CELLULAR ROLES OF DNA TOPOISOMERASES: A MOLECULAR PERSPECTIVE

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DNA topoisomerases are the magicians of the DNA world — by allowing DNA strands or double helices to pass through each other, they can solve all of the topological problems of DNA in replication, transcription and other cellular transactions. Extensive biochemical and structural studies over the past three decades have provided molecular models of how the various subfamilies of DNA topoisomerase manipulate DNA. In this review, the cellular roles of these enzymes are examined from a molecular point of view.

Many a spectator of the linking-ring trick has been fascinated by the magician's seemingly impossible feat of linking solid rings into a chain, or separating a chain into individual rings. In reality, the magician creates an illusion of interconversion by a sleight of hand: some of the rings are permanently linked and the others are permanently separate — the one exception being a 'key ring' with a hidden opening, through which other rings can be inserted or removed.

DNA topoisomerases are the true magicians of the DNA world. In their presence, DNA strands or double helices can pass through each other as if all physical boundaries had disappeared: the intertwined parental strands of a replicating DNA ring can come apart, interlocked double-stranded DNA rings (catenanes) can become unlinked and knots can be introduced or removed from DNA rings. In contrast to the hocus-pocus of the magician, however, the DNA topoisomerases accomplish their feats by the simple and elegant chemistry of transesterification.

In the strand-breakage reaction by a DNA topoisomerase, a tyrosyl oxygen of the enzyme attacks a DNA phosphorus, forming a covalent phosphotyrosine link and breaking a DNA phosphodiester bond at the same time (FIG. 1). Rejoining of the DNA strand occurs by a second transesterification, which is basically the reverse of the first — the oxygen of the DNA hydroxyl group that is generated in the first reaction attacks the phosphorus of the phosphotyrosine link, breaking the covalent bond between the protein and DNA, and reforming the DNA backbone bond. These reactions create transient enzyme-mediated gates in the DNA for the passage of another DNA strand or double helix.

DNA topoisomerases fall into two categories — type I and type II. For the type I enzymes, the DNA strands are transiently broken one at a time; for the type II enzymes, by contrast, a pair of strands in a DNA double helix are transiently broken in concert by a dimeric enzyme molecule. The two types can be further divided into four subfamilies: IA, IB, IIA and IIB (TABLE 1). Members of the same subfamily are structurally and mechanistically similar, whereas those of different subfamilies are distinct.

The purpose of this review is to provide a perspective of the cellular roles of these remarkable enzymes from a vista of their basic reaction characteristics — a more comprehensive coverage of the literature can be found in several recent reviews[1–3]. To provide the necessary backdrop, some unique aspects of reactions that are catalysed by the different subfamilies of the DNA topoisomerases are summarized first. For clarity, reactions that are catalysed by these enzymes are often described for DNA rings. However, similar reactions occur in linear chromosomes owing to their organization into intracellular structures that contain multiple loops, or because their ends are immobile.

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NEGATIVELY AND POSITIVELY SUPERCOILED DNA
A loop of a double-stranded DNA segment will become contorted if one end of it is turned around its helical axis at that end while the other end is kept stationary in space (much like the spatial coiling of a rubber tubing when similarly handled). The number of supercoils that are introduced into this loop is a parameter that is used to quantify the distortion of the looped DNA segment; one negative supercoil is said to be introduced into the DNA loop by each full turn of the rotating end in the direction that tends to unwind the right-handed double helix; one positive supercoil is said to be introduced by each full turn of the rotating end in the direction that tends to overwind the right-handed double helix.

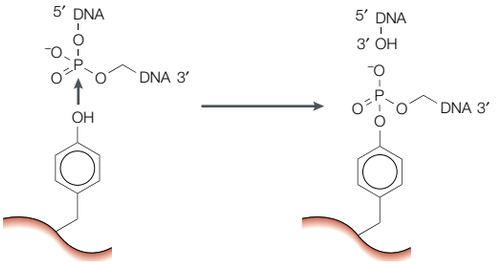


Figure 1 | **Catalysis of transient breakage of DNA by DNA topoisomerases.** Transesterification between an enzyme tyrosyl and a DNA phosphate group leads to the breakage of a DNA backbone bond and the formation of a covalent enzyme–DNA intermediate. Rejoining of the DNA backbone bond occurs by the reversal of the reaction shown. In the reaction that is catalysed by a type IA or a type II enzyme, a 3′-OH is the leaving group and the active-site tyrosyl becomes covalently linked to a 5′-phosphoryl group, as depicted. In the reaction that is catalysed by a type IB enzyme (not shown), a 5′-OH is the leaving group and the active-site tyrosyl becomes covalently linked to a 3′-phosphoryl group.

Reactions catalysed by topoisomerases

Type IA. In the relaxation of an underwound or NEGATIVELY SUPERCOILED DNA by a type IA enzyme, a short stretch of double-stranded (ds)DNA is first unpaired by the binding of the enzyme, and a transient break is introduced in this single-stranded region[1,4,5]. The less negatively supercoiled the DNA is, the more difficult it is for the enzyme to unpair the dsDNA; and so, the proficiency of the enzyme progressively decreases during the course of the reaction. Overwound or POSITIVELY SUPERCOILED DNA is refractive to the type IA enzymes unless a pre-existing single-stranded region is present[5].

The type IA DNA topoisomerases can also pass one DNA double helix through another if at least one of the pair contains a nick or gap[6]; in this reaction, an enzyme probably introduces a transient break across from the nick or gap. The type IA enzymes are believed to catalyse DNA strand passage by an 'enzyme-bridging' mechanism (FIG. 2a), in which the DNA ends that are created in the DNA breakage reaction are bridged by the topoisomerase[1,7,8], and movements of the enzyme-bound DNA ends relative to each other mediate the opening and closing of the DNA gate[7].

Type IB. The type IB enzymes are thought to act by a 'DNA rotation', rather than by an enzyme-bridging, mechanism[3]. When a DNA-bound type IB enzyme (FIG. 2b) transiently cleaves one of the DNA strands, only the side of the DNA double helix that is upstream of the nick — the side containing the protein-linked 3′ end of the broken strand — is tightly bound to the enzyme. Interaction between the downstream side of the dsDNA and the enzyme is mostly ionic in nature, so it presents a low barrier to rotation between the DNA and protein[3]. The DNA segments that flank a transient nick can therefore rotate relative to each other by turning around one of the single bonds that opposes the nick[3].

The type IB enzymes are very efficient at relaxing both positively and negatively supercoiled DNA. Although catenation or decatenation of nicked dsDNA rings by a type IB enzyme *in vitro* has been reported[8], it remains unclear how the enzyme carries out intermolecular strand passage. A linear dsDNA intermediate, with the enzyme covalently linked to one end of it, could be formed in such a reaction; if so, these reactions are probably not significant *in vivo*.

There is another mechanistically important difference between the two type I enzymes. In the type IA-enzyme-catalysed reactions, breakage and rejoining of the DNA strand occur in a single-stranded region[1,4,5]. In the reactions that are catalysed by the type IB enzymes, the nick is generated in a dsDNA segment[3]. Cleavage by a type IB enzyme in the single-stranded region of a dsDNA with a single-stranded gap could occur, but the 5′ end of the transiently broken DNA might readily detach from the enzyme, yielding a linear DNA intermediate.

Type II. In contrast to the type IA and type IB enzymes, the type IIA and IIB DNA topoisomerases catalyse the ATP-dependent transport of one intact DNA double helix through another[1–3,9]. Before the first type IIB enzyme was identified in the archaeon *Sulfolobus shibatae*[10], all type II DNA topoisomerases were thought to belong to a single subfamily. It is now clear that both type IIA and type IIB DNA topoisomerases are widely distributed[11] (BOX 1). FIGURE 2c depicts a molecular model for the transport of one DNA double helix through another by a type IIA enzyme[9,12]. The less-extensively studied type IIB enzymes share several common mechanistic features with the type IIA enzymes[10], but there are distinct structural differences between the two subfamilies[13].

The ATP-dependent transport of one DNA double helix through another by a type II DNA topoisomerase is manifested in several topological transformations, including catenation and decatenation of dsDNA rings, and the relaxation of positively or negatively supercoiled DNA[1–3,9,12]. The relative efficiencies of a given type II enzyme in catalysing these reactions depend on the structural features of the DNA substrates and the enzyme–DNA complexes. Bacterial gyrase (DNA topoisomerase II), for example, is unique in that a

Table 1 | **Subfamilies of DNA topoisomerases**

Subfamily	Representative members
IA	Bacterial DNA topoisomerases I and III Yeast DNA topoisomerase III *Drosophila melanogaster* DNA topoisomerases IIIα and IIIβ Mammalian DNA topoisomerases IIIα and IIIβ
IB	Eukaryotic DNA topoisomerase I Mammalian mitochondrial DNA topoisomerase I Pox virus topoisomerase
IIA	Bacterial gyrase, DNA topoisomerase IV Phage T4 DNA topoisomerase Yeast DNA topoisomerase II *Drosophila* DNA topoisomerase II Mammalian DNA topoisomerases IIα and IIβ
IIB	*Sulfolobus shibatae* DNA topoisomerase VI (subunit A homologous to yeast Spo11)

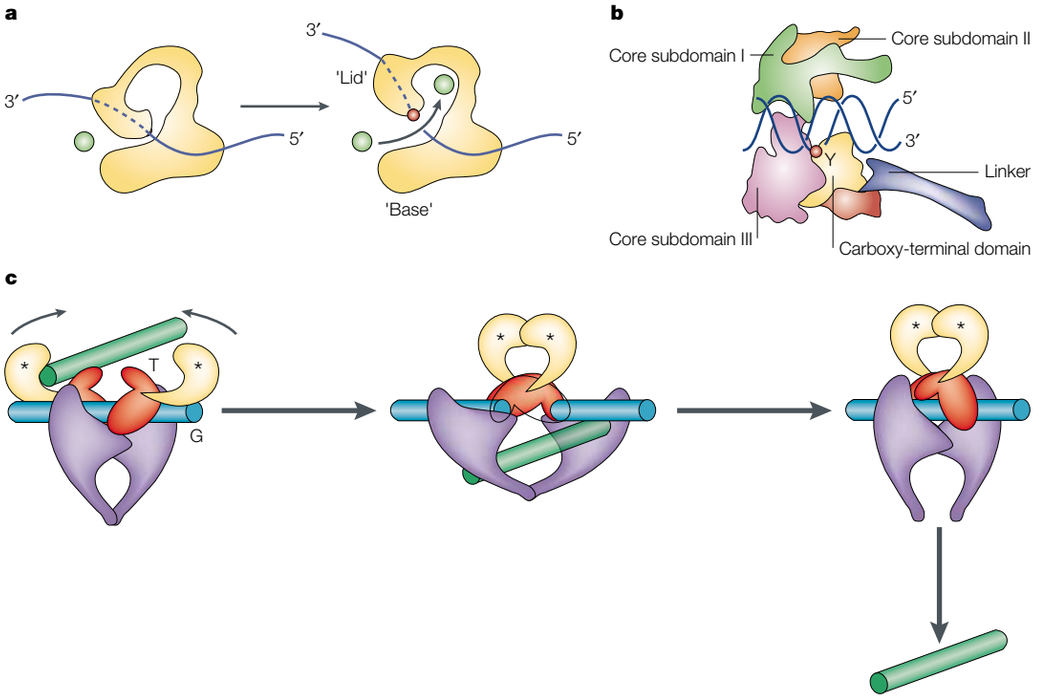


Figure 2 | **Molecular models for the passage of one DNA strand or double helix through another by different subfamilies of DNA topoisomerases. a** | Type IA topoisomerases. On transient breakage of a DNA strand (blue line), the 5′ end of the broken DNA strand is covalently attached to the active-site tyrosyl group (red circle) in the 'lid' of the enzyme, and the 3′ end is noncovalently bound to the 'base' of the enzyme. Lifting the lid away from the base opens a gate in the DNA for the passage of another strand (green circle). The location of the second strand, either before or after its passage through the DNA gate, is largely unknown. Once the second strand has entered the central cavity of the enzyme, it must exit the cavity, after the rejoining of the broken strand, without passing through the rejoined DNA strand[7,113]. **b** | Type IB enzymes. The covalent intermediate between a 22-base-pair DNA fragment and a type IB DNA topoisomerase is shown. The 3′ end of the broken DNA scissile strand is covalently linked to the active-site tyrosyl group (Y) of the enzyme (red circle). For clarity, a portion of the enzyme is sectioned off to reveal the entire DNA fragment. The enzyme-generated nick divides the DNA fragment into two segments: the DNA segment to the left of the nick is tightly held by the enzyme, but interaction between the enzyme and the DNA segment to the right of the nick is mostly ionic, so it permits rotation of the DNA segment to the right of the nick relative to the protein. The illustration is based on the crystal structures of several complexes that are formed between DNA and human DNA topoisomerase I (REF. 3). This DNA-rotation mechanism allows multiple strand-passage events for each strand breakage–rejoining cycle[114]. **c** | Type IIA enzymes. The protein structure shown is based on structures of the ATPase domain of *E. coli* GyrB protein[115] and a fragment of yeast DNA topoisomerase II containing the domains that are required for DNA breakage and rejoining[12]. The G-segment — the double-stranded DNA segment that contains the enzyme-mediated DNA gate — is depicted as a blue rod. The DNA T-segment being passed through the G-segment is depicted as a green rod. The asterisks represent the ATP-binding sites. Reproduced with permission from *Nature*[12] © (1996) Macmillan Magazines Ltd. See REFS 9 and 12 for further details.

140-base-pair DNA segment wraps around the enzyme, in a right-handed orientation, to position the DNA segment to be transported (the T-segment in FIG. 2c) and the DNA segment to be transiently cleaved (the gate- or G-segment in FIG. 2c) in a particular way[9]. This right-handed wrapping of DNA around the enzyme is closely related to its preferential relaxation of positively supercoiled DNA, as well as its ability to mediate negative supercoiling of a relaxed DNA ring or loop[9,14].

The DNA topoisomerases evolved to solve the topological problems of DNA, all of which are deeply rooted in its double-helix structure. The topological problems of DNA in its various cellular transactions are reviewed below.

DNA replication

Elongation of replicating DNA chains. In FIG. 3a, a DNA segment is depicted, together with a region that has been replicated (a 'replication bubble'), and the replication machinery at one replication fork is represented by a rod passing in-between the two DNA strands that are being duplicated. The topological consequences of an advancing fork, and the roles of different DNA topoisomerases, depend on whether the replication machinery (the blue rod depicted in FIG. 3a) is allowed to rotate in the cellular milieu.

Imagine that the rod is not allowed to rotate around the helical axis of the unreplicated DNA ahead of the replication fork (the machinery can be membrane-attached and therefore immobile[15]). As the replication

Box 1 | **How many DNA topoisomerases in various organisms?**

DNA topoisomerases are among the most conserved proteins in the DNA world. Their ubiquity is borne out by extensive biochemical data and by genomic sequences of numerous organisms from all kingdoms of life (reviewed in REF. 3; see also REFS 11,98). In humans, two enzymes of each of the subfamilies IA (DNA topoisomerases IIIα and IIIβ), IB (DNA topoisomerase I and mitochondrial DNA topoisomerase I[99]) and IIA (DNA topoisomerases IIα and IIβ), are known. Yeasts have three DNA topoisomerases — I, II and III — which belong to the type IB, IIA and IA subfamilies, respectively. *Escherichia coli* has two type IA enzymes (DNA topoisomerases I and III) and two type IIA enzymes (gyrase and DNA topoisomerase IV). *Drosophila melanogaster* also has four — two type IA enzymes, one type IB enzyme and one type IIA enzyme. In plants, all subfamilies of DNA topoisomerases IA, IB, IIA and IIB seem to be present in one or more forms.

Searches of genomic sequences show that all organisms have at least one type IA enzyme. All organisms also have at least one type II enzyme, usually of the IIA subfamily, but archaea with no type IIA and one type IIB enzyme (DNA topoisomerase VI) are known[10,11]. The type IB enzymes are widely present in eukarya, archaea and bacteria, but there are exceptions — notably, their absence in *E. coli* and many other bacteria. Indeed, bacteria such as *Campylobacter jejuni* and *Treponema pallidum* seem to have only two DNA topoisomerases, a type IA and a type IIA enzyme[3,98]. So, the minimal requirement for DNA topoisomerases in living organisms is probably one type IA and one type II enzyme. Under laboratory conditions, however, a single type IIA enzyme has been shown to sustain viability. Growth of *Saccharomyces cerevisiae Δtop1 Δtop3* double mutants that lack both type I enzymes can be maintained, albeit rather poorly[55].

For multicellular organisms, different demands during different developmental stages and in different tissues tend to increase the number of DNA topoisomerases. For the six known mouse DNA topoisomerases, none is dispensable (although information on the requirement for the mitochondrial type IB enzyme is not yet available). Targeted gene-disruption experiments show that inactivation of DNA topoisomerase I leads to embryonic death between the 4- and 16-cell stage[100], inactivation of DNA topoisomerase IIβ leads to death at birth[97] and inactivation of DNA topoisomerase IIIα leads to embryonic death shortly after implantation[101]. Inactivation of DNA topoisomerase IIIβ causes no apparent embryonic or neonatal abnormalities, but the mutant animals have a shortened average life span[102]. Although no knockout studies have been carried out for the *TOP2α* gene, which encodes DNA topoisomerase IIα, various studies indicate that inactivation of this enzyme is lethal, even in cell lines[2].

fork advances during SEMICONSERVATIVE REPLICATION, the rod forces the helical intertwines of the DNA ahead of it into a progressively shortened region, and the DNA becomes overwound or positively supercoiled; behind the advancing fork, the replicated bubble becomes progressively larger (FIG. 3b). If the rod is permitted to rotate, the positive supercoils ahead of it can be redistributed to the region behind it, leading to intertwining of the pair of replicated DNA segments (FIG. 3c) and/or positive supercoiling of the unreplicated DNA behind the fork.

The mechanisms of the various subfamilies of DNA topoisomerases predict that the positive supercoils that are generated by replication can be removed by a type IB or a type II enzyme[1–3]. If the replication machinery could readily rotate, a type II enzyme might also act behind the fork to remove the intertwines between the newly synthesized DNA double helices[16] (FIG. 3c). The type IA enzymes, however, are inefficient at removing positive supercoils[1,4] that do not have a pre-existing single-stranded region in the DNA[5]. Neither a type IA nor a type IB enzyme can pass an intact DNA double helix through another, although the presence of a single-stranded nick or gap[6,8], or perhaps an unpaired bubble in the duplex[17], might enable the enzyme to do so. Hence, the type IA enzymes are expected to be less suitable than type IB or type II enzymes for solving the topological problems that are associated with DNA chain elongation in replication.

Studies in various organisms generally support the above predictions[1,2]. In the budding yeast *Saccharomyces cerevisiae*, either the type IB enzyme (DNA topoisomerase I) or the type IIA enzyme (DNA topoisomerase II), but not the type IA enzyme (DNA topoisomerase III), can support the elongation step of DNA replication[18]. In *Drosophila melanogaster*, DNA topoisomerase I is essential in all developmental stages of the embryo that are actively engaged in cell proliferation[19], consistent with the idea that one key function of the type IB enzyme is to serve as a replication swivel. *Escherichia coli* DNA topoisomerase III, a type IA enzyme, can support plasmid replication in a purified system[20], but its ability to support DNA chain elongation *in vivo* is uncertain.

Segregation of newly replicated chromosomes. Distinct topological problems occur when two replication forks converge (FIG. 4a). As the unreplicated segment of parental DNA becomes very short, a type IB DNA topoisomerase is probably unable to remove the last few parental intertwines, because its action requires that it binds to a short stretch of dsDNA (FIG. 2b)[3,21]. The residual intertwines between the parental strands can be converted to intertwines between the newly replicated daughter molecules (FIG. 4b)[22], however, so that a type II enzyme can accomplish the final segregation of the newly replicated pair. A type IA enzyme might also be able to resolve the structures shown in FIG. 4a, by transiently cleaving a single strand at the junction between ssDNA and dsDNA[23].

There is strong evidence, especially from studies of *E. coli* and the yeasts *S. cerevisiae* and *Schizosaccharomyces pombe*, to indicate that the type II DNA topoisomerases are indispensable in chromosome segregation[1,2]. Significantly, when yeast cells undergo mitosis, the longer chromosomes are more likely to suffer loss or breakage in the absence of the type II enzyme[24]. This finding supports the idea that the DNA topoisomerases evolved to solve the topological problems of DNA as it became progressively longer, or when ring-shaped DNA emerged; for short linear chromosomes, the topological problems can be alleviated by movements of their ends.

Initiation of the replication bubble. The initiation of replication is marked by the opening of a short unpaired region of DNA. In plasmid-replication systems reconstituted from purified *E. coli* proteins, a negatively supercoiled template is usually required for initiation[25]. Because of the unique ability of bacterial gyrase to negatively supercoil a DNA[1,14], the requirement for a negatively supercoiled DNA template *in vitro* is suggestive of a role for DNA gyrase in the initiation of bacterial DNA replication. However, negative supercoiling of a topologically isolated DNA segment can also be

SEMICONSERVATIVE REPLICATION
A common mode of replication in which both strands of a DNA double helix are copied by the replication machinery to give a pair of progeny DNA molecules.

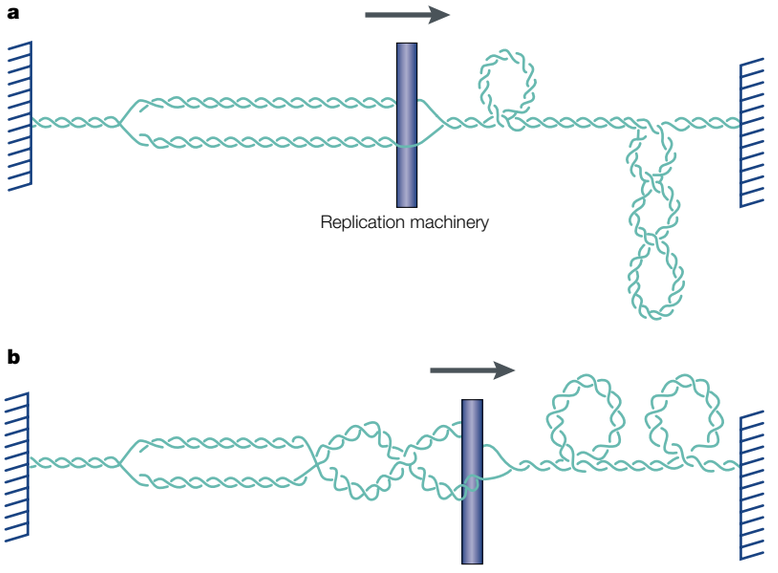


Figure 3 | **Topological problems associated with an elongating replication fork.** The replication machinery is illustrated as a rod, and the topological consequences of DNA chain elongation depend on whether the replication machinery can readily rotate in the cellular milieu. The ends of the DNA are attached to a hypothetical immobile structure, which could be the nuclear or cell membrane. **a** | The replication machinery is immobile; the DNA turns as it is passed through the machinery, and positive supercoils accumulate ahead of the advancing replication fork. **b** | The replication machinery is allowed to rotate around the helical axis of the unreplicated DNA. This rotation allows the redistribution of positive supercoils ahead of the advancing fork into the region behind it, and leads to the intertwining of the pair of duplicated double helices (as depicted) and/or positive supercoiling of that region (not shown). In either **a** or **b**, a type IB or type II DNA topoisomerase, but probably not a type IA enzyme, can solve the topological problems.

effected by transcription or chromatin remodelling (see below), and different replication systems could differ in their dependence on DNA negative supercoiling for initiation.

Transcription

The topological problems that are encountered during the elongation of a nascent transcript[26] (FIG. 5) resemble those of the elongation step in replication (FIG. 3). However, the two processes differ in that the elongation step of transcription, unlike that of replication, does not involve a continuous separation of the parental DNA strands. This difference is best appreciated in the case of a DNA ring.

In the replication of a DNA ring, the pair of intertwined parental strands must progressively untwine and become completely unlinked at the end of replication, so that a DNA topoisomerase is mandatory. In transcription, the generation of oppositely supercoiled domains does not alter the intertwines between the complementary strands of the DNA template, and any requirement for a topoisomerase would be to modulate the local supercoiled state of the DNA, rather than fulfilling a topological necessity (see below).

As for the elongation step in replication, the requirement for DNA topoisomerases in transcription also depends on whether the transcription apparatus R that is depicted in FIG. 5 (which includes the RNA polymerase, proteins associated with the polymerase, and the nascent transcript and its associated proteins) can rotate without encountering a large potential barrier. Several possibilities that might prevent the rotation of R around its DNA template were proposed in the 1987 'twin-supercoiled-domain model' of transcription[26]. Of these possibilities, cotranscriptional insertion of nascent polypeptides into the cell membrane (termed 'transertion' in REF. 27) seems to be the most important in prokaryotes[28–30].

Because the expression of membrane proteins and proteins for export constitutes a considerable portion of the total programme of cellular synthesis, the removal of positive and negative supercoils that are generated by transcription is an important function of the DNA topoisomerases in prokaryotes. The type IIA enzymes in prokaryotes, especially DNA gyrase, shoulder the responsibility of removing positive supercoils, whereas the type IA enzyme DNA topoisomerase I is important in the removal of negative supercoils[1,2]. In *E. coli topA* mutants, which lack DNA topoisomerase I, excessive negative supercoiling in the wake of the moving transcription machinery might cause base-pairing between the nascent RNA and its template strand ('R-LOOPING'), which would be detrimental to the cells[31]. *E. coli topA* mutants are not viable unless they acquire compensatory mutations[32,33]. Inactivation of *Salmonella typhimurium* and *Shigella flexneri topA*, however, does not lead to loss of viability[34,35]. Perhaps, the cellular level of another topoisomerase, such as DNA topoisomerase IV, is high enough in the latter organisms to fulfil the role of removing negative supercoils[2,36].

There is also evidence for transcriptional supercoiling in the budding yeast[2]. Mobility of the transcription 'factory' might be hindered or prevented because of its association with the nuclear membrane[15], or because of the recently reported coupling between transcription and translation in the nucleus[37]. The presence of eukaryotic DNA topoisomerase I in actively transcribed regions is well documented[1,2], and this association involves the amino-terminal region of the enzyme[38].

Because deletion of yeast *TOP1* has no principal effects on cell growth[39,40], DNA topoisomerase II could presumably substitute for any role of DNA topoisomerase I in solving the topological problems of transcription. Yeast *top1 top2* temperature-sensitive double mutants have reduced ribosomal RNA synthesis by RNA polymerase I at non-permissive temperatures, but the reduction of the RNA-polymerase-II-mediated synthesis of messenger RNA is more moderate[39,40]. Because an RNA polymerase is a powerful motor[41], it can probably overcome substantial frictional resistance while translocating along its DNA template in the absence of DNA topoisomerase activities. The molecular nature of the observed differences between the effects of the DNA topoisomerases on transcription that is mediated by RNA polymerases I and II is unclear, but it might be related to the very robust expression of the ribosomal DNA gene cluster, or the organization of discrete substructures of the nucleus[42,43].

R-LOOPING
Refers to a structure in a double-stranded DNA segment in which a single-stranded RNA pairs with a portion of one DNA strand to displace a portion of the other DNA strand in a single-stranded state.

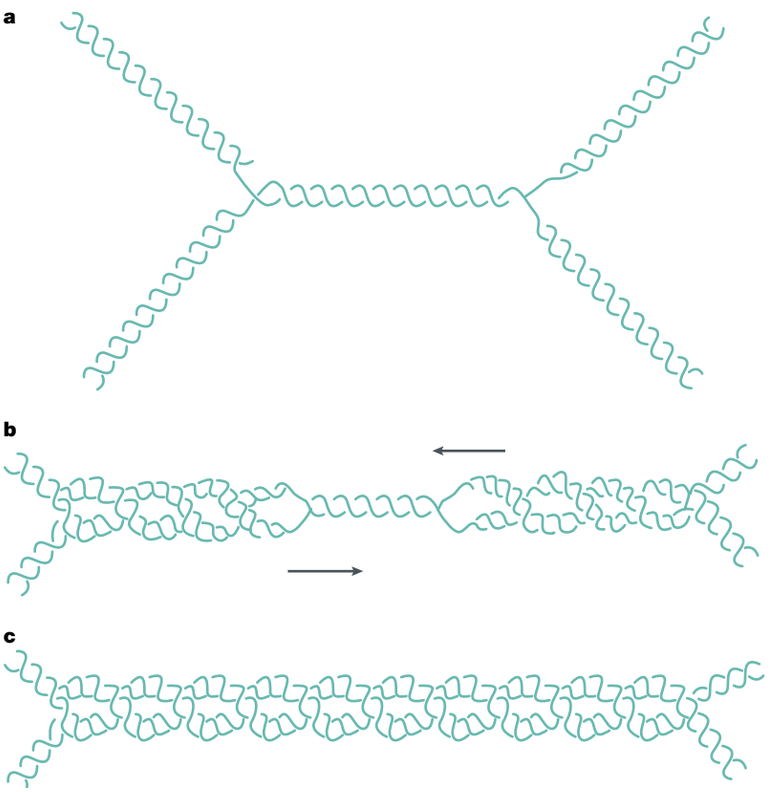


Figure 4 | **A distinct topological problem occurs when two replication forks converge.** When the unreplicated DNA segment becomes very short (**a**), the type IB topoisomerase enzyme cannot remove the last few intertwines between the parental strands. These single-stranded intertwines can probably be removed by a type IA enzyme[23], or be converted to double-stranded intertwines (**b** and **c**) for removal by a type II DNA topoisomerase[22]. Modified from REF. 22. © (1981), with permission from Elsevier Science.

Transcription of a chromatin template dotted with nucleosomes poses additional topological problems. Passage of an advancing RNA polymerase through a nucleosome has been postulated to periodically enclose a DNA loop containing both the partially unravelled nucleosome and the polymerase[44]. A DNA topoisomerase might be involved in continued translocation of the polymerase in such a loop. Interestingly, a recent study[45] indicates that efficient transcription of a chromatin — but not a DNA — template requires the presence of a DNA topoisomerase in the transcription complex. However, all postulates that invoke a stringent topoisomerase requirement in transcription must be reconciled with the finding that, in yeast, inactivation of both DNA topoisomerases I and II does not drastically reduce mRNA synthesis[39,40].

DNA topoisomerase I has also been shown to act as a co-activator in purified mammalian transcription systems[46–48]. The topoisomerase seems to facilitate the formation of an active TFIID–TFIIA protein complex on the promoter[48], but the DNA strand breakage and rejoining activity of the topoisomerase is not required in this co-activator role[46,48].

There are further problems of transcription that might involve topoisomerases, although no experimental results are available yet to support these ideas. For a very long transcript, or for closely spaced transcripts along a very actively transcribed gene, entanglement between a transcript and its template[15] (or between the transcripts themselves) could present a problem. Also, in cases where a stably base-paired region between a nascent RNA and its template strand has formed, a topoisomerase-mediated untwining of the transcript from the template strand, rather than the nucleolytic removal of the DNA-bound RNA by RNase H, could also be used to salvage the transcript.

DNA recombination

Many fundamental steps in different cellular processes often share common features. The structure that is shown in FIG. 4a to illustrate the problem of resolving a pair of intertwined parental strands between two converging replication forks, for example, can also be used to illustrate the pairing of two gapped DNA molecules to form a recombination intermediate. Resolution of a pair of newly replicated chromosomes and of an intermediate of recombinational repair clearly share common topological features, and might involve the same DNA topoisomerases[49].

The roles of DNA topoisomerases in modulating the frequency of recombination events have received much interest. Inactivation of any one of the three yeast DNA topoisomerases increases genome instability[50], and the effects of inactivating the type IA enzymes are particularly striking (see below). Equally significant is the finding that a key player in meiotic recombination is related to one subunit of the type IIB DNA topoisomerases[10,51]. The recombination field itself is undergoing a sea change, and the importance of recombinational repair of DNA damage, and in restoring stalled replication forks, has received increasing attention[52,53].

Type IA enzymes in recombinational repair. As summarized in BOX 1, at least one type IA enzyme is present in all living organisms. Why this omnipresence? What are the cellular roles of the type IA DNA topoisomerases? Studies of the past decade point to an important role for these enzymes in the resolution of intermediates that are found in recombinational repair.

E. coli cells that lack both type IA DNA topoisomerases are non-viable, even in the presence of a *topA* compensatory mutation[54]. These cells have extensive filamentation and possess an abnormal nucleoid structure. The viability of *topA topB* double-mutant cells that carry a *topA* compensatory mutation can be restored, however, by a further deletion of the *recA* gene[54]. These findings indicate that, in bacteria, the type IA enzymes might be involved in RecA-mediated recombination and that they could specifically resolve recombination intermediates before chromosome segregation[54].

In the yeasts, inactivating the single type IA enzyme, DNA topoisomerase III, leads to loss of viability in *S. pombe* but not in *S. cerevisiae*[55–57]. *S. cerevisiae top3* nulls show a complex phenotype, including slow growth and reduced viability, hyper-recombination between repetitive sequences, hypersensitivity to DNA-damaging

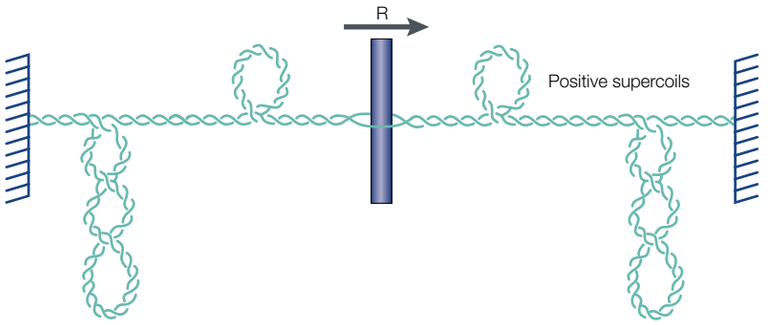


Figure 5 | **Generation of oppositely supercoiled domains by transcription.** The transcription apparatus R — including the RNA polymerase and its associated proteins, the nascent RNA and RNA-bound proteins — is represented by a rod. When R cannot rotate around the helical axis of the DNA template, overwinding or positive supercoiling of the DNA template ahead of R is accompanied by underwinding or negative supercoiling of the DNA template behind R. The topology of replicative elongation (FIG. 3) can be viewed as a special case of the generation of twin supercoiled domains by a machinery tracking along a DNA double helix. The separation of two intertwined strands behind the replication machinery can be considered as a special case of negative supercoiling (two unlinked single-stranded DNA rings of complementary sequences, for example, can be considered as the most negatively supercoiled form of a duplex DNA ring).

agents and inability to sporulate[2,55]. Genetic screens for suppressors of *S. cerevisiae top3* null mutants led to the identification of mutations that map to the gene *SGS1*[58]. This gene was shown to encode a helicase of the RecQ family[58,59], the members of which include *E. coli* RecQ protein, *S. pombe* Rqh1 protein, and the human Bloom syndrome and Werner syndrome proteins, BLM and WRN, respectively[60–62]. A link between another human RecQ helicase RECQL4 and the Rothmond–Thomson syndrome has also been proposed[63]. The three human syndromes show signs of genome instability, and are variously characterized by growth abnormality, predisposition to different cancers and signs of premature ageing[60–62]. There is strong evidence that Sgs1 helicase physically interacts with DNA topoisomerase III[58,64,65], and interaction between other members of the RecQ family and type IA DNA topoisomerases has also been reported[60–62,66].

Several studies have implicated *SGS1* in DNA recombination and repair[67,68]. Significantly, it is the inactivation of *SGS1* that suppresses the requirement for a type IA enzyme, which suggests that a type IA enzyme might be needed to resolve a structure that is formed during recombination or repair[58]. What might be the molecular nature of such a structure? Why is a type IA DNA topoisomerase specifically required? Could this unique role of the type IA enzymes be related to their omnipresence in all organisms? In BOX 2, the plausible involvement of a type IA DNA topoisomerase in the resolution of Holliday structures, especially the double HOLLIDAY JUNCTION, is discussed.

HOLLIDAY JUNCTION
A DNA structure named after Robin Holliday, who first described it in 1964 as a plausible recombination intermediate between a pair of homologous DNA molecules.

A type IIB enzyme in meiotic recombination? The amino-acid sequence of the type IIB enzyme DNA topoisomerase VI indicated the presence of its homologues in various organisms, of which the *SPO11* gene product of *S. cerevisiae* is of particular interest[10,69]. The Spo11 protein links covalently to the 5′ ends of dsDNA breaks at the hot-spots of meiotic recombination[69]. Although Spo11 seems to catalyse DNA breakage as well as rejoining during meiosis, it differs from the archetypal type IIB DNA topoisomerases in that there is, as yet, no evidence for its involvement in DNA strand passage[69].

Interestingly, although the A subunit of a type IIB enzyme seems to contain the counterparts of all structural motifs of the type IIA DNA topoisomerases that are known to be important in the catalysis of DNA breakage and rejoining (including the active-site tyrosine region and a Rossmann fold termed the 'toprim'), it does not cleave DNA in the absence of the B subunit[70]. This finding raises several questions. Does DNA cleavage by Spo11 require a second subunit? Does its mechanism resemble that of a type IIB DNA topoisomerase, at least in terms of DNA breakage and rejoining? If the answer is yes, how is the rejoining reaction prevented when a stable double-stranded break is finally formed?

Topoisomerases and chromosome condensation

Chromatin compaction, chromosome segregation and DNA topology are intricately interrelated[71–74]. In eukaryotes, earlier genetic studies of the fission yeast, cytological studies of various cells treated with topoisomerase inhibitors, and biochemical studies of chromosome condensation in cell extracts have all implicated DNA topoisomerase II in chromatin and chromosome condensation during mitosis[1,2,75]. Mammalian DNA topoisomerase II has also been implicated in apoptotic chromatin condensation[76].

Eukaryotic DNA topoisomerase I has also been proposed to have a role in chromosome condensation[77,78]. In yeast, DNA topoisomerase I and the product of the *TRF4* gene seem to share such a role in mitotic cells[77]; *trf4 top1* double mutants are defective in chromosome condensation, spindle elongation and nuclear segregation[77], and they also fail to establish the condensed state of the rDNA locus at mitosis[78].

The idea that at least one DNA topoisomerase is required for chromosome condensation and decondensation is consistent with the expected changes in the twist and writhe of a long DNA when it undergoes protein-mediated compaction. Recent studies of the condensins — ubiquitous ATP-dependent protein complexes that are crucial for chromosome condensation — have also implicated a requirement for a DNA topoisomerase in chromosome condensation[74].

In eukaryotes, either a type II or a type IB enzyme should be able to solve the topological problems of coiling a DNA into a compact form (or in decondensation). Historically, eukaryotic DNA topoisomerase II has been the focus of attention in chromosome condensation[75], but its precise role in the many steps of morphologically distinct changes between an interphase chromosome and a highly condensed metaphase chromosome is unclear. Whether DNA topoisomerase II has a role during meiotic chromosome condensation in *S. pombe* has also been questioned[79].

In bacteria, it seems that DNA supercoiling *per se* can also affect chromosome compaction and segregation. The efficiency of *E. coli* plasmid partition seems to be

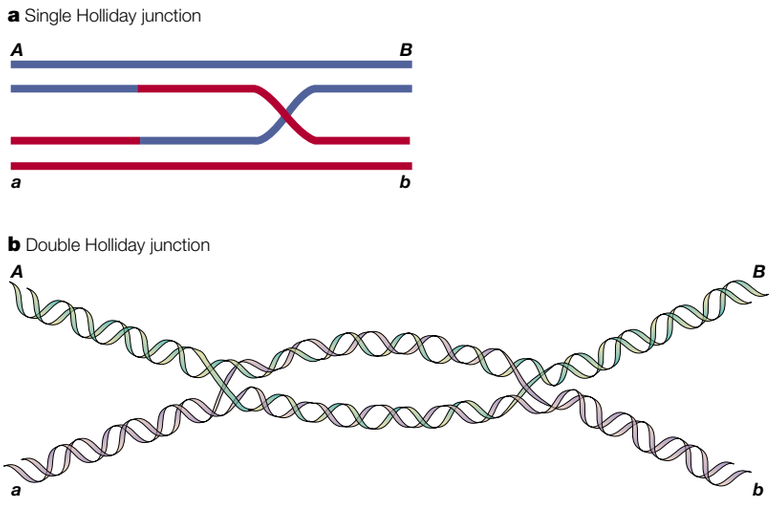
increased by the presence of a strong gyrase-binding site on a plasmid[80,81], or by a reduction of the intracellular activity of DNA topoisomerase I[82]. A higher degree of negative supercoiling was thought to make the plasmid molecules more compact, and hence more easily segregated by random partition[82]. *E. coli topA* mutants were also found to suppress the production of anucleate cells caused by mutations in the *muk* genes, which encode proteins that resemble the core subunits of the condensins[83].

DNA topoisomerases and chromosome structure

In general, whenever a long chromatin fibre undergoes a structural change, any accompanying changes in its twist and writhe could require the catalytic action of one or more DNA topoisomerases. Several studies have also implicated DNA topoisomerases in chromatin structure and organization. The association of *Drosophila* DNA topoisomerase II and human DNA topoisomerase IIβ with ATP-using chromatin remodelling complexes has

Box 2 | **Type IA DNA topoisomerase in Holliday-junction resolution?**

The presence of at least one type IA DNA topoisomerase in all organisms (BOX 1) **indicates that these enzymes have a key cellular function, and recent studies have pointed to their role in recombinational repair and in chromosome segregation. Which DNA structures might specifically require a type IA DNA topoisomerase to be processed?**



In answer to this question, from a molecular point of view, a double Holliday junction is particularly appealing. Single and double Holliday junctions are sketched in parts a and b of the figure, respectively. The importance of these structures in recombination, including meiotic recombination, and the repair of DNA lesions and restart of stalled replication forks, is well documented[52,103–107]. A single Holliday junction can be resolved, for example, by specific ENDONUCLEASES **or site-specific** DNA STRAND-TRANSFERASES**. Interestingly, pox-virus topoisomerases, which belong to the type IB DNA topoisomerase family, have also been shown to resolve Holliday junctions in a way that is similar to the site-specific DNA strand-transferases[108]. (Historically, a type IB enzyme was also postulated to catalyse the formation of a double Holliday junction from a pair of homologous DNA molecules[109].) Resolution of a Holliday structure by either class of enzyme can proceed in two ways — depending on which pair of the DNA strands are cleaved at the junction — to yield either a product in which the original ends of the DNA duplexes are exchanged (recombinant with crossover), or a non-crossover structure in which the original ends are not exchanged.**

Resolution of a single Holliday junction without breakage and rejoining of DNA strands is also possible; for example, by BRANCH MIGRATION **of the junction to a pre-existing nick or molecular end[105]. Branch migration might encounter topological problems similar to those discussed for replication and transcription, and the involvement of a topoisomerase is plausible. It is not clear, however, why a type IA enzyme should be specifically required; one possibility is through association with a protein that recognizes a Holliday junction (see below).**

Several arguments can be made for a specific requirement for a type IA DNA topoisomerase in the resolution of a double Holliday junction. First, in part b, the middle part of the drawing contains two helices that are formed by intertwining of the separate strands of the pair of DNA molecules *AB* and *ab*. By successive passage of an *AB* strand through an *ab* strand, or *vice versa*, these intermolecular intertwines can be completely resolved by a type IA enzyme, but not by either a type IB or a type II enzyme. Second, the topoisomerase-mediated resolution of a double Holliday junction can occur locally, without strand exchanges, thereby yielding only products without a crossover. This strategy would allow cells to repair DNA lesions and restart replication forks without the risk of undesirable genetic exchanges. Third, in the cases of yeast Sgs1 and human BLM helicases, each of which forms a complex with a type IA DNA topoisomerase, binding of the helicases to Holliday junctions has been shown[110,111]. So, these helicase–topoisomerase complexes could preferentially bind to Holliday junctions. In the case of *Schizosaccharomyces pombe rqh1* mutants, which lack the Sgs1 homologue, expression of a bacterial enzyme that could resolve Holliday structures partially suppressed the *rqh1* phenotype[112]. Fourth, bacteria possessing a type IB enzyme resembling a pox-virus topoisomerase seem to lack the type IA enzyme DNA topoisomerase III[98]. Because of notable differences between the type IA and IB enzymes, it is intriguing whether this curious negative correlation might be related to the ability of both enzymes in resolving Holliday junctions.

ENDONUCLEASE
An enzyme that catalyses hydrolytic cleavage of DNA in the middle of a DNA strand or double helix.

DNA STRAND-TRANSFERASE
An enzyme that transfers a donor group of one DNA strand (for example, a 5′ phosphoryl group) to a receiving group of another DNA strand (for example, a 3′ hydroxyl group).

BRANCH MIGRATION
The movement of a junction formed by multiple DNA or RNA strands of complementary nucleotide sequences that results from nearly simultaneous breakage and formation of base pairs among them.

been reported[84,85], although the functionality of this association is yet to be established.

A structural role for eukaryotic DNA topoisomerase II in the higher-order organization of chromosomes has also been postulated[86]. Mammalian DNA topoisomerase II (presumably the α-isoform) is a main non-histone protein in the axial core or scaffold of metaphase chromosomes[87]. The co-localization of *Drosophila* DNA topoisomerase II with a protein called Barren, which is involved in chromosome condensation[88], is also consistent with a direct role for the topoisomerase in chromosome organization. However, recent studies of fluorescently tagged human DNA topoisomerases IIα and IIβ indicate that neither enzyme is an immobile structural component of the chromosomal scaffold[89].

Topoisomerases as targets of therapeutic agents

DNA topoisomerases have been shown to be the molecular targets of many antimicrobial and anticancer agents. Among topoisomerase-targeting drugs in clinical use at present, most (if not all) act by trapping the covalent DNA–enzyme intermediates to convert a normal cellular enzyme to a DNA-damaging agent. Several recent reviews of DNA topoisomerases in pharmacology and clinical medicine have been published[90–92].

Conclusions

In the three decades since the discovery of the first DNA topoisomerase[4], extensive studies of these enzymes have led to a much better understanding of their reaction mechanisms and cellular roles. The identification of many chemically distinct toxins[93–95], natural products and synthetic compounds[90–92] that convert DNA topoisomerases to DNA-damaging agents also underscores nature's dilemma — it must solve the topological problems of DNA that come with its double-helical structure, yet the solution utilizing the DNA topoisomerases comes with the risk of creating weak spots in the DNA. Although, on the one hand, DNA topoisomerases are key targets for the development of better therapeutics, on the other hand these enzymes might also suffer assaults by natural and synthesized products, sometimes with devastating consequences. A plausible role for DNA topoisomerase II in carcinogenesis, for example, has been proposed[96]. Further studies of a potential role for the DNA topoisomerases in preventive medicine are urgently needed.

The many tasks of the DNA topoisomerases often make it a real challenge to establish the links between their molecular roles and the physiological consequences of their inactivation. For example, deletion of the mouse *TOP2β* gene, which encodes DNA topoisomerase IIβ, leads to defects in the formation of neuromuscular junctions[97]. How does an enzyme that acts on DNA in the nucleus affect the formation of neuromuscular junctions? We still do not know.

Interactions between various DNA topoisomerases and other proteins — for example, between a type IA DNA topoisomerase and a RecQ-family helicase[58,60–62,64–66] — and the mechanistic and functional consequences of these interactions, have not yet been sufficiently explored to shed light on the many questions still remaining. Clearly, the study of these fascinating enzymes has not yet passed the point of diminishing returns.

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Online links

DATABASES
The following terms in this article are linked online to:
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SGS1 | *SPO11* | topoisomerase I | topoisomerase II | topoisomerase III | *TRF4* |
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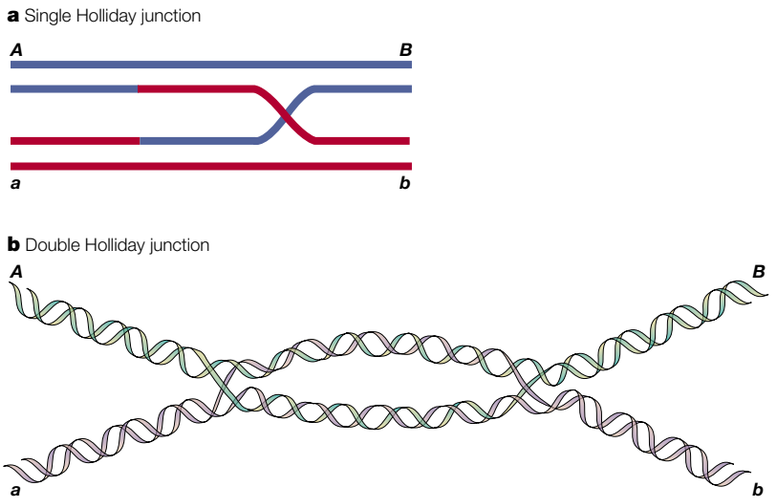
Corrections (shown in red)

Box 2 | **Type IA DNA topoisomerase in Holliday-junction resolution?**



The presence of at least one type IA DNA topoisomerase in all organisms (BOX 1) indicates that these enzymes have a key cellular function, and recent studies have pointed to their role in recombinational repair and in chromosome segregation. Which DNA structures might specifically require a type IA DNA topoisomerase to be processed?

In answer to this question, from a molecular point of view, a double Holliday junction is particularly appealing. Single and double Holliday junctions are sketched in parts **a** and **b** of the figure, respectively (the DNA double helix is represented by two parallel lines in **a** and two intertwined ribbons in **b**). The importance of these structures in recombination, including meiotic recombination, and the repair of DNA lesions and restart of stalled replication forks, is well documented[52,103–107]. A single Holliday junction can be resolved, for example, by specific ENDONUCLEASES or site-specific DNA STRAND-TRANSFERASES. Interestingly, pox-virus topoisomerases, which belong to the type IB DNA topoisomerase family, have also been shown to resolve Holliday junctions in a way that is similar to the site-specific DNA strand-transferases[108]. (Historically, a type IB enzyme was also postulated to catalyse the formation of a double Holliday junction from a pair of homologous DNA molecules[109].) Resolution of a Holliday structure by either class of enzyme can proceed in two ways — depending on which pair of the DNA strands are cleaved at the junction — to yield either a product in which the original ends of the DNA duplexes are exchanged (recombinant with crossover), or a non-crossover structure in which the original ends are not exchanged.

Resolution of a single Holliday junction without breakage and rejoining of DNA strands is also possible; for example, by BRANCH MIGRATION of the junction to a pre-existing nick or molecular end[105]. Branch migration might encounter topological problems similar to those discussed for replication and transcription, and the involvement of a topoisomerase is plausible. It is not clear, however, why a type IA enzyme should be specifically required; one possibility is through association with a protein that recognizes a Holliday junction (see below).

Several arguments can be made for a specific requirement for a type IA DNA topoisomerase in the resolution of a double Holliday junction. First, in part **b**, the middle part of the drawing contains two helices that are formed by intertwining of the separate strands of the pair of DNA molecules *AB* and *ab*. By successive passage of an *AB* strand through an *ab* strand, or *vice versa*, these intermolecular intertwines can be completely resolved by a type IA enzyme, but not by either a type IB or a type II enzyme. Second, the topoisomerase-mediated resolution of a double Holliday junction can occur locally, without strand exchanges, thereby yielding only products without a crossover. This strategy would allow cells to repair DNA lesions and restart replication forks without the risk of undesirable genetic exchanges. Third, in the cases of yeast Sgs1 and human BLM helicases, each of which forms a complex with a type IA DNA topoisomerase, binding of the helicases to Holliday junctions has been shown[110,111]. So, these helicase–topoisomerase complexes could preferentially bind to Holliday junctions. In the case of *Schizosaccharomyces pombe rqh1* mutants, which lack the Sgs1 homologue, expression of a bacterial enzyme that could resolve Holliday structures partially suppressed the *rqh1* phenotype[112]. Fourth, bacteria possessing a type IB enzyme resembling a pox-virus topoisomerase seem to lack the type IA enzyme DNA topoisomerase III[98]. Because of notable differences between the type IA and IB enzymes, it is intriguing whether this curious negative correlation might be related to the ability of both enzymes in resolving Holliday junctions.

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Citations to FIG. 3c throughout the main body text should read FIG. 3b.