

## DNA topoisomerases

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### 5.1 Introduction

Virtually every reaction that occurs in biological systems is catalysed by an enzyme. The interconversions of the different topological forms of DNA are no exceptions. The enzymes that catalyse these processes are known as DNA topoisomerases and constitute a widespread and fascinating group.

The first DNA topoisomerase was discovered by James Wang in 1971, the so-called  $\omega$  protein from *Escherichia coli* (1). This enzyme, now called DNA topoisomerase I, was found to reduce the number of negative supercoils in bacteriophage  $\lambda$  DNA, as measured by changes in sedimentation coefficient (see Chapter 2, Section 2.2.2). This finding was followed closely by the discovery of a ‘nicking-closing’ activity from nuclear extracts of mouse cells by James Champoux (2), which could remove supercoils from closed-circular polyoma virus DNA. Although it is also referred to as DNA topoisomerase I, this enzyme turns out to have a mechanism of action quite distinct from the prokaryotic enzyme found by Wang. These two enzymes are now classified in different groups: Type IA for the prokaryotic enzyme and type IB for the eukaryotic enzyme (see Section 5.2). An enzyme capable of introducing supercoils, DNA gyrase, was discovered a few years later by Martin Gellert and co-workers, during a search for host co-factors that support site-specific recombination by bacteriophage  $\lambda$  (3). They showed that the  $\lambda$  DNA substrate had to be supercoiled in order to support the recombination reaction. This substrate could be replaced by relaxed closed-circular DNA only if it was incubated with an *E. coli* cell fraction and ATP. An activity was purified from *E. coli* extracts that could supercoil DNA in the presence of ATP. The rather non-systematic but euphonic name, DNA gyrase, has stuck, although the enzyme is also referred to as DNA topoisomerase II, and its eukaryotic homologues are known by this name.

Every cell type so far examined contains DNA topoisomerases, and where a genetic test has been possible, at least one is essential for cell growth. Examples of the type of organisms in which topoisomerases have been studied include the bacteria *E. coli* and *Staphylococcus aureus*, yeasts, the model plant *Arabidopsis*, *Drosophila*, and man. In addition, several viruses are known to encode a topoisomerase, for example, bacteriophage T4 and the animal virus vaccinia. Examples of DNA topoisomerases are given in *Table 5.1*. Perhaps the most important aspect of topoisomerases for those interested in DNA topology is the mechanism of topoisomerase action: How do these enzymes achieve the complex interconversions of DNA supercoils, knots, and catenanes? The current understanding of how these enzymes work is summarized in Section 5.3.

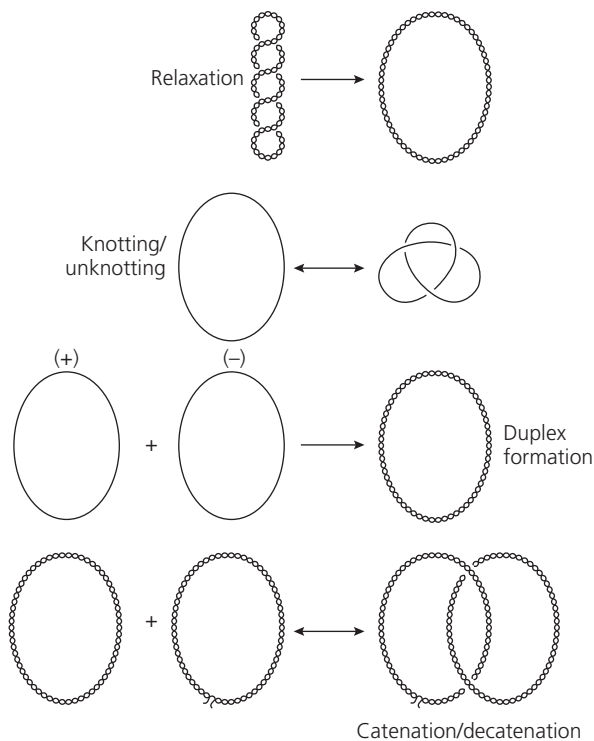
**Table 5.1 DNA topoisomerases**

Enzyme	Type	Source	Subunit size (kDa) and composition	Remarks
Bacterial topoisomerase I ( $\omega$ protein)	IA	Bacteria (e.g. <i>E. coli</i> )	97 Monomer	Cannot relax positive supercoils
Eukaryotic topoisomerase I	IB	Eukaryotes (e.g. human)	91 Monomer	Can relax both positive and negative supercoils
Vaccinia virus topoisomerase I	IB	Vaccinia virus	37 Monomer	ATP stimulates topoisomerase activity
Topoisomerase III <sup>a</sup>	IA	Bacteria (e.g. <i>E. coli</i> )	73 Monomer	Potent decatenating activity
Reverse gyrase	IA	Thermophilic Archaea (e.g. <i>Sulfolobus acidocaldarius</i> )	143 Monomer	Can introduce positive supercoils into DNA (ATP-dependent)
DNA gyrase	IIA	Bacteria (e.g. <i>E. coli</i> )	97 and 90 A <sub>2</sub> B <sub>2</sub>	Can introduce negative supercoils into DNA (ATP-dependent)
T4 topoisomerase	IIA	Bacteriophage T4	58, 51, and 18 2 copies of each subunit	Can relax, but not supercoil, DNA (ATP-dependent)
Eukaryotic topoisomerase II	IIA	Eukaryotes (e.g. human topoisomerase II $\alpha$ )	174 Homodimer	Can relax, but not supercoil, DNA (ATP-dependent)
Topoisomerase IV <sup>a</sup>	IIA	Bacteria (e.g. <i>E. coli</i> )	84 and 70 C <sub>2</sub> E <sub>2</sub>	Can relax, but not supercoil, DNA, potent decatenase (ATP-dependent)
Topoisomerase VI	IIB	Archaea (e.g. <i>Sulfolobus shibatae</i> )	45 and 60 A <sub>2</sub> B <sub>2</sub>	Can relax, but not supercoil, DNA (ATP-dependent)

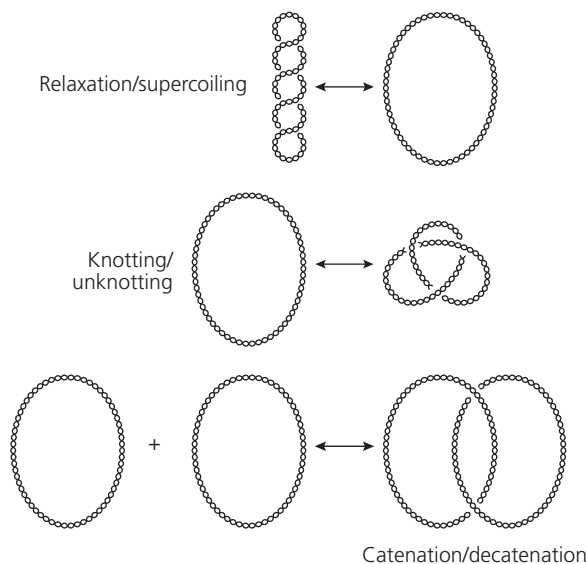
<sup>a</sup> Note that topoisomerases III and IV do not represent 'types' of topoisomerase mechanism (cf. type I and type II).

## 5.2 Reactions of topoisomerases

A reaction common to all topoisomerases is the ability to relax negatively supercoiled DNA; that is to convert it into a less-supercoiled form, or increase its linking number (Lk). It should be clear from the discussion in Chapter 2 that this conversion must involve the transient breaking and rejoining of DNA strands. A number of other reactions are also known and these are illustrated in *Figures 5.1 and 5.2*. During mechanistic studies it became clear that topoisomerases could be divided into two main types. Type I enzymes are able to carry out reactions involving the breaking of only one strand of the DNA (*Figure 5.1*), while type II enzymes can carry out those involving the breaking of both strands (*Figure 5.2*). Perhaps the best illustration of this distinction is the catenation and decatenation of double-stranded DNA circles. These interconversions can be achieved by type II topoisomerases but not by type I enzymes, unless one of the circles already contains a break in one strand (4). More recently topoisomerases have been further subdivided into type IA and B, and type IIA and B.



**Figure 5.1** Reactions of type I topoisomerases (redrawn from ref. 48).



**Figure 5.2 Reactions of type II topoisomerases**  
(redrawn from ref. 48).

Not all topoisomerases can carry out the full range of reactions shown in *Figures 5.1 and 5.2*. For example, the type I topoisomerase from *E. coli* can relax only negatively supercoiled DNA, while the type I enzyme from calf thymus will relax both negatively and positively supercoiled DNA (1, 2). This turns out to be a consequence of the inability of *E. coli* topoisomerase I to bind to positively supercoiled DNA. Indeed, the *E. coli* enzyme can relax a positively supercoiled DNA molecule if it is constructed to contain a single-stranded loop (5), suggesting that it preferentially binds single-stranded DNA. A further example concerns the ability of topoisomerases to introduce supercoils into DNA. Only one type II enzyme, DNA gyrase, is able to do this. Gyrase is found in bacteria (and more recently plants (50)) and can introduce negative supercoils into DNA using the free energy from ATP hydrolysis (3). Thermophilic archaea and eubacteria, such as *Sulfolobus* and *Thermotogales* contain an enzyme called reverse gyrase that can introduce positive supercoils into DNA (6); reverse gyrase is actually a type I enzyme and is not a homologue of DNA gyrase (*Table 5.1*). Type II enzymes from archaea, bacteriophage, and most eukaryotes are unable to catalyze DNA supercoiling.

In addition to topoisomerase I and DNA gyrase (topoisomerase II), *E. coli* has two further topoisomerases (*Table 5.1*). Topoisomerase III is another type IA enzyme that is more active as a decatenating enzyme than as a DNA-relaxing enzyme (7). Topoisomerase IV is a type II enzyme with a high degree of sequence similarity to DNA gyrase, which can relax, but

not supercoil, DNA and again shows efficient decatenation activity (8, 9). Topoisomerase V, originally identified in *Methanopyrus kandleri* is the only known example of a type IB enzyme in bacteria. This organism possesses a number of other topoisomerases, including a novel heterodimeric reverse gyrase. Multiple topoisomerases are also found in other organisms, including humans (Table 5.1 (10)). Mammalian cells possess two type II enzymes, topoisomerase II $\alpha$  and topoisomerase II $\beta$ ; these isoforms differ in their patterns of expression with the  $\alpha$ -isoform being expressed preferentially in proliferating cells. Archaea (and plants) possess a type II enzyme called topo VI (11), which appears to be distinct from the other type II enzymes, and its discovery has caused these enzymes to be subdivided into types IIA and IIB (Table 5.1). The enzyme from *Sulfolobus* consists of two subunits (A and B) one of which (A) has similarity to Spo11, a yeast protein involved in meiotic recombination (11). Sequence analysis of the *Arabidopsis thaliana* genome suggests that topoisomerase VI occurs in plants as well. Experimental evidence supports the idea that this enzyme is important in plant growth and development, possibly in the process of endoreduplication, in which the DNA in plant cells is duplicated without going through mitosis (12–14).

There are now a large number of enzymes that have been shown to perform reactions of the type shown in Figures 5.1 and 5.2. Some of these were not originally identified as topoisomerases but as enzymes involved in recombination reactions. Examples include resolvase proteins involved in the process of transposition and the Int protein involved in bacteriophage  $\lambda$  integration (see Chapter 6, Section 6.6). These proteins carry out reactions involving the breaking of DNA and the transfer of the broken end to another DNA molecule. As we will see, this is mechanistically very similar to a topoisomerase reaction except that topoisomerases rejoin the broken end to its original partner.

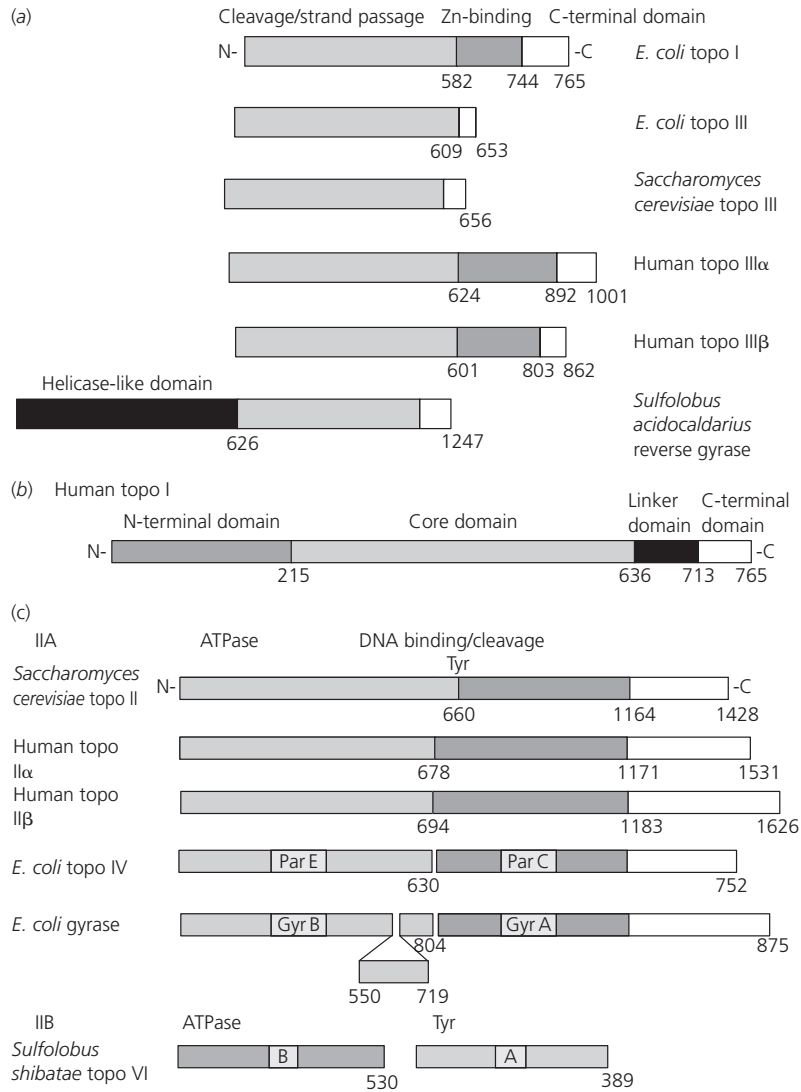
### 5.3 Structures and mechanisms of topoisomerases

If we consider the DNA relaxation reaction in the light of our discussions about linking number changes in Chapter 2 (see Sections 2.2.1, 2.3.1), it seems logical that topoisomerases should work by a swivel mechanism. This might involve breaking one (or both) strands of the DNA, allowing the free end (or ends) to rotate about the helix axis, and resealing the break. This would alter the linking number of the DNA as required by the relaxation reaction. However, if we consider the knotting/unknotting and catenation/decatenation

reactions (*Figures 5.1 and 5.2*), it should be clear that a swivel mechanism cannot account for the full range of topoisomerase actions. In fact a different type of mechanism, called strand passage, can account for the ability of topoisomerases to catalyse all these interconversions. In its most general form, strand passage involves the cleavage of one or both strands of the DNA by the enzyme and the passing of a single- or double-stranded segment of DNA through the break, which is then resealed. Topoisomerases stabilize the DNA break by forming a covalent bond between the enzyme (via a tyrosine hydroxyl group) and the phosphate at the break site. The strand passage event can involve segments of DNA from the same DNA molecule, in the case of relaxation/supercoiling and knotting/unknitting, or from separate DNA molecules, in the case of catenation/decatenation. The details of the strand-passage mechanism differ from one enzyme to another. The reactions of type I enzymes proceed through single-strand breaks in DNA and involve covalent attachment to either the 5'-phosphate (type IA) or the 3'-phosphate (type IB) at the break site. The reactions of type II enzymes proceed via double-strand breaks and all involve covalent attachment at the 5'-phosphate. Intramolecular topoisomerase reactions (e.g. relaxation/supercoiling) that occur via passage of a single strand of DNA through a transient single-strand break change the linking number in increments of 1; those that occur via passage of a double strand of DNA through a transient double-strand break change Lk in increments of 2 (15). This turns out to be an effective diagnostic test for distinguishing type I and type II topoisomerases.

Perhaps the most important advance in relation to understanding topoisomerase mechanism has been the application of x-ray crystallography to determine the structures of some of these enzymes. In only one case has the structure of an intact topoisomerase been solved, but there are also several examples of protein fragments whose structures have given us key insights into the likely mechanisms of action of the intact enzymes. Many of these fragments correspond to protein domains, and alignment of topoisomerases from the same group shows how these domains are conserved (*Figure 5.3*). This also demonstrates the evolutionary relationships among these enzymes.

Structures of some DNA topoisomerases are shown in *Figure 5.4*, and examples of the proposed mechanisms for certain enzymes are shown in *Figures 5.5–5.8*. Most of these structures have been solved in the absence of DNA and only models of the protein–DNA complexes are available in these cases. The only exception is human DNA topoisomerase I where structures of covalent and non-covalent complexes with DNA have been solved.

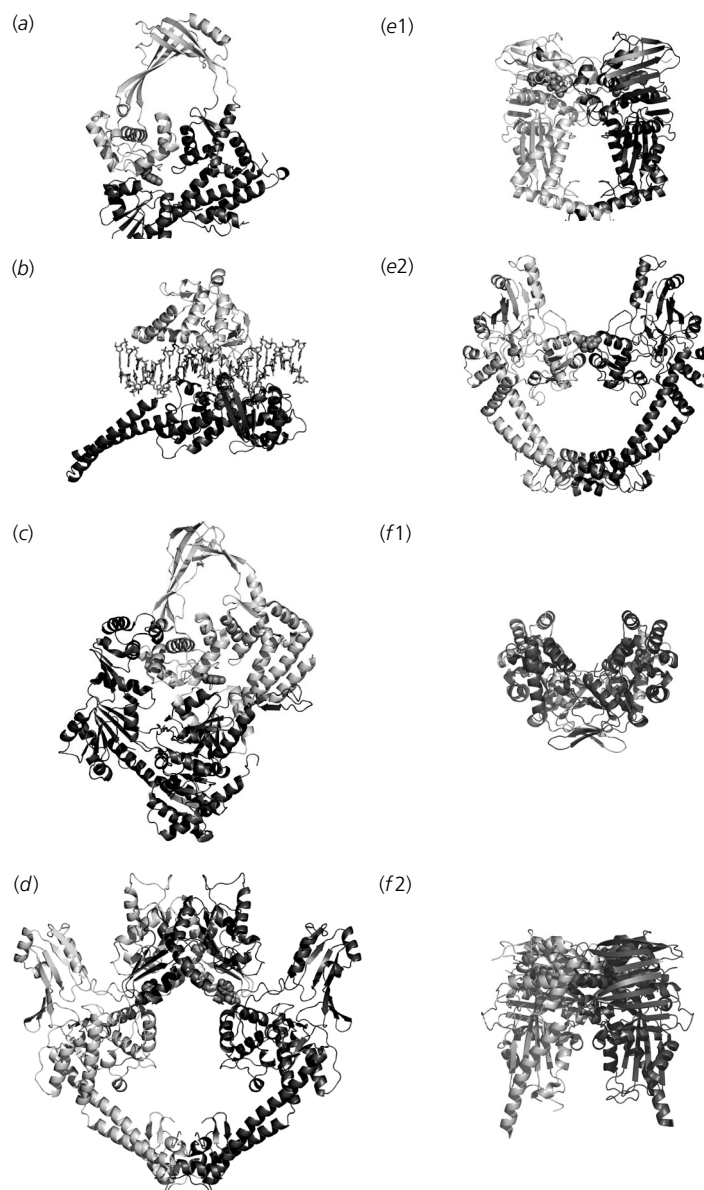


**Figure 5.3 Domain alignments of type I and type II topoisomerases.** (a) Domain alignments of type IA topoisomerases. (b) Domain alignments of type IB topoisomerases. (c) Domain alignments of type II topoisomerases. (Redrawn from ref. 49 with permission; copyright (2001) Annual Reviews www.annualreviews.org).

### 5.3.1 Type I topoisomerases

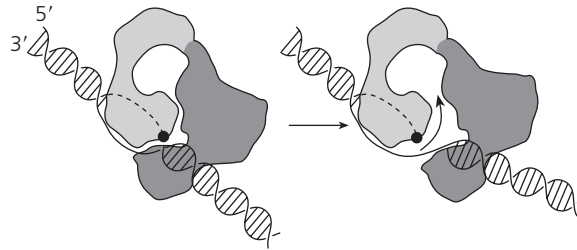
The first type I topoisomerase structure to be solved was that of a 67 kDa N-terminal fragment of *E. coli* DNA topoisomerase I (16), an example of a type

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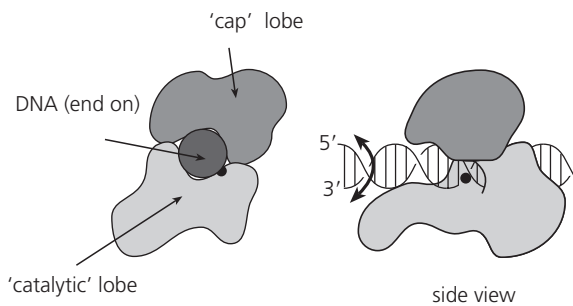


**Figure 5.4 Structures of DNA topoisomerases.** Crystal structures of selected topoisomerase fragments are shown in ribbon representation. (a) N-terminal 67 kDa fragment of *E. coli* topoisomerase I (16); (b) C-terminal 70 kDa fragment of human topoisomerase I complexed with DNA (49); (c) reverse gyrase (21); (d) 92 kDa fragment of yeast topoisomerase II (22); (e1) N-terminal 43 kDa fragment of the DNA gyrase B protein (26); (e2) N-terminal 59 kDa fragment of the DNA gyrase A protein (23); (f1) Topo VI A subunit (30); (f2) Topo VI B subunit (33). The images were generated using PyMol (51) using crystallographic co-ordinates acquired from the PDB (52).





**Figure 5.5 A proposed mechanism for *E. coli* topoisomerase I.** The enzyme binds DNA and cleaves one strand forming a 5'-phosphotyrosine linkage (black circle). The complementary strand is passed through the gap and into the central cavity of the enzyme. Resealing of the nick and release of the passed strand changes the linking number by  $\pm 1$ , resulting in relaxation of the DNA. If the initial cleavage is opposite a nick or gap in a duplex circle, then passage of another duplex segment could lead to a catenation or knotting reaction.



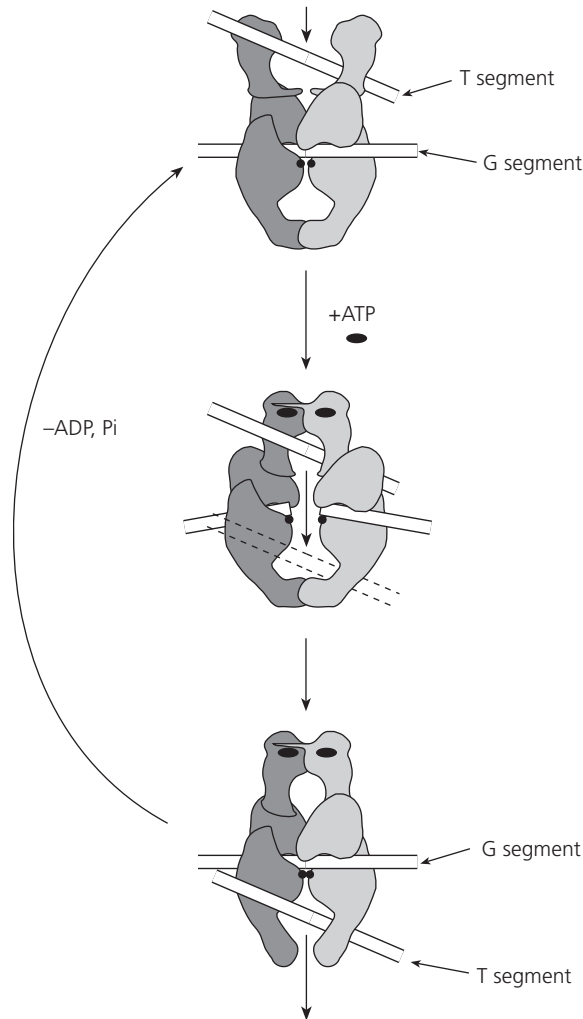
**Figure 5.6 A proposed mechanism for human topoisomerase I.** The enzyme binds duplex DNA and cleaves one strand forming a 3'-phosphotyrosine linkage (black circle). The free 5'-OH can then rotate (controlled rotation) before the break is resealed, resulting in DNA relaxation.

IA enzyme (Table 5.1). An important feature of the structure of this fragment (Figure 5.4) is the presence of a central cavity, large enough to accommodate a segment of double-stranded DNA. The active-site tyrosine is at the interface between two domains at the opening of this cavity, and this suggests a mechanism in which DNA (either single- or double-stranded) can enter the interior of the enzyme through the DNA break resulting from cleavage of a single-stranded segment (Figure 5.5). Cavities such as the one present in the *E. coli* DNA topoisomerase I turn out to be a common feature of topoisomerase structures and provide strong support for the concept of strand passage in topoisomerase mechanisms.

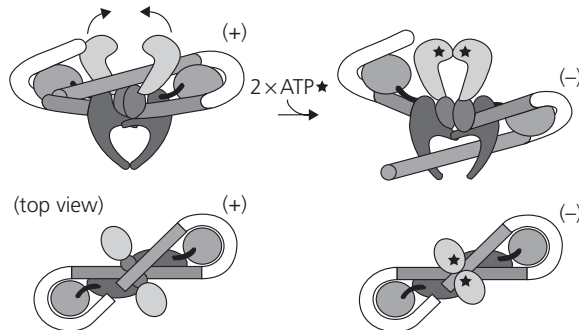
Although the idea of strand passage is now firmly established, it seems quite likely that one group of topoisomerases, the type IB enzymes, operate by a swivel mechanism, the 'logical' mechanism we mentioned earlier. One of

**Figure 5.7 A proposed mechanism for yeast topoisomerase II.**

A segment of DNA (the G segment) is bound to the enzyme in the vicinity of the active-site tyrosines (black circles). Cleavage of the G segment (to yield 5'-phosphotyrosine linkages) allows passage of a second DNA segment (the T segment) through the G segment. The T segment is captured by ATP-dependent dimerization of the N-terminal (ATPase) domains of the enzyme, which form a 'clamp'. The T segment exits from the bottom of the enzyme as shown. ATP hydrolysis releases the clamp and allows the enzyme to reset.



the members of this group for which we have structural information is human topoisomerase I (*Figure 5.4*). The structure of a 70 kDa fragment of the human enzyme bound to DNA shows no tell-tale cavity appropriate to a strand-passage step; instead it surrounds the DNA double helix and, following cleavage and covalent attachment of the 3'-phosphate to the protein, the 5'-hydroxyl end may be able to swivel (17, 18). This has led to a mechanistic model for type IB enzymes termed 'controlled rotation' (*Figure 5.6*). Experimental evidence for this type of mechanism has also been obtained from work on another member of this group, vaccinia virus topoisomerase I (19).



**Figure 5.8 A proposed mechanism for *E. coli* DNA gyrase.** Gyrase operates by a similar mechanism to topoisomerase II (Figure 5.7) except that the G segment is wrapped around the enzyme (as illustrated in the figure) so as to present the T segment to the DNA gate. This wrap strongly favours intramolecular strand passage and leads to the enzyme's ability to introduce negative supercoils into DNA. The asterisk represents ATP. (Reproduced from ref. 53 with permission; copyright (2004) National Academy of Sciences, USA.)

Reverse gyrase, which is a type IA enzyme, may act via a mechanism that is distinct from those of the more conventional (DNA-relaxing) type I enzymes. The enzyme contains a helicase-like domain and a type IA topoisomerase domain in the same polypeptide (20). The crystal structure of the 120 kDa reverse gyrase from *Archaeoglobus fulgidus* has been solved with and without a bound ATP analogue (21). The enzyme has an N-terminal domain resembling helicase structures and a C-terminal domain equivalent to *E. coli* topoisomerase I (Figure 5.4). The structures suggest a strand-passage mechanism, similar to *E. coli* topoisomerase I (Figure 5.5), except that in the case of reverse gyrase it is adapted to give only a positively supercoiled product (21).

### 5.3.2 Type II topoisomerases

The crystal structure of a large (92 kDa) internal fragment of yeast DNA topoisomerase II has been solved (22). This structure again reveals a large cavity that could accommodate a double-stranded DNA (Figure 5.4). This structure is complemented by that of a 59 kDa N-terminal fragment of the *E. coli* DNA gyrase A protein, which is homologous to yeast topoisomerase II (23). These two structures show a high degree of similarity in their secondary and tertiary folds (Figure 5.4), but are significantly different in terms of their quaternary structures. It is thought that they represent two conformational states that occur in the reaction cycle of the type II topoisomerases. Mechanistic studies of yeast topoisomerase II have defined two segments of DNA that are bound to

the enzymes: a G (or gate) segment, which is cleaved, and a T (or transport) segment, which is passed through the break in the G segment (24). Coupled with the structural information on yeast topoisomerase II and additional structural information on DNA gyrase (see below), these mechanistic studies suggest a two-gate model for topoisomerase II (Figure 5.7), in which a T segment is captured by the enzyme and passes through two protein gates: one associated with the cleaved G segment, and one controlling the exit of the T segment from the protein. An ATP-operated clamp, comprising the N-terminal domain of the enzyme dimer, effects the initial capture of the T segment. The structure of this domain was first determined for the corresponding region of bacterial gyrase (see below) and has also been solved for yeast topoisomerase II (25). It is proposed that this domain dimerizes in the presence of ATP capturing the T segment so that it can be passed through the cleaved G segment (Figure 5.7).

Bacterial DNA gyrase differs from its eukaryotic counterpart in that it can actively introduce negative supercoils into DNA. Apart from the structure of an N-terminal fragment of the gyrase A protein mentioned above, the structure of an N-terminal fragment of the *E. coli* B protein is also known (Figure 5.4). The structure of this 43 kDa fragment was solved with a bound ATP analogue (5'-adenylyl- $\beta$ ,  $\gamma$ -imidodiphosphate) (26). As with the other type II topoisomerase fragments, this structure is dimeric and reveals a cavity large enough to accommodate DNA. Mechanistically, gyrase is thought to operate much like topoisomerase II, in that a captured T segment is passed through a cleaved G segment. Gyrase's unique ability to catalyse DNA supercoiling is thought to derive from the wrapping of a segment of DNA (which contains the G segment) around the enzyme in such a way as to present a T segment to the ATP-operated clamp (27) (Figure 5.8). The wrapping function resides in the C-terminal domain of the gyrase A protein, which shares little sequence similarity with the corresponding region of topoisomerase II. This ability to wrap DNA is reminiscent of other systems in which DNA is wrapped around proteins, such as the nucleosome (Chapter 3, Section 3.5) and RNA polymerase (Chapter 6, Section 6.4.1). The ATP-operated clamp is a common feature of all type II topoisomerases and appears to function to pass the T segment through the enzyme-stabilized break in the G segment (24). The structure of the N-terminal domain of GyrB indicates homology to a diverse range of other proteins, which have become known as the GHKL superfamily (28). These proteins include MutL (a mismatch repair protein), Hsp90 (a chaperone protein), histidine kinases, and the B subunit of topoisomerase VI (see below). The GHKL proteins share four conserved motifs involved in nucleotide binding and hydrolysis.

DNA supercoiling is an energetically unfavourable process and gyrase is somehow able to transform the chemical energy derived from the hydrolysis

of a phosphodiester bond in ATP into the torsional stress of supercoiling. Although it must be assumed that this process involves conformational changes in the protein, little is known of the detailed mechanism of this energy-coupling process. It is interesting to note that other type II topoisomerases hydrolyse ATP but are able only to relax, not supercoil, DNA (Table 5.1). Gyrase can also relax supercoiled DNA, but this reaction does not require ATP. The requirement for ATP hydrolysis for the reactions of topoisomerase II, most of which are apparently energetically favourable, has been something of a puzzle. Experiments have suggested that it is likely that this energy requirement enables topoisomerase II to catalyse reactions in which the products are a non-equilibrium distribution of topoisomers (29). For example, *E. coli* topoisomerase IV can relax supercoiled DNA to yield a distribution of relaxed topoisomers that is narrower than that obtained by relaxation by topoisomerase I under the same conditions (see Chapter 2, Section 2.6.2). This implies that type II enzymes can use the energy of ATP hydrolysis to shift the distribution of the relaxed topoisomer products away from equilibrium. In the case of gyrase this results in products that have negative supercoiling ( $\sigma$ ; see Chapter 2, Section 2.2.2); in the case of topoisomerase II, the energy of ATP hydrolysis is serving to relax the DNA more than at equilibrium. A further illustration of this phenomenon occurs with catenated DNA. Rybenkov *et al.* (29) used a system that allows equilibration of catenanes with uncatenated DNA to show that topoisomerase IV, and other type II enzymes, are able to generate products with a much lower proportion of catenanes than those formed at equilibrium in the absence of enzyme, that is, the enzymes strongly favour the decatenation reaction.

The structure of a fragment of the A subunit of topoisomerase VI from *Methanococcus jannaschii* (Figure 5.4) suggests that the type IIB enzymes are distinct from the IIA enzymes (30). However, comparison of the structures of the type IA, IIA, and IIB enzymes has shown that there is structural similarity between these enzymes, specifically in a region containing a so-called Rossmann-like fold that is thought to co-ordinate metal ions (31). Sequence alignments have suggested that this homology extends to DNA primases and other enzymes and this structural feature has been termed the 'toprim' fold (32). In addition, the structure of a truncated form of the topoisomerase VIB subunit from *Sulfolobus shibatae* has been solved, both with and without an ATP analogue bound (33). Although the only region of topoisomerase VIB with a sequence apparently related to type IIA enzymes is a GHKL-type motif (see earlier), this structure shows impressive similarity to the N-terminal domain of GyrB (Figure 5.4). The structure reveals similarities with parts of the GyrB fragment involved in nucleotide hydrolysis and signalling of hydrolysis to other parts of the protein. This work reinforces the idea that type IIA

and IIB enzymes are evolutionarily related and are likely to share a similar mechanism of strand passage, and nucleotide binding and hydrolysis.

## 5.4 Topoisomerases as drug targets

In the opening section of this chapter we noted that topoisomerases are essential for cell growth. As a consequence, these enzymes are potential targets for cytotoxic drugs (34–38). For example, DNA gyrase and its close relative topoisomerase IV are targets for two groups of antibacterial compounds: the quinolones and the coumarins (*Table 5.2*). In the context of topoisomerase mechanisms it is interesting to consider how these drugs might work. The quinolones (e.g. nalidixic acid and ciprofloxacin) are thought to act by interrupting the DNA breakage–reunion step of the gyrase reaction (39). Indeed, under certain conditions, quinolones can lead to trapping of the gyrase–DNA intermediate in which the A subunits of the enzyme are covalently bound to the 5′-phosphates of DNA at the break site. The coumarin drugs (e.g. novobiocin and coumermycin A<sub>1</sub>) act in an entirely different way by inhibiting the hydrolysis of ATP by gyrase and thus preventing DNA supercoiling. Although coumarins are structurally dissimilar to ATP, crystal structure information on complexes between gyrase and coumarins show that the drugs bind at a site that overlaps the ATP-binding site on the enzyme, and are therefore competitive inhibitors (40, 41).

The eukaryotic topoisomerases are also drug targets (*Table 5.2*). The anti-tumour drug camptothecin, whose derivatives are widely used in cancer chemotherapy, acts on eukaryotic topoisomerase I. The drug stabilizes the complex between the enzyme and DNA in a manner analogous to the action of quinolones on DNA gyrase. Crystal structure information on human topoisomerase I suggests a model in which the drug interacts with the bound

**Table 5.2 Inhibitors of DNA topoisomerases**

Inhibitor	Target enzyme	Therapeutic value
Quinolones (e.g. ciprofloxacin)	DNA gyrase and topoisomerase IV	Effective antibacterial agents
Coumarins (e.g. novobiocin)	DNA gyrase and topoisomerase IV	Antibiotics, but not widely used
Camptothecin (e.g. topotecan)	Human topoisomerase I	Anticancer drug
Amsacrine (mAMSA)	Human topoisomerase II	Anticancer drug
Epipodophyllotoxins (e.g. teniposide)	Human topoisomerase II	Anticancer drug

DNA by ring stacking and with the protein by hydrogen bonding (17). Several other antitumour drugs have been shown to be inhibitors of eukaryotic topoisomerase II. These include acridines (e.g. amsacrine), ellipticines (e.g. 2-methyl-9-hydroxy-ellipticinium acetate), and epipodophyllotoxins (e.g. teniposide). Again these drugs are thought to act by stabilizing the covalent complex between the enzyme and DNA, and in many cases this is thought to be mediated by intercalation of the drug into the DNA at the site of enzyme binding. Other topoisomerase II-targeted drugs act in a different way; for example, the bisdioxypiperazine ICRF-193 is thought to act by stabilizing the closed clamp form of the enzyme (42). A structure of the ATPase domain of yeast topoisomerase II complexed with the related compound ICRF-187 shows that the drug stabilizes a transient dimer interface between two ATPase domains (25).

As well as being the targets of antibacterial agents and anticancer drugs, topoisomerases can also be the targets of toxins; the bacterial toxins CcdB and microcin B17 both act on DNA gyrase (43). CcdB is a small protein (11.7 kDa) that is part of a toxin–antitoxin system, forming a complex with CcdA, for maintaining the F plasmid in *E. coli*. Loss of the F plasmid leads to loss of CcdA, which is relatively unstable, and release of CcdB from the complex, which then kills the host cell (44). Microcin B17 is a glycine-rich peptide (3.2 kDa) containing oxazole and thiazole rings, which is produced by some strains of *Enterobacteriaceae* to inhibit phylogenetically related species (45). Both CcdB and microcin B17 kill cells by stabilizing the complex between gyrase and DNA, in a manner reminiscent to that of quinolones, but using distinct mechanisms. Study of the mode of action of such proteinaceous inhibitors may yield new ideas for the design of novel inhibitors of gyrase.

## 5.5 Biological role of topoisomerases

As we will discuss in detail in Chapter 6, changes in DNA topology occur in many cellular processes and can have profound biological consequences. Therefore, it is not surprising to find that DNA topoisomerases are involved directly or indirectly in these processes. This fundamental requirement for DNA topoisomerases derives from the double-helical structure of DNA (Chapter 1; Chapter 2, Section 2.2.1), insofar as most processes involving DNA must involve the unwinding of the DNA helix to access the bases (at least temporarily). For example, the two strands of DNA must become completely unlinked during DNA replication; topoisomerases are thought to be involved in various stages of this process. DNA gyrase is important for initiation of

replication in prokaryotes; this requirement is likely to reflect the need for negative supercoiling prior to initiation. During the elongation steps of replication, the parental DNA is being continuously unwound and topoisomerases are required to prevent the accumulation of positive supercoils (see Chapter 2, Section 2.2.1). Both type I and type II topoisomerases can act to relieve the torsional stress generated during elongation. At the termination of DNA replication, the progeny DNA molecules are frequently catenated and both type I and type II topoisomerases can, in principle, unlink the intertwined molecules (see Chapter 6, Section 6.3.3).

Unlike replication, transcription could theoretically proceed without any topological problems. However, in bacteria, transcription can lead to the supercoiling of the DNA, when, for example, the DNA is anchored to fixed points or two genes on a circular plasmid are being transcribed in opposite directions (see Chapter 6, Section 6.5). Transcription can lead to positive supercoiling ahead of the transcription complex and negative supercoiling behind it. Experiments have suggested that DNA gyrase can relax the positive supercoils and topoisomerase I can relax the negative supercoils (46). For example, highly positively supercoiled plasmids have been isolated from *E. coli* treated with coumarin drugs (which inhibit DNA gyrase), and plasmids isolated from *E. coli* strains carrying mutations in *topA* (the gene encoding topoisomerase I) can exhibit high levels of negative supercoiling (see Chapter 6, Section 6.5).

An important function of topoisomerases within the cell is the maintenance of DNA supercoiling. In bacteria the level of intracellular supercoiling influences the rate of transcription of many genes. Given that RNA polymerase unwinds DNA on binding to promoters it is to be expected that negative supercoiling should stimulate transcription. In fact negative supercoiling can both increase and decrease gene expression (see Chapter 6, Section 6.4.1). The topoisomerase genes themselves are affected by supercoiling. Lowering negative supercoiling raises the expression of the gyrase genes and reduces the expression of *topA*. It is thought that this represents a homeostatic mechanism for the control of DNA supercoiling within the bacterial cell (47).

## 5.6 Conclusions

The existence of an essential class of enzymes whose function is to interconvert different topological isomers of DNA demonstrates the vital importance of DNA topology in cells. Apart from their relevance to DNA topology, topoisomerases are of great interest from the standpoint of their mechanistic enzymology and because of their potential as drug targets.



## 5.7 Further Reading

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