3B can also resolve RNA catenanes by single-strand passage¹⁹⁷. See Supplementary Fig. 1 for biochemical, molecular and structural details.

DNA supercoiling

The amount of DNA twist, which is the number of crossovers of the two strands across each other; writhe is a measure of the double helix winding around itself. Positive DNA supercoiling (Sc^+) is defined by increased twist and/or writhe; negative DNA supercoiling (Sc^-) is the opposite.

Hemicatenanes

Crossovers of two strands of DNA originating from different DNA molecules.

supercoiling (Sc^-) behind (upstream) them. Excessive torsional strain first changes the helical twist (number of crossovers of the two strands across each other) until the entire duplex of DNA (or RNA) winds around itself (writhe) and flips into alternative plectonemic structures with crossover segments and entanglements. TOP1, TOP1MT, TOP2A and TOP2B readily remove both Sc^+ and Sc^- (FIG. 1; Supplementary Fig. 1) until the torsional strain is eliminated. The other two topoisomerases, TOP3A and TOP3B, just relax hyper-negative supercoiling, as they can only form cleavage complexes in single-stranded nucleic acids (FIG. 1a,e–g).

Other topological difficulties arise during replication and chromatin loop formation when duplex DNA molecules form catenanes. Decatenation reactions are

carried out by TOP2 enzymes¹ (FIG. 1c; Supplementary Fig. 1d,m). TOP2 enzymes can also resolve precatenanes formed behind replication forks (FIG. 1b) and unknot duplex DNA molecules (FIG. 1d). Such knots have been proposed to contribute to chromatin organization^{9,10} and recombination¹¹ in yeast models.

The eukaryotic type IA topoisomerases, TOP3A and TOP3B, catalyse a unique range of topological changes requiring the passage of a single strand of nucleic acid through another (FIG. 1e–g). DNA hemicatenanes are the preferential substrate for TOP3A⁶ (FIG. 1e). A transcript isoform of TOP3A is also essential for mtDNA replication and segregation^{12,13} (BOX 1). Unique among the human topoisomerases, TOP3B — similar to its yeast and bacterial paralogues Top3 and Topo III, respectively — acts as a

Holliday junctions

Branched DNA structures consisting of four double-stranded arms joined together. Double Holliday junctions form hemicatenanes that are resolved by the Bloom syndrome protein (BLM)–topoisomerase 3A (TOP3A)–RecQ-mediated genome instability proteins (RMI1/2) (BTR) dissolvasome complex.

dual DNA and RNA topoisomerase^{14,15}. RNA knots and catenanes are the likely cellular substrates for TOP3B¹⁴ (FIG. 1f,g).

Topoisomerase biochemistry. All topoisomerases act by cleaving and rejoining the nucleic acid backbone using a tyrosine nucleophilic residue¹ (Supplementary Fig. 1a,b). Each break results from the formation of a covalent bond between the topoisomerase catalytic Tyr residue and one end of the broken nucleic acid. The polarity is specific for each topoisomerase: 3' end bonding for TOP1 and TOP1MT, and 5' end bonding for the other topoisomerases. The covalent catalytic intermediates are referred to as the TOPccs. Vertebrate TOP2A and TOP2B and yeast Top2 (type II topoisomerases) function as homodimers and generate DNA double-strand breaks (DSBs) with a canonical 5' base overhang^{1,16,17} (Supplementary Fig. 1d).

Four main biochemical characteristics differentiate topoisomerases: polarity (3' versus 5' tyrosyl linkage); substrate specificity (single-stranded versus double-stranded DNA or RNA); nucleic acid relaxation mechanisms (DNA strand rotation in the case of TOP1 and TOP1MT versus crossover inversion in the case of TOP2A, TOP2B, TOP3A and TOP3B, and RNA crossover inversion in the case of TOP3B); and cofactor

requirements (ATP and/or magnesium) (Supplementary Fig. 1d,e; Supplementary Table 1).

TOP1 and TOP1MT (type IB topoisomerases) cleave only one strand of double-stranded DNA by forming the 3'-phosphotyrosyl linkage (3' DNA–protein crosslinks (DPCs)) (Supplementary Fig. 1a; Supplementary Table 1), which relates them to the prokaryotic Tyr recombinases^{18,19}. Unlike type IA topoisomerases (TOP3A and TOP3B) (see below), TOP1 and TOP1MT only process double-stranded DNA and relax DNA supercoils processively and without metal or protein cofactor by controlled rotation of the broken strand around the intact strand^{20,21}, with a preference for nucleosome-free DNA^{10,22} (Supplementary Fig. 1c). Hence, TOP1 and TOP1MT are also referred to as 'swivelases' or 'DNA untwisting enzymes'²³.

TOP2A and TOP2B (type IIA topoisomerases) act as 'writhases' or 'crossover invertases', which enables them to resolve not only supercoils but also catenanes and knots by passing one DNA duplex through the DSB made in another duplex at crossover points between the two duplexes (FIG. 1c,d; Supplementary Fig. 1d,m; Supplementary Table 1). Note that TOP2A and TOP2B also generate DNA single-strand breaks (SSBs) when only one protein of the dimer breaks the DNA¹. The main differences between TOP2A and TOP2B are in the polypeptide sequence of their carboxy-terminal domains (CTDs) (Supplementary Fig. 1g), their cell cycle regulation (TOP2A increases in abundance throughout S phase, is degraded at the end of mitosis and is dispensable in terminally differentiated cells)^{8,24} and the fact that TOP2A preferentially relaxes Sc⁺ whereas TOP2B relaxes Sc⁻ and Sc⁺ similarly^{17,25}.

Both the type IIA topoisomerases and the type IA topoisomerases — TOP3A and TOP3B — cleave nucleic acids by forming 5' DPCs with the help of magnesium positioning nucleic acids in their active site^{3,6} (Supplementary Fig. 1g,h). However, type IA topoisomerases differ from type IIA topoisomerases in their selectivity for binding single-stranded rather than double-stranded nucleic acids, their lack of ATP requirement and the activity of TOP3B as a dual DNA and RNA topoisomerase^{6,14,26} (FIG. 1e–g; Supplementary Table 1). TOP3 enzymes are also dependent on protein scaffolding cofactors: RecQ-mediated genome instability protein 1 (RMI1) and RMI2 for TOP3A, and Tudor domain-containing protein 3 (TDRD3) for TOP3B^{6,15,27}. TOP3A, RMI1 and RIM2 form the heterotrimeric Bloom syndrome protein (BLM)–TOP3A–RMI1/2 (BTR) dissolvasome complex for recombination²⁸ and resolution of double Holliday junctions associated with DNA replication⁶. TOP3A can also associate with other helicases and translocases including the helicase Fanconi anaemia group M protein (FANCM) to suppress sister chromatid exchanges and promote replication restart²⁹. The TOP3B partner TDRD3 forms a multimeric RNA regulator complex with fragile X mental retardation protein¹⁵. In addition to its role in TOP3B recruitment, TDRD3 has also been shown to increase the processivity of TOP3B^{27,30} (Supplementary Fig. 1n).

TOPccs are normally transient and 'self-reversible', as the deoxyribose hydroxyl ends of the cleaved DNA

Box 1 | Mitochondrial topoisomerases

Each human mitochondrial DNA (mtDNA) molecule consists of circular DNA of 16,569 bp assembled in a nucleoid, which is tethered to the inner mitochondrial membrane^{338,367}. Replication of the G-rich 'heavy' strand originates from the OriH sequence in the ~1 kb non-coding region. Replication proceeds unidirectionally for approximately two thirds of the mitochondrial genome until it reaches the light strand replication origin (OriL), after which it becomes bidirectional^{338,367}. Bidirectional replication and transcription of mtDNA represent model systems for DNA supercoiling, R-loops, G quadruplexes and decatenation. Termination of replication in the non-coding region generates hemicatenated daughter molecules¹². The mitochondrial isoform of topoisomerase 3A (TOP3A)^{186,366} is required at the end of replication to decatenate the daughter mtDNA molecules, which are intertwined across their OriH regions, a process that is facilitated by mitochondrial TOP1 (TOP1MT) and is independent of the usual TOP3A-interacting Bloom syndrome protein (BLM)–TOP3A–RecQ-mediated genome instability proteins (RMI1/2) (BTR) dissolvasome complex¹². Mutations in TOP3A have been reported in individuals with combined Bloom and mitochondrial syndromes characterized by dilated cardiomyopathy, mtDNA depletion in muscles and progressive external ophthalmoplegia syndrome^{13,187}. In the fruit fly, inactivation of mitochondrial TOP3A results in defective genome integrity and mitochondrial functions with accelerated ageing and infertility^{186,193} (TABLE 1).

TOP1MT efficiently relaxes both positive DNA supercoiling (Sc⁺) and negative DNA supercoiling (Sc⁻)^{369,370}, and therefore complements TOP3A, which can only remove Sc⁻. Cells lacking TOP1MT accumulate Sc⁻ mtDNA¹⁷³, and dual depletion of TOP3A and TOP1MT further reduces mtDNA decatenation and the number of nucleoids¹². TOP1MT is not required for mtDNA transcription^{175,176,324} and for mouse development^{173,325}, implying that other topoisomerase(s) relax Sc⁺ in mtDNA¹⁷³. TOP1MT also facilitates mitochondrial translation^{176,177}.

TOP2A and TOP2B, which efficiently remove Sc⁺, have been reported in mitochondria of human cells, and in bovine mitochondria and mouse brain and sperm mitochondria^{173,370,371}. However, mitochondrial TOP2 polypeptides were undetectable in another study following stringent biochemical mitochondrial fractionation¹². Hence, mitochondrial localization and activity of TOP2A and TOP2B may depend on specific cell growth conditions and tissue, purification procedures or are present at the surface of mitochondrial membranes.

Both tyrosyl-DNA phosphodiesterase 1 (TDP1)³⁷² and TDP2 (REF.³⁷³) are active in mitochondria. However, similar to the majority of mitochondrial proteins, they do not bear identifiable mitochondrial targeting sequences.

act as nucleophiles towards the tyrosyl–DNA phosphodiester bonds¹. Efficient substrate resealing requires the alignment of the ends of the broken DNA inside the TOPcc (Supplementary Fig. 1a,b). Such alignment is provided both by the base pairing and stacking of duplex DNA and by atomic interactions with the topoisomerase. If the TOPccs fail to reseat, they become topoisomerase DNA–protein crosslinks (TOP-DPCs): stalled (abortive or irreversible) TOPccs consisting of a DNA–protein (topoisomerase) crosslink at the end of a break, which require DNA repair for their resolution (see below). Trapping of TOP-DPCs is the mechanism of action of widely used anticancer and antibacterial chemotherapies^{17,31,32} (Supplementary Box 1). Multiple endogenous and environmental factors also induce TOP-DPCs^{1,17}.

Roles in replication and transcription

Transcription and replication constantly change DNA topology, and thus require the activities of topoisomerases.

Replication of the nuclear and mitochondrial genomes.

As expected for genes involved in regulating DNA replication, *TOP1*, *TOP2A* and *TOP3A* transcript levels are linked with cell proliferation^{6,33–35}, and their expression is correlated with the proliferation marker Ki67 and replicative polymerases and helicases in the cancer cell line encyclopaedia database³⁶ (Supplementary Fig. 1).

During replication fork progression, topoisomerases dissipate the supercoiling generated by DNA unwinding³⁷. Without topoisomerase activity, the formation of Sc⁺ in front of the replication fork and the resulting tightening of the DNA duplex with the potential formation of plectonemes would block replisome movement. Being a highly processive swivelase, TOP1 can remove supercoils in short DNA segments devoid of nucleosomes, whereas TOP2A, acting as a writhase, likely acts at crossover points where the DNA enters and exits nucleosomes^{22,38} (FIG. 1b). Yeast Top2 has also been shown to efficiently remove Sc⁺ in single-molecule model systems³⁹, and yeast Top1 was shown to act at replication pause sites in ribosomal gene arrays⁴⁰. Unexpectedly, yeast Top1 (and Top2 when acting as a Top1 backup) enforces replication pausing by the topoisomerase 1-associated factor (Tof1) at replication fork barriers by binding to Tof1 and preventing head-on collisions between replication and transcription machineries⁴⁰. Whether the vertebrate paralog of Tof1 has the same coordinated functions with TOP1 and TOP2A requires testing⁴¹.

The topology of DNA behind replisomes depends on whether the frontal Sc⁺ forces the replisome to rotate around the DNA axis. Such rotation diffuses the Sc⁺ behind the replisome, generating braided daughter strands that are referred to as precatenanes³⁷ (FIG. 1b), and can only be resolved by TOP2 (or potentially by TOP3 if the precatenanes contain a single-stranded segment of DNA)⁴². Rotation of the replisome is believed to be rare during replication due to the large protein complexes assembled around the replisome and the removal of Sc⁺ by TOP1 and TOP2. However, rotation

has been reported in yeast^{43,44} as replisomes converge or in mammalian cells at common fragile sites, which are chromosomal regions that are sensitive to replication stress and prone to DNA breaks^{45,46}. In addition to its role in replication fork pausing, Tof1 can also stabilize replisomes by preventing fork rotation⁴³.

The role of TOP2A in replication termination appears to be limited, as newly replicated DNA is decatenated during replication (FIG. 1b), and most decatenation is complete upon entry into prophase⁸. Nevertheless, Top2 and TOP2A may be crucial for replication termination in highly repetitive sequences, such as telomeres, centromeres and ribosomal DNA, where Top2 and TOP2A activity may maintain segments of sister chromosomes catenated to assist in their condensation and cohesion, while subsequently allowing segregation at anaphase⁸.

As replication proceeds within replication domains (of 400–800 kb) that match topologically associating domains (TADs)⁴⁷, daughter DNA molecules are catenated due to the helical structure of the replicating DNA. TOP2A is essential for decatenating daughter DNA molecules and resolving sister chromatid intertwinings^{1,8,48} (FIG. 1c). In *Xenopus laevis* oocytes, following dissociation of the replicative CMG helicase at converging replisomes, catenated DNA loops are disentangled at their crossover points by TOP2A⁴⁹.

TOP3A is a key enzyme for the completion of replication because it is the sole topoisomerase capable of removing hemicatenanes arising from converging replication forks⁴² and from recombination intermediates^{1,6} (FIG. 1e). The BTR dissolvasome complex has been shown to sense replication protein A (RPA)-coated single-stranded DNA, which may explain its role in restarting stalled replication forks^{50,51}. At the end of S phase, in addition to its role in resolving ultra-fine DNA bridges between sister chromatids⁵², TOP3A in coordination with the helicase Plk1-interacting checkpoint helicase (PICH; also known as ERCC6L) has been shown to generate replicated DNA Sc⁺ segments, which are preferential substrates for TOP2A-mediated decatenation, thereby enabling the segregation of chromatin fibres during anaphase⁵³. These observations suggest that TOP3A can couple its activity with different helicases: BLM in the dissolvasome, FANCM at stalled replication forks and PICH during mitosis. The potential activity of TOP3A for removing hemicatenanes during replication in human cells needs to be further established.

In mitochondria, TOP3A acts as a hemicatenane resolvase; in its absence, newly replicated mtDNA molecules remain intertwined at the mtDNA replication origin, a phenotype which is further accentuated in the absence of TOP1MT¹² (BOX 1). Notably, TOP3A acts independently of the BTR dissolvasome (as BLM, RMI1 and RMI2 do not enter mitochondria), which could imply that a mitochondrial helicase replaces the dissolvasome in working together with TOP3A.

Topoisomerases and transcription. Topoisomerases are needed to resolve the topological tensions arising during transcription. The classical twin domain model

Topologically associating domains

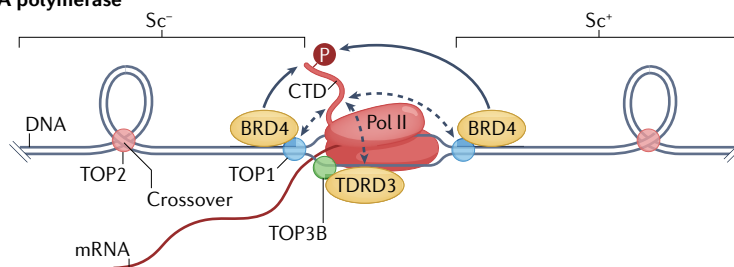
(TADs). In interphase chromosomes, genomic regions in which interactions between loci are enriched compared with interactions with loci outside the domain.

predicts a requirement for topoisomerase activity to relax Sc^+ ahead of the RNA polymerase and Sc^- behind it^{54,55} (FIG. 1a). Both TOP1 and TOP2 can act behind transcription complexes to remove Sc^- (FIG. 2a): TOP1 in underwound duplex DNA segments and TOP2 at crossovers between DNA duplexes. Whereas the Sc^+ generated ahead of the RNA polymerase II (Pol II) complex is readily removed by TOP1 (REF.⁵⁶), the Sc^- behind Pol II (FIG. 2a) appears to be less efficiently processed. This disparity provides an explanation for the generation of ‘transcription-induced supercoiling’⁵⁷. Transcription-induced Sc^- has been mapped genome-wide using psoralen, which binds DNA proportionally to Sc^- levels^{58–61}. It is plausible, but not formally demonstrated, that the accumulation of Sc^- could be attenuated when transcription complexes follow each other in

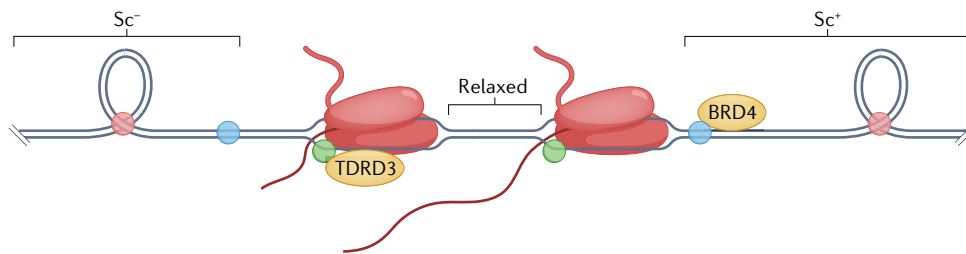
tandem, as the Sc^- generated by a transcription complex could be absorbed by (and facilitate the translocation of) the following transcription complex (FIG. 2b).

TOP1 has recently been shown to bind the heptad repeats of the Pol II CTD in association with the chromatin regulator bromodomain-containing protein 4 (BRD4)⁵⁶ (FIG. 2a). Based on these data, a model was proposed in which TOP1 is recruited to promoters, but kept inactive until phosphorylation of Pol II CTD Ser2 by BRD4 releases Pol II and TOP1 from promoter-proximal pausing into productive transcript elongation⁵⁶. The TOP1 requirement in front of transcription complexes to remove Sc^+ (FIG. 2a) may account for its role in activating long genes such as neuronal and inflammatory genes^{62–65}. Accordingly, TOP1 inhibition by topotecan (Supplementary Box 1) has been reported

a Elongating RNA polymerase



b RNA polymerases in tandem



Enhancer-promoter interactions

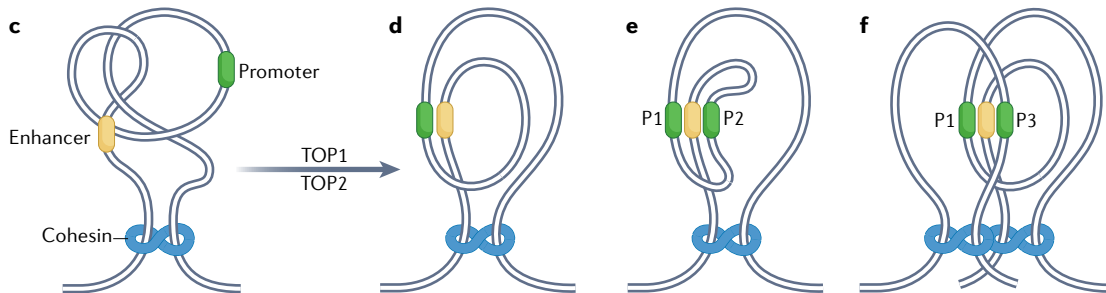


Fig. 2 | Functions of topoisomerases in transcription. **a** | Transcription induces positive DNA supercoiling (Sc^+) ahead of RNA polymerase II (Pol II) and negative supercoiling (Sc^-) behind it⁵⁴. Topoisomerase 1 (TOP1) directly binds the carboxyl terminus domain (CTD) of Pol II (dashed arrows) and is activated by bromodomain-containing protein 4 (BRD4), which phosphorylates the Pol II CTD (P)⁵⁶. This interaction can efficiently remove Sc^+ and allow translocation of Pol II. Behind the transcription complex, excessive Sc^- must be removed to prevent formation of R-loops and alternative DNA structures¹⁶⁹ (not shown). Recruitment of TOP3B by Tudor domain-containing protein 3 (TDRD3), which interacts with the Pol II CTD, suppresses R-loops²⁷. TOP1 deficiency also leads to increased levels of R-loops owing to Sc^- accumulation behind Pol II¹⁶⁹. **b** | In theory, polymerases transcribing in tandem could cancel supercoiling between them, which would facilitate transcript elongation. **c–f** | Removal of topological constraints by TOP2 and TOP1 (part **c**) facilitates close interactions between enhancers and promoters: enhancer–promoter interaction within a topologically associating domain (TAD) (part **d**); a single enhancer activating two promoters (P1 and P2) in the same TAD (part **e**); and activation of two promoters (P1 and P3) in different TADs (part **f**).

to suppress transcription-mediated lethal inflammation during SARS-CoV-2 infection⁶⁶. TOP2 also appears to be required for the transcription of long genes, both in mammals⁶² and in yeast⁷.

Behind the transcription complex, hyper-negative supercoiling resulting from insufficient TOP1 activity has been proposed as a source of R-loops^{67–69}, G quadruplex structures⁷⁰, left-handed Z-DNA⁷¹ and nucleosome destabilization^{59,61}. The accumulation of R-loops in the context of TOP1 deficiency is a potential source of DNA breaks and genomic instability^{72,73}. Excessive Sc^- — similar to excessive Sc^+ — can block transcription⁵⁵, suggesting that TOP1, in addition to its role in removing Sc^+ , has a preferential role for removing Sc^- behind transcription complexes (FIG. 2a,b).

TOP3B is also likely to suppress excessive transcription-induced Sc^- and R-loops by passing a single strand of DNA through a break made in another DNA strand (single-strand passage). The recruitment of TOP3B has been attributed to its interaction with TDRD3, which binds methylated Arg residues: on histones H3 and H4 (H3R17me2a and H4R3me2a) at active promoters, and on the Pol II CTD (R1810me2a)^{27,74} through its Tudor domains (FIG. 2a,b). However, it is not known whether the potential role of TOP3B in resolving R-loops is related to its dual DNA and RNA topoisomerase activities.

In addition to its topological activity, TOP2 has been shown to regulate neuronal immediate-early response genes^{75,76} and the transcription of hormone-responsive genes (including genes activated by androgens, oestrogens, corticoid hormones, retinoic acid and more) by forming TOP2Bccs at promoters^{75–83}. Both TOP2B and TOP2A have been proposed to regulate the promoter-proximal pause and release of Pol II at immediate-early response genes^{84–87}, as does TOP1 (REF. 56). TOP2 has also been shown to activate the transcription of *MYC* by oestrogens, which has been linked with oncogenesis^{80,85–88}. Further research is needed to determine why TOP2ccs tend to be stalled at promoters and whether the TOP2 cleavage-rejoining activity responds to post-translational modifications, such as phosphorylation and oxidation. The potential role of DNA repair factors (including TDPs, ataxia telangiectasia mutated (ATM) and DNA-dependent protein kinase (DNA-PK)) in ligand-induced gene activation also remains to be elucidated.

Because genes are activated by enhancers that are generally located quite a distance in *cis* from promoters, enhancer–promoter interactions require the formation of chromatin loops⁸⁹. Such chromatin loops generate DNA topological and torsional constraints, which are substrates for topoisomerases (FIG. 2c–f).

Roles in genome organization

Advanced microscopy methods and chromosome conformation capture-based techniques that identify DNA sequences in close proximity, owing to chromatin folding, have revealed the high-order organization and dynamic folding of the genome in interphase cells and led to the identification of chromatin loops and TADs^{89–91}. Structural maintenance of chromosomes (SMC) complexes are crucial for the formation of chromatin

loops and TADs. They comprise condensins, cohesins and the SMC5–SMC6 complexes^{89,92–94}. Cohesin and condensin are loaded onto DNA in nucleosome-depleted promoter regions during interphase, and were proposed to move in the same direction as transcription⁹³. Whereas condensins organize chromatin loops and provide compaction and elasticity to chromosomes during mitosis, cohesins establish chromatin loops in interphase and sister chromatid cohesion from S phase to mitosis (FIG. 3). The functions of the SMC5–SMC6 complex are less known and are discussed only briefly here. SMC5–SMC6 has been proposed to function in promoting Top2-mediated resolution of sister chromatid intertwinings, and for recruiting the yeast dissolvosome complex^{94,95}. The human SMC5–SMC6 complex has also been proposed to resolve TOP2A-mediated DSBs during replication⁹⁶, and to promote homologous recombination between sister chromatids by recruiting the cohesin SMC1–SMC3 complex to DSBs⁹⁷.

Because TOPccs are protein-associated DNA strand breaks, they can be mapped by pulling down the topoisomerase polypeptides to retrieve the bound DNA segments or by directly mapping the DNA breaks (the different techniques used to map TOPccs are summarized in Supplementary Box 2). Genome-wide mapping techniques relying on massive parallel sequencing have been used to locate the sites of action of topoisomerases in relationship to SMC complexes and high-order chromatin structures.

Fig. 3 | Functions of topoisomerases in genome organization. a–d

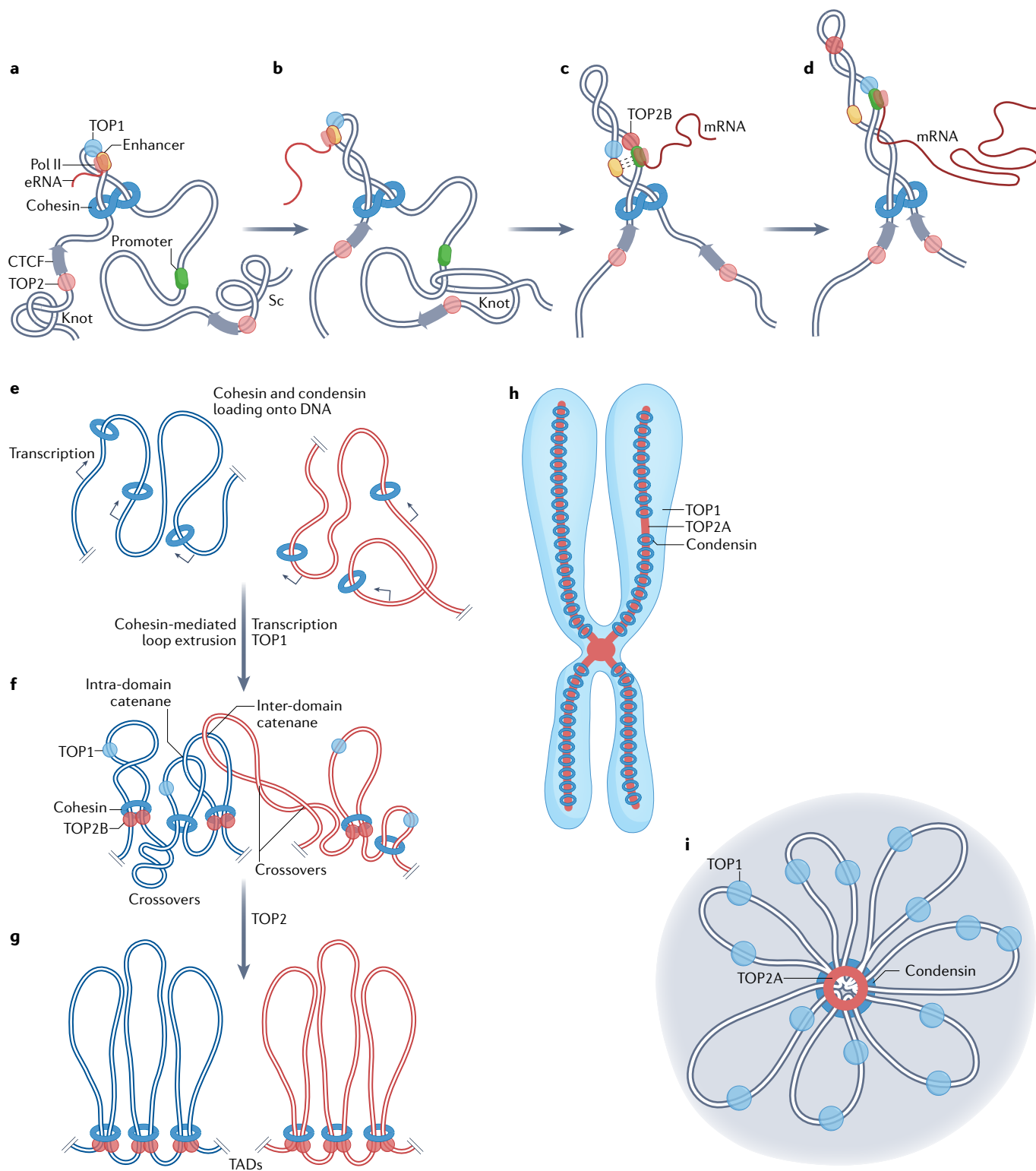
The loop extrusion model, with proposed roles for topoisomerase 1 (TOP1) and TOP2B⁵⁷: cohesin holds two ends of a chromatin loop containing an enhancer, TOP1 preferentially removes positive DNA supercoiling induced by enhancer RNA (eRNA) synthesis, the resulting negative DNA supercoiling is proposed to pull the ends of DNA through the cohesin complex, and TOP2B bound to CCCTC-binding factor (CTCF) may allow this translocation by removing topological obstacles such as knots and supercoils (part a); as eRNA transcription continues, the extruded loop increases in size, and extrusion of one end (left) is arrested when cohesin encounters CTCF (part b); following further loop extrusion, the enhancer comes into contact with a promoter, and mRNA synthesis begins (part c); CTCF at the second end of the loop comes into contact with the cohesin complex and the chromatin loop is fully extruded, with TOP1 shown acting next to the promoter and TOP2 at DNA crossovers (part d). e–g | Proposed roles of cohesins, TOP1 and TOP2B in assembly of chromatin loops and topologically associating domains (TADs): cohesin and condensin complexes are loaded onto DNA during G1 phase of cell cycle, along with transcription resumption after mitosis (part e); transcription-driven negative DNA supercoiling is proposed to extrude chromatin loops (parts a–d) and form TADs, with TOP2B removing associated topological barriers such as DNA crossovers and catenanes (part f), resulting in TAD formation (part g). h | During mitosis, TOP2A is part of the chromosome scaffold comprising condensin complexes, whereas TOP1 is present in loop domains. i | Transversal axial view of chromatin scaffold around TOP2A and condensins, with TOP1 in loop domains to remove supercoiling tension. Pol II, polymerase II. Sc, supercoiling.

Structural maintenance of chromosomes (SMC). SMC complexes are ATPase complexes that tether and organize chromatin by forming chromatin loops and ensuring sister chromatid cohesion.

TADs and roles of TOP1 and TOP2 in loop extrusion. Cohesins extrude chromatin into loops that are delineated by CCCTC-binding factor (CTCF) sites (FIG. 3a–d). Loop extrusion requires the ATPase activity and the translocation of cohesins, which stops upon cohesin encountering a CTCF protein bound to DNA in a convergent orientation^{98–101}. When CTCF sites are convergent, the C termini of their bound CTCFs are positioned to interact with the cohesins at TAD borders⁵⁷

(FIG. 3d). The tightness of the cohesin central pore may not allow free axial rotation of the DNA¹⁰², and the two DNA segments at the basis of TADs were proposed to be held together by two cohesin rings in a ‘handcuff-like’ arrangement¹⁰³ (FIGS 2c–f, 3a–g). Hence, CTCF and cohesin define the borders of TADs¹⁰⁴.

TOP2 chromatin binding is dependent on and proportional to cohesin binding¹⁰⁰. However, cohesin binding is not TOP2-dependent, indicating that TOP2



is recruited to chromatin downstream of cohesins¹⁰⁰. Genome-wide mappings of TOP2B sites by chromatin immunoprecipitation followed by sequencing (ChIP-seq) and End-seq (Supplementary Box 2) show a significant overlap between TOP2B, CTCF and cohesin binding sites^{75,99–101,105–107}. These sites co-localize with the ChIP-seq peaks of the cohesin subunit SCC1 (also known as RAD21)⁸⁹. The coincidence of TOP2B locations with TAD borders has been related to an interaction of TOP2B with the amino terminus of CTCF^{99,100,106} (FIG. 3a–d). Although the TOP2Bccs at CTCF-cohesin sites may form independently of transcription, their conversion to DSBs appears to be enhanced by transcription, and by the ‘debulking’ of the TOP2ccs by the ubiquitin-proteasome system (UPS) and TOP2 excision by TDP2 (REF.¹⁰⁰). The preferential location of TOP2B at TAD boundaries coincides with chromosomal translocations^{100,101}, and the repair of aborted TOP2cc by TDP2 was shown to suppress genomic breaks induced by androgens in prostate epithelial cells¹⁰⁸.

It is plausible that the presence of TOP2B at TAD borders resolves the topological issues arising from loop extrusion⁵⁷. As the DNA is threaded through the cohesin complex, TOP2B would be strategically positioned to eliminate topological barriers and DNA entanglements such as knots, plectonemes and catenanes before the DNA can translocate through the cohesin complexes (FIG. 3a–d). Transcription-induced Sc^- within TADs has been proposed to be generated by RNA production at enhancers^{57,58,109–111} (FIG. 3a,b). Sc^- and plectonemes can be viewed as facilitators of promoter-enhancer contacts within the same TAD^{57,112,113} (FIG. 3c,d), as observed in bacterial systems⁵⁷.

Mitotic chromosomes and the decatenation checkpoint.

The removal of cohesin at prophase through the so-called ‘prophase pathway’ is crucial for successful mitosis as condensins replace cohesins⁹³. In fission yeast, this pathway depends on the Smc5-SMC6 complex, replication signals and Top2 activity¹¹⁴. The prophase pathway is poorly understood in human cells. TOP2A dysfunction has been proposed to activate a decatenation checkpoint that arrests cells at G2–M phase transition to protect them against chromosomal damage⁸.

Top2 activity is required during mitosis¹¹⁵ and vertebrate cells treated with the TOP2 inhibitor ICRF-193 (Supplementary Box 1) die with severe chromosomal alterations¹¹⁶. TOP2A, but not TOP2B, is cell cycle regulated^{1,16,24,34}; it increases sharply from mid S phase through mitosis through transcriptional activation of TOP2A, stabilization of TOP2A mRNA and activation of the deubiquitylase USP15, which prevents the targeting of TOP2A for proteasomal degradation^{8,117}. Following mitosis, TOP2A is rapidly degraded following its ubiquitylation by the anaphase promoting complex and its activator CDH1, and USP15 is inactivated^{8,117}.

During prophase, condensin is the primary driver of chromosome condensation as it scaffolds DNA loops (FIG. 3h,i). Condensin also generates Sc^+ in an ATP-dependent manner within those loops, which further contributes to chromosome compaction. TOP2A, but not TOP2B, readily relaxes this Sc^{+25} , suggesting the existence of a fine-tuned balance between TOP2A and

condensins⁸. TOP2A may minimize DNA entanglements as condensin organizes mitotic DNA loops^{9,118–121}. However, yeast Top2 has recently been shown to promote sister chromatid intertwines during prophase while removing them at the onset of anaphase¹²². Whereas TOP2A and Top2 are essential components of the chromosome mitotic scaffold, TOP1 has also been observed within mitotic chromosomal loops, where it may act to dissipate excessive supercoiling^{123,124} (FIG. 3h,i).

Centromeric and telomeric regions and ribosomal DNA appear to retain catenated segments until anaphase⁸. In addition to TOP2A and condensins, resolution of ribosomal DNA regions requires the activity of the helicase PICH, of the poly(ADP-ribose) polymerase tankyrase 1 and of TOP3A¹²⁵. The translocase activity of PICH was recently reported to extrude Sc^- DNA loops, which are relaxed by TOP3A to provide Sc^+ substrates for sister chromatid disjunction by TOP2A and for the rapid disjunction of sister centromeres at anaphase⁵³.

Recent studies provide evidence that, as part of the dissolvosome complex, the helicase activity of BLM coupled with the single-strand passage activity of TOP3A ensures telomere integrity both in alternative lengthening of telomeres cells, which represent approximately 10–15% of cancers, and in normal cells that use telomerase to maintain their telomeres^{126,127}. These data are consistent with the prior finding that TOP3A is involved in the alternative lengthening of telomeres pathway of telomere maintenance¹²⁸. TOP2A has also been shown to cooperate with telomeric repeat-binding factor 2 and Apollo to protect telomeres from replicative damage¹²⁹. The roles of topoisomerases in centromeres and telomeres warrant further investigations, as these regions consist of DNA repeats prone to recombination and formation of non-canonical DNA structures^{130,131}.

It has been proposed that cells possess a ‘decatenation checkpoint’ that limits chromosomal instability and aneuploidy in response to TOP2A dysfunction⁸. However, the molecular pathways that activate this checkpoint are poorly understood; they may be related to abnormal regulation of TOP2A by SUMOylation, ubiquitylation and protein kinases that modify its C terminus^{8,132–134}.

Chromatin remodelling and heterochromatin. In addition to their role in organizing chromatin and chromosomes and minimizing DNA entanglements, topoisomerases associate with chromatin remodelling complexes. TOP2A was found associated with the SWI/SNF complex BRG1-associated factor to facilitate decatenation¹³⁵ and chromatin remodelling for both resolution and formation of facultative heterochromatin¹³⁶. TOP2A has also been found in association with cohesin and BAZ2A, an essential component of the chromatin remodeller nucleolar remodelling complex, to regulate chromatin accessibility and expression of developmental genes in mouse embryonic stem cells¹³⁷.

Studies with TOP2B-deficient cells³⁴ are warranted to explore the potential connection between chromatin remodelling and TOP2B activity and the role of TOP2B in the formation of chromatin loops and TADs.

TOP3B has also been implicated in heterochromatin formation by interacting with the RNAi

machinery to promote transcriptional gene silencing in *Drosophila melanogaster*^{4,138}, and TOP3A has been suggested to act with PICH to condense chromatin at centromeres⁵³. BRG1 (also known as SMARCA4) itself has also been shown to recruit TOP1 to suppress transcription-associated genomic instability¹³⁹.

It is tempting to speculate that persistent TOPccs may provide anchors for tethering DNA segments to nuclear structures. Such ‘functional’ DPCs would be self-reversible, and if not reversible they would be removed by the repair pathways described below. Post-translational modifications of topoisomerases such as phosphorylation^{133,140}, oxidation^{141,142}, acidification¹⁴³ and acetylation¹³³ could regulate the stability and reversibility of the TOPcc, and thus maintain such genomic anchor points.

Topoisomerases and genome instability

Topoisomerases can both damage the DNA when they abort their catalytic cycle in response to anticancer drugs, environmental agents or reactive metabolites^{1,17}

and contribute to DNA repair. Consequently, topoisomerases modulate genome stability, and their dysfunction causes chromosomal rearrangements and contributes to cancer.

Roles in DNA repair and genome stability. TOP1 is a potent ribonuclease at single ribonucleotides or short stretches of ribonucleotides incorporated into the genome by DNA polymerases^{144,145}. The canonical pathway of ribonucleotide excision repair is primarily carried out by RNase H2 (REF.¹⁴⁶). However, the frequency of ribonucleotide misincorporation is so high^{147,148} that, when embedded ribonucleotides are not removed by RNase H2, TOP1 converts them into SSBs with 3’ ends bearing a 2’,3’-cyclophosphate¹⁴⁵ (FIGS 4,5). Such blocked ends are processed by apurinic endonuclease 2 (APE2), as a potential backup gap repair pathway for the ribonucleotide excision repair pathway^{149,150}. Subsequently, a TOP1cc formed 5’ adjacent to the ribonucleotide (possibly by the same TOP1 molecule sliding back on

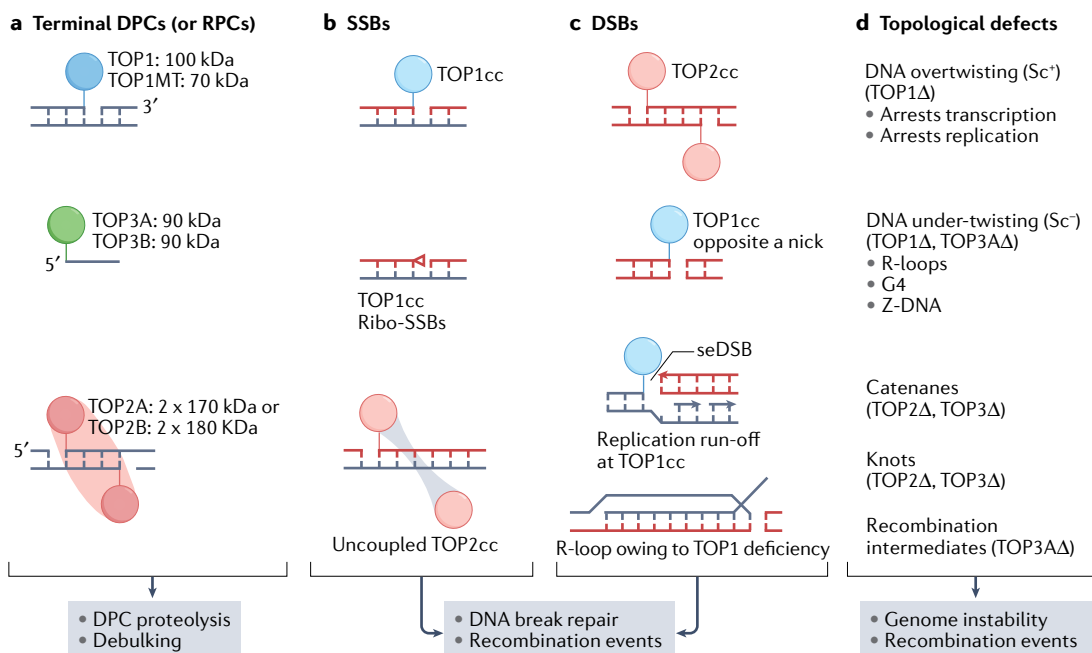
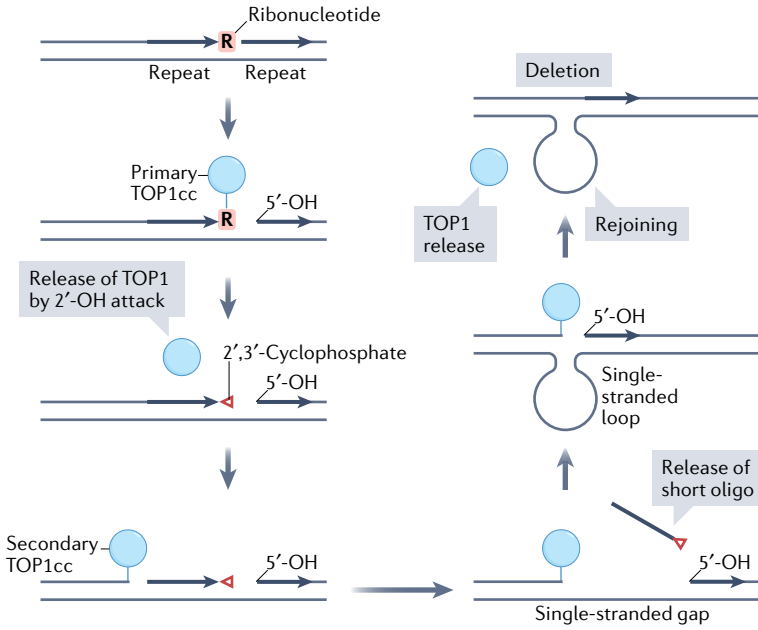
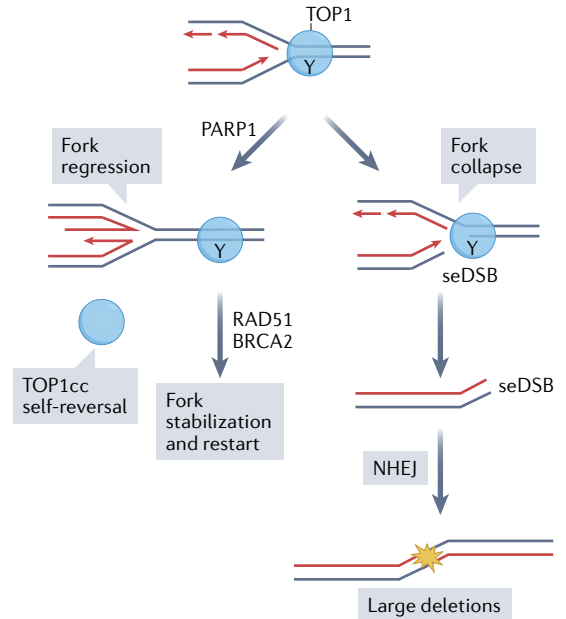


Fig. 4 | Genotoxic and pathogenic topoisomerase lesions. Catalytic intermediates of topoisomerases are normally transient because topoisomerase cleavage complexes (TOPccs) are self-reversible (Supplementary Fig. 1). Irreversible TOPccs are generated by trapping of TOPccs by anticancer drugs (Supplementary Box 1) and by pre-existing DNA alterations^{1,17}. TOPccs produce complex nucleic acid alterations, including DNA–protein crosslinks (DPCs) and RNA–protein crosslinks (RPCs), DNA breaks and topological defects. **a** | DPCs form either at 3’ DNA ends (topoisomerase 1 (TOP1) or mitochondrial TOP1 (TOP1MT)) or 5’ DNA ends (TOP2A, TOP2B, TOP3A or TOP3B). They also form at 5’ RNA ends for TOP3B⁷. TOP1, TOP1MT, TOP2A and TOP2B bind double-stranded DNA; TOP3A and TOP3B bind single-stranded DNA or RNA. DPCs and RPCs generally need to be proteolysed or debulked (denatured) before their excision^{201,244}. **b** | DNA single-strand breaks (SSBs) formed by stalled or irreversible TOP1ccs (top). TOP1 can also generate SSBs by converting ribonucleotides incorporated by DNA polymerases into nicks with 2’,3’-cyclophosphate blocking ends (red triangle) (middle)^{144,145,211}. Cleavage of DNA by TOP2 can be asymmetrical, with only one component of the TOP2 homodimer forming a TOP2cc (bottom). This situation is commonly observed following treatment with etoposide^{227,358}. **c** | DNA double-strand breaks (DSBs) formed by trapping of TOP2A and TOP2B^{132,359} following their proteasomal degradation (top)^{201,249}. TOP1 can also generate DSBs, when it nicks the DNA opposite to a nick^{211,360} or when collision with a replisome produces ‘replication run-off’ with a single-ended DSB (seDSB) (middle)²²¹. R-loops forming due to insufficient TOP1 activity induce DSBs (bottom)⁷². **d** | Insufficient topoisomerase activity can result in excessive positive DNA supercoiling (Sc⁺) that arrests transcription and replication, and in negative supercoiling (Sc⁻) that induces formation of R-loops and alternative DNA structures, including G quadruplexes (G4) and Z-DNA^{67,165}. Catenanes and knots, which also stop DNA (and possibly RNA) transactions, increase in conditions of TOP2 and TOP3 deficiency. Unresolved recombination intermediates owing to TOP3A deficiency lead to sister chromatid exchanges and genomic instability⁶.

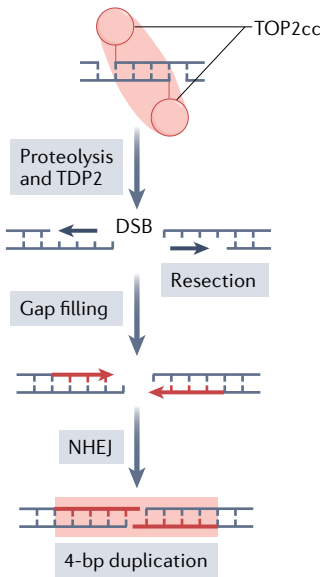
a TOP1-mediated short deletions



b TOP1-mediated large deletions



c TOP2-mediated short duplications



d TOP2B-mediated chromosomal rearrangement model

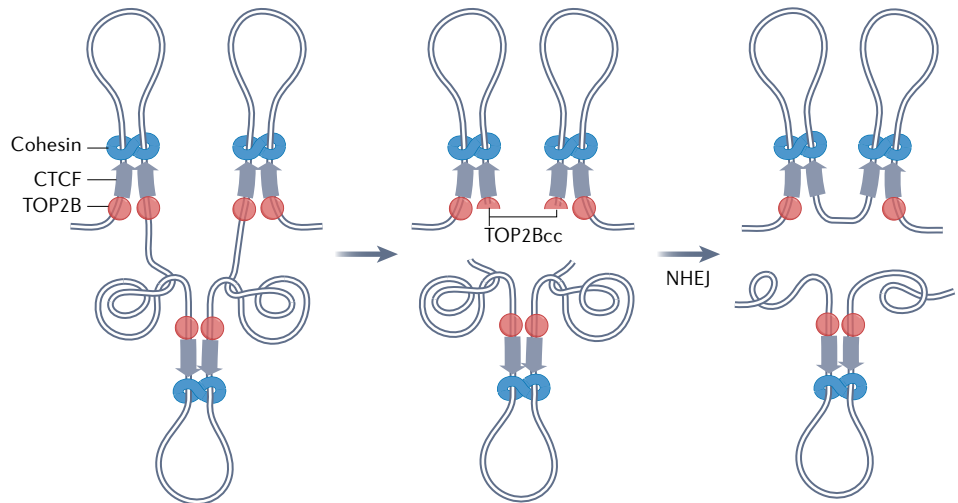


Fig. 5 | Topoisomerase-induced mutagenesis and recombination events.

a | Model for formation of topoisomerase 1 (TOP1)-mediated deletions at sites of ribonucleotide incorporation within short tandem repeats. Incorporation of ribonucleotides by DNA polymerases is one of the most common abnormalities in the DNA⁴¹⁸. A ‘primary’ TOP1 cleavage complex (TOP1cc) forms on a ribonucleotide. The 2′-hydroxyl of the ribose sugar reverses the bond with TOP1 (not shown) and generates a nick with a 2′,3′-cyclophosphate end. A ‘secondary’ TOP1 forms a TOP1cc 5′ of the nick, and the resulting short oligonucleotide bearing the 2′,3′-cyclophosphate is released. The 5′ end of DNA is captured by TOP1, which is followed by rejoining of the two ends and release of TOP1, thus generating a short deletion^{144,151,152}. The ribonuclease activity of TOP1 has been linked with the ‘Indel Signature 4’ (ID4) in the Catalogue of Somatic Mutations in Cancer (COSMIC) database³⁶¹, which consists of 2–5 base pair deletions. The ID4 signature has been proposed to be named the ID-TOP1 mutational signature³⁶². **b** | TOP1-mediated large deletions. A replication fork collides with a TOP1cc on the leading (bottom) strand (Y represents the covalently linked catalytic Tyr at the 3′ end of the

break). Replication fork regression induced by poly(ADP-ribose) polymerase 1 (PARP1)³⁶³ promotes TOP1cc self-reversal or fork stabilization and replication restart with RAD51 and breast cancer-associated type 2 (BRCA2). Alternative to fork regression, ‘replication run-off’⁷²¹ generates a single-ended DNA double-strand break (seDSB), and ligation of two distant seDSBs by non-homologous end joining (NHEJ) produces large deletions²²⁶. **c** | TOP2-mediated short duplications²⁰³. Processing of TOP2cc by proteolysis and tyrosyl-DNA phosphodiesterase 2 (TDP2) produce DSBs. The 3′ ends of the break can undergo resection followed by gap filling. Ligation through NHEJ results in 4-bp duplications. An indel signature consisting of 2–4 base pair duplications and due to a TOP2A mutation (K473N) that traps TOP2A has been found in patient tumours and proposed to be named as the ID-TOP2A signature³⁶⁴. **d** | Simplified model of TOP2B-mediated chromosomal rearrangements based on the loop extrusion model¹⁰⁰. Schematic depicts chromatin loops with cohesin, CCCTC-binding factor (CTCF) and TOP2B at base of loops. Stalled or irreversible TOP2Bccs generate DSBs that disjoin the loops. Rejoining of two adjacent DSBs produces a translocation.

the DNA) (FIG. 5a) can release a short 2–5 bp DNA fragment and form a single-stranded gap (FIG. 5a). Following the excision of this secondary TOP1-DPC by TDP1 or APE2, gap filling has been suggested to restore the DNA^{1,146,149,151–153}. However, the flexibility of the single-stranded DNA segment, especially in DNA sequences with short repeats, may also allow the 5'-hydroxyl (5'-OH) to attack the TOP1-tyrosyl-DNA bond and reverse the TOP1cc¹⁴⁴ (FIG. 5a). Although this reaction is primarily viewed as a source of genomic instability^{151,152}, the ribonuclease activity of TOP1 may serve as a backup pathway for the removal of ribonucleotides in DNA^{152–154}.

The above TOP1 sequential cleavage model has also been invoked for TOP1-mediated repair of ultraviolet-induced DNA damage in nucleotide excision repair-deficient cells and for base excision repair and removal of 6–4 photoproducts (pyrimidine dimers)¹⁵⁵. Because of its potent ligase activity across non-canonical and non-homologous structures^{152,156,157}, including Holliday junctions¹⁵⁸, TOP1 may have additional DNA repair and rejoining activities, warranting further investigations. In addition, a recent study showed that TOP1 interacts with cyclic cGMP-AMP synthase and promotes its binding to DNA, thereby eliciting innate immune responses and cell senescence¹⁵⁹.

TOP3A, in addition to its roles in resolving double Holliday junctions and suppressing sister chromatid exchanges, is involved in DNA end resection¹⁶⁰ and homology-directed repair (HDR)^{6,28,161,162}. Recent studies suggest that TOP3A senses RPA-coated single-stranded DNA and contributes to the restart of replication forks⁵⁰. In addition, by resolving recombination intermediates, TOP3A may prevent telomere exchanges and crossovers, leading to telomere erosion^{126,127}.

To our knowledge, there is no evidence for a direct repair role of TOP2 enzymes. Yet their association with chromatin remodelling complexes could contribute to DNA repair^{135–137}. Moreover, TOP2B binds to DSB sites¹⁶³ and its activity at chromatin loops could serve to regulate DNA repair domains¹⁶⁴.

Genome instability related to insufficient topoisomerase activity. Insufficient activity of the topoisomerases has profound effects on DNA and RNA metabolism (transcription and replication), and on the accumulation of non-canonical DNA structures such as R-loops, G-quadruplexes, Z-DNA, catenanes and knots^{67,165} (FIG. 4d).

TOP1 is essential both for early development in mice and for viability of vertebrate cells¹⁶⁶ (TABLE 1; Supplementary Table 1). Studies in mice with neuron-specific TOP1 inactivation demonstrate the crucial roles of TOP1 in postmitotic cells⁶⁵. The cerebral cortex develops normally, but neurons exhibit reduced transcription of long genes and, ultimately, undergo degeneration at postnatal day 7, which is accompanied by accumulation of the DNA damage response biomarker γ H2AX¹⁶⁷. DNA breakage results, at least in part, from the formation of R-loops^{72,168}. Indeed, TOP1 depletion increases R-loop abundance in heterochromatin domains of human HEK293 cells¹⁶⁹ and at transcription termination sites of highly expressed genes in

HeLa cells⁶⁹. Although R-loops induce DSBs mainly by interfering with DNA replication forks¹⁷⁰, they can also generate DSBs in postmitotic cells^{72,73,171,172}.

Unlike TOP1, TOP1MT is dispensable for mouse development¹⁷³ (TABLE 1). Yet knocking out *TOP1MT* impairs liver regeneration and tumour growth by reducing the translation of genes encoded in mitochondria^{174–177}. Notably, TOP1MT is transcriptionally activated by the oncogene MYC¹⁷⁸.

Genetic studies show that TOP2 is required for chromosome condensation and segregation and for heterochromatin assembly in cell line models¹⁷⁹. The essential role of TOP2A in mitosis is consistent with the embryonic lethality of TOP2A-deficient mice at the four-cell or eight-cell stages¹⁸⁰.

TOP2B-deficient mice develop in utero, as TOP2A can compensate for the loss of transcriptional gene regulation in cycling cells^{33,181}. However, TOP2B-deficient mice die at birth because the nerves that innervate their diaphragm do not form dendrites to connect to the muscles. The mice also fail to form laminar structures in their cortex^{182,183} (TABLE 1).

TOP3A is essential for the nuclear and mitochondrial genomes^{184–186} (BOX 1; TABLE 1). Bloom syndrome has recently been linked with genetic alterations in *TOP3A*¹⁸⁷ and in the dissolvosome component *RMI2* (REF. 188), which is consistent with the fact that TOP3A and BLM cooperate in the dissolvosome complex^{6,189} to suppress sister chromatid exchanges^{161,190} and HDR¹⁹¹ and contribute to the resolution of stalled replication forks¹⁹². The mitochondrial isoform of TOP3A is crucial for mtDNA replication¹², fertility^{186,193} and, owing to its mitochondrial functions, the viability of postmitotic cells¹⁸⁵ (BOX 1; TABLE 1).

TOP3B is not essential, but mice lacking *TOP3B* have a shortened lifespan, a higher incidence of aneuploidy in germ cells, increased autoimmunity^{194–196}, abnormal synapse formation¹⁹⁷ and behavioural impairments¹⁹⁸ (TABLE 1). Individuals with genetic inactivation of *TOP3B* suffer from severe neurological symptoms, including cognitive impairment, which have been related to the RNA topoisomerase activity of TOP3B^{26,197}. *TOP3B* inactivation has also been reported in a person with renal carcinoma¹⁹⁹. The molecular mechanisms underlying these phenotypes and how they are related to the RNA and DNA functions of TOP3B^{2,4,15,138,198,200} are important issues for future studies.

Topoisomerase trapping by metabolic and environmental perturbations and drugs. Many environmental and metabolic perturbations induce TOPcc trapping^{1,17,201}, which produces highly deleterious DNA lesions emanating from TOP-DPCs associated with SSBs and DSBs (FIG. 4). Studies with anticancer drugs that target TOPccs and with self-poisoning topoisomerases provide complementary approaches to elucidate the DNA damaging effects of trapped TOPccs and the repair pathways and of cellular responses to TOP1ccs³¹, TOP1MTccs²⁰², TOP2ccs^{203,204} and TOP3Bccs².

Trapping of TOP1ccs is the mechanism of action of the anticancer drugs derived from camptothecins and indenoisoquinolines^{205,206} (Supplementary Box 1),

Homology-directed repair (HDR). A replication-associated DNA double-strand break (DSB) repair pathway that uses a homologous sequence as a template for resynthesizing a missing DNA segment. Its classical form is homologous recombination and its common effector is RAD51.

γ H2AX
Histone H2AX phosphorylated at Ser139; a sensitive biomarker of DNA double-strand breaks (DSBs).

TOPcc trapping
Stabilization of topoisomerase cleavage complexes (TOPccs) by inhibition of DNA end rejoining by a drug molecule bound at the interface of the DNA break and the enzyme or by DNA lesions that misalign the broken DNA ends, thereby preventing the release of the topoisomerase.

Table 1 | Pathogenicity of topoisomerase malfunctions and phenotypic dysfunctions

Gene	Clinical manifestations	Vertebrate cell line models	Mouse knockout phenotypes	Fruitfly knockout phenotype	Yeast knockout phenotypes
TOP1	TOP1 antibody (SCL70) autoimmune syndromes Aicardi–Goutières syndrome ¹⁴⁷ Autism ⁶²	Reduced TOP1 expression causes replication stalling, R-loops and genome instability ^{67,165}	Essential for early embryogenesis ¹⁶⁶ Genomic instability and early neurodegeneration in the brain ⁶⁵	TOP1 is essential for multiple cellular functions ^{319,320}	Top1 knockout causes slow growth and accelerated ageing phenotype (ribosomal DNA circles) ³²¹
TOP1MT	Unknown. SNPs are frequent ³²²	TOP1MT overexpression reduces mtDNA transcription ^{323,324} Expression of TOP1MT (mouse T546-N550H; human T554A-N558H) producing irreversible TOP1MTccs induces mtDNA degradation ²⁰²	Dispensable ^{174,176,325,326} Reduced tissue regeneration ¹⁷⁴ Carcinogenesis ¹⁷⁶ Reduced spermatogenesis ³²⁷ Reduced mtDNA replication ¹⁷⁴ Reduced mitochondrial transcription ^{175,324} Reduced mitochondrial translation ¹⁷⁶		
TOP2A	Amplified in HER2-positive breast cancers (chromosome 17q amplicon) TOP2 antibodies found in the autoimmunity syndrome lupus ³²⁸ and in cancer ³²⁹	Selectively expressed and essential in proliferating cells ^{33,330} Non-essential in quiescent cell lines	–	–	Top2 is essential for termination of DNA replication and chromosome segregation at mitosis ^{115,321}
TOP2B	Mutations associated with B cell deficiency, global developmental delay and autism spectrum disorder ^{331–333}	Non-essential in cell lines ^{34,334}	Perinatal lethality owing to defects in neuronal differentiation and connectivity ^{182,183,335} Conditional knockout: role in corticogenesis ¹⁸² , and retina ¹⁷³ and ovarian development ³³⁶ Depletion by local injection: defective consolidation of fear memory ³³⁷		
TOP3A	Bloom syndrome ^{13,187,188} Mitochondrial disease ^{12,13,338} mtDNA deletions ¹² MLL gene fusion in AML ³³⁹	TOP3A inactivation produces sister chromatid exchanges, defective chromosome segregation, ultrafine anaphase bridges and mitotic catastrophe ^{6,52,187} Resolves stalled replication forks with FANCM ¹⁹²	Essential for early embryogenesis ¹⁸⁴	TOP3A is essential ^{185,186} ; the mitochondrial isoform is required for fertility and maintenance of mtDNA ^{186,193}	Top3 knockout causes low growth and hyper-recombination ^{340,341} Rescued by knocking out Sgs ¹ , the yeast RECQ orthologue ³⁴²
TOP3B	Neurological disorders, intellectual deficiency and psychosis ^{26,197} Carcinogenesis ¹⁹⁹ Premature ageing ⁴	Genomic instability (R-loops) ¹⁹⁹ Defective neuronal synapses ³⁴³ Genetically engineered TOP3B (R338W) producing irreversible TOP3Bccs induces DNA damage and R-loops ²	Dispensable ^{194–196} Splenomegaly, immune infiltrates ¹⁹⁴ and autoimmunity ¹⁹⁶ Infertility and aneuploidy ¹⁹⁵ Neurological defects ¹⁹⁸ Defective synapse formation ¹⁹⁷ Tumours	Defective synapse formation ¹⁹⁷ Defect in heterochromatin formation ¹³⁸	
TDP1	Spinocerebellar ataxia with axonal neuropathy ^{344,345}	Knockout reduces mtDNA transcript abundance ³²³ Deficiency causes broad sensitivity to DNA damaging agents ³⁴⁶	Age-dependent cerebellar atrophy ³⁴⁷ Potentiates with ATM neurodegeneration ²⁵⁰	Reduced lifespan and climbing ability in females ³⁴⁸	TDP1 knockout (there is no TDP2) is hyper-sensitive to TOP1ccs ^{349,350}
TDP2	Seizures, intellectual deficiency and ataxia ^{264,351}	TDP2 repairs TOP1ccs in the absence of TDP1 (REFS ^{264,352}) Required for picornavirus replication ^{353,354} Suppresses genomic instability induced by androgens and oestrogens ¹⁰⁸	Genomic instability and neuronal defects ^{280,355} Increased incidence of thymic cancers in <i>Atm</i> ^{-/-} mice ³⁵⁶		

AML, acute myeloid leukaemia; ATM, ataxia telangiectasia mutated; FANCM, Fanconi anaemia group M protein; MLL, myeloid/lymphoid or mixed-lineage leukaemia; mtDNA, mitochondrial DNA; TDP, tyrosyl-DNA phosphodiesterase; TOP1, topoisomerase 1; TOP1cc, topoisomerase cleavage complex; TOP1MT, mitochondrial TOP1.

Non-homologous end joining

(NHEJ). The prominent DNA double-strand break (DSB) repair pathway, which rapidly joins adjacent DNA ends. Its main effectors are Ku70–Ku80 and DNA-dependent protein kinase (DNA-PK).

which are classically used to study the DNA damaging effects of and cellular responses to TOP1ccs in cellular models. Topotecan and irinotecan are widely used for cancer therapy and tumour-targeted TOP1 inhibitors are promising novel chemotherapies³¹. Local structural DNA perturbations can trap TOP1ccs as well^{207,208}. These include the incorporation of ribonucleotides and of anti-cancer nucleoside analogues such as cytarabine (Ara-C) and gemcitabine, and DNA adducts by platinum derivatives such as cisplatin and carboplatin^{209–212}. TOP1cc can also be stabilized near DNA mismatches, abasic sites and 7,8-dihydro-8-oxoguanine (8-oxoG) base oxidation, ultraviolet damage and single-strand nicks in the strand not cleaved by TOP1 (REFS^{1,155,201,213–217}) (FIG. 4c).

Trapped TOP1ccs are a well-established source of mutations and genomic instability (FIGS 4, 5). TOP1-mediated deletions at short repeated sequences (FIG. 5a) can be initiated by ribonucleotides incorporated into the DNA by replicative polymerases^{148,218} (FIG. 5a). TOP1 converts them to SSBs (FIG. 4b), and following the formation of a second TOP1cc on the same DNA strand (FIG. 5a), TOP1 readily generates short deletions^{144,151,152} due to its potent DNA ligation activity^{154,213,219,220}. This recombination property is routinely used for molecular cloning with TOP1 (REF.¹⁴⁵). The occurrence of TOP1ccs across nicks can also induce recombinogenic

DSBs^{1,211,213} (FIG. 4c). Another mutagenic consequence of irreversible TOP1ccs results from their collisions with replication forks (FIGS 4c, 6). When the TOP1cc is on the leading strand, it produces ‘replication run-off’²²¹ resulting in the formation of a single-ended DSB (seDSB) (FIGS 4c, 5b). Such seDSBs are highly cytotoxic²⁰⁴, unless the replication fork reverses (FIG. 5b), and can produce large deletions and chromosomal translocations through non-homologous end joining (NHEJ)^{222–226} (FIG. 5b).

TOP2ccs are also a well-established source of DNA damage and carcinogenic mutations by endogenous and environmental agents¹⁷. Their trapping by TOP2 poisons is the target of the anticancer drugs etoposide, doxorubicin, mitoxantrone and amsacrine (Supplementary Box 1), which generate TOP2-DPCs coupled with DSBs and SSBs. These drugs are classically used to map TOP2ccs and study their DNA damaging effects and repair pathways^{32,48,206,227–230}. In addition, the TOP2 catalytic inhibitors merbarone and ICRF-193 (or ICRF-187) prevent the enzymatic activity of TOP2 by stabilizing topological TOP2–DNA complexes^{32,231}, which are highly cytotoxic or require the intervention of DNA repair pathways^{196,222,231}. The conformation of TOP2 is sensitive to temperature and biochemical perturbations^{141–143,232}. Exposure to etoposide either at an elevated temperature (39 °C) or together with an

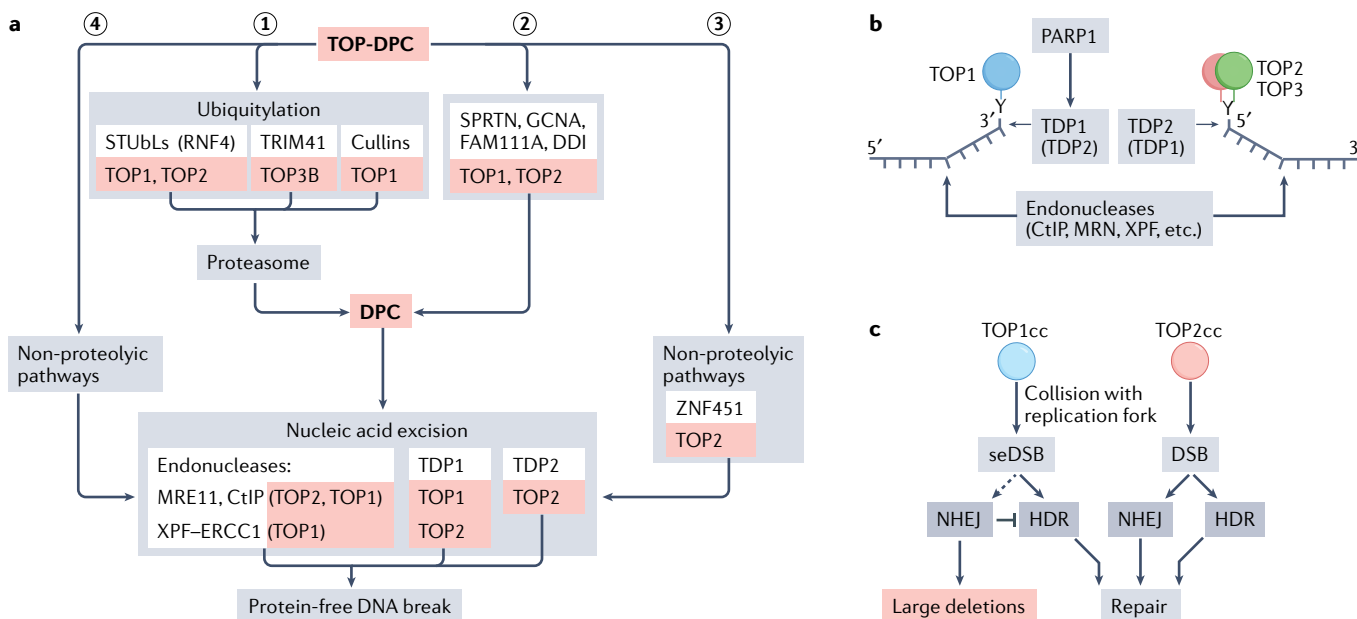


Fig. 6 | Main repair pathways for trapped topoisomerases in humans.

a Overall scheme for conversion of topoisomerase DNA–protein crosslinks (TOP-DPCs) into protein-free DNA breaks²⁰¹. Association of TOP-DPCs with replication or transcription complexes and phase of the cell cycle (S phase versus G1 phase) are likely determinants of pathway choice. Debulking of TOP-DPCs by the three ubiquitin–proteasome pathways includes the conserved SUMO-targeted ubiquitin ligase (STUbl) pathway, in which RNF4 is the human E3 ubiquitin ligase for TOP1-DPCs, TOP2A-DPCs and TOP2B-DPCs²⁴⁹; the TRIM41 E3 ligase pathway for crosslinks between TOP3B and DNA or RNA²; and the cullin pathway for TOP1-DPCs^{365,366} (step 1). Non-proteasomal TOP-DPC proteolytic pathways²⁴⁵. The proteases Spartan (SPRTN), GCNA (also known as ACRC), FAM111A and DDI debulk TOP1-DPCs and TOP2-DPCs (step 2). Non-proteolytic pathway for TOP2, driven by SUMO E3 ligase ZNF451 (REF.²⁵⁹) (step 3). Nucleic-acid excision

pathways for TOP1 and/or TOP2 include excision by the endonucleases MRE11, CtIP and XPF–ERCC1, or excision by tyrosyl-DNA phosphodiesterase 1 (TDP1) and TDP2 (step 4). **b** | TDP1 is activated by poly(ADP-ribose) polymerase 1 (PARP1)^{268,269,275}, and upon cleaving DNA leaves a 3'-phosphate that is further processed by polynucleotide kinase phosphatase (not shown). TDP2 leaves a 5'-phosphate that can be directly ligated or extended by DNA polymerases (not shown). Both TDP1 and TDP2 require debulking of TOP-DPCs to gain access to the tyrosyl–DNA links. Additional excision pathways involve endonucleases. **c** | Differential roles of non-homologous end joining (NHEJ) and homology-directed repair (HDR) in repair of TOP1-induced single-ended DNA double-strand breaks (seDSBs) and TOP2-DPC-induced DSBs. Whereas seDSB repair by NHEJ is toxic²²², possibly by inducing large deletions owing to illegitimate end joining of distant seDSBs (FIG. 5b), NHEJ is crucial for repair of TOP2-DPCs. TOP1cc, TOP1 cleavage complex.

inhibitor of heat shock protein 90 (HSP90) enhances the abundance of TOP2-DPCs^{233,234}. Thus, HSP90 appears to be required for maintaining the conformation of TOP2 and avoiding TOP2 misfolding and the stalling of TOP2-DPCs. In addition to misfolding, TOP2 trapping can occur at sites of endogenous base damage¹⁷, including abasic sites, oxidized and alkylated bases and base mismatches^{235–238}, and at secondary DNA structures²³⁹. This relation with endogenous base damage suggests that physiological TOP2cc trapping occurs more frequently than is commonly appreciated²⁴⁰. This conclusion is supported by the finding that loss of MRE11, the nuclease that removes TOP2 adducts (FIG. 6a,b), causes the endogenous accumulation of TOP2cc^{80,241–243}.

Genomic damage owing to inaccurate repair of TOP1ccs and TOP2ccs. Due to the inaccessible location of the covalent tyrosyl–DNA bonds inside the TOPccs (Supplementary Fig. 1i–k), the removal of topoisomerase adducts requires a debulking step²⁰¹ so that excision repair enzymes can access the topoisomerase tyrosyl–DNA junctions^{201,244} (FIG. 6; Supplementary Fig. 1a,b). To that effect, TOP-DPCs are subjected to proteolytic degradation^{2,201,244,245} (FIG. 6a). The UPS is highly active in cancer cells, which is exploited for the treatment of multiple myeloma with proteasome inhibitors. Moreover, it has recently become possible to examine proteolysed TOP1-DPCs using a specific antibody that recognizes the TOP1–DNA cleavage junctions^{246–249}.

The endogenous accumulation of TOP1-DPCs has been detected in mice deficient in the kinase ATM or in the protease Spartan (SPRTN)^{247,250}. Cancer cells treated with camptothecin or etoposide undergo rapid ubiquitin-dependent degradation of TOP1 and TOP2 (REFS^{249,251,252}). Likewise, human embryonic kidney cells proteolyse more than 50% of their cellular TOP1, TOP2A and TOP2B upon exposure to camptothecin or etoposide²⁴⁹. Accordingly, proteasome and ubiquitin inhibitors delay TOP-DPC removal^{2,249,253}. The UPS-mediated removal of TOP1-DPCs is variable in different cell lines and tends to be more robust in drug-resistant cancer cells than in normal cells^{251,252}. Recently, we showed that TOP3B-DPCs are also removed by the UPS pathway².

TOP2-DPCs can also be proteolysed by non-proteasomal pathways^{245,248,254–256}, and non-proteolytically by TDP2 following their SUMOylation and unfolding by ZNF451 (REFS^{257–259}) (FIG. 6). Non-proteasomal proteolysis by SPRTN repairs replication-associated TOP1-DPCs and TOP2-DPCs²⁴⁵, and genetic inactivation of *SPRTN* causes Ruijs–Aalfs syndrome with progeroid features and hepatocellular carcinomas^{245,247}. Further studies are warranted to explore whether the sensitivity of malignant tumours to camptothecin and etoposide and the carcinogenic consequence of TOPccs are related to the UPS or SPRTN pathways, and whether the genomic instability of Ruijs–Aalfs syndrome is related to the accumulation of unrepaired TOP-DPCs.

FIGURE 6a outlines TDP1-dependent, TDP2-dependent and exonuclease-dependent repair of TOP1-DPCs, TOP2-DPCs and TOP3B-DPCs, and TABLE 1 lists the disorders and cellular defects related to

TDP1 and TDP2 deficiencies. Human and yeast recombinant TDP1 proteins hydrolyse phosphodiester linkages between Tyr residues and 3'-phosphates of DNA^{207,260–262} (FIG. 6b; Supplementary Fig. 1a,b). Although purified TDP2 protein has weak activity as a 3' TDP^{263,264}, human TDP2 contributes to the removal of TOP1-DPCs in vivo. Recent analyses in human B cells show that more than 90% of TOP1-DPCs are removed during a 15-min repair time in wild type and *TDP2*^{-/-} cells, 70% in *TDPI*^{-/-} cells and only 40% in *TDPI*^{-/-}*TDP2*^{-/-} cells²⁵³. Thus, both TDP1 and TDP2 are important for TOP1-DPC removal. The stability of TDP1 is regulated by the deubiquitylase UCHL3 (REF. 265). TDP1 activity is also controlled by its SUMOylation²⁶⁶, its phosphorylation by ATM and DNA-PK²⁶⁷, and its recruitment by poly(ADP-ribose) polymerase 1 (PARP1)^{268,269}. ATM-deficient mice exhibit endogenous accumulation of TOP1-DPCs and neurodegeneration²⁵⁰.

TOP2-DPCs can also be directly excised by endonucleases^{100,241,270} (FIG. 6a). Additionally, the endonuclease complexes MUS81–EME1 (REFS^{271,272}) and XPF–ERCC1 (REFS^{273–275}) can excise TOP1-DPCs (FIG. 6). Hence, cancer and genetic deficiencies of such nucleases or their scaffolding proteins (for example, SLX4 in the case of XPF–ERCC1) may predispose to genomic instability by defective excision of TOP-DPCs^{275,276}. Somatic mutations in tumours may also provide therapeutic opportunities related to synthetic lethality, such as the use of PARP inhibitors to block the TDP1 repair pathway in ERCC1-deficient cancers treated with TOP1 inhibitors²⁷⁵ (FIG. 6b).

Following the excision of TOP-DPCs, cells restore the DNA by filling the gaps and resealing the DNA breaks. Camptothecin generates DSBs in cycling cells when replication forks encounter TOP1-DPC (FIGS 4c,6c). HDR, but not NHEJ, repairs the DSBs using the intact sister chromatid as a template. However, error-prone NHEJ frequently occurs in camptothecin-treated cells upon inhibition of ATM^{226,277}, suggesting that genome instability resulting from endogenous TOP1cc is accentuated in the context of ATM and HDR deficiencies.

In contrast to the dominant role for HDR in repairing TOP1-induced DSBs, NHEJ is preferentially used for TOP2-induced DSBs even in cycling cells²²² (FIG. 6c). Whereas DSB end resection by HDR efficiently removes TOP2-DPCs²⁷⁸, NHEJ can only ligate TOP2-DPCs after the removal of 5' TOP2 adducts, which restores the 3'-OH and 5'-phosphate ends^{279–281}. Abnormal rejoining of TOP2-induced DSBs has recently been reported to account for short duplications²⁰³ (FIG. 5c). Following hydrolysis of the 5'-tyrosyl bond by TDP2 (Tdp1 in yeast), gap filling by DNA polymerase generates a blunt-ended DSB, which is ligated by NHEJ.

Both TDP2 and MRE11 remove TOP2 adducts (FIG. 6b), and their relative contribution varies in different conditions. TDP2 removes 50–70% of TOP2 adducts generated by 2 h of etoposide exposure in mouse primary B cells. In mouse embryonic fibroblasts, loss of ATM and TDP2 together (but not separately) causes a significant delay in the repair of DSBs induced by etoposide²⁸². ATM may phosphorylate MRE11 and its cofactor, CtIP (also known as RBBP8), as these proteins

are known to be phosphorylated by ATM and promote NHEJ of etoposide-induced TOP2-DPCs in G1 phase²⁸³. More than 99% depletion of MRE11 and inactivation of its nuclease activity is required for the accumulation of endogenous TOP2ccs in human B cells²⁴². Thus, a residual amount of MRE11 activity appears sufficient for the repair of TOP2ccs. Similar to the complete loss of MRE11, neuron-specific disruption of Nijmegen breakage syndrome 1 (*NBS1*), an essential cofactor of MRE11 (with RAD50), also causes the accumulation of endogenous TOP2cc²⁴². Overexpression of TDP2 partially rescues the lethality of MRE11-deficient cells, indicating that stalled TOP2ccs generate lethal DSBs in cycling cells. Considering the viability of TDP2-deficient mice²⁸⁰ and the pivotal role of TDP2 in the repair of etoposide-induced TOP2ccs^{264,280,284}, future studies are warranted to understand why TDP2 fails to suppress the endogenous accumulation of lethal TOP2ccs. Another unsolved question is the molecular mechanism(s) linking MRE11 with NHEJ to ensure the accurate repair of TOP2ccs, as NHEJ cannot accurately rejoin DSBs carrying the 3' overhangs generated by the endonucleolytic removal of TOP2 adducts.

DSB end resection during HDR generates 3' single-strand tails, and thereby removes 5' blocking adducts such as TOP2-DPCs. A recent study shows that breast cancer-associated type 1 (BRCA1) and the ubiquitin-associated proteins RAP80 and UBC13 (also known as UBE2N), which are involved in DSB end resection, contribute to the removal of etoposide-induced 5' TOP2-DPCs independently of their function in HDR^{243,281}. RAP80 and UBC13 promote the physical interaction between BRCA1 and MRE11 in G1 phase as well as in S/G2 phase²⁸⁵. These findings suggest that BRCA1 and ubiquitylation promote MRE11-dependent removal of TOP2ccs prior to NHEJ during subsequent G0 or G1 phases.

The redundancy and complexity of the pathways that repair irreversible TOPccs and their associated DNA breaks are consistent with the occurrence of such lesions under physiological conditions. Somatic mutations in the pathways that repair these lesions (including in XPF, ERCC1, BRCA1 and BRCA2), in MRE11 and in other factors, may provide opportunities for synthetic lethal interaction treatment of cancers.

Chromosomal translocations and therapy-related acute myeloid leukaemia. Anticancer drugs targeting TOP2 enzymes (Supplementary Box 1) are associated with long-term undesirable side effects including therapy-related acute myeloid leukaemia (t-AML)^{17,286,287}. Although rare compared with the high lethality of the initial cancers, t-AML can be devastating for those individuals affected. t-AML occurs following treatment for a wide range of primary neoplasia. Breast cancer accounts for about 35% of t-AML cases, presumably owing to the relative number of breast cancer cases and the age profile of the individuals, and prior haematological malignancies account for approximately 30% of t-AML cases²⁸⁸. As cancer survival statistics have improved, t-AML cases have increased; up to 15% of all acute myeloid leukaemia (AML) cases can be classified as t-AML²⁸⁹.

Therapy-related leukaemia following treatment with TOP2 poisons is presumed to result from genetic damage to haematopoietic progenitor cells leading to the emergence of clones exhibiting a differentiation block and/or an increase in self-renewal and proliferation. The precise point or points during haematopoietic differentiation where progenitors can be transformed in this way is open to debate, but the 'window of opportunity' is likely between multipotential progenitor cells and granulocyte-macrophage progenitors^{290–294}.

Therapy-associated AML appearing after exposure to TOP2 poisons often contains one of a set of recurrent chromosomal translocations giving rise to fusion genes that disrupt blood cell development and differentiation^{17,228,295}. Thus, errors in the cellular processing and repair of TOP2-DPCs in haematopoietic cells are viewed as a common cause of t-AML. Indeed, translocation junction sequences are consistent with the erroneous joining of heterologous chromosome segments by NHEJ, or by the variant pathway alternative end joining^{296,297}. The most frequent translocations observed in t-AML involve fusion between the gene myeloid/lymphoid or mixed-lineage leukaemia (*MLL*; also known as *KMT2A*) at 11q23 and the genes *AF9* (*MLLT3*) at 9p21, *AF4* (*AFF1*) at 4q21 or, less frequently, numerous other genes^{17,101,295,298}. Breakage at the *MLL*–*AF9* or *MLL*–*AF4* fusion genes is associated with TOP2 cleavage sites⁹⁹. Other recurrent translocations include t(15;17)(*PML-RARA*), t(8;21)(*AML-ETO*), t(11q23, var) and inv(16)(p13;q22)(*CBFB-MYH11*)^{295,299,300}. Thus, a simple model for the aetiology of t-AML associated with TOP2 poisons involves illegitimate end joining between DNA repair intermediates, resulting in the formation of leukaemogenic chromosomal translocations^{292,295,299} (FIG. 5c). In addition, there appears to be some preference for which translocation (and therefore which AML subtype) is induced by different TOP2 poisons. For example, etoposide is associated with *MLL* translocations, whereas t(15,17)(*PML-RARA*) translocations resulting in treatment-associated acute promyelocytic leukaemia have been associated with mitoxantrone and epirubicin^{300–305}.

Chromosome break points involved in *MLL* translocations in AML fall within an 8 kb break-point cluster region, and those derived from t-AML are over-represented in the distal 1 kb of this break-point cluster region. This break-point clustering suggests that a specific mechanism is associated with therapy-related, but not de novo, AML translocations. The co-localization of TOP2B and CTCF^{99,106,306} provides one possible explanation for the over-representation of t-AML translocation break sites in the distal 1 kb of the *MLL* break-point cluster region, as this region is adjacent to a CTCF binding site within the *MLL* gene^{299,307}. The proximity to nascent mRNAs suggests that the translocations occur within transcription hubs^{228,299,308}. Furthermore, exposure of cells to large doses of TOP2 poisons results in chromosomal breakage in this 1 kb region that can be detected by Southern blotting^{309–312}, which strongly implicates TOP2 either directly or indirectly^{313–317} in the generation of DSBs that are the precursor of the chromosomal translocations.

Conclusions and future perspective

Topoisomerases are magicians of DNA and RNA^{15,318}, and their full range of functions remain to be discovered. Unknown functions such as the manipulation of RNA topology and how cells use and resolve knots, and the roles of topoisomerases in metabolism and mitochondria, are partially understood. Because topoisomerases are exploited as targets for a wide range of anticancer and antibacterial drugs, understanding the repair pathways of TOPccs can provide opportunities for improving the rational use of topoisomerase inhibitors and their combination with other drugs in cancer treatment.

Topoisomerases are an increasingly recognized source of genome instability. In this Review we have discussed the emerging range of (redundant) repair pathways that handle the failures of topoisomerases in physiological conditions and following their therapeutic targeting. These pathways have to repair highly damaging lesions, consisting of DPCs, SSBs and DSBs. The existence of redundant mechanisms that repair topoisomerase-induced

genomic damage begs the question of repair pathway choice and selection. It is likely that the sites of damage and whether topoisomerases are trapped during transcription or replication, or whether the damage occurs in highly proliferative cells such as haematopoietic progenitors or in highly differentiated cells such as neurons, are germane to repair pathway choice.

Because of the potential danger of topoisomerases, it is likely that their activity is controlled and restricted to proper sites of action. In that context, further studies are warranted to elucidate the molecular partners of topoisomerases within molecular machines and their regulation by post-translational modifications.

Finally, the detailed location and roles of topoisomerases at centromeres and telomeres, and how topoisomerases are coordinated with chromatin remodelling factors and the architecture of chromatin and chromosomes, represent promising areas of investigation.

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