DNA topoisomerase II and its growing repertoire of biological functions

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Abstract | DNA topoisomerases are enzymes that disentangle the topological problems that arise in double-stranded DNA. Many of these can be solved by the generation of either single or double strand breaks. However, where there is a clear requirement to alter DNA topology by introducing transient double strand breaks, only DNA topoisomerase II (TOP2) can carry out this reaction. Extensive biochemical and structural studies have provided detailed models of how TOP2 alters DNA structure, and recent molecular studies have greatly expanded knowledge of the biological contexts in which TOP2 functions, such as DNA replication, transcription and chromosome segregation — processes that are essential for preventing tumorigenesis.

Catenane
Circles linked as in a chain: the two links cannot be separated without breaking one of the two molecules.

The double-stranded nature of DNA creates a special set of problems for processes that require strand unwinding such as transcription and replication. The unwinding that occurs during these processes creates a topological problem because the unwinding must be compensated by overwinding elsewhere in the DNA molecule. DNA topoisomerases are enzymes that solve these difficulties by introducing transient breaks in DNA. The transient breaks allow changes in DNA topology that eliminate the overwinding. There are two classes of topoisomerases: type I enzymes, which introduce single strand breaks in DNA, and type II topoisomerases, which introduce double strand breaks[1,2]. As a single unrepaired double strand break has potentially lethal consequences, type II topoisomerases might be viewed as a particularly dangerous way of dealing with the topological problems of DNA. The work of Sundin and Varshavsky showed that there were topological problems arising on the completion of replication that absolutely required a type II topoisomerase to separate replicated molecules[3,4]. Studies in a wide range of eukaryotes have confirmed these initial notions and have shown that type II topoisomerases are required to segregate replicated chromosomes. Moreover, type II topoisomerases participate in many of the nuclear processes that generate topological problems.

The past few years have seen an explosion in findings concerning the biochemistry and biology of type II topoisomerases. The biochemical steps in the TOP2 reaction have been demonstrated, and structural studies have provided an underpinning for understanding the enzyme reaction cycle. At the same time, the number of processes that are known to use type II topoisomerases, especially in gene expression, has multiplied. This was driven by efforts to understand why mammalian cells express two TOP2 isozymes (TOP2α and TOP2β) (BOX 1), whereas most other eukaryotes have only a single TOP2 enzyme. There has been continued interest in how cells regulate when and where type II enzymes act, as well as how cells insulate themselves when type II enzymes fail to function properly. This Review highlights recent work on the role of TOP2 in replication, transcription and chromosome structure that may be relevant to the phenotypes of cancer cells.

How the TOP2 machine works

Topological changes in DNA require the introduction of DNA strand breaks, and topoisomerases provide a safe mechanism for introducing these changes. Because the strand breaks are protected (covalently bound to protein) they neither generate ends that are subject to rearrangement or recombination, nor generate DNA damage responses. A simple topological change, illustrated in FIG. 1, is the decatenation of a singly linked catenane. Catenanes are the simplest topological change that can be visualized. Another important change is in the regulation of DNA supercoiling. Topoisomerases can convert DNA that is underwound to the energetically more stable state of no superhelical turns. Although it is most rigorous to discuss topological changes in the context of a circular DNA molecule, topological considerations also apply to long linear molecules, as the break is too far away to allow the changes in winding to occur with reasonable kinetics. This is a brief description of topological changes in DNA, and more complete and careful descriptions are available[5].

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At a glance

- Type II topoisomerases change DNA topology by generating transient DNA double strand breaks and are essential for all eukaryotic cells.
- Mammalian cells have two topoisomerase II (TOP2) isoforms, TOP2α and TOP2β. TOP2α is essential for all cells, and is essential for separating replicated chromosomes. TOP2β is required for normal development, but is dispensable in some cell types. Type II topoisomerases are required for other processes such as transcription, and the precise roles of the two isoforms in these processes are a subject of current studies.
- Type II topoisomerases use a two gate mechanism for carrying out topological changes in DNA. The enzyme requires ATP hydrolysis for its reaction. ATP hydrolysis is used for for conformational changes of the enzyme, and is not directly involved in DNA breakage or resealing.
- Crystal structures of several domains of yeast Top2 have provided additional information about how the enzyme carries out its reactions. A recent structure of the breakage reunion domain of yeast Top2 bound to DNA has shown that the enzyme induces a large bend in the DNA that is cleaved by the enzyme.
- Biological functions of TOP2 isoforms are modulated by a variety of protein–protein interactions. Some of these interactions may affect enzyme activity, stability and localization.
- TOP2 activity is also modulated by post-translational modification. In addition to phosphorylation, a crucial post-translational modification of TOP2 is sumoylation. Failure to sumoylate TOP2α or to remove the SUMO modification disrupts the ability of TOP2α to separate replicated chromosomes.
- TOP2β has a key role in the survival of some neural cells. TOP2β is important in transcriptional regulation, and it is likely that TOP2β enzyme activity is specifically required.
- Some aspects of TOP2 function during the cell cycle are monitored by checkpoints. It has been hypothesized that a major role of checkpoints is to monitor the completion of decatenation. If so, then TOP2-dependent checkpoints may be crucial for normal chromosome segregation and genome stability.

The catalytic reaction. Eukaryotic type II topoisomerases are large homodimeric enzymes. The overall reaction strategy is the generation of a transient double strand break, with each subunit breaking one DNA strand. The enzyme will pass an unbroken strand through the transient break, and then reseal the break. The detailed reaction mechanism of TOP2 is presented in FIG. 1. DNA cleavage by TOP2 uses a tyrosine that is activated to attack the phosphodiester backbone of DNA and form a phosphotyrosine linkage. For TOP2, cleavage requires a collaboration between the active site tyrosine and other residues, including the TOPRIM domain[6]. The TOPRIM domain includes an acidic triad of residues that is involved in complexing a divalent cation, which is absolutely required for the cleavage reaction. Because the energy of the phosphodiester bond is conserved in the phosphotyrosine bond, the cleavage reaction can be reversed without a high-energy cofactor, leading to restoration of the phosphodiester bond and the free enzyme. This mechanism of DNA cleavage provides several distinct advantages, including the protection of DNA ends and the ability to quickly and efficiently re-ligate the DNA strand break. It is this reaction that is exploited by many drugs that target TOP2. Agents such as etoposide and mAMSA inhibit the re-ligation step and trap TOP2 as a complex in which the enzyme is covalently bound to DNA with broken strands. An important property of the covalent complex is that in most instances it remains freely reversible. Removal of the drug allows the enzyme to rapidly and efficiently reseal the DNA break.

Early studies of TOP2-targeting drugs relied on enzyme denaturation to trap the drug-stabilized complex. As the breaks and covalently bound protein were only efficiently detected in the presence of a protein denaturant, it was formally possible that the denaturant somehow induced the strand breaks. Therefore, the intermediate was termed a cleavable complex. As many studies have demonstrated the presence of cleaved DNA in the absence of denaturants, terms such as cleaved complex are more precise, although cleavable complex continues to be used for historical reasons.

Structural analyses of TOP2. Although the mechanics of DNA cleavage and strand passage were originally studied biochemically, a series of elegant structural studies has provided support and elaboration of the enzyme mechanisms described above. The model for most structural studies has been the type II topoisomerase from yeast. However, eukaryotic type II topoisomerases are highly conserved, and the structural insights from the yeast enzyme are also likely to apply to the human enzyme. The amino-terminal domain of the protein carries the ATP-binding domain. A central portion of the protein includes the TOPRIM domain, followed by the breakage reunion domain, which carries the active site tyrosine. The carboxy-terminal domain of the protein is not conserved between the type II topoisomerase from different species, nor is it conserved between TOP2α and TOP2β. The C-terminal domain is probably required for nuclear localization, regulation of enzyme activity by post-translational modification, and regulation of enzyme function by protein–protein interactions. The size and flexibility of TOP2 has prevented the determination of the structure of the intact enzyme. Therefore, much of the structural information has been obtained from structures of portions of the protein. The structures that have been determined for yeast Top2 include the N-terminal domain of the protein[7] (the human α-amino-terminal domain has also been reported[8]), and three separate structures of the breakage reunion domain, including a recent structure that includes this domain bound to DNA[9]. These protein structures have provided a rich source of insights into TOP2 function and have been reviewed comprehensively[10,11]. Therefore, only key highlights of the structures are described (FIG. 2).

The N-terminal domain of the ATPase region consists of a GHKL (gyrase, HSP90, histidine kinase, MutL) fold that is found in a variety of ATPases[10]. An important characteristic of the ATP-binding site is that both subunits contribute to its overall architecture[7]. The collaboration between the subunits couples ATP binding to dimer formation. Similarly, after ATP hydrolysis and release of ADP and inorganic phosphate, dimerization at the N terminus is destabilized. The C-terminal part of the ATPase has been termed the transducer domain. The transducer domain signals ATP binding to the breakage reunion domain. It appears to do this by undergoing a

TOPRIM domain
A conserved domain found in topoisomerases, primases and other DNA metabolic enzymes. The TOPRIM domain adopts a Rossman fold and is involved in divalent cation binding.

B DNA
DNA exists in many possible conformations, but only A-DNA, B-DNA and Z-DNA have been observed in organisms. Which conformation DNA adopts depends, for example, on the sequence of the DNA, or the amount and direction of supercoiling. The B form is most common under the conditions found in cells.

Boltzmann distribution
A certain distribution function or probability measure for the distribution of the states of a system.

shift in position following ATP binding that may trigger other conformational changes in the breakage reunion domain. Importantly, the transducer domain contributes amino acids that participate in ATP binding and hydrolysis. This may allow ATP hydrolysis to influence progression of the enzyme through the catalytic cycle (in addition to its role in N-terminal dimerization).

The breakage reunion domain consists of a large heart-shaped structure with a large central cavity. The N-terminal portion of the protein consists of the TOPRIM domain. The active site tyrosine is part of a winged helix domain that is similar to the catabolite activator protein (CAP-like domain). Adjacent to the CAP-like domain is a 'tower' that leads into a long coiled coil that terminates in another dimer interface[12]. This initial structure, confirmed by a subsequent crystal structure, exhibited several features consistent with the two gate model described above, especially in the C-terminal dimer interface that was likely to represent the exit point for the T segment. The crystal structure raised several questions, including the significance of the separation between the tyrosine residues of the two subunits that was too great for interaction with B-DNA. A reasonable interpretation was that this structure shows an open state in which the enzyme has introduced a break in the DNA and separated the two strands to allow for passage of the T segment. This point of view was supported by a second structure with a reduced separation between the active site tyrosines[13]. This second structure also showed a substantial conformational shift, suggesting that this structure represented an intermediate between the open structure and the structure before cleavage. A second aspect of the two structures was that the TOPRIM domains were located too far from the active site tyrosines to participate in DNA cleavage, as suggested by biochemical data. Some of the questions raised were answered in a third structure that included a nicked DNA molecule bound to the breakage reunion domain. In this structure, the TOPRIM domain is brought near to the active site tyrosine, allowing collaboration for DNA cleavage.

There are two other noteworthy aspects of the TOP2–DNA binary complex[9]. First, the DNA bound to TOP2 is bent by 150°. The DNA between the active site tyrosines is in an A form helix. The detection of a bend in the DNA provided strong support for a model explaining a peculiar property of type II topoisomerases. DNA topoisomerase I, when carrying out relaxation, generates a series of topoisomers centred around the lowest free-energy state. The distribution of topoisomers follows a Boltzmann distribution, consistent with the free energy associated with DNA supercoiling. By contrast, the distribution of topoisomers formed by TOP2 relaxation is much narrower than expected. The biological significance of this reaction is that TOP2 needs to perform complete separation of catenated molecules before mitosis and the presence of a single link would be sufficient to prevent proper segregation. Therefore this property of TOP2, termed topology simplification, helps to ensure a complete decatenation reaction[14]. Cozzarelli and colleagues proposed a model for topology simplification, which required that TOP2 introduce a strong bend in the G segment[15]. The reported structure fulfils this expectation. Finally, the TOP2 structure shows the C-terminal dimerization found in the other two structures to be disrupted. In other words, this structure shows the C-terminal gate to be open, in support of the prediction that the T segment exits the enzyme through this gate.

Box 1 | **The complement of type II topoisomerases in eukaryotic cells**

There are two broad classes of type II topoisomerases: type IIA topoisomerases, which include prokaryotic DNA gyrase, prokaryotic topoisomerase IV and eukaryotic topoisomerase II (TOP2), and type IIB topoisomerases, which include TopoVI from plants[98] and homologues of *Saccharomyces cerevisiae* Spo11, which are required to introduce double strand cleavage that initiates meiotic recombination[99]. In lower eukaryotes, including single-cell organisms such as yeast, insects and vertebrates such as *Xenopus laevis*, there is a single TOP2 isoform. Mammals have two TOP2 isoforms termed α and β[100]. Expression of TOP2α is cell cycle regulated, and this enzyme is essential for the viability of all dividing cells. Many non-dividing cells lack detectable TOP2α. The TOP2β isozyme is required for viability in mouse, and has a key role in neuronal development. Embryos lacking TOP2β fail to innervate the diaphragm and die at or before birth. As the embryos develop almost to term, it possible to isolate viable cells completely lacking TOP2β. The roles of TOP2α and TOP2β appear to be dictated mainly by their carboxy-terminal domains. In a conditional knockout cell system, expression of TOP2β fails to complement a deficiency of TOP2α, although fusion of the catalytic domains of TOP2β to the C-terminal domain of TOP2α does complement the deficiency. Conversely, fusion of the C-terminal domain of TOP2β to TOP2α catalytic domains does not complement the conditional deficiency of TOP2α[101]. The second class of type II topoisomerases, type IIB enzymes, are homologous to archaebacterial type II topoisomerases. Mammals and lower eukaryotes have a type IIB homologue: SPO11 (REFS 102,103). This enzyme is required to initiate meiotic recombination by the generation of an enzyme-mediated double strand break. Type IIB topoisomerases have diverse physiological functions in plants[98,104].

Biological functions of TOP2

A key question in the biology of TOP2 proteins is how the protein is localized to where it needs to perform its important functions. As genetic analysis by loss-of-function mutants is difficult for proteins that are essential for all cells, proteomic approaches have frequently been used to dissect processes that use TOP2 isozymes. Recently, small interfering RNA (siRNA) directed against TOP2 isozymes has been applied for studying the effects of loss of topoisomerase functions, with particular success in *Drosophila melanogaster* systems[16–19]. An additional difficulty is that ectopic expression of TOP2α has been difficult to achieve, and overexpression of the enzyme induces apoptosis[20]. This problem has been ameliorated by expression of N-terminal enhanced green fluorescent protein–TOP2 fusions[21]; however, it remains possible that the N-terminal tag significantly affects the function of the protein. TABLE 1 presents a compilation of proteins that have been described in the literature to physically interact with TOP2 isozymes. Not all of the interactions listed in TABLE 1 have been demonstrated to have physiological relevance.

Role of TOP2 in replication. One of the central roles of DNA topoisomerases is to solve the topological problems associated with replication. Semi-conservative

Precatenane
A structure related to a catenane that results from the interwinding of DNA strands behind a replication fork. Precatenanes interconvert with positive supercoils that arise in front of a replication fork.

replication involves the unwinding of duplex DNA and copying of each strand. In the absence of a topoisomerase activity the unwinding of the parental duplex leads to the accumulation of positively supercoiled DNA in front of the replication fork, which can be relaxed by either TOP1 or TOP2. In addition to the generation of positive supercoiling in front of the fork, the positively supercoiled DNA at the replication fork can isomerize by migration of the positive supercoiling into wrapping of the two replicated strands (FIG. 3). This structure, called a precatenane, is a substrate for TOP2-mediated DNA catenation, and may represent a plausible mechanism for TOP2 action during replication elongation[22]. Studies in bacterial replication have provided clear evidence for precatenane formation[23] and it is likely that this mechanism is also important in eukaryotic cells[22,24]. In the latter stages of replication, when two replication forks impinge on each other, there is no longer room for a type I topoisomerase to relax positive supercoils, and completion of replication leads to two interlinked catenanes. This catenated dimer requires TOP2 for resolution (FIG. 3).

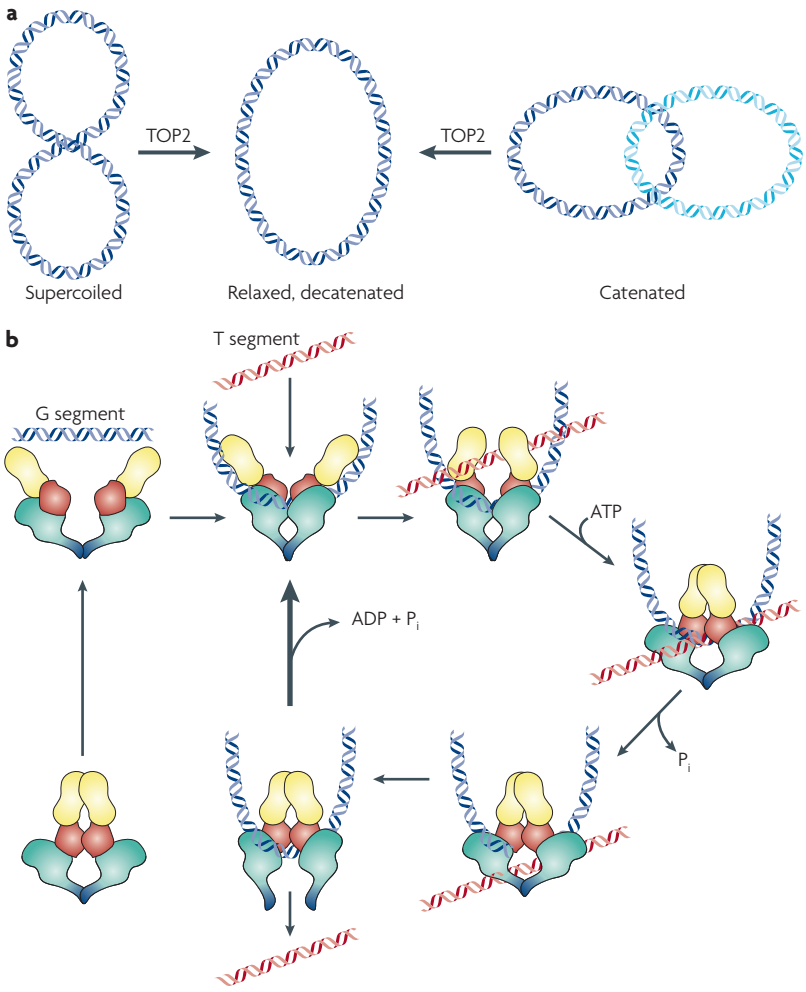

Figure 1 | **Mechanism of strand passage by type II topoisomerases. a** | Reactions catalysed by eukaryotic topoisomerase II (TOP2) include decatenation of linked intact double stranded DNA and relaxation of supercoiled DNA. The reaction formally requires introduction of a double strand break, strand passage and break resealing. **b** | Topoisomerase II interacts with two DNA strands to effect strand passage. The enzyme introduces a double strand break in one DNA strand, termed the G or gate segment, and will pass a second strand termed the T segment through the break. In the presence of Mg^{2+}, the enzyme can cleave the DNA, forming a phosphotyrosine linkage between each single strand and a tyrosine in each subunit. ATP binding causes the enzyme to form a closed clamp. The closed clamp may also capture another strand (the T strand) that will pass through the break made in the G strand. After passing through the break in the G strand, the T strand exits the enzyme through the carboxy terminus (the bottom of the enzyme as drawn). ATP hydrolysis occurs at two steps in the reaction cycle[105]. The first ATP hydrolysed may assist in strand passage. The second hydrolysis step (along with release of ADP and inorganic phosphate (P_i)) allows the clamp to reopen, and allows release of the G segment (for a distributive reaction). Alternately, the enzyme may initiate another catalytic cycle without dissociating from the G strand. The figure is modified, with permission, from *Nature* REF. 12 © (2002) Macmillan Publishers Ltd. All rights reserved.

The products of the replication of a small circular DNA *in vitro* in the absence of TOP2 are catenated dimeric plasmids. A requirement for TOP2 in this reaction with chromosomal DNA was first observed in yeast. Yeast cells that have Top2 as the only active topoisomerase are viable, and undergo normal DNA replication[25,26] without activation of any S phase-dependent checkpoints[27]. In the absence of TOP2 (for example, using temperature-sensitive yeast mutants), cells complete DNA replication and die when they enter mitosis[28]. Interestingly, the effects on replication differ between yeast cells completely lacking any Top2 protein and cells carrying an enzymatically inactive protein[29]. Cells depleted of Top2 using a conditionally degradable Top2 protein were able to complete replication, but not chromosome decatenation and, in agreement with results obtained with temperature-sensitive proteins, lost viability at mitosis. Expression of a catalytically dead protein generated a different phenotype: a failure to complete replication at sites where two replication forks meet. A plausible model for these results is that TOP2 is normally recruited to act where replication forks meet. In the complete absence of the protein, replication is complete, with the products of the reaction being catenated sister chromatids. The presence of a catalytically inactive protein interferes with the completion of replication, leading to checkpoint induction.

Experiments in mammalian cells using conditionally expressed TOP2α[30] or siRNA knockdown of TOP2α[31] (BOX 1) support this model for TOP2 action. As many cell types can be recovered from TOP2β homozygous knockout mice and grown in culture, it is unlikely that TOP2β has a crucial role in replication. Studies using RNA interference (RNAi) directed against TOP2α generally fail to reveal an indispensable role during replication, although recent experiments suggest that phosphorylation of TOP2α during S phase is required for normal S phase progression[31]. Interestingly, biochemical analysis of the human TOP2α has shown that the protein is much more active in relaxing positively supercoiled substrates than in relaxing negatively supercoiled substrates. This property is not found in TOP2β, nor is it seen with lower-eukaryotic type II topoisomerases[32]. As positive supercoiling is expected to be generated in advance of a replication fork, this preferential activity has been suggested to imply an important role for TOP2α at some point in replication.

The role of TOP2 in chromosome separation and segregation. Although catenation of replicated chromosomes is presented as a problem, the generation of catenated sister chromatids may assist in the proper segregation of duplicated chromosomes[33]. After replication, sister chromatids must stay together until mitosis. Early separation leads to inaccurate chromosome transmission. Although early models of sister chromatid cohesion posited a role for catenanes in cohesion maintenance, subsequent studies showed that specialized protein complexes called cohesins were essential for keeping sister chromatids together[34,35]. Surprisingly, although mutation of cohesins diminished cohesion, some cohesion was still evident[36,37]. One possible explanation for these results is that cohesion can be maintained by multiple mechanisms, with catenanes representing one of several mechanisms.

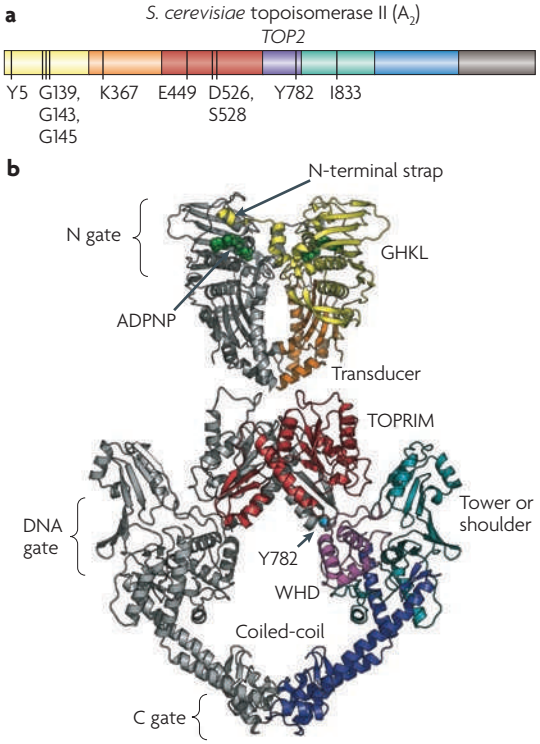

Figure 2 | **Structure of eukaryotic topoisomerase II (TOP2). a** | The figure shows the domain structure of a eukaryotic TOP2 (specifically that of *Saccharomyces cerevisiae* binding the non-hydrolysable ATP analogue ADPNP (5′-adenylyl-β,γ-imidodiphosphate)). Domains are indicated in colour (see below), and key residues are indicated. The residues marked include G139, G143 and G145 in the ATP binding domain; K367 — a transducer domain residue that contributes to the ATPase; E449, D526 and D528 — the acidic triad involved in binding a divalent cation; Y782 — the residue that makes a covalent attachment with DNA; and I833 — a tower domain residue that is involved in DNA interaction. **b** | The figure shows the structure of yeast Top2 based on structures for the ATPase domain and the breakage reunion domain[7,106]. The GHKL (gyrase, HSP90, histidine kinase, MutL) and transducer domains are shown in yellow and orange, TOPRIM, winged helix domain (WHD), tower, and coiled coil are shown in red, purple, teal and blue, and Tyr782 is shown as a cyan sphere. The figure is reproduced, with permission, from REF. 11 © (2008) Cambridge University Press.

The accurate programme of chromosome decatenation probably requires that TOP2 act at specific chromosome regions following a precise temporal programme. The localization of TOP2 to specific chromosome regions relies both on protein–protein interactions[38,39] and post-translational modification of TOP2 (discussed below). Earlier studies had indicated localization of TOP2α to centromeric regions[40–42]. The ability to specifically deplete TOP2 isozymes in model systems that are amenable to cytological analysis has been crucial for testing where and when TOP2 must act during mitosis. For example, in *D. melanogaster* S2 cells, RNAi directed against TOP2 clearly leads to a failure in chromosome separation[16,19]. The details of chromosome behaviour in the absence of TOP2 protein include abnormal localization of chromosome arms[16] and a failure to establish amphitelic kinetochore attachment[19] (that is, a failure to attach centromeres to opposite poles of the spindle). Coelho and colleagues also observed that depletion of TOP2 led to reduced activity of Aurora kinase B, an effect that could be ameliorated by co-depletion of TOP2 and the spindle checkpoint protein BubR1. The observations of Coelho and colleagues clearly connect TOP2 protein to the dynamics of events at mitosis, particularly as regulated by checkpoints that assess tension at kinetochores[43–45]. Recent studies in mammalian cells also support the hypothesis that TOP2 has crucial functions at centromeres[46], although the molecular details remain to be elaborated.

Protein modification of TOP2α. Support for a role for catenation in chromosome cohesion came from seminal studies on the regulation of yeast Top2 by the ubiquitin-like modifier SUMO[47]. Mutation of the *SMT4*, the isopeptidase that deconjugates SUMO, leads to precocious sister chromatid separation. The defect in cohesion was specific for regions near the yeast centromere. This defect could be suppressed either by overexpression of yeast Top2, or by mutating all candidate sites on Top2 that could be modified by SUMO. One explanation for these results is that SUMO modification blocks the ability of TOP2 to maintain cohesion at chromosomes. An economical explanation is that SUMO modification inhibits decatenation (or promotes catenation) by TOP2. As TOP2 has roles in chromosome structure, the SUMO modification may impart a structural alteration that is required for maintaining cohesion at centromeres.

SUMO modification of TOP2α is crucial in mammalian cells. Initial experiments in *Xenopus laevis* suggested that PIASγ (also known as PIAS4) is a major SUMO E3 ligase[48]. Depletion of PIASγ from *X. laevis* extracts leads to metaphase arrest, and depletion of sumoylated proteins from the inner centromere. Support for this hypothesis was obtained using siRNA directed against PIASγ in human cells, finding a lack of TOP2α localization to centromeres in PIASγ-deficient cells[49]. Surprisingly, a chromosome segregation defect was not seen in a mouse knockout of *Pias4*[50,51]. Other recent

Hypomorphs
Organisms expressing alleles that result in a reduction, but not the elimination, of wild-type levels of a gene product or activity, often causing a less severe phenotype than a loss-of-function (or null) allele.

results have called into question the role of PIASγ in sumoylation of TOP2. RANBP2 is a nucleoporin with SUMO E3 ligase activity. The gene is essential in mouse, and hypomorphs show defects in chromosome segregation, generation of anaphase bridges, induction of high levels of aneuploidy, and increased spontaneous and chemical-induced tumorigenesis[52]. *In vitro* analysis demonstrated that RANBP2 hypomorphs are defective in SUMO modification of TOP2α, with a failure to localize TOP2α to centromeres. Ectopic expression of either RANBP2 or a SUMO–TOP2 fusion restores TOP2 localization to centromeres. The same authors also showed that PIASγ-deficient mouse embryonic fibroblasts do not show a defect in TOP2α localization, nor do they show a defect in TOP2α sumoylation. These results provide overwhelming support for the hypothesis that RANBP2 is the major SUMO E3 ligase for TOP2α. What might be the importance of PIASγ? Although it may not participate in sumoylation of TOP2, many other centromere proteins are also sumoylated, and the defects observed in *X. laevis* extracts may reflect roles in sumoylating other proteins. The experiments with RANBP2 highlight the potential

Table 1 | **Proteins and protein complexes interacting with TOP2 in mammalian cells**

Protein	Postulated function with TOP2	TOP2 isozyme involved	Refs
14-3-3ε	Modulates TOP2 cleavage activity by an unknown mechanism; 14-3-3 proteins have roles in cell signalling	α	107
APC	Important regulator of mitotic proteins	α	108
Aurora B kinase	Regulator of mitotic events; TOP2α is a substrate of Aurora B	α	19,109
BRCA1	Tumour suppressor, activates TOP2 decatenation activity perhaps by monoubiquitylination	α	110
CAPH	Condensin I-associated subunit, required for sister chromatid resolution	α	38,111
CK2	Protein kinase with diverse functions, activates TOP2 (phosphorylation by the enzyme not required)	α and β	112–117
CDC2	Protein kinase regulator of cell cycle progression	α	118
CHRAC1	Chromatin-remodelling complex	Found in *Drosophila melanogaster* but not human cells	119,120
CRM1	Nuclear export protein, may stimulate elimination of TOP2 from the nucleus under some conditions	α	121,122
HDAC1	Gene repression	α and β	123,124
HDAC2	Gene repression	α and β	123,124
Jab1 or CSN5	Regulation of TOP2 stability	α	125
Ku70 or Ku80	Non-homologous end joining, telomere metabolism, transcription	α and β	69,70,126
MDC1	Checkpoint protein reported to bind to phosphorylated TOP2α	α	89
p53	Tumour suppressor with diverse functions	α and β	127
PARP	Multiple cellular functions including DNA repair	β	69,70
PCNA	DNA clamp required for DNA replication and repair	α	128
PIASγ	SUMO E3 ligase, modifies TOP2α in *Xenopus laevis*	α	48
PIN1	Interacts with both CK2 and TOP2α, involved in replication termination and chromosome condensation	α	54,129,130
PLCR1	Unknown	α and β	131
RANBP2	SUMO E3 ligase, modifies TOP2α	α	52,132
RARα	Gene regulation	β	71,116
RHA1	Gene regulation	α	133
SUMO1, SUMO2 and SUMO3	Small ubiquitin-like modifier with diverse cellular functions	α and β	48,134–136
TCF4	Part of the β-catenin–TCF4 nuclear complex, transcription	α	137
TOPBP1	DNA damage checkpoint protein, homologue of the yeast replication protein Dpb11	β	95,138
Toposome	Multiprotein complex that includes RHA1, a protein kinase SRPK1, and other proteins; function not yet determined.	α	139

APC, adenomatous polyposis coli; CAPH, condensin complex subunit 2; CDC2, cell division cycle 2; CHRAC1, chromatin accessibility complex protein 1; CK2, casein kinase II; CRM1, chromosome region maintenance 1; HDAC, histone deacetylase; MDC1, mediator of DNA damage checkpoint 1; PARP, poly(ADP-ribose) polymerase 1; PCNA, proliferating cell nuclear antigen; PLCR1, phospholipid scramblase 1; RANBP2, RAN-binding protein 2; RARα, retinoic acid receptor-α; RHA1, RNA helicase I; TCF4, T-cell factor 4; TOP2, topoisomerase II; TOPBP1, topoisomerase binding protein 1.

importance of TOP2 in chromosome stability. RANBP2 probably has other important targets besides TOP2α that may contribute to high levels of aneuploidy and tumorigenesis. However, the results suggest the interesting possibility that prevention of aneuploidy rightly qualifies TOP2α as a tumour suppressor.

TOP2 and chromosome structure. Classical studies indicated that TOP2 has a key role in chromosome structure and chromosome condensation[53,54]. Early studies using specific extraction procedures identified a chromosome scaffold that included TOP2α and an additional complex now termed condensin[34,55–57]. A role for TOP2 in condensation was certainly plausible on the basis of possible topological constraints as chromatin is compacted. A detailed description of current issues related to chromosome structure and condensation is beyond the scope of this Review. Many current issues relate to what steps absolutely require TOP2, given that chromosome condensation can occur in many contexts in which TOP2 is absent. The ability to examine the roles of TOP2 in physiological settings by RNAi or by conditional replacement using mutant alleles of TOP2 will be crucial in understanding these processes and how they connect to other cellular events including decatenation and faithful chromosome segregation.

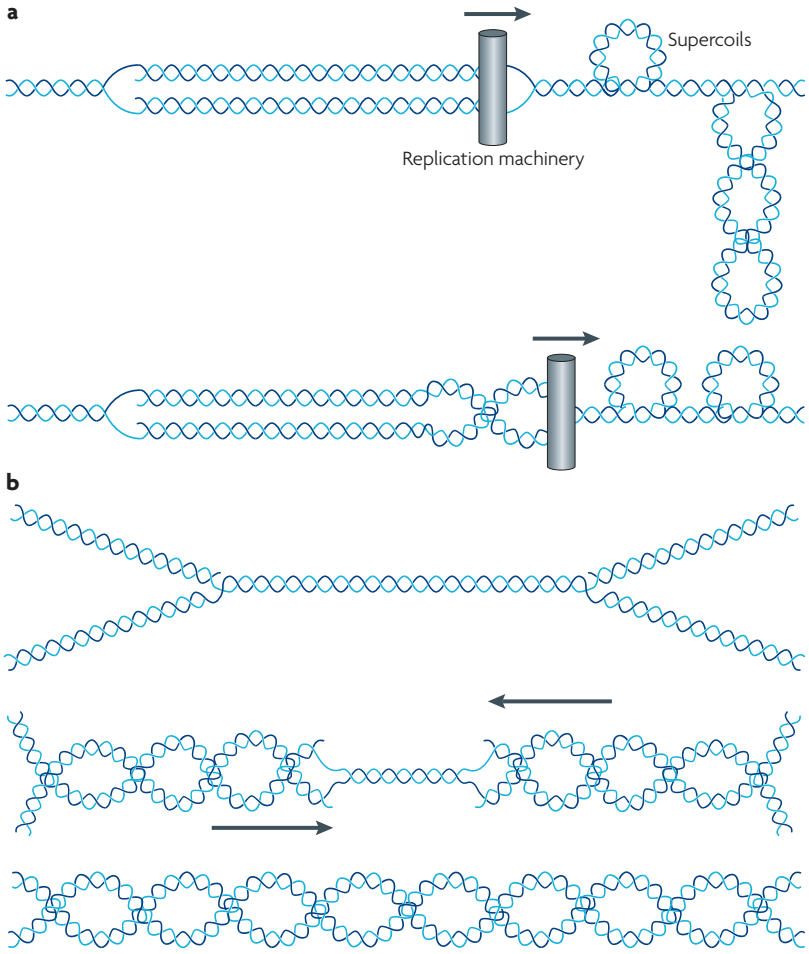

Figure 3 | **Roles of topoisomerase II (TOP2) in replication. The figure shows the** partition of superhelical strain during replication fork progression *in vivo*. During replication, helicase action on DNA creates positive superhelical stress on the DNA. This results in positive supercoils in front of the fork (**a**). This structure can isomerize into intertwinings of the daughter duplexes, generating precatenanes. At early steps in replication, when forks are widely separated (**b**), either TOP1 or TOP2 can function as a replication swivel. TOP1 acts by relaxing positive supercoils whereas TOP2 unlinks precatenanes. Note that TOP2 also should be able to relax positive supercoils, and does not require the isomerization to precatenanes for unlinking replicated strands. As the replication forks converge, there is a limited ability to generate positive supercoiling, and complete unlinking absolutely requires TOP2. The figure is reproduced, with permission, from *Nature Reviews Molecular Cell Biology* REF. 68 © (2002) Macmillan Publishers Ltd. All rights reserved.

Transcription

In yeast, loss of either topoisomerase does not block DNA replication or transcription, but both processes are strongly inhibited if both enzyme activities are absent[25,26,58–60]. The effect on transcription in yeast is mainly on transcription by RNA polymerase I; overall levels of polymerase II transcription are affected to a lesser extent. It has recently been suggested that, in yeast, Top2 may be more active in relaxing supercoils in chromatin than topoisomerase I[61], although this property has not yet been associated with any unique phenotypical consequences. It was initially reported that there may be a unique requirement for a type II topoisomerase for transcription *in vitro* on chromatin templates, based in part on the association of TOP2α with a multi-subunit RNA polymerase II holoenzyme[62]. Subsequent work indicated that either a type I or a type II topoisomerase could support transcription on chromatin templates[63]. This finding suggests that the crucial function provided by the topoisomerase is relaxation of DNA supercoiling. It should be noted that TOP1 functions as a basal transcription factor *in vitro*, but this function can also be carried out by TOP1 protein that is catalytically inactive owing to an active site mutation[64–66]. Therefore, these functions of topoisomerases in transcription differ from the structural role of TOP1 previously described. An important model for generation of supercoiling during transcription has been described by Liu and Wang, and posits that the tracking of RNA polymerase leads to transient positive supercoiling ahead of the transcriptional machinery and negative supercoiling behind it; the generation of supercoils could reasonably be exacerbated by the presence of chromatin[67,68]. The transcriptional supercoiling model provides an important basis for a requirement of a topoisomerase during transcriptional elongation. Whether there are contexts in which a specific topoisomerase is preferentially used remains to be determined.

Recent work has also provided evidence for a specific role for mammalian TOP2β in transcription initiation. Ju *et al.* used chromatin immunoprecipitation to demonstrate that TOP2β localizes to promoters of genes of which expression is activated by nuclear hormone receptors (but not to many other promoters undergoing active transcription)[69,70]. They showed that TOP2β associates with signal-dependent promoters as part of a complex that includes several proteins important for DNA repair, and that the enzymatic activity of TOP2β

Bisdioxopiperazines. A class of small molecules, including ICRF-159, ICRF-187 and MST-16, that inhibit the catalytic activity of TOP2 and do not stabilize the TOP2 cleaved complex. Bisdioxopiperazines are the most commonly used catalytic inhibitors of type II topoisomerases.

was required for efficient transcriptional activation. It is important to note that, although the complex that associates with the promoter includes several proteins that have key roles in DNA repair — such as poly(ADP-ribose) polymerase, DNA-dependent protein kinase, and Ku70–Ku86 — the presence of the repair proteins does not seem to be required to repair the TOP2β-induced break. Rather, TOP2β is recruited to a subset of promoters, in a complex that includes DNA repair proteins. The enzymatic function of TOP2β is required at the promoter, rather than the enzyme acting in a purely structural role as occurs with TOP1. The break induced by TOP2β is the normal cleavage of the enzyme reaction cycle, as shown in FIG. 1. The enzymatic function of poly(ADP-ribose) polymerase also appears to be required, but it may function in ways that are distinct from the function of this enzyme in DNA repair. The importance of this finding is that it establishes a specific role for the enzymatic activity of a type II topoisomerase in transcriptional regulation. Several interesting challenges are raised by this work, including identifying the determinants that lead to recruitment of TOP2β and assessing whether recruitment of a topoisomerase other than TOP2β can also lead to transcriptional activation.

Although it is easy to appreciate that TOP2 activity may be required for activation of transcription[68], a recent result suggests that TOP2β can participate in repression of transcription. Miller and colleagues showed that TOP2β can negatively regulate RARα transcriptional activation[71]. They hypothesize that in this context TOP2β is part of a complex that is distinct from the one described by Ju and colleagues.

Further support for a specific role of TOP2β in transcriptional regulation has been provided by Lyu and colleagues[72]. As TOP2β has key roles in neural development[73,74], they reasoned that loss of function of TOP2β might lead to alterations in gene expression in neural tissue. Mice carrying homozygous deletions of *Top2b* are unviable owing to multiple neuronal deficits, including a failure of motor neurons to innervate the diaphragm. Using microarray analysis, they determined that approximately 1–4% of expressed genes showed changes in expression in *Top2b*$^{-/-}$ mice. Importantly, they were also able to demonstrate localization of TOP2β to various genes, including many developmentally regulated genes. Taken together the studies described above clearly indicate important contexts in which TOP2β influences regulation of gene expression. It will be interesting to determine in what other contexts type II topoisomerases contribute to gene regulation, especially in pathways related to cancer development.

Checkpoints for ensuring correct TOP2 function

Key events in progression through the cell cycle are monitored through a series of checkpoints. Checkpoints assess the integrity of crucial events during the cell cycle, such as the completion of DNA replication and the presence of an appropriate mitotic spindle[75]. As topoisomerase II carries out a reaction that is essential for chromosome separation at mitosis, a plausible hypothesis is that cells can monitor the successful completion of topoisomerase II decatenation, and arrest cell cycle progression if decatenation (or chromosome condensation) is incomplete. Early studies using *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* argued against this possibility, as conditional Top2 mutants showed minimal cell cycle delay, and instead accumulated broken chromosomes at the time of mitosis[28,76–78]. Topoisomerase II poisons generate DNA damage, in addition to inhibition of enzyme activity, and would be expected to delay cell cycle progression by means of DNA damage checkpoints.[79–81] The demonstration by Andoh and colleagues that bisdioxopiperazines were specific catalytic inhibitors of eukaryotic topoisomerase II[82] allowed a test in mammalian cells for the presence of a checkpoint for topoisomerase II function. Downes and colleagues found that bisdioxopiperazines such as ICRF-187 and ICRF-193 were able to elicit a caffeine-sensitive delay of entry into mitosis[83]. Subsequent work using ICRF-187 demonstrated a mitotic delay that was dependent on ATR (ataxia telangiectasia and Rad3-related) and BRCA1 (REF. 84), but apparently independent of both DNA damage checkpoints and the spindle checkpoint[85]. As noted by Downes and colleagues, the checkpoint they identified depended on the properties of bisdioxopiperazines[83]. As they were able to show distinct differences between etoposide (as a standard TOP2 poison) and ICRF-193, they concluded that the effects of ICRF-193 arose from a lack of TOP2 activity.

An alternate approach to assessing whether cells monitor the completion of TOP2 function is to completely deplete TOP2 protein before mitosis. This has been done in both yeast and mammalian cells. As described above, a complete depletion of TOP2 does not lead to a delay in mitosis in yeast cells[29], whereas expression of an inactive TOP2 does lead to a mitotic delay. This finding is in agreement with a previous hypothesis that yeast cells carrying a temperature-sensitive Top2 fail to arrest at mitosis because the presence of TOP2 is needed to trigger the delay[86]. However, the results of Diffley and colleagues suggest that the arrest seen in yeast is due to a problem with replication rather than decatenation. In mammalian cells, whether mitotic delay is induced by TOP2α depletion is a point of controversy. Removal of TOP2 using a conditional tetracycline (Tet)-off system showed that loss of TOP2 protein led to mitotic delay. By contrast, no delay was seen in cells depleted for TOP2α using siRNA[31]. In the latter experiments, loss of cell viability was clearly seen in cells depleted for TOP2α. In experiments from other laboratories using siRNA directed against TOP2α, no phenotype was observed, presumably because the knockdown of TOP2α was insufficient. Additional experiments, perhaps with primary cells, will be useful in demarcating the types of cells that can carry out mitotic delay in response to insufficient TOP2 activity. It is interesting to note that recent experiments indicate a lack of a mitotic delay induced by bisdioxopiperazines in embryonic stem cells and haematopoetic progenitor cells[87,88].

The hypothesis that TOP2 protein may be required for induction of a checkpoint that assesses TOP2 function

has recently gained additional support from observations that TOP2 interacts with the DNA damage checkpoint protein MDC1 (REF. 89). A prominent phosphorylation site in TOP2α is S1524 (REF. 90), and phosphorylation of this site is required for interaction with MDC1. Interestingly, cells expressing a S1524A mutant are defective in checkpoint arrest induced by bisdioxopiperazines[89]. MDC1 was also shown to be required for a G2 delay following exposure to bisdioxopiperazines. MDC1 is also required for DNA damage signalling pathways[91], although Luo and colleagues did not observe other hallmarks of DNA damage responses. The possible connections of checkpoints for TOP2 function with other checkpoints that ensure replication is completed provide interesting clues to the relevant signalling pathways involved in assessing TOP2 function.

The checkpoint induced by bisdioxopiperazines is termed a decatenation checkpoint, but it would more accurately be termed a TOP2 checkpoint, as there is no direct evidence that the mitotic delay monitors chromatid decatenation. It is not clear how the cell could assess sister chromatid catenation. The presence of catenanes is a property of a chromosomal domain, and it does not generate obvious local consequences. For example, cells might assess DNA supercoiling by 'counting' crossing of the DNA double helix (formally known as writhe), but there is no obvious way to assess writhe that is specifically associated with catenanes. Assessment of catenation state may depend more on structural alterations, perhaps at centromeres.

There has been interest in determining whether the topoisomerase II checkpoint can be exploited for cancer therapy. A small molecule inhibitor of a bisdioxopiperazine-induced checkpoint has been described, although the molecular target of the small molecule is unknown[92]. This may be of particular use in concert with potent TOP2 catalytic inhibitors. In any case, perturbation of TOP2 checkpoints is unlikely to be a major determinant of response to TOP2 poisons, which depend more on DNA damage checkpoints for their efficacy.

TOP2 is required in many biological contexts

The original impetus for studying TOP2 came in part from the unexplained and complicated reactions the enzyme carries out. Therefore, early studies concentrated especially on the biochemical and structural aspects of the enzyme. These studies have now concluded with a detailed understanding of many crucial issues of TOP2 biochemistry. TOP2 was expected to be important in chromosome replication and segregation, but the recent work suggests that decatenation of replicated chromosomes requires a precise choreography that includes regulating TOP2 action both spatially and temporally. Importantly, cells may have the means of ensuring that these processes have occurred correctly, although how cells assess proper TOP2 function remains unclear.

A recent surprise has been the unique roles of TOP2β. It was surprising that TOP2β is specifically required in certain neuronal cells, and the finding that this enzyme is required for transcription of some genes will lead to further unappreciated biological roles for both TOP2 isoforms. Although not discussed in detail here, TOP2 has also been proposed to have roles in DNA repair[93–95], especially in the ability of DNA lesions such as abasic sites to generate enzyme-mediated DNA damage[96]. Other possible functions of TOP2 will depend on a better understanding of the protein complexes that include TOP2. As with other proteomic studies, identification of the relevant protein complexes is only the first step in understanding the relevant biological processes.

As described in the accompanying Review[97], TOP2 is especially relevant in cancer because it is the target of many active anticancer agents. At present, most drugs targeting TOP2 kill cells by generating enzyme-mediated DNA damage, rather than by inhibiting enzyme activity. The importance of TOP2 in proliferating cells, as well as its roles in transcription, suggest that catalytic inhibition may also be a useful anticancer strategy. If this strategy proves successful, a more complete understanding of TOP2 biological functions will be crucial.

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DATABASES

National Cancer Institute Drug Dictionary: http://www.cancer.gov/drugdictionary/etoposide | mAMSA

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ATR | BRCA1 | MDC1 | PIAS4 | RANBP2 | TOP2α | TOP2β

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