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Untangling bacterial DNA topoisomerases functions

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Abstract

Topoisomerases are the main enzymes capable of resolving the topological constraints imposed by DNA transactions such as transcription or replication. All bacteria possess topoisomerases of different types. Although, bacteria with circular replicons should encounter similar DNA topology issues, the distribution of topoisomerases varies from one bacterium to another, suggesting polymorphic functioning. Recently, several proteins restricting, enhancing or modifying the activity of topoisomerases were discovered opening the way to new area of understanding DNA topology management during the bacterial cell cycle. In this review, we discuss the distribution of topoisomerases across the bacterial phylum and current knowledge on the interplay between the different topoisomerase to maintain topological homeostasis.

Keywords: Topoisomerase, DNA topology, Bacteria, Supercoiling, Decatenation, Antibiotics

Subjects: Microbiology, Topology

Introduction

In prokaryotic cells, DNA is compacted in the cytoplasm to form the nucleoid, a dynamic structure that undergoes various topological changes during the cell cycle. To maintain DNA homeostasis, bacteria use specialized enzymes called topoisomerases. These enzymes are essential, ubiquitous and modify DNA supercoiling by introducing transient breaks in DNA strand(s), allowing the double helix to unwind or coil. This process is essential for many cellular functions, such as DNA replication and transcription, and is therefore vital for the bacterium. Bacteria possess two main types of topoisomerases: type I DNA topoisomerases, which cut a single strand of DNA, and type IIA DNA topoisomerases, which transiently cut both strands. The number of topoisomerases varies from one bacterium to another, but all have at least one type I DNA topoisomerase, Topo I, and one type II DNA topoisomerase, DNA Gyrase. Many bacteria possess a second type IIA topoisomerase, Topo IV, and sometimes a second type IA topoisomerase, Topo III. Some bacteria possess uncommon topoisomerases such as Topo III β , Topo IB, reverse Gyrase or TopoNM. Topoisomerases have been extensively studied individually, defining their main functions *in vitro* and then *in vivo*. Current studies are now looking at topoisomerase's consortiums and topoisomerase's partners to explore their mechanistic relationships. In this review, we focus on the distribution across the bacterial phylum and intriguing aspects of the relationships between topoisomerases.

Distribution and functional diversity of topoisomerases in bacterial species

In the tree of life, topoisomerases are highly conserved, but their number varies from one organism to another. Bacterial genomes encode at least a minimum essential set composed of 2 topoisomerases, a DNA Gyrase and a Topo I, and up to 5 topoisomerases (Fig. 1A). In addition, genes encoding for topoisomerases are also found on plasmids (1,2) and bacteriophages (3) suggesting their evolution by horizontal gene transfer and adaptation to the regulation of supercoiling homeostasis.

Type IA topoisomerases

Type IA topoisomerases are monomeric enzymes able to relax negatively supercoiled DNA by causing a transient single strand break and pass another single strand of DNA through this break, then close it. Among them, Topo I, encoded by the *topA* gene, is conserved in all bacterial species, while Topo III encoded by the *topB* gene, and Reverse Gyrase (RG) are present only in some species (fig. 1A). Topo III and RG are the result of horizontal gene transfer (4–6).

In vitro, Topo I and Topo III can relax negative supercoils, but Topo I is more efficient (7). In contrast, Topo III is more efficient in decatenation of ssDNA than Topo I and can therefore support DNA replication (8). In addition to the Topo III α , some *Bacillus*, *Clostridium*, *Enterobacter* and *Staphylococcus* possess an atypical Topo III, named Topo III β (9). Unlike Topo III α , Topo III β only partially relaxes negatively supercoiled DNA and does not decatenate single strand rings (9) (Fig. 1AB). RG are present in all hyperthermophilic genomes both bacteria and archaea (5,10). They play an important role in the adaptation of life to very high temperatures. RG are made up of a type IA topoisomerase domain and a helicase domain working in collaboration (11), they introduce positive supercoils in an ATP-dependent reaction (Fig. 1AB) and are therefore the only topoisomerases capable of generating positive supercoils in DNA.

Type IB topoisomerases

Deinococcus radiodurans, *Pseudomonas aeruginosa*, some *Bordetella*, *Xanthomonas* and *Mycobacterium* possess a Topo IB related to vaccinia virus topoisomerase and thought to be the result of horizontal gene transfer (12,13) (Fig. 1A). Topo IB is only found in bacteria lacking TopoIII (13). As type IA topoisomerases, it creates a single strand cut but allows the DNA to

move freely around the intact strand. In this way, it can relax both positive and negative supercoils (Fig. 1B) (14).

Type IIA topoisomerases

Type IIA DNA topoisomerases, namely Gyrase and Topo IV, are tetrameric enzymes composed of two subunits, GyrA/ParC and GyrB/ParE. Among bacterial species, Gyrase is ubiquitous, whereas Topo IV is partially conserved (Fig. 1A). Although the evolution of bacterial type IIA topoisomerases is not clearly established, it is suggested that the first enzyme was a Gyrase, from which Topo IV emerged following a genetic duplication event and subsequently lost the canonical GyrA box (15). Both introduce double-strand breaks in an ATP-dependent manner leading to similar relaxation of positive supercoils (Fig. 1B). However, Gyrase is the only enzyme that can introduce negative supercoils (16). By contrast, *E. coli* Topo IV is much more efficient for dsDNA decatenation than Gyrase (17,18). These activities suggest that *E. coli* Type IIA topoisomerases are specialized. Unlike *E. coli*, *Mycobacterium tuberculosis* has only one type IIA topoisomerase, Gyrase, which not only introduces negative supercoils, but also has enhanced cleavage and decatenation activities (19). Unlike other *Mycobacterium* possessing a minimal set of topoisomerases, *Mycobacterium smegmatis* has an additional unique type II topoisomerase, called TopoNM, which is composed of two subunits (TopoN and TopoM) homologous to DNA Gyrase and Topo IV (20). In addition to relaxing supercoils and decatenating circular DNA, it has the unique ability to introduce positive supercoils to substrate relaxed (20).

Type IIB

Topo VI, initially identified in the archaeal hyperthermophile *Sulfolobus shibatae*, has since been found throughout the archaea, a few bacterial species, and intriguingly, in eukaryotes such as plants and algae. It relaxes positive and negative supercoils, and decatenates DNA (21). Its physiological function(s) in bacteria remains unknown but interestingly it shares high homology with Spo11, which is responsible for the formation of dsDNA breaks during meiosis in eukaryotes.

DNA topoisomerase VIII, a new class of topoisomerases, has recently been identified in plasmids in bacteria and archaea (22,23). Topo VIII is distinct from topo VI as it is more common in bacteria than archaea, exhibits dramatic sequence divergence and is usually a homodimer. Their activities and cellular functions are yet poorly characterized.

Topoisomerase balance: antagonistic and synergistic effects on viability

The study of few bacterial models show that most topoisomerases are essential for their survival. Topo III and Topo IB, whose genes can be deleted without significant physiological alteration in *E. coli* or *B. subtilis* for Topo III (24,25) and in *Deinococcus radiodurans* for Topo IB (26,27) may only be important for bacteria in specific conditions.

The essentiality of Topo I differed from that of Gyrase or Topo IV. To our knowledge the deletion of Gyrase or Topo IV are simply not possible. Heat-sensitive alleles, isolated because of their nucleoid partition phenotype (*par*), have therefore been extensively used for *in vivo* studies of Gyrase and Topo IV. In this context, overexpression of Topo III restored survival when Topo IV is inactivated (28).

In *E. coli* or *B. subtilis* deletion attempts of *topA* resulted in compensatory mutations in the Gyrase genes (29–31) or in an amplification of the region carrying the *parC* and *parE* genes encoding Topo IV (32,33). Interestingly, *topA* null mutants could not be obtained in *Mycobacterium tuberculosis* and *Helicobacter pylori*, maybe because they do not possess Topo IV (34,35). The balance between Topo I and Gyrase seems to be a crucial point. Indeed, when *E. coli* Gyrase is dysfunctional Topo I might be inactivated by acetylation. The CobB deacetylase is involved in this regulation (36). The GyrA:Topo I ratio was also studied in *Streptococcus pneumoniae* with a depletion strategy, it revealed that imbalances lead to growth defects and viability issues (37). Supercoiling homeostasis was perturbed in a more

dramatic way in an archaeon that only encoded Topo I and RG by the introduction of a functional DNA Gyrase. Although plasmid supercoiling shifted from positive to negative, this introduction has limited impact on growth and physiology (38). Altogether, these genetic insights suggest extensive cross talks between Topoisomerases to maintain supercoiling/catenation homeostasis.

Topoisomerase involvement during transcription

Transcription modulates the topological state and dynamics of DNA. According to the twin-domain model of DNA supercoiling, the transcription machineries generate an accumulation of negative supercoils behind and positive supercoils ahead (39). Supercoiling waves impose mechanical perturbations on the DNA that propagate kilo bases away from the transcribed genes (40,41) and perturbation of the expression of the flanking genes (42). Topoisomerases, recruited to these transcribed zones, are therefore essential to recover an adequate supercoiling density ($\sigma \sim -0.06$ in *E. coli* for instance).

The antagonistic roles of Topo I and DNA Gyrase during transcription have been most extensively studied. In the current model, Topo I act upstream of transcription by relaxing negative supercoils, while Gyrase removes positive supercoils induced by transcription and introduces negative supercoils (Fig. 2A). This model is supported by ChIP-seq studies examining genome-wide the association of topoisomerases around expressed genes. Topo I colocalizes with RNA polymerase (RNAP) in *E. coli* (43), *Streptococcus pneumoniae* (44) and *Mycobacterium tuberculosis* (45). DNA Gyrase cleavage sites were enriched in extended regions located downstream of highly transcribed genes in *E. coli* (46) and in *Mycobacterium tuberculosis* (45). The superposition of the Topo I ChIP-Seq signal with that of RNAP ChIP-Seq (46,47), as well as the reduced binding of RNAP to promoter in presence of a drug inhibiting Topo I (44) confirmed the hypothesis that the interaction of Topo I with RNAP stabilizes the RNAP-DNA complex.

As illustrated by transcriptomic analyses of Topo I and Gyrase inhibitions respectively with seconeolistine, and novobiocine, in *D. dadantii* the consequences of Topoisomerase functioning or malfunctioning on gene expression is particularly difficult to decipher (48). Each drug impacts significantly about 500 genes, but their expression level, their genomic context and the growth phase of the bacteria influence their response to Topoisomerase inhibition and perhaps to local supercoiling changes. Several studies attempted to rationalize these observations with controlled artificial systems. For instance, the introduction of a strong Gyrase binding site limits positive supercoiling diffusion and restore correct expression of a supercoiling sensitive promoter placed downstream of a highly expressed gene (42). More recently, Boulas and colleagues built a first-principle biophysical model from *in vivo* gene expression data. They highlighted the antagonist effect of Topo I on transcription: as the removal of negative supercoiling promote elongation, the change in supercoiling density can repress transcription initiation (49). *In vitro* single-molecule assays suggested that Gyrase dissociation from and rebinding to DNA segment, catalyzing changes in its supercoiling level, was associated with transcriptional bursting (50).

Although it is mainly known for its decatenation activity, Topo IV is reported to be more active on positively supercoiled regions downstream of highly active transcription units (41,51) ; it may therefore help to resolve topological stress during transcription. However, the consequence of Topo IV alteration on transcription has not yet been directly described. To our knowledge, the influence of Topo III, Topo III β , RG and Topo IB on transcription has not yet been documented.

During replication, several partners control type IIA topoisomerase activities

During DNA replication, DNA is copied into two sister chromatids. This process generates positive supercoils downstream and catenation links upstream of the replication fork (52). By removing positive supercoils ahead of replication forks, DNA Gyrase is an essential driver of replication progression (53). When Gyrase becomes limiting Topo IV can support replication with a smaller speed and frequent fork arrests (54). In *Caulobacter crescentus*, the stimulation of replication by DNA Gyrase and Topo IV requires GapR, a nucleoid associated protein (NAP),

that binds positive supercoils DNA. The clamping of positive supercoils by GapR stimulates Gyrase and Topo IV (55). In *E. coli*, YejK a protein that clamps DNA, interacts with TopoIV and Gyrase and modifies their activities may play a similar role as GapR (56,57).

The main enzyme involved in decatenation is Topo IV (58,59). Studies on bacteria harboring heat-sensitive alleles of ParC and ParE have shown that inactivation of Topo IV leads to *Par* phenotype (60) and drastic chromosome conformation changes (61). Topo IV activity appeared to be regulated by different factors. First, it is inhibited by the binding of SeqA to newly replicated DNA (62). Second, it is stimulated by FtsK (63,64) and XerCD (65) for a maximum of activity in the replication terminus region at the end of the cell cycle (66). Finally, Topo IV, MukBEF (the *E. coli* SMC complex) and MatP (the protein defining the Ter macrodomain) (67,68) play together a particular ballet. MatP and Topo IV compete for MukB. MukB is dislodged from the terminus by MatP (67,69) this delays the segregation of the terminus region presumably because MukB fails to bring Topo IV to this region (70–72). *In vitro* and *in vivo* assays suggest that this interplay has strong consequences on Topo IV activity and chromosome conformation but the rationale of these changes is still unclear (61,73–76). Many factors are therefore controlling Topo IV activity, some of them are conserved in most bacterial phyla (XerCD, FtsK) while some of them are phylum specific (SeqA, MatP, MukB, GapR/YejK), this suggests that Topo IV control may be under different selective pressure according to the environment of the bacteria. In *Mycobacterium tuberculosis*, which does not encode Topo IV, Gyrase is specifically recruited to the ter region (45). It is tempting to speculate that a partner might assist in this original targeting and to promote daughter chromosome decatenation (Fig. 2B).

Type I topoisomerases also act during replication

The lack of severe phenotypes associated with *topB* deletion in *E. coli* suggested that it only plays accessory or condition specific roles. Topo III deletion exacerbates the *Par* phenotype when Topo IV is inactivated (25) and conversely, Topo III overexpression restores chromosome decatenation and improves the survival of *parE* or *parC* thermosensitive mutants (28). High-resolution live cell cycle imaging revealed that Topo III speed-up sister nucleoids segregation. Presumably, because it has access to newly formed precatenanes through an interaction with the DnaX complex of the replisome (77). Hi-C experiments revealing that the lack of Topo III favor short range chromatin contacts, perhaps between sister chromatids, also support a role in precatenanes removal near replication forks (61) (Fig. 2A). Finally, the RecQ helicase and Topo III may collaborate to resolve converging replication forks (78).

Some bacteria, such as *Deinococcus radiodurans*, have a Topo IB that interacts with genome partition proteins and the initiation of DNA replication (DnaA). Topo IB is located in the septal regions and is thought to be involved in genome stability in dividing populations (26). In addition, the Topo IB mutant showed a higher accumulation of ssDNA after irradiation, suggesting that Topo IB may play a role in DNA replication stress and radioresistance (27). In addition to its prominent role in transcription, in *Mycobacterium segmentis*, Topo I has been shown to bind and cleave the ter region, suggesting an involvement in chromosome segregation (79) (Fig. 2B).

These different observations open avenues for future studies investigating type I topoisomerases' roles during replication and their cross talks with type IIA topoisomerases in bacteria.

Topoisomerases are essential to prevent Replication – Transcription conflicts

Since transcription and replication take place simultaneously, this can lead to conflicts between the replication complex and the transcription complex. These conflicts can be codirectional if the two machineries move in the same direction, or head-on if they move towards each other. Lang and Merrikh, using an engineered head-on and codirectional conflicts system, have shown that the relaxation of positive supercoils by type II topoisomerase, DNA Gyrase and Topo IV, is required to resolve head-on conflict between transcription and replication but not

codirectional conflict in *Bacillus subtilis* (80). During transcription, the nascent transcribed RNA can hybridize with the complementary DNA strand from which it was transcribed, forming a structure known as a R-loop. In addition, R-loops formation can be facilitated by negative supercoiling (81) and head-on conflict (80). Interestingly, R-loops had been shown to promote constitutive stable DNA replication (cSDR) which leads to transcription and replication conflicts, impact replication and therefore the failure of R-loop removal becomes lethal (82). Topo I deficiency leads to increased negative supercoiling that promote R-loop formation, stabilization, genome instability and cSDR (83–86). Thus, essentiality of TopoI can be due to aberrant chromosomal DNA replication and excessive transcription-replication conflicts in its absence (87). In addition, Topo III have a synergic effect with Topo I on prevention of unregulated replication by inhibiting R-loop formation (32). Type IA topoisomerase deficiency can be compensated for by overproduction of Topo IV, perhaps by controlling R-loop replication (32). Thus, topoisomerase I, III, IV and Gyrase could help prevent transcription and replication conflicts.

Topoisomerases are involved ecological competition

The crucial role of topoisomerases in DNA homeostasis makes them natural targets for various bacterial growth inhibition strategies such as antibiotics and phages. The first strategy to be characterized was antibiotics produced by bacteria to compete in their ecological niche. *Streptomyces*, for instance, produce novobiocin, coumermycin and clorobiocin as DNA Gyrase inhibitors (88,89). Even recently, new antibiotics targeting Gyrase, Simocyclinone from *Streptomyces* species and evybactin from *Photobacterium noenieputensis* have been identified (90,91). Second, phages encode proteins that can interfere with bacterial topoisomerases for their own benefit. Recently, a peptide called Igy, encoded by *Pseudomonas* bacteriophage LUZ24, was found to inhibit the *Pseudomonas* Gyrase. In vivo, overproduction of Igy blocks DNA replication and leads to cell death (92).

On the other hand, topoisomerases can also be the targets of toxins from toxin-antitoxin systems (TAs), which are widespread in prokaryotes and play a key role in plasmid maintenance, stress management, bacterial persistence and anti-phage defense (93,94). Some TAs toxins (CcdB, ParE and TbsT) target Gyrase (95–97), the FicT toxin targets both Gyrase and Topo IV (98). TopAI inhibits Topo I in *E. coli* (99). These TAs systems targeting topoisomerases are involved in plasmid maintenance (100) and bacterial adaptation to stresses (97,101) but their eventual role in phage defense is not characterized.

Perspectives

1 : Topoisomerases are crucial for maintaining DNA homeostasis, enabling essential processes like replication, transcription, and chromosome segregation. In bacteria, their distribution, functions and regulations differs suggesting adaptation to intrinsic and extrinsic factors.

2 : Recent observations shows that topoisomerases have multiple functions and acts in complementary ways to maintain supercoiling homeostasis. Many, new topoisomerase partners contribute to specify these functions.

3 : Many cross-talks between topoisomerases and topoisomerase's partners remain to be discovered. This will highlight the diversity of ecological adaptations and open avenues for therapeutic strategies with new drugs or rejuvenating old antibiotics.

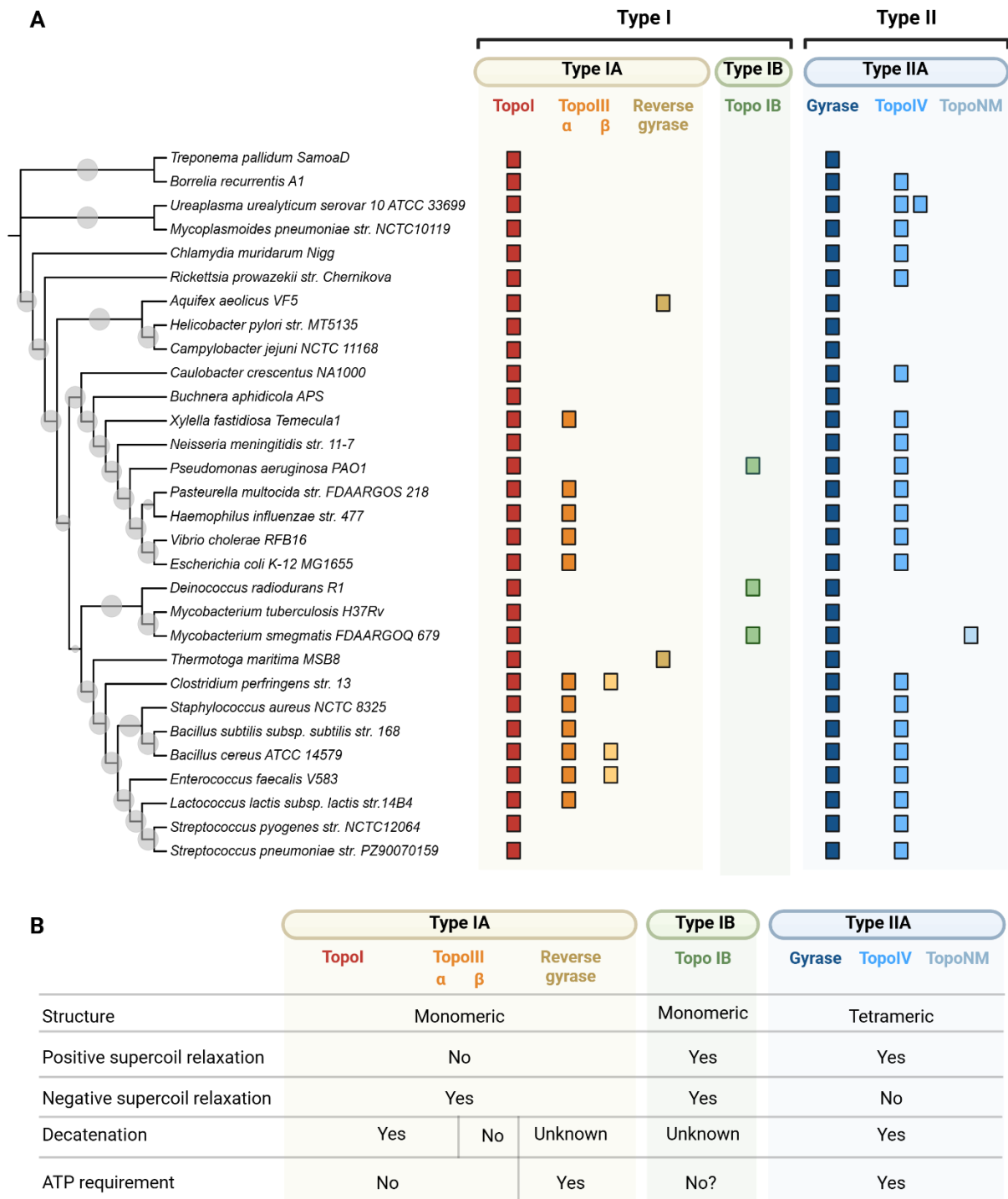


Figure 1: Distribution and function of bacterial topoisomerases.

(A) Analysis of the distribution of topoisomerases in different bacteria. The tree was generated from the binary gene presence-absence matrix using Roary tool with a threshold of 99% to define core genes. The presence of type I and type II bacterial topoisomerases are determined by BLAST with a 40% threshold of identity in the different bacterial genomes. Topoisomerase presence is symbolized by squares. **(B)** Comparative table of the function of bacterial topoisomerases.

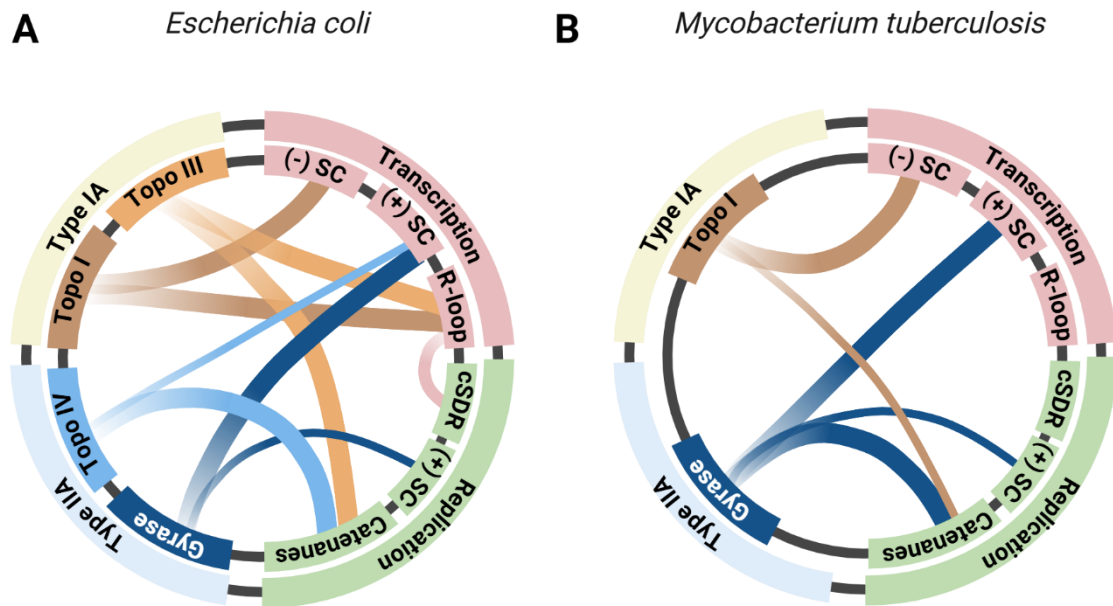


Figure 2: Illustration of the different functions of topoisomerases during transcription and replication in *E. coli* with four topoisomerases (**A**) and *M. tuberculosis* with two topoisomerases (**B**).

The topoisomerases are shown on the left (yellow for type IA and blue for type IIA) and the functions on the right (pink for transcription and green for replication). The thickness of the links between the topoisomerases and the biological functions represents the extent of the documentation for each topoisomerase function.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

cSDR	constitutive stable DNA replication
RNAP	RNA polymerase
Topo	Topoisomerase
TAs	Toxin Antitoxin system

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