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REVIEW

The regulation of DNA supercoiling across evolution

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Abstract

DNA supercoiling controls a variety of cellular processes, including transcription, recombination, chromosome replication, and segregation, across all domains of life. As a physical property, DNA supercoiling alters the double helix structure by under- or over-winding it. Intriguingly, the evolution of DNA supercoiling reveals both similarities and differences in its properties and regulation across the three domains of life. Whereas all organisms exhibit local, constrained DNA supercoiling, only bacteria and archaea exhibit unconstrained global supercoiling. DNA supercoiling emerges naturally from certain cellular processes and can also be changed by enzymes called topoisomerases. While structurally and mechanistically distinct, topoisomerases that dissipate excessive supercoils exist in all domains of life. By contrast, topoisomerases that introduce positive or negative supercoils exist only in bacteria and archaea. The abundance of topoisomerases is also transcriptionally and post-transcriptionally regulated in domain-specific ways. Nucleoid-associated proteins, metabolites, and physicochemical factors influence DNA supercoiling by acting on the DNA itself or by impacting the activity of topoisomerases. Overall, the unique strategies that organisms have evolved to regulate DNA supercoiling hold significant therapeutic potential, such as bactericidal agents that target bacteria-specific processes or anticancer drugs that hinder abnormal DNA replication by acting on eukaryotic topoisomerases specialized in this process. The investigation of DNA supercoiling therefore reveals general principles, conserved mechanisms, and kingdom-specific variations relevant to a wide range of biological questions.

KEYWORDS

DNA gyrase, DNA replication, histones, nucleoid-associated proteins, topoisomerases, transcription

1 | INTRODUCTION

Deoxyribonucleic acid (DNA) is the genetic material of living cells. Its linear succession of nucleotides defines an organism by specifying the products that are made and the circumstances in which they are made. However, some genetic information is encoded outside of the DNA sequence, in the structure of the DNA itself, in what is called DNA supercoiling. In all domains of life, DNA supercoiling plays a critical role in key cellular processes such as transcription, DNA replication and repair, and recombination. This article examines the regulation of DNA supercoiling across the domains of life.

2 | DNA SUPERCOILING IS A PHYSICAL PROPERTY OF DNA THAT EMERGES FROM THE NATURE OF THE DOUBLE HELIX

The DNA molecule is typically organized as a double helix. DNA can present in several forms (see Reference 1 for a review on the topic). This review focuses on the more common B-form DNA. In the absence of stress, the B-form double helix is right-handed, with a periodicity of $h_0 = 10.6$ bp.[2] One can define a linking number at rest Lk_0 for such molecule as how often the two strands of DNA cross each other. It therefore follows that for a DNA molecule of length N:

$$Lk_0 = \frac{N}{h_0}$$

DNA supercoiling defines the phenomenon whereby the actual linking number Lk differs from Lk_0. The handedness of supercoils is positive when they are in the same direction as the double helix (right-handed), that is, DNA is over-wound ($Lk > Lk_0$), and negative when DNA is under-wound ($Lk < Lk_0$). Supercoils can take different physical forms, most commonly twist (when the two strands cross each other) and writhe (when the double helix crosses itself; Figure 1a). These two forms are spontaneously interconvertible.[3] A common measurement for DNA supercoiling is the supercoiling density σ, defined as follows:

$$\sigma = \frac{(Lk - Lk_0)}{Lk_0}$$

For example, an exponentially growing *Escherichia coli* cell will have a supercoil density of −0.06, meaning that for every 100 turns the double helix should have in the rest state, 6 are missing.

One key property naturally emerges from the double helix structure: DNA supercoiling is invariant as long as the two strands of DNA are intact. The corollary is that a linear, unconstrained DNA molecule cannot have DNA supercoiling: any attempt to introduce DNA supercoiling would be lost in a freely rotating end. Likewise, an unconstrained single- or double-strand break dissipates all DNA supercoiling. The various kingdoms of life have evolved both divergent and shared ways to maintain DNA supercoiling, from circular chromosomes (bacteria and archaea) to proteins constraining DNA supercoiling (archaeal and eukaryotic histones and, to a lesser extent, bacterial nucleoid-associated proteins [NAPs]) and barriers to supercoil diffusion, thereby allowing the

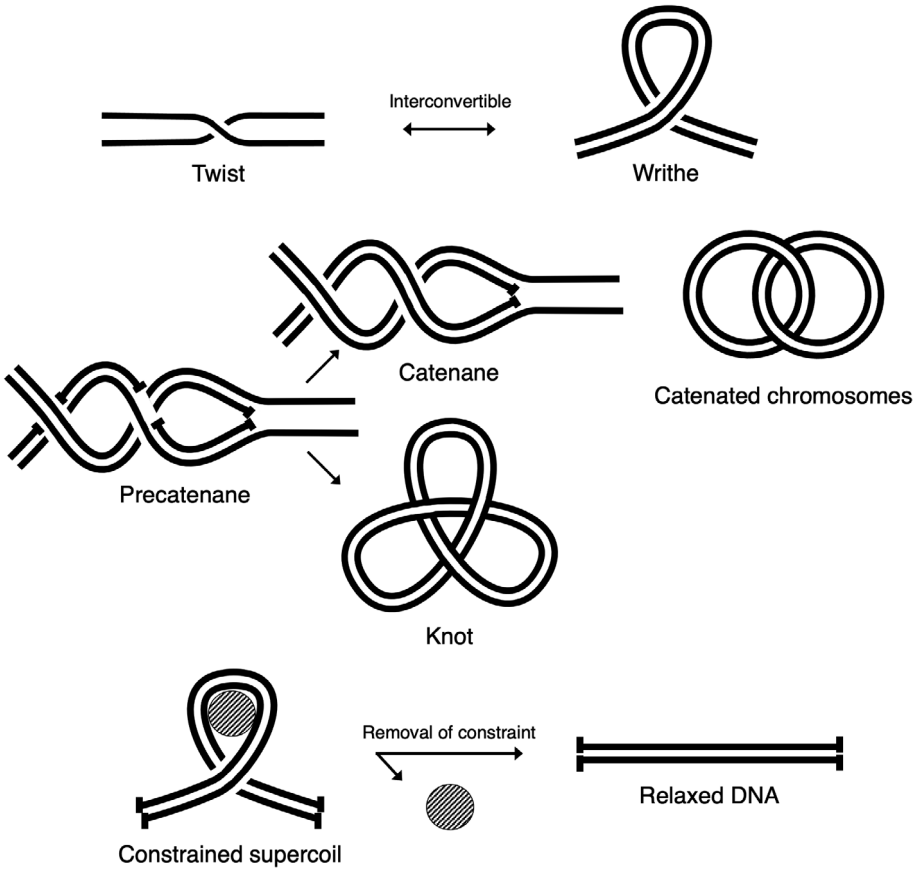

FIGURE 1 Forms of DNA supercoiling and processes that generate supercoiling. (a) DNA supercoiling is present in two interconvertible forms, namely twist (each strand crossing itself) and writhe (the two strands crossing each other). (b) Precatenanes are formed during replication and can become catenanes (intermolecular entanglements) or knots (same-molecule entanglements). (c) Supercoils can be constrained by DNA-binding proteins (represented as a circle) but are lost upon removal of the constraint

maintenance of local, unconstrained DNA supercoiling, as detailed in the rest of this review.

3 | UNIVERSAL, ESSENTIAL CELLULAR PROCESSES NATURALLY CREATE UNCONSTRAINED SUPERCOILS AS A BYPRODUCT

DNA supercoiling emerges naturally from certain cellular processes. Namely, transcription and DNA replication generate supercoils in eukaryotes and bacteria. Transcription is carried out by enzymes called RNA polymerases that melt the DNA double helix to separate the two DNA strands and form an open complex, and then transcribe RNA from the template DNA. During transcription, bacterial RNA polymerase applies torque to the DNA,[4] which causes rotation of the DNA.[5] However, as described in Section 4, DNA is constrained, meaning that this rotation is hindered and therefore converted to DNA supercoiling.[6] Torque causes negative supercoiling behind RNA polymerase, favoring melted DNA,[4] and positive supercoiling ahead of RNA polymerase without melting DNA, as positively supercoiled DNA is more resistant to melting.[7] Though not directly demonstrated, torque generation by eukaryotic RNA polymerases is postulated to exist due to their similarity with the transcription elongation mechanisms carried out by bacterial RNA polymerases.[8] This model of opposite supercoil generation by transcription is called "twin-domain."[6]

During transcription, it is estimated that one positive supercoil and one negative supercoil are generated for every 10 transcribed base pairs.[9] Accordingly, in bacteria, a template transcribed in the presence of bacterial topoisomerase I, which only relaxes negative supercoils, becomes positively supercoiled,[10] and a template transcribed in the presence of DNA gyrase, which has a strong preference toward positive supercoils, becomes negatively supercoiled.[11] Both phenomena were observed in a mutant yeast deprived of endogenous topoisomerases and instead expressing bacterial topoisomerases,[12] consistent with the existence of twin-domain supercoil generation in eukaryotes. Genome-wide transcription data in bacteria[13] and eukaryotes[14] are consistent with gene expression creating DNA supercoiling and impacting genes neighboring the one being transcribed (Table 1).

DNA replication also generates DNA supercoiling. Unlike transcription, DNA replication involves full separation of the two DNA strands and use of each strand as a template to synthesize a new strand.[15] In bacteria, DNA replication generates melted DNA behind the fork, that is, a strong negative DNA supercoiling.[16] Because there is no DNA strand break, total supercoiling must be

TABLE 1 Distribution and properties of topoisomerases in the various kingdoms of life

Type	Common name	Cofactors	Main role
Eukaryotes			
1B	TOP1	Mg^{2+}[a], polyamines[a]	Remove both positive and negative supercoiling
IIA	TOP2[b]	Mg^{2+}, ATP	Decatenation
1A	TOP3[b]	Mg^{2+}	Decatenation?
Bacteria			
IA	Topo I	Mg^{2+}	Remove negative supercoiling
IIA	Gyrase	Mg^{2+}, ATP, polyamines[a]	Introduce negative supercoiling
IA	Topo III	Mg^{2+}	Decatenation?
IIA	Topo IV	Mg^{2+}, ATP	Remove positive supercoiling and decatenation
Archaea			
IA	Topo III	Mg^{2+}	Decatenation?
IA	Reverse gyrase[b]	Mg^{2+}, ATP	Introduce positive supercoiling
IIB	Topo VI	Mg^{2+}, ATP	Remove both positive and negative supercoiling and decatenation.

[a]Significantly enhances enzymatic activity but not an absolute requirement.
[b]Duplications exist in certain species.

conserved, leading to the formation of positive supercoils ahead of the fork,[17] which can be visualized in vitro by electron microscopy.[18] However, positive supercoils hinder DNA replication, and a fraction are dissipated in the form of precatenanes behind the fork[18] (Figure 1b). Precatenanes result from the intertwining of the two replicated DNA strand pairs and are made possible by the single-strand breaks originating from Okazaki fragments.[17] If not dissipated, precatenanes become catenanes (two chromosomes linked together and incapable of segregation) or knots (entanglements within the same chromosome) until resolved (Figure 1b). Dissipation of positive supercoils, precatenanes, catenanes, and knots is performed by topoisomerases (discussed in Section 6).

Because the fundamental mechanisms of DNA replication are the same in bacteria and eukaryotes, positive supercoils ahead of the replication fork and precatenanes behind the fork are also formed and resolved in the latter.[19] Generation of supercoils by transcription and DNA replication has not been formally demonstrated in archaea. Nevertheless, their RNA polymerase is related to the eukaryotic Pol II,[20] and their DNA replication resembles that of eukaryotes.[21] Both processes generate supercoils as described above. It is therefore probable that archaea likewise generate supercoils during DNA replication and transcription.

DNA damage and repair also contribute to DNA supercoiling. Unlike transcription and replication, in which no unconstrained strand breaks occur, DNA damage and repair can cause single- and double- strand breaks, which result in DNA relaxation and are commonly believed to be the reason why DNA supercoiling changes during these processes. For example, X-ray-mediated DNA damage causes transient DNA relaxation in mitochondria.[22] Oxidative[23] and radiation[24] stress, as well as double-strand breaks caused by restriction enzymes,[25] do likewise in the bacterium *E. coli*. These changes may be the direct effects of DNA damage, repair, and/or signaling pathways sensitive to DNA damage. Moreover, they raise the questions of whether and how repair machineries affect DNA supercoiling.

The eukaryotic minor mismatch repair complex Mlh1-Mlh3 actively causes nicks in supercoiled DNA, resulting in relaxation.[26] Many other repair mechanisms involve the degradation of damaged DNA, resulting in a transient single-stranded DNA,[27] which is expected to relax DNA. However, an in vitro system evaluating eukaryotic DNA supercoiling by whole cell extracts found an increase in DNA supercoiling in repaired DNA compared to nonrepaired damaged DNA.[28] Likewise, an in vivo system evaluating the repair of nicks on the F-plasmid DNA in bacteria found that nicked and repaired DNA molecules had a nonzero supercoiling,[24] indicating the existence of a mechanism to preserve preexisting supercoils. Given that the F-plasmid is 100 kb long, one would expect ~10 independent supercoiling domains (see Section 5), and lack of supercoil diffusion may be the reason why supercoiling is partially conserved. Because repair machineries and pathways differ among bacteria, archaea, and eukaryotes, establishing whether DNA repair machineries actively contribute to the maintenance of DNA supercoiling may open new therapeutic avenues aimed at perturbing their role in maintaining DNA supercoiling.

4 | KINGDOM-SPECIFIC DNA-BINDING PROTEINS GENERATE CONSTRAINED SUPERCOILS

All domains of life use constrained supercoiling, which differs from all the unconstrained supercoils discussed above. Constrained supercoils are linked to the binding and wrapping of DNA around a protein and lost when the protein is removed (Figure 1c).

In eukaryotes and archaea, constrained supercoils are mediated by histones, a family of four proteins that assemble in heterooctamers (for a detailed review on histone assembly and structure, see Reference 29), typically organized as $(H2A\text{-}H2B)(H3\text{-}H4)_2(H2A\text{-}H2B)$. In eukaryotes, one histone octamer normally constrains ~1.5 negative superhelical turns over a length of 146 bp.[30] This number can vary based on the acetylation status of histones: a highly acetylated nucleosome constrains ~20% fewer supercoils than a nonacetylated one.[31] In addition, the H3-H4 heterotetramer is capable of constraining positive supercoiling in vitro,[32] although in vivo constraining of positive supercoils rarely occurs[33] because the H2A-H2B dimers favor the constraining of negative supercoils.[34]

Most Archaea have histones. Though the monomeric forms of archaeal and eukaryotic histones are structurally similar, archaeal histones exist as dimers or tetramers.[35] Dimers are capable of bending DNA without wrapping,[36] whereas tetramers are capable of wrapping DNA like a eukaryotic octamer, capturing ~130 bp and 1.5 supercoils.[37] Depending on physiochemical conditions, archaeal histones can trap both positive and negative supercoils.[38]

All Bacteria and some Archaea use a distinct class of molecules to constrain supercoils: NAPs. Despite being structurally unrelated to histones, NAPs are often referred to as "histone-like" due to the similar function they perform. NAPs are defined as abundant DNA-binding proteins capable of altering chromosomal structure. Bacterial proteins universally recognized as NAPs

are HU (Heat-Unstable nucleoid structuring protein), H-NS (Heat-Stable Nucleoid Structuring Protein, also referred to Histone-Like Nucleoid Structuring Protein), FIS (Factor for Inversion Stimulation), Dps (DNA protection in starved cells), and IHF (Integration Host Factor). ~10–12 HU dimers bound in 9 bp intervals can constrain one negative supercoil.[39] Similarly, ~6 H-NS dimers bound in 50 bp intervals can constrain one negative supercoil.[40] FIS is also capable of constraining negative supercoils, though less efficiently than HU or H-NS: ~40 FIS dimers in 40 bp intervals can constrain one negative supercoil.[41] Computer simulations suggest that any bacterial NAP capable of bending DNA (like Dps or IHF) can affect supercoiling.[42] If confirmed experimentally, this would imply that constraining of DNA supercoiling in organisms that depend on NAPs is achieved modularly, by the cooperative action of many different proteins that also perform other functions, rather than by a single, specialized group of molecules like histones in eukaryotes. It may be hard to develop an antibacterial strategy based on the disruption of constrained supercoils because multiple NAPs would need to be simultaneously inhibited.

5 | TRANSCRIPTION CREATES BARRIERS TO SUPERCOILING DIFFUSION THAT PRESERVE SUPERCOILING DESPITE DNA DAMAGE

Single- and double-strand DNA breaks dissipate all supercoiling. The frequency with which DNA breaks occur suggests that it should be extremely difficult for organisms to maintain any DNA supercoiling. However, living organisms divide their DNA into supercoiling domains that are topologically insulated from each other, so that a break in one domain does not impact supercoiling of a neighboring domain. The existence of such domains was demonstrated in *E. coli*[25] and *Caulobacter crescentus*[43] and is hypothesized to exist in all bacteria. These domains likely correspond to the side loops on isolated nucleoids observed by electron microscopy.[44] The size of these domains ranges from 2 to 65 kb, with an average size of ~10 kb.[25,44] These domains are dynamic and defined, in part, by regions of high transcription,[43] which presumably reflects the drag imposed by the heavy transcription-translation machinery in bacteria in which transcription and translation are generally coupled. Recent technical breakthroughs, such as the development of a fluorescent method to examine DNA supercoiling in living bacteria,[45] will allow easy measurement of local DNA supercoiling and therefore lead to advances in our understanding of topological domains and their boundaries.

Eukaryotes also maintain highly structured, topologically insulated domains with a median size of 100 kb.[46] The nature of the domain boundaries remains unclear. They have a weak correlation with boundaries between GC-rich and AT-rich regions, suggesting a contribution from the transcription factor CTCF, which binds to such boundaries in isolated domains.[46] A correlation was also found between RNA polymerase occupancy and the negative supercoiling state of a domain, leading to the hypothesis that transcription also shapes domains in eukaryotes.[46] This correlation may not represent causation since negative supercoiling facilitates gene transcription.

The existence of stable, unconstrained positive supercoiling in many Archaea strongly suggests the existence of barriers to supercoiling diffusion. Archaeal barriers to supercoiling diffusion have not been demonstrated yet but likely follow the same principles as in eukaryotes and bacteria, where a combination of regions of high transcription and specific insulating proteins would prevent supercoil diffusion.

6 | TOPOISOMERASES COMPRISE A WIDE VARIETY OF KINGDOM-SPECIFIC PROTEINS THAT DIRECTLY CHANGE DNA SUPERCOILING

Transcription and DNA replication are supercoiling-sensitive processes that generate DNA supercoiling.[47,48] How, then, do cells manage the supercoils generated by these cellular processes? And how do bacteria and archaea manage their unconstrained DNA supercoiling? These functions are carried out by a class of enzymes designated topoisomerases that introduce breaks in DNA to change supercoiling. Topoisomerases are divided into two major types based on the nature of the DNA break: type I topoisomerases (Figure 2a,b) make single-strand breaks, and type II topoisomerases (Figure 2c,d) make double-strand breaks. Further subclasses are summarized in Table 1 and detailed in the text below.

All kingdoms have at least one topoisomerase dedicated to dissipating the excessive negative supercoils caused by transcription. In bacteria and eukaryotes, this task is performed by a type I enzyme (IA for bacteria and IB for eukaryotes). Types IA and IB are not structurally, mechanistically, or evolutionarily related but share the ability to operate in an adenosine triphosphate (ATP)-independent manner. The type IA bacterial enzyme Topo I (Figure 2a) relaxes negative supercoils but not positive

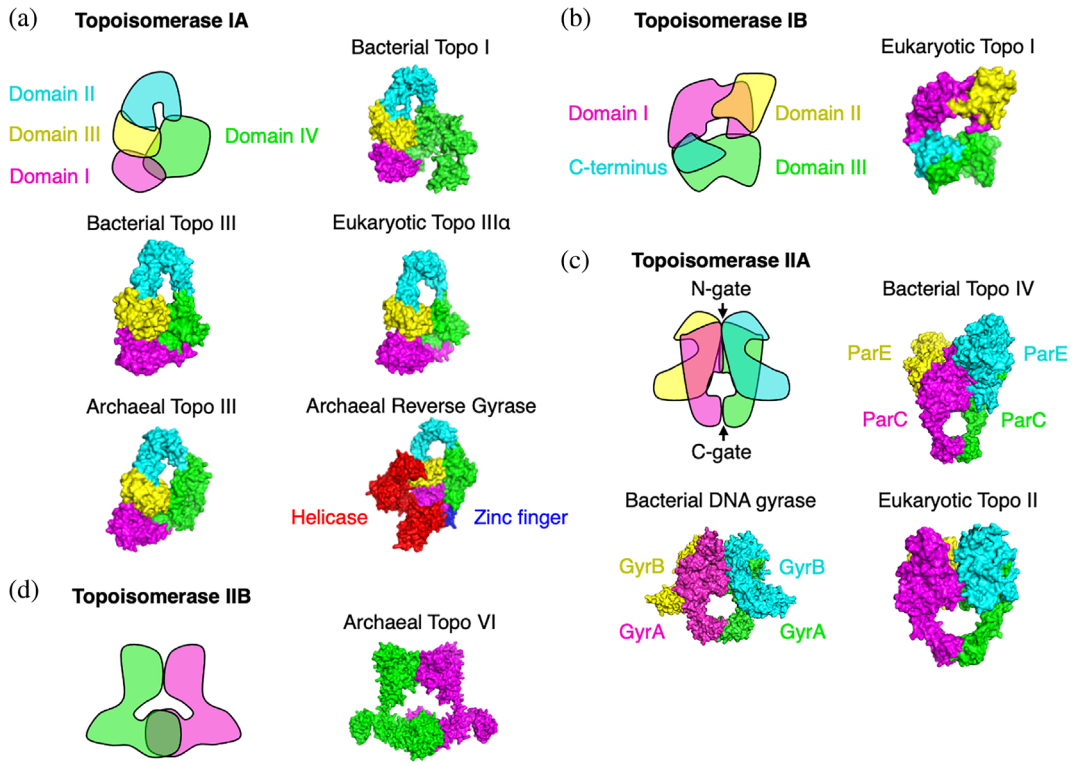

FIGURE 2 Comparison of the structures of the main topoisomerases. For each type, a general schematic is given, followed by representative experimental X-ray structures for each kingdom. (a) Type IA topoisomerases. (b) Type IB topoisomerases. (c) Type IIA topoisomerases. Note that DNA gyrase and Topo IV are heterotetramers, unlike the eukaryotic Topo II, which is a homodimer, with the GyrA-like and GyrB-like domains fused together in each monomer. (d) Type IIB topoisomerases. PDB structures used in this figure: 1EJ9, 2O5C, 2Q2E, 4CGT, 4DDX, 4I3H, 4RUL, 5GWK, 6K8O, and 6RKV

supercoils, unless there is an exposed region of single-stranded DNA.[49] Topo I cuts and binds to a 5′ phosphate group on DNA and performs strand passage, resulting in the removal of one supercoil.[50] In contrast to bacterial Topo I, the type IB eukaryotic Topo I (Figure 2b) relaxes both types of supercoils, binds to a 3′ phosphate group on DNA, and performs strand rotation, resulting in the removal of one supercoil.[51] DNA topoisomerases are generally Mg^{2+} dependent.[52] Bacterial Topo I follows this general rule,[53] but a notable particularity of the eukaryotic Topo I is that, while 16-fold activated by Mg^{2+}, it does not strictly require it.[54] Furthermore, Mg^{2+} can be substituted by Mn^{2+}, Ba^{2+}, or Ca^{2+} without loss of activity.[54] The Topo I enzymes also differ in that the eukaryotic Topo I is stimulated by the polyamines spermidine and spermine,[54] whereas bacterial Topo I is insensitive to spermidine and its precursor putrescine.[55]

Archaea generally lack an equivalent type I topoisomerase. The dissipation of excessive supercoils is instead dependent on a type IIB enzyme—called Topo VI (Figure 2d)—that differs greatly from its bacterial and eukaryotic counterparts. Topo VI is an ATP-dependent enzyme capable of relaxing both positive and negative supercoils.[56] In addition, Topo VI exhibits significant decatenase activity,[56] and its activity is Mg^{2+} dependent,[56] like that of other type II enzymes. A notable exception is the Archaea *Methanopyrus kandleri*, which has a type I topoisomerase called Topo V that is fully Mg^{2+}- and ATP-independent and relaxes both positive and negative supercoils.[57] Because this enzyme has yet to be found in other species, its evolutionary origin remains unknown.

Mobile genetic elements in bacteria and archaea sometimes encode Topo VIII, a distant relative of Topo VI that has weak relaxation and decatenation activities.[58] How does a mobile genetic element benefit from encoding Topo VIII when the host genome species specifies one or more topoisomerases? One possibility is that the Topo VIII is uniquely suited to operate with the DNA replication proteins, such as transposases, specified on the mobile genetic element.

Bacteria and eukaryotes use a dedicated enzyme for decatenation. Thus, they differ from Archaea, which use Topo VI for both DNA relaxation and decatenation. In bacteria, decatenation and unknotting are primarily performed by Topo IV, a type IIA enzyme (Figure 2c). Beyond its primary role in decatenation and unknotting,[59] Topo IV shares with Topo I the ability to

relax excessive supercoils, exhibiting 3-[60] to 20-fold[61] higher in vitro activity on positive supercoils compared to negative supercoils. In eukaryotes, decatenation and unknotting are performed by the TOP2A and TOP2B proteins, which are homologs of topoisomerase IV and often collectively referred to as Topo II (Figure 2(C)). Topo II has significant DNA relaxation activity,[62] is required for the chromosome condensation taking place before cell division,[63] and is involved in a variety of cell division-related activities alongside the proteins cohesin and condensin (for an in-depth recent review, see Reference 64).

Bacteria also have a unique type IIA topoisomerase called DNA gyrase[65] (Figure 2c). The particularity of this enzyme among other type II topoisomerases is that it is capable of introducing negative supercoils into DNA at the expense of ATP,[66] which results in bacteria maintaining a negatively supercoiled DNA at all times. DNA gyrase can also relax positive[60] and negative[67] supercoils and perform decatenation and unknotting,[68] albeit with a low efficiency compared to its paralog Topo IV.[59,69] DNA gyrase is also specified in certain eukaryotic genomes, such as in the plant *A. thaliana*[70] and the apicomplexan parasite *Plasmodium falciparum*,[71] as well as in the euryarchaea *Thermoplasma acidophilum*[72] and *Archaeblobus profundus*.[73] The sporadic distribution of DNA gyrase-specifying genes suggests acquisition of these genes via horizontal gene transfer.[74] Notably, the DNA gyrases of *A. thaliana* and *P. falciparum* are targeted to organelles[70,71] rather than acting on nuclear DNA.

Archaea have a unique type IA enzyme called reverse gyrase (Figure 2a) that relaxes negative (but not positive) supercoils and introduces positive supercoils in a relaxed template at the expense of ATP.[75] The enzyme is Mg^{2+} dependent[75] and generally possesses two zinc finger domains[76] that contribute to its enzymatic activity.[77] The N-terminus of reverse gyrase is unique in that it resembles a eukaryotic-like helicase[78] (Figure 2a). While helicase activity could not be measured for reverse gyrase, the helicase-like domain contributes to reverse gyrase function.[79] Reverse gyrase is found in hyperthermophilic bacteria such as *Thermotoga maritima*,[80] which likely acquire it by horizontal gene transfer.[74]

Topo III is a type IA topoisomerase that exists in all kingdoms of life (Figure 2a). It is a Mg^{2+}-dependent yet ATP-independent enzyme. It displays decatenase activity in both bacteria[68,81] and eukaryotes.[82] Though it is also capable of relaxing negatively supercoiled DNA,[49,83] the bacterial Topo III operates at a slower rate than Topo I.[49,84] Topo III is incapable of relaxing positive supercoils.[49] Unlike Topo I, Topo IV, and gyrase, Topo III is not essential in bacteria.[85] A Topo III null mutant has increased frameshift mutation rates,[86] suggesting a role related to DNA replication. Yeasts have a single TOP3, but higher eukaryotes have two paralogs of the protein—TOP3α and TOP3β—only one of which (TOP3α) is essential.[87] The widespread presence of Topo III across all kingdoms suggests an early appearance in the evolution of living cells, indicating that it likely performed a key function in early cellular life. That Topo III is generally nonessential in current organisms raises intriguing evolutionary questions: was Topo III an early, generic topoisomerase that performed DNA relaxation and decatenation but was later supplanted by specific, more efficient topoisomerases? Or did it perform a different function yet to be identified that other topoisomerases cannot, explaining its conservation to this day?

7 | EXPRESSION OF CERTAIN TOPOISOMERASES IS REGULATED ACCORDING TO THEIR FUNCTION

The essentiality of many topoisomerases implies that they must be present at least at a basal level all of the time. However, organisms tune the expression of some topoisomerases in response to specific signals (summarized in Table 2 and detailed in the text below). As discussed in the previous section, the transcriptional regulation of eukaryotic topoisomerases reflects their restricted roles in managing supercoils created as a result of transcription and DNA replication.

TOP1 encodes the eukaryotic type I topoisomerase. The *TOP1* regulatory region bears binding sites for the ubiquitous transcription factors SP1 and OCT-1.[88] SP1 is expressed at a basal level and activated by phosphorylation in response to DNA damage,[89] whereas OCT-1 is fully constitutive.[90] SP1-mediated induction is generally considered minor, and therefore TOP1 is overall constitutively expressed, in agreement with its role in the housekeeping process of preventing supercoil accumulation.

TOP2 encodes the single yeast Topo II. Unlike Topo I, Topo II is both more specialized in supporting DNA replication, thanks to its decatenation and unknotting activity, and subjected to more regulation. TOP2 ensures proper chromosome segregation during mitosis[91] and meiosis.[92] The *TOP2A* and *TOP2B* genes encode two distinct Topo II in higher eukaryotes. TOP2A appears to play critical roles in embryonic development and cell proliferation. By contrast, TOP2B behaves more as a housekeeping enzyme. Both *TOP2A* and *TOP2B* display tissue-, developmental-, and cell cycle-dependent expression and are highly expressed in the thymus of both rats and mice.[93,94] *TOP2A* also shows strong expression in the spleen and sporadic expression in other tissues, while

TABLE 2 Summary of known regulation for the main topoisomerases in bacteria and eukaryotes

	Name	Gene expression	Transcript	Enzyme
	DNA relaxation			
Bacteria	Topo I	Induced by supercoiled DNA	N/D	Constitutive, possibly inhibited by anaerobiosis
Eukaryote	Topo I	Constitutive	N/D	Activated by polyamines
	DNA compaction			
Bacteria	DNA gyrase	Induced by relaxed DNA	N/D	Activity correlates with [ATP]/[ADP] ratio, activated by polyamines. Putative chaperone
	Decatenases			
Bacteria	Topo IV	Cell cycle-regulated in *Caulobacter*	N/D	N/D
Eukaryote	TOP2A	Peaks in G2/M phase, regulated by many cell cycle- and tissue-dependent transcription factors	Stabilized in G2/M phases	Activity and stability regulated by ubiquitinylation, SUMOylation, and phosphorylation
	TOP2B	Mostly constitutive, some tissue dependence	N/D	Activity regulated by SUMOylation and phosphorylation

Abbreviation: N/D, no data available.

TOP2B is more broadly expressed.[93,94] *TOP2A* is expressed in rapidly proliferating cell types and tissues.[93,95] In addition, *TOP2A* (but not *TOP2B*) is cell cycle regulated, with a peak of expression in the G2 and M phases.[96] This peak is ascribed to a combination of transcriptional and post-transcriptional regulation. The transcription factors SP1,[97] NF-Y,[97,98] ICBP90,[99] MYB,[100] P53,[101] ATF,[102] and others not yet identified[103] regulate *TOP2A*. Some of these factors, notably ICBP90, display the same tissue-dependent expression as *TOP2A*,[99] which may account, in part, for its expression behavior. Though the *TOP2A* regulator P53 exhibits cell cycle-dependent activity and abundance, the cell cycle control of *TOP2A* expression remains unclear because P53 acts on a minimal *TOP2A* promoter deprived of any regulatory element,[101] which argues that its regulatory effect is nonspecific. An alternative hypothesis has been proposed whereby NF-Y, despite being constitutively expressed and binding to the *TOP2A* promoter region, is responsible for the cell cycle-dependent regulation by recruiting histone acetyltransferases to indirectly activate *TOP2A* expression in the G2/M phases.[98] Post-transcriptional regulation is also hypothesized to contribute to the cell cycle-dependent expression of *TOP2A* because transcript stability depends on the cell cycle.[104]

As far as the eukaryotic Topo III is concerned, the *TOP3A* and *TOP3B* genes are expressed constitutively across all investigated tissues. Though *TOP3A* is subject to alternative splicing,[105] the regulatory consequences of splicing are unknown. Understanding how *TOP3A* and *TOP3B* are regulated, especially in comparison with their conserved archaeal and bacterial counterparts, could shed light on the function of Topo III.

Bacterial topoisomerases have a key function not performed by their eukaryotic counterparts: maintaining a certain level of global negative DNA supercoiling. This key function accounts for the regulation of the bacterial Topo I and DNA gyrase differing from that of other topoisomerases. Expression of the Topo I-encoding *topA* gene increases when bacteria experience high negative DNA supercoiling, a condition that promotes repression of the DNA gyrase-specifying *gyrA* and *gyrB* genes. It makes physiological sense for transcription of the enzyme promoting DNA relaxation to increase when global DNA supercoiling increases. Mechanistically, *topA* transcription follows the general rule that the more supercoiled a DNA is, the easier it is to melt and thus transcribe.[106]

By contrast, the supercoiling sensitivity of *gyrA* and *gyrB* transcription is less clear because the −10 element of the *gyrA* and *gyrB* promoters is necessary and sufficient to confer supercoiling sensitivity[107,108] despite sharing many features with promoters that are not supercoiling sensitive. In addition to sequence features of these two promoters, the relative positioning of the −10 and −35 elements appears to play a role in their activity because mutations that reduce promoter flexibility (i.e., the physical capacity of the regulatory region to bend) also reduce their DNA supercoiling sensitivity.[109] Solving the mechanism responsible for the DNA supercoiling sensitivity of

a promoter would represent a foundational advance in understanding regulatory networks in bacteria, allowing the prediction and modeling of expression behaviors that depend on DNA supercoiling, thereby greatly enhancing our knowledge of the contribution of DNA supercoiling to cellular physiology.

DNA supercoiling induction of Topo I transcription by high negative supercoiling is also displayed by *Mycobacterium tuberculosis,* and *M. smegmatis,*[110] and *Streptococcus pneumoniae*.[111] Furthermore, the opposite sensitivity of Topo I and DNA gyrase transcription to DNA supercoiling was reported in *Streptomyces coelicolor*.[112] Conservation of the transcriptional regulation of topo I and DNA gyrase across bacterial evolution suggests an ancestral, conserved mechanism by which bacteria homeostatically maintain DNA supercoiling.

Topo III is encoded by the *topB* gene, which forms an operon with the upstream *selD* gene. Though *selD* is generally considered constitutively expressed in *E. coli*,[113] expression of *topB* and *selD* decreases during late stationary phase.[114] The operon organization of the *selD* and *topB* genes is not conserved beyond the *Escherichia*, *Shigella*, and *Salmonella* genera, and therefore regulation of *topB* beyond these genera is unknown.

The Topo IV-encoding *parC* and *parE* genes are cell cycle regulated in *C. crescentus*.[115] The promoters of both genes are activated right before DNA replication,[115] consistent with the role of Topo IV in decatenating replicated chromosomes.[59] It would be interesting to learn whether this regulation exists in bacterial species in which, unlike *C. crescentus*, DNA replication and cell division happen simultaneously.

The investigation of archaeal topoisomerases has revealed expression responses to environmental changes. In the extreme salinity-inhabiting *Halobacterium salinarum*, the Topo VI-encoding *top6A* and *top6B* genes are mildly induced in response to UV light,[116] suggesting a role for Topo VI in DNA repair. In the extreme thermophilic bacterium *Sulfolobus solfarticus*, both the transcript[117] and protein[118] amounts of the reverse gyrase TopR1 are anti-correlated with temperature. Because TopR1-specific activity increases with temperature,[118] changes in TopR1 amounts may be a homeostatic mechanism to maintain constant total activity.

8 | POST-TRANSLATIONAL FACTORS ACT ON TOPOISOMERASES TO ALTER DNA SUPERCOILING

Most topoisomerases are Mg^{2+}-dependent enzymes,[52] suggesting that changes in the intracellular Mg^{2+} concentration should affect DNA supercoiling. However, the difficulty of measuring bioavailable Mg^{2+} has prevented a direct test of this hypothesis. Nevertheless, starvation for extracellular Mg^{2+} impacts DNA supercoiling in a variety of organisms, presumably by altering the Mg^{2+} concentration in the cytosol or specific organelles. For example, the formation of DNA complexes by the human Topo II decreases in cells experiencing Mg^{2+} starvation.[119]

In enteric bacteria, the activities of both Topo I[53] and DNA gyrase[120] require Mg^{2+}. That these enzymes exert opposite effects on DNA supercoiling precludes a straightforward relationship between DNA supercoiling and Mg^{2+} concentration. In *S. Typhimurium*, Mg^{2+} starvation causes DNA relaxation,[55,121] which may reflect that DNA gyrase is more sensitive to Mg^{2+} availability than Topo I. Curiously, excess Mg^{2+} also causes relaxation in *S. Typhimurium*.[55] The latter effect results from a decrease in the concentration of polyamines,[55] which are required for DNA gyrase activity.[55,120]

The in vitro activities of bacterial DNA gyrase[55] and eukaryotic Topo I[54] are polyamine dependent. In vivo, bacterial DNA supercoiling is directly correlated with the concentration of the polyamines putrescine and spermidine.[55] Thus, DNA supercoiling is intimately connected with polyamine metabolism, import, and export. The identity of the specific polyamine controlling DNA gyrase in vivo often differs across closely related bacterial species. For example, spermidine and putrescine are the main polyamines controlling DNA supercoiling in *E. coli* and *S. Typhimurium*, respectively.[55] Putrescine is converted into spermidine by the enzyme SpeE.[122] Thus, inactivation of the *speE* gene has opposite effects on *E. coli* and *S. Typhimurium*: it increases global DNA supercoiling in the former but decreases it in the latter.[55]

Bacteria are unique in that the enzymes that govern global DNA supercoiling exhibit a different dependence on ATP: the DNA-compacting DNA gyrase is ATP dependent, whereas the DNA-relaxing Topo I is not. In addition, DNA gyrase activity is inhibited by ADP.[123] Therefore the [ATP]/[ADP] ratio is a key driver of DNA supercoiling in bacteria,[124] as it shifts the equilibrium between the activities of DNA gyrase versus Topo I. A similar dependence has not been reported in other kingdoms, presumably because the archaeal reverse gyrase and topo VI are both ATP dependent, whereas eukaryotes do not maintain global supercoiling.

In eukaryotes, Topo II activity and abundance are controlled post-translationally. The vertebrate TOP2A can be directed for degradation by ubiquitinylation.[125] The opposite action of the anaphase-promoting complex that promotes ubiquitinylation[126] versus the deubiquitylase USP15[127] controls TOP2A ubiquitinylation and therefore

degradation in a cell cycle-dependent manner, with TOP2A stability being maximal in G2/M phases. By contrast, neither TOP2B[128] nor the unique TOP2 found in non-vertebrates[129] appears to have a similar regulation. Topo II activity is also dependent on other post-translational modifications, namely SUMOylation (a polypeptidic modification) and phosphorylation, which both contribute to the cell cycle dependence of Topo II activity (for a recent review on the topic, see Reference 64).

9 | BACTERIA AND ARCHEAE CHANGE DNA SUPERCOILING IN RESPONSE TO EXTRACELLULAR STRESSES

Bacterial DNA supercoiling is sensitive to a wide variety of extracellular stresses. For example, an increase in osmolarity generally increases DNA supercoiling. However, the molecular mechanism(s) responsible for this phenomenon is unclear because NaCl shock causes a transient increase in DNA supercoiling in *E. coli* that closely correlates with the [ATP]/[ADP] ratio,[130] whereas performing the shock with KCl causes the same transient increase in DNA supercoiling independently of the [ATP]/[ADP] ratio.[131] Similar responses have been reported in *S. Typhimurium*,[132] *B. subtilis*,[133] and *S. aureus*,[134] indicating widespread conservation of the phenomenon, but its link with the [ATP]/[ADP] ratio remains unknown.

Oxidative stress decreases bacterial DNA supercoiling transiently. *E. coli* experiences a decrease in DNA supercoiling upon treatment with H_2O_2 that is followed by a return to pretreatment levels within 30 min.[23] In the phytopathogenic enterobacterium *Dickeya dadantii*, the same effect is dependent on the NAPs FIS and H-NS.[135] The dependence on FIS and H-NS suggests the participation of specific regulatory networks in the changes in DNA supercoiling resulting from oxidative stress. Conversely, oxygen limitation and anaerobiosis increase DNA supercoiling in *E. coli*.[136,137] In the latter case, however, DNA supercoiling is not correlated with the [ATP]/[ADP] ratio.[136] Increased supercoiling appears to result from a decrease in Topo I activity, which was lower in crude extracts from cells subjected to anaerobiosis than in control bacteria.[138] The concurrent decrease in Topo I activity and increase in DNA supercoiling triggered by anaerobiosis have also been observed in *S. Typhimurium*,[139] but the mechanism(s) by which they occur remains unknown.

A mildly acidic pH durably relaxes DNA in *S. Typhimurium*[140] and *D. dadantii*.[135] In the latter, this relaxation is H-NS dependent.[135] In vitro, DNA gyrase activity is inhibited at pH <5.[140] It has been proposed that a decrease in external pH causes a drop in cytoplasmic pH, explaining the observed decrease in DNA supercoiling.[140] This model, however, suffers from major inconsistencies. First, mesophilic bacteria such as *E. coli* and *S. Typhimurium* maintain an intracellular pH no lower than 6.7 even when the extracellular pH drops to 5.[141,142] Second, an acidic pH favors entry of the drug commonly used to decrease DNA supercoiling in vivo—novobiocin—into the bacterial cell.[143] Therefore, the reported synergy in bacterial growth inhibition resulting from novobiocin and acidic pH may result from increased permeability to novobiocin rather than from direct inhibition of DNA gyrase by a decrease in pH.[140] Surprisingly, *E. coli* did not significantly change its DNA supercoiling in conditions where *S. Typhimurium*'s did,[144] indicating species-specific changes in the underlying regulatory network. A supercoiling response to alkaline pH has not been reported.

An increase in temperature from 17 to 47°C results in increased negative DNA supercoiling in *E. coli*.[145,146] This increase is attributed to changes in the [ATP]/[ADP] ratio, which correlates with DNA supercoiling during an upshift from 30 to 47°C.[145] Oddly, DNA gyrase is 90% inactivated within 20 min of treatment at 46°C in vitro,[147] implying that a factor such as a protein chaperone maintains DNA gyrase activity in organisms experiencing high temperatures. DNA gyrase is essential in all investigated bacteria, and therefore factors that protect DNA gyrase may be an attractive target for antibacterial agents.

As discussed above, many archaea and notably hyperthermophiles maintain a positively supercoiled DNA due to the high resistance of positively supercoiled DNA to melting.[7] However, positively supercoiled DNA is detrimental to transcription and DNA replication, so archaea must find a balance between DNA stability and capacity to carry out these essential processes. The archaeal topoisomerase VI, which conducts most of the DNA relaxation, is inhibited by high temperatures,[56,148] while reverse gyrase activity is stimulated.[148] Therefore, opposing variations in enzymatic activities likely cause increased DNA supercoiling at higher temperatures. This is reminiscent of the transcriptional homeostatic control of the balance between topoisomerase I and DNA gyrase in bacteria to maintain negative supercoiling, although the archaeal equivalent for positive supercoils appears due to enzymatic activity rather than transcription.

Extracellular factors influencing DNA supercoiling in vivo in eukaryotes have yet to be reported. This likely stems from the fact that eukaryotic DNA supercoiling is only local, and suitable methods to measure it are

laborious.[149] Studying this control especially in pathogenic fungi may lead to benefits, notably in the agricultural sector, where fungal infections are common.

10 | CONCLUDING REMARKS: EXPLOITING SIMILARITIES AND DIFFERENCES IN DNA SUPERCOILING REGULATION FOR THERAPEUTIC PURPOSES

DNA supercoiling is a fundamental property of DNA that exists in some form across all domains of life. It likely emerged alongside the DNA molecule itself and the machineries that transcribe and replicate it, and which still create and depend on supercoiling to this day. Importantly, each kingdom has evolved distinct strategies for regulating supercoiling, some even co-opting it for gene regulation: from the opposition of the ATP-dependent bacterial DNA gyrase to the ATP-independent topoisomerase I, to the eukaryotic topo II associated with cell division proteins, and the polyamine dependence of only certain specific topoisomerases. Taken together, the essentiality and the kingdom-specific (sometimes species-specific) regulation of DNA supercoiling present extraordinary therapeutic potential. It may be possible to target the regulatory adaptations that have evolved only in certain branches, as has been done with fluoroquinolone antibiotics targeting the bacteria-specific DNA gyrase. Similarly, the requirement of eukaryotic topoisomerases for fast proliferation has opened avenues for anticancer drugs like anthracyclines. Ultimately, deeper knowledge of the regulatory networks that underlie the maintenance of DNA supercoiling in all kingdoms has the potential to lead to new, transformative medicines.

AUTHOR CONTRIBUTIONS

Alexandre Duprey: Conceptualization (lead); funding acquisition (supporting); visualization (lead); writing—original draft (lead); writing—review and editing (equal). **Eduardo A. Groisman:** Funding acquisition (lead); writing—review and editing (equal).

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