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Molecular Evolution of DNA Topoisomerases: Mechanisms, Diversification, and Functional Adaptations

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Abstract

Topoisomerases are one of the essential enzymes that regulate DNA topology. They enable crucial processes such as replication, transcription and chromosomal segregation across bacteria, archaea and eukaryotes. This review traces their emergence from a hypothesized RNA world, where proto-enzymes may have managed RNA entanglements, to their pivotal role in DNA-based life following the RNA-to-DNA transition. Genomic and phylogenetic data suggest their diversification into Type I (IA, IB, IC) and Type II (IIA, IIB) families, likely resulting from independent evolutionary events driven by horizontal gene transfer (HGT), gene duplication, and modular innovation.The conserved Toprim domain in Type IA and IIA enzymes indicates a shared catalytic ancestry. However, HGT, particularly from viruses, introduced variants such as Type IB, thereby enhancing topological versatility.These adaptations-Type IA's magnesium-driven simplicity for early cells, Type IIA's ATP-powered catenation resolution for complex genomes, and unique forms such as reverse gyrase for extremophiles-mirror genomic complexity and ecological pressures. First elucidated by James Wang's 1970s discovery in Escherichia coli, their universal presence suggests origins tied to the last universal common ancestor- Luca. Today, the evolutionary legacy of TOP2 proteins guides medical and biotechnological advancements. For instance, TOP2 inhibitors are employed as cancer treatments, while gyrase-specific antibiotics are utilized to combat bacterial infections. This article synthesizes molecular, phylogenetic, and functional insights to illuminate how topoisomerases evolved to overcome DNA's topological challenges. By doing so, it provides a window into life's ancient past and its modern applications.

Keywords: Topoisomerase, Evolution, DNA Topology, Horizontal Gene Transfer, Type I, Type II, Genomic Complexity

1. Introduction

DNA topoisomerases are universal enzymes that manage the topology of nucleic acids. Apart from this, it plays a significant role in enabling replication, transcription, recombination, and chromosome segregation across bacteria, archaea, and eukaryotes. Their catalytic cycles transiently cleave DNA and pass strands or allow controlled rotation to dissipate torsional stress. Therefore, it prevents deleterious supercoiling, knots, and catenanes that would otherwise stall genome transactions. Classic biochemical and structural work established two mechanistic classes: Type I (single-strand break) and Type II (double-strand break) - with five major families (IA, IB, IC, IIA, IIB) that differ in chemistry, architecture, and distribution across the tree of life [1].

An evolutionary perspective reveals a mosaic origin, rather than a single lineage tracing uniformly from the last universal common ancestor (LUCA). Distinct topoisomerase families appear to have emerged independently, sharing modular catalytic elements such as the TOPRIM fold between Type IA and Type II while lacking global homology, and then spreading via vertical descent and extensive HGT [1]. Phylogenomic syntheses suggest that the most recent common ancestors of the three cellular domains had different sets of topoisomerases. Viruses also contributed unique lineages, especially for Type IB, which helps explain the mismatch between enzyme phylogenies and organismal trees. These observations have led to ideas about how topoisomerases evolved alongside the transition from simpler to more complex DNA genomes and replication systems. This might have happened

in an ancestral virosphere before being introduced into primitive cells [1,2].

Functionally, the enzyme repertoire reflects genomic architecture and ecological constraints. Bacteria rely on DNA gyrase to introduce negative supercoils and on Topo IV to decatenate sister chromosomes, pairing with Type IA enzymes to maintain homeostatic supercoiling. Eukaryotes deploy TOP1 for chromatin-associated transcriptional stress and TOP2A/B for chromosome condensation and segregation. Archaea adds distinctive solutions including Topo VI (Type IIB) and reverse gyrase, the latter a hallmark of hyperthermophiles that stabilizes duplex DNA via ATP-dependent positive supercoiling [3]. Recent structural advances, such as cryo-EM snapshots of human TOP3B complexes, are refining mechanistic models for strand passage and RNA/DNA topological control by Type IA enzymes, expanding the evolutionary and functional scope of topoisomerases beyond DNA alone [4,5].

Topoisomerases are super important drug targets in medicine. Quinolones target bacterial gyrase and Topo IV enzymes [6,7]. Scientists are still figuring out how these mechanisms work and how bacteria can resist them. At the same time, studies also indicate how topoisomerase activity is linked to tumor gene regulation and R-loop dynamics. These enzymes play a crucial role in how cells respond to stress and maintain their genetic integrity. This review will go into detail about how topoisomerases are classified, their mechanisms, their origins, and how they have evolved over time. It will also explore how different topoisomerase domains have diversified and provide evidence for the existence of Type IIB systems (Topo VI and TopoVIL) [8].

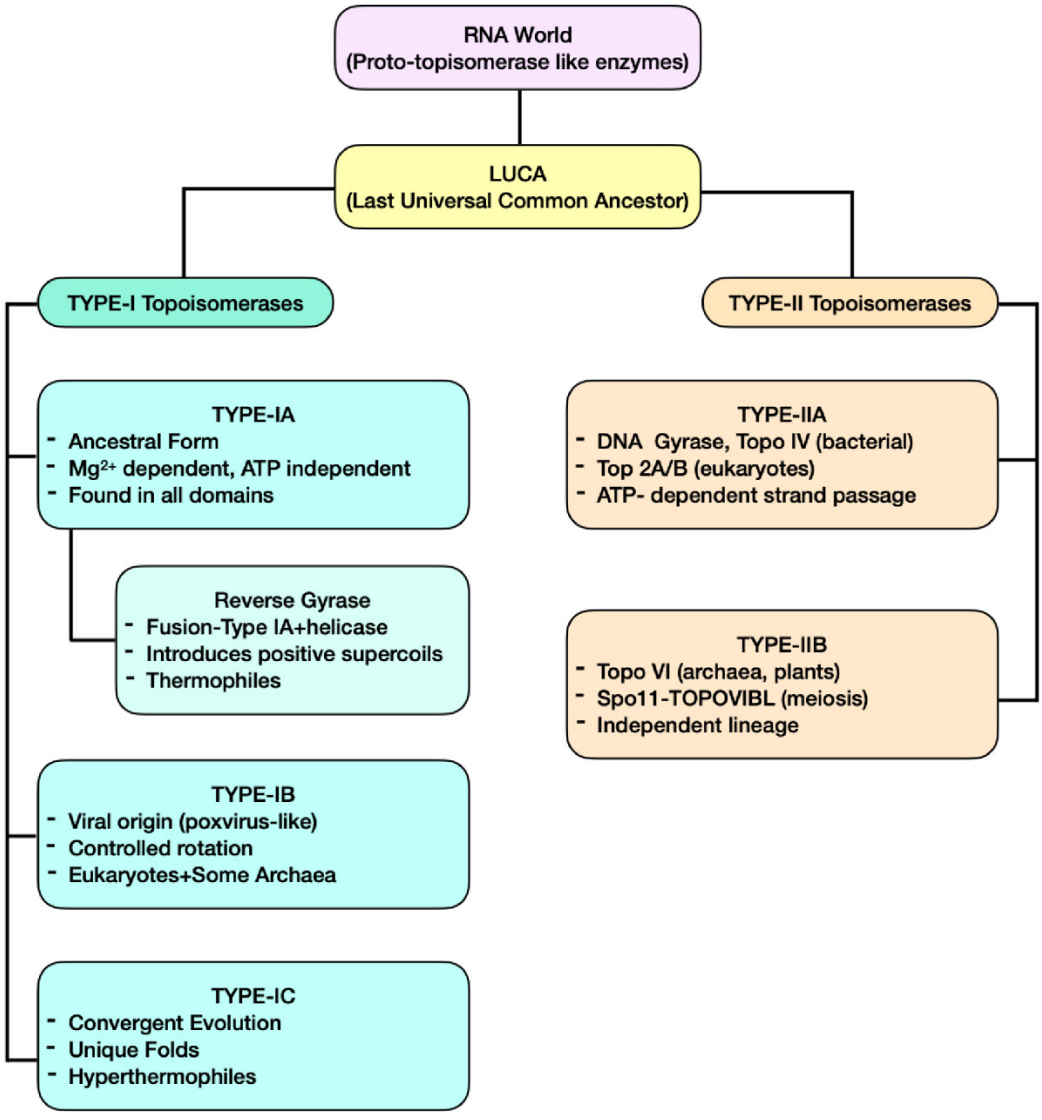

Figure: Schematic Showing Evolution of Topoisomerases

1.1. Classification and Functional Diversity of Topoisomerases

DNA topoisomerases are categorized into two mechanistic classes based on whether they introduce single- or double-strand breaks, with five principal families that exhibit distinct chemistries, architectures, and evolutionary distributions: Type I (IA, IB, IC) and Type II (IIA, IIB). These families solve common topological problems-supercoiling, knotting, and catenation—through convergently evolved solutions and, in some cases, shared catalytic modules such as the Toprim fold between Type IA and Type II enzymes. Recent structural and phylogenomic advances further refine mechanistic models and clarify lineage-specific innovations, especially in archaeal IIB systems and eukaryotic specializations [2,3,9].

1.2. Type I Topoisomerases

Type I enzymes transiently cleave one DNA strand to regulate the linking number without necessitating ATP hydrolysis. They employ either strand passage (Type IA) or controlled rotation/ swivel (Type IB, IC) to dissipate torsional stress. This group is subdivided into three distinct families: Type IA, Type IB, and Type IC. Each family possesses unique mechanisms and evolutionary histories that marks their adaptability to diverse cellular demands [2].

1.3. Type IA Topoisomerases

Type IA topoisomerases are widely distributed across bacteria and eukaryotes (e.g., human TOP3A and TOP3B), as well as in bacteria (e.g., *E. coli* Topo I) and archaea. These enzymes plays a crucial role in relaxing negatively supercoiled DNA, a process essential for alleviating the torsional strain generated ahead of replication forks or during transcription. Structurally, Type IA enzymes employ a magnesium-dependent, ATP-independent mechanism. They utilize a conserved tyrosine residue within their active site to form a covalent phosphotyrosine intermediate with the 5' end of the cleaved DNA strand. This cleavage allows the intact strand to rotate around the break, effectively unwinding negative supercoils before the strand is religated. This restores the DNA's integrity [10].

The simplicity of this mechanism relies on magnesium as a cofactor rather than ATP. The Divalent ion - Magnesium and Manganese ion, coordinate acidic residues facilitating the the cleavage and overall efficiency. Therefore, it positions Type IA as a candidate for one of the earliest topoisomerases in evolutionary history [1]. Phylogenetic analyses suggest that Type IA enzymes, found in both prokaryotes and eukaryotes, likely trace back to the LUCA. It reflects their foundational role in early DNA-based life [2]. In bacteria like *E. coli,* Topo I work in concert with DNA gyrase to maintain an optimal level of supercoiling, counteracting the positive supercoils introduced during replication [6]. In eukaryotes, TOP3A and TOP3B play specialized roles, such as resolving recombination intermediates like Holliday junctions, which is indeed a function critical for genomic stability during DNA repair and meiosis [11].

The structural conservation of Type IA enzymes, particularly the Toprim domain a magnesium-binding motif shared with Type IIA topoisomerases—provides further evidence of their ancient lineage [9]. This domain facilitates the coordination of metal ions necessary for catalysis, a feature that likely predates the divergence of bacterial and archaeal lineages [11]. The enzyme's reliance on a single-strand break mechanism, without the energy-intensive requirements of ATP, suggests an evolutionary adaptation suited to the resource-limited environments of early cells, where simplicity and efficiency were paramount.

1.4. Type IB Topoisomerases

Type IB topoisomerases, in contrast, represent a mechanistically distinct subfamily, predominantly found in eukaryotes (e.g., human TOP1), some archaea, and certain viruses [4,11]. Unlike Type IA, these enzymes employ a "controlled rotation" mechanism to relax both positive and negative supercoils, a versatility that suits the complex chromatin environment of eukaryotic nuclei. Structurally, Type IB enzymes lack the magnesium dependency of Type IA, instead it relies solely on a tyrosine residue to cleave the DNA strand at the 3'-end, forming a covalent intermediate [11]. After cleavage, the enzyme remains tethered to the DNA that allows the intact strand to swivel around the break in a controlled manner. The following mechanism is guided by the enzyme's protein clamp, before religation occurs [10].

This ATP-independent swivel mechanism is a significant evolutionary departure from Type IA,that reflects an adaptation to the larger, more densely packed genomes of eukaryotes [12]. In human cells, TOP1 is essential for transcription, relaxing supercoils generated by RNA polymerase as it tracks along DNA, and its disruption can lead to genomic instability, a hallmark of cancer [11]. The absence of Type IB in most bacteria, coupled with its presence in eukaryotes and some archaea, suggests a later evolutionary origin, possibly acquired through HGT from viral ancestors, such as poxviruses, which encode similar enzymes [1]. This hypothesis is strengthened by the enzyme's structural divergence, which lacks the Toprim domain and instead possesses a unique cap domain that stabilizes the DNA during rotation [4,11]. The adaptability of Type IB to eukaryotic chromatin dynamics, where DNA is wound around histones, highlights its evolutionary refinement. Its ability to handle both positive and negative supercoils, unlike the negative-specific Type IA, highlights a functional specialization that likely co-evolved with the emergence of nuclear organization and transcriptional complexity in eukaryotic cells [11].

1.5. Type IC Topoisomerases (Topo V)

Type IC topoisomerases, exemplified by Topo V from the hyperthermophilic archaeon *Methanopyrus kandleri,* are a rare and enigmatic subfamily. They are only found in specific extremophiles [13]. Structurally distinct from both Type IA and IB, Topo V features a novel fold that combines a topoisomerase domain with helix-turn-helix motifs typically associated with DNA-binding proteins [13]. Like other Type I enzymes, Topo V cleaves a single

strand to relax supercoils. However, its mechanism and cofactor requirements remain less understood due to its limited distribution and unique architecture [1,13].

Type IC holds evolutionary significance as a product of convergent evolution, rather than direct descent from a shared Type I ancestor [13]. Its presence in *Methanopyrus kandleri,* an organism thriving in extreme heat and pressure, suggests an adaptation to stabilize DNA under harsh conditions, similar to the role of reverse gyrase in other thermophiles [3]. Moreover, the absence of widespread homologs across domains further supports the idea that Type IC emerged independently, possibly through the recruitment of distinct protein domains to address topological challenges in extreme environments [2,13,14]. This rarity and uniqueness make Type IC an intriguing case study in evolutionary innovation, though its limited representation hinders broader conclusions.

1.6. Type II Topoisomerases

Type II topoisomerases can be distinguished by their ability to cleave the DNA strands simultaneously. They are ATP- dependent enzymes critical for resolving more complex topological entanglements, such as catenanes and knots. This group is subdivided into Type IIA and Type IIB, each with distinct structural features and evolutionary trajectories that reflect their roles in diverse cellular contexts [2].

1.7. Type IIA Topoisomerases

Type IIA topoisomerases are ubiquitous enzymes found in both bacteria and eukaryotes. In bacteria, they include DNA gyrase and Topo IV, while in eukaryotes, they include human TOP2A and TOP2B. These enzymes perform energy-intensive tasks that require ATP hydrolysis [6]. They operate through a double-strand break mechanism, cleaving both strands with two tyrosine residues—one per subunit—to form a transient DNA-enzyme gate [10]. Another DNA segment, called the T-segment, is then passed through this gate, resolving catenanes, knots, or supercoils. Finally, the cleaved strands are religated [12]. The process is powered by ATP, which drives conformational changes in the enzyme's multi-subunit structure, including the opening and closing of a protein clamp known as the C-gate [2].

In bacteria, Type IIA enzymes exhibit functional specialization. DNA gyrase, a unique topoisomerase, introduces negative supercoils into relaxed or positively supercoiled DNA. This capability is essential for compacting the bacterial chromosome and facilitating the progression of replication forks [6]. Gyrase and Topo IV are enzymes that differ in their supercoiling patterns. Gyrase's negative supercoiling distinguishes it from Topo IV, which primarily decatenates daughter chromosomes after replication to ensure proper segregation during cell division. In eukaryotes, TOP2A and TOP2B share the strand-passage mechanism but have distinct roles. TOP2A is crucial for mitotic chromosome disentanglement, while TOP2B supports transcription in non-dividing cells. The evolutionary divergence of these isoforms likely occurred through gene duplication, adapting their functions to meet the specific demands of the eukaryotic cell cycle [2,11,12].

The ATP dependency and structural complexity of Type IIA enzymes suggest a later evolutionary origin than Type IA, likely co-evolving with larger genomes and the need for robust topological resolution [1]. The presence of the Toprim domain, shared with Type IA, indicates a common catalytic ancestry, though Type IIA's multi-domain architecture including ATPase and DNA-binding regions reflects significant elaboration [9]. In bacteria, the tandem evolution of gyrase and Topo IV highlights an adaptation to rapid division, while in eukaryotes, TOP2's mitotic role underscores its refinement for linear chromosome management [6].

1.8. Type IIB Topoisomerases

Type IIB topoisomerases, exemplified by Topo VI in archaea and some plants, share the double-strand cleavage and strand-passage mechanism of Type IIA but differ in subunit composition and evolutionary lineage [1,2]. Structurally, Type IIB enzymes lack the C-gate found in Type IIA, relying instead on a simpler two-subunit design: an A subunit for DNA cleavage and a B subunit for ATP hydrolysis. This streamlined architecture still enables catenation resolution and supercoil relaxation, though its functional scope appears narrower than Type IIA's [2]. In archaea, Topo VI is prevalent among extremophiles, suggesting an adaptation to high-temperature environments where DNA stability is paramount [3]. In plants, it supports chromosome segregation during meiosis, a role analogous to eukaryotic TOP2A but achieved through a distinct mechanism [12]. The phylogenetic separation of Type IIB from Type IIA, coupled with its absence in most bacteria and animals, points to an independent evolutionary origin, possibly arising through convergence or HGT in specific lineages [1].

This distinctiveness underscores the plasticity of topoisomerase evolution, adapting similar functional solutions to diverse ecological niches. Eukaryotic TopoVIL (Spo11–TOPOVIBL) is a TopoVI-derived complex essential for meiotic DSB formation. Spo11 is a conserved TopoVIA ortholog with an added N-terminal regulatory region, whereas TOPOVIBL exhibits remarkable plasticity, including ATPase-motif divergence and lineage-specific truncations [15,16]. Structural and biochemical work indicates TOPOVIBL variants can bind DNA with geometry preferences and do not necessarily bind ATP, reflecting functional repurposing from a topoisomerase to a programmed DSB generator in meiosis [16,17].

2. Functional Roles and Evolutionary Adaptations

Functionally, Type I and Type II topoisomerases complement each other within cellular contexts. Type I enzymes, particularly IA and IB, manage supercoiling ahead of replication forks and during transcription, preventing excessive torsional stress that could stall polymerases. Type II enzymes, with their strand-passage capability, excel at decatenating chromosomes post-replication and resolving knots, tasks critical for cell division and genomic integrity [12].

This division of labor reflects evolutionary adaptations to specific cellular needs. Bacterial replication efficiency relies on the interplay of Topo I and gyrase, while eukaryotic chromosome segregation demands the robust action of TOP2A [11]. The diversity among these subfamilies highlights their evolutionary divergence, driven by genomic complexity and environmental pressures. Type IA's simplicity suits early, resource-scarce cells, while Type IB's versatility addresses eukaryotic chromatin [11]. Type IIA's ATP-driven power meets the demands of large genomes, and Type IIB's streamlined design fits extremophiles and plants [2]. Together, these adaptations illustrate a spectrum of evolutionary solutions to the universal challenge of DNA topology, from ancient origins to modern specialization [10].

2.1. Evolutionary Origins of Topoisomerases

The evolutionary origins of topoisomerases are pivotal in comprehending the molecular machinery that facilitated life's transition from an RNA-based existence to the DNA-dominated world of modern organisms. This transformative shift, spanning billions of years, presented unprecedented challenges in nucleic acid topology, particularly supercoiling, knotting, and catenation aspects that RNA's inherent flexibility largely circumvented. During this period, topoisomerases likely emerged as critical solutions due to their ability to introduce transient breaks in DNA and facilitate strand rotation or passage. Over time, these rudimentary precursors evolved into the diverse and sophisticated enzymes we observe today [1].

2.2. Emergence During the RNA-to-DNA Transition

The RNA world hypothesis posits that early life relied on RNA as both a genetic repository and a catalytic agent, with ribozymes driving replication, transcription, and metabolic processes [1]. In this primordial setting, RNA's single-stranded nature and inherent flexibility minimized the topological constraints that characterize DNA. However, replication of RNA genomes-particularly if they formed circular or concatenated structures-likely generated entanglements which indeed requires resolution. Proto-enzymes, possibly RNA-based ribozymes or simple protein catalysts with topoisomerase-like activity, may have emerged to unwind these structures or separate intertwined strands, laying the groundwork for later DNA-specific enzymes [2]. These precursors, though speculative, represent the evolutionary seeds from which topoisomerases could have sprouted, driven by the need to manage nucleic acid dynamics in pre-DNA life.

The transition to DNA as the primary genetic material marked a seismic shift in molecular biology. DNA's double-helical structure offered greater stability and fidelity for storing genetic information, enabling the development of larger, more complex genomes [10]. Yet, this stability came at a cost. DNA's rigidity and tendency to supercoil during unwinding posed topological challenges significantly greater than those encountered in RNA replication. As helicases unwind the strands of a double helix during replication, positive supercoils are generated ahead of the fork. Conversely, catenation of daughter molecules occurs after replication. These issues were rarely encountered in RNA replication, which relies on less structured intermediates. This transition likely occurred incrementally, with early DNA-based organisms inheriting replication machinery from RNA systems, necessitating new tools to handle these novel constraints [1].

Topoisomerases are hypothesized to have co-evolved with DNA polymerases during this period, emerging as indispensable partners in replication and transcription [10]. The universal distribution of topoisomerases across bacteria, archaea, and eukaryotes strongly suggests their presence predates the LUCA, a hypothetical organism from which all modern life diverged [2]. As DNA took root, the selective pressure to manage its topology would have been immense, favoring the refinement of proto-topoisomerases into enzymes capable of precise strand cleavage and religation. The diversity of modern topoisomerase families—Type I (IA, IB, IC) and Type II (IIA, IIB) hints at a complex evolutionary trajectory, rooted in this ancient need to adapt to DNA's unique properties [1].

2.3. Type IA as the Ancestral Form

Among the topoisomerase families, Type IA emerges as the most likely candidate for the ancestral form, owing to its structural simplicity and mechanistic elegance. Found in bacteria (e.g., E. coli Topo I), archaea, and eukaryotes (e.g., human TOP3A and TOP3B), Type IA enzymes relax negative supercoils through a magnesium-dependent, ATP-independent mechanism. The process begins with a conserved tyrosine residue in the enzyme's active site cleaving one DNA strand, forming a covalent phosphotyrosine intermediate with the 5'-end [10]. This break allows the intact strand to rotate around the cleavage site, unwinding negative supercoils, after which the enzyme religates the strand, restoring DNA continuity [11]. This straightforward approach, eschewing the energy demands of ATP, suggests Type IA could have functioned in early cells with limited metabolic resources, a hallmark of an ancestral enzyme [1].

Phylogenetic analyses bolster this view, revealing Type IA's deep conservation across bacteria and archaea, with homologs exhibiting sequence and structural similarities that trace back to the earliest divergences of life [2]. This ubiquity implies a role in LUCA, where Type IA likely managed supercoiling in small, circular DNA genomes structures common among early prokaryotes and plasmids [1]. The Toprim domain, a magnesium-binding motif shared with Type IIA topoisomerases, provides a critical clue to their shared catalytic ancestry [9]. This domain, which coordinates metal ions to stabilize the cleavage reaction, is a conserved feature across Type IA enzymes, from bacterial Topo I to eukaryotic TOP3, suggesting it was an early evolutionary innovation retained through billions of years [9]. Structurally, the Toprim domain's presence in both Type IA and IIA hints at a common origin, with Type IA's simplicity predating the ATP-dependent complexity of Type II [11].

Functionally, Type IA's role in relaxing negative supercoils aligns

with the topological needs of early replication. As primitive helicases unwound DNA, negative supercoils accumulated behind the fork, potentially stalling replication if unresolved. In modern bacteria, Type IA collaborates with DNA gyrase to maintain supercoiling homeostasis, a dynamic that may echo an ancient partnership between topoisomerases and replication machinery [6]. In eukaryotes, TOP3A and TOP3B have evolved specialized roles, such as resolving Holliday junctions during recombination, but their core mechanism retains the simplicity of their bacterial counterparts [11]. This evolutionary continuity, coupled with Type IA's minimal energy requirements, positions it as a foundational enzyme, likely present in the earliest DNA-based organisms before the diversification of other families.

Extremophiles like *Thermotoga maritima* offer a living testament to topoisomerase antiquity, bridging ancient adaptations with modern observations. This hyperthermophilic bacterium possesses both Type IA and reverse gyrase, suggesting these enzymes were critical in early, harsh environments resembling Earth's primordial conditions [2]. Type IA's presence across extremophiles—from thermophiles to halophiles reinforces its role as an ancient, versatile enzyme, capable of managing supercoiling under diverse stresses [1]. Reverse gyrase, found in thermophilic archaea and bacteria, points to an early divergence driven by ecological pressures, with its positive supercoiling protecting DNA from thermal denaturation [3]. The coexistence of these enzymes in *Thermotoga* suggests an evolutionary snapshot, where Type IA provided a baseline function, and reverse gyrase emerged as a specialized adaptation, reflecting a dynamic history of topological innovation [2].

2.4. Co-Evolution with DNA Replication and Later Divergence

The interdependence of topoisomerases and DNA replication systems underscores their co-evolutionary origins. As DNA polymerases and helicases evolved to synthesize and unwind the double helix, they generated topological stress i.e. positive supercoils ahead of the fork and catenanes behind it, that required resolution [10]. Type IA likely addressed negative supercoiling, a byproduct of fork movement, while the absence of Type IB in most bacteria but its presence in eukaryotes and some archaea suggests a later evolutionary divergence [1]. Type IB, with its "controlled rotation" mechanism, relaxes both positive and negative supercoils without ATP or metal ions, a sophistication that suits the chromatin-rich environment of eukaryotic nuclei [11]. This capability, absent in Type IA, indicates Type IB emerged to meet the needs of larger, more complex genomes, marking a significant evolutionary leap [12].

The phylogenetic distribution of Type IB prevalent in eukaryotes and some archaea but rare in bacteria—suggests it was not part of the original topoisomerase repertoire but rather acquired later, possibly through HGT or viral transfer [2]. Structurally, Type IB lacks the Toprim domain, instead featuring a unique cap domain that stabilizes DNA during rotation, a divergence from Type IA's conserved features [11]. This structural shift, combined with its functional versatility, supports the idea that Type IB evolved as genomes grew and cellular processes like transcription within chromatin demanded more flexible topological management. The RNA-to-DNA transition thus seeded Type IA as a universal solution, with Type IB arising subsequently to address emerging complexities, reflecting a dynamic interplay of inheritance and innovation.

3. Viral Contributions and Modular Evolution

Viral contributions offer a compelling twist to the topoisomerase origin story, suggesting that some families, notably Type IB and Type IC, may have viral roots. Researchers propose that Type IB, found in eukaryotes and some archaea, could have originated from viral enzymes, with poxvirus Topo IB as a potential ancestor [3]. Poxviruses encode a Type IB-like enzyme that mirrors eukaryotic TOP1 in structure and mechanism, cleaving DNA at the 3'-end and facilitating controlled rotation [1]. This similarity raises the possibility that early DNA viruses, infecting primitive cells, introduced Type IB genes via HGT, which were then integrated and refined by host genomes, particularly in eukaryotic and archaeal lineages [2]. This viral hypothesis challenges a purely vertical descent model, suggesting topoisomerase evolution involved lateral gene exchange, enriching cellular repertoires with exogenous innovations.

Reverse gyrase in thermophilic archaea provides a striking example of modular evolution, further supporting the role of gene fusion in topoisomerase origins. This enzyme, a hybrid of a Type IA topoisomerase and a helicase domain. It introduces positive supercoils to stabilize DNA at high temperatures, a unique adaptation among topoisomerases [1]. Its chimeric structure likely arose through recombination or HGT, possibly from viral or bacterial sources, illustrating how topoisomerases evolved by assembling functional modules [3]. The helicase domain unwinds DNA, while the Type IA component manages topology, a synergy that enhances stability in extreme environments [2]. Similarly, Type IC (Topo V) in *Methanopyrus kandleri,* with its novel fold combining topoisomerase and DNA-binding motifs, suggests convergent evolution or lateral acquisition, tailored to extremophile needs [13]. These modular constructs highlight a patchwork evolutionary process, where topoisomerases were shaped by both endogenous refinement and external genetic contributions.

3.1. Type IIB lineage and meiotic repurposing

The Type IIB family (TopoVI) is prominent in archaea and is found in some eukaryotes. In eukaryotes, its subunits duplicated and diverged, leading to the formation of the meiotic TopoVIL complex. This complex consists of SPO11 (the TopoVIA ortholog) and TOPOVIBL (the TopoVIB-like ortholog). Comparative genomics suggests that BIN4 and RHL1 co-evolve with TopoVI in plants, indicating the presence of a broader catalytic complex in certain eukaryotes. While SPO11 is deeply conserved, TOPOVIBL exhibits striking plasticity, often diverging or truncating ATPase motifs [17]. This suggests specialized regulation for programmed

DSB formation rather than classical strand passage. Structural and biochemical work indicates that TOPOVIBL variants can bind DNA with specific geometry preferences and frequently lack canonical ATP-binding sites. These findings reinforce the evolutionary shift from a decatenating topoisomerase to a regulated DSB generator during meiosis. Additionally, further studies have identified direct regulatory interactions, such as those between TOPOVIBL and REC114, which help to time and tune meiotic DSB formation. These interactions demonstrate how subunit divergence and partner recruitment rewired a topoisomerase ancestor for recombination initiation [15-17].

3.2. Diversification of Topoisomerases Across Domains

The evolution of topoisomerases across the three domains of life—bacteria, archaea, and eukaryotes—reflects a remarkable interplay of genomic complexity, ecological pressures, and evolutionary mechanisms such as gene duplication and HGT. These enzymes, crucial for managing DNA topology during replication, transcription, and chromosome segregation, have evolved into distinct profiles that cater to the unique requirements of each domain. In bacteria, topoisomerases facilitate rapid division and compact circular genomes; in archaea, they adapt to extreme environments; and in eukaryotes, they address the intricacies of large, linear chromosomes and complex cellular processes like mitosis and meiosis [1]. This diversification defies a simplistic three-domain model, revealing a fluid evolutionary history shaped by both vertical inheritance and lateral gene exchange.

3.3. Topoisomerase Evolution in Bacteria

Bacterial genomes, typically small, circular, and rapidly replicating, have driven the evolution of a streamlined yet efficient set of topoisomerases, primarily Type IA and Type IIA enzymes, to maintain topological homeostasis under the pressures of fast cell division.

3.4. Topo I and Gyrase

In bacteria, Type IA topoisomerases, such as *E. coli* Topo I, play a foundational role in relaxing negative supercoils, counteracting the torsional stress generated during replication and transcription. Topo I employs a magnesium-dependent, ATP-independent mechanism, cleaving one DNA strand with a tyrosine residue to form a phosphotyrosine intermediate, allowing the intact strand to rotate and relieve supercoiling before relegation [10]. This simplicity reflects an ancient adaptation, likely inherited from early prokaryotes, suited to the metabolic constraints of bacterial cells [2]. Complementing Topo I is DNA gyrase, a Type IIA topoisomerase unique to bacteria and some archaea, which introduces negative supercoils into relaxed or positively supercoiled DNA. Gyrase's mechanism involves cleaving both DNA strands with two tyrosine residues, passing another segment (the T-segment) through the break, and religating the strands, a process powered by ATP hydrolysis [6]. This ability to actively underwind DNA compacts the bacterial chromosome, facilitating replication fork progression and fitting the genome into the confined space of the cell. In E. coli, gyrase's negative supercoiling is critical for initiating replication by creating a topological state conducive to helicase activity, a function that distinguishes it from other topoisomerases [6].

Topo IV, another bacterial Type IIA enzyme, shares structural similarities with gyrase but specializes in decatenating daughter chromosomes post-replication, ensuring proper segregation during cell division. Like gyrase, Topo IV uses an ATP-dependent strand-passage mechanism, but it preferentially resolves catenanes rather than introducing supercoils [10]. Phylogenetic evidence suggests Topo IV diverged from a gyrase-like ancestor through gene duplication, with subsequent functional specialization tailoring it to decatenation rather than supercoiling [6]. This divergence is evident in their distinct subunit compositions: both enzymes consist of GyrA/GyrB (gyrase) or ParC/ParE (Topo IV) subunits, but their active sites and regulatory regions have evolved to prioritize different topological tasks [6].

4. Adaptation to Rapid Division

The reliance on ATP-dependent Type IIA enzymes in bacteria reflects an adaptation to rapid division and circular genomes. The circular nature of bacterial DNA, unlike the linear chromosomes of eukaryotes, predisposes it to catenation during replication, necessitating robust decatenation mechanisms like Topo IV. Meanwhile, gyrase's negative supercoiling enhances chromosome compaction, a critical advantage for bacteria with high replication rates, such as *E. coli,* which can divide every 20 minutes under optimal conditions [6]. The interplay of Topo I and gyrase maintains a dynamic equilibrium of supercoiling, with Topo I relaxing excess negative supercoils introduced by gyrase, a partnership that likely evolved to optimize replication efficiency in resource-limited environments. This streamlined system underscores bacteria's evolutionary focus on speed and simplicity, contrasting with the complexity seen in eukaryotes.

4.1. Topoisomerase Evolution in Archaea

Archaea, often thriving in extreme environments, exhibit a diverse topoisomerase repertoire that blends bacterial and eukaryotic features, shaped by ecological pressures and HGT. This diversity includes unique enzymes like reverse gyrase, alongside Type IB, Type IC (Topo V), and Type IIB (Topo VI), reflecting a complex evolutionary history.

4.2. Reverse Gyrase with its Thermophilic Adaptability

Reverse gyrase, exclusive to thermophilic and hyperthermophilic archaea (e.g., Sulfolobus), is a standout adaptation, introducing positive supercoils to stabilize DNA at high temperatures [3]. Unlike typical topoisomerases that relax supercoils, reverse gyrase actively overwinds DNA, counteracting thermal denaturation that could unwind the double helix in extreme heat [1]. Structurally, it is a fusion of a Type IA topoisomerase domain and a helicase domain, with the helicase unwinding DNA and the topoisomerase domain religating it into a positively supercoiled state, all powered by ATP [2,14].

This chimeric design suggests modular evolution, likely arising through gene fusion or HGT from bacterial or viral sources, tailored to the ecological niche of thermophiles [3]. The evolutionary significance of reverse gyrase lies in its role as a survival mechanism in environments mimicking early Earth, such as hydrothermal vents. Positive supercoiling increases DNA's melting temperature, protecting it from heat-induced strand separation, a function absent in mesophilic organisms [1]. Its presence in both thermophilic archaea and bacteria (e.g., *Thermotoga maritima*) suggests either convergent evolution or ancient HGT, highlighting the fluidity of topoisomerase evolution across domains [2].

4.3. Diversity and Horizontal Gene Transfer

Archaea also harbor Type IB, Type IC (Topo V), and Type IIB (Topo VI), a diversity that defies a simple bacterial or eukaryotic origin. Type IB, found in some archaea, resembles eukaryotic TOP1 with its "controlled rotation" mechanism, relaxing both positive and negative supercoils without ATP [11]. Its presence alongside Type IA suggests HGT, possibly from eukaryotes or viruses, rather than vertical descent [1]. Type IC, exemplified by Topo V in *Methanopyrus kandleri,* is a rare outlier with a unique fold combining topoisomerase and DNA-binding domains, hinting at convergent evolution in extremophiles [13]. Topo VI (Type IIB), prevalent in many archaea, uses an ATP-dependent strand-passage mechanism akin to Type IIA but with a simpler subunit structure, suggesting an independent lineage [2].

This eclectic mix likely arose through HGT, with archaea acquiring genes from bacteria, eukaryotes, or viruses, facilitated by their ecological proximity in extreme habitats [13]. The absence of a uniform topoisomerase profile across archaea underscores their evolutionary plasticity, adapting to diverse conditions like high temperature, salinity, or acidity [3]. Topo VI (type IIB) is prevalent in archaea and facilitates ATP-dependent double-strand cleavage and duplex passage through an A/B subunit organization lacking the IIA C-gate. This unique scaffold is independently adapted to the chromosome biology of archaea [15-17]. Eukaryotic meiosis repurposes a Topo VI-derived system (TopoVIL), in which Spo11 (TopoVIA ortholog) catalyzes programmed DSBs and TOPOVIBL (TopoVIB-like) exhibits remarkable sequence plasticity, including frequent losses of canonical ATPase motifs and altered dimer interfaces, consistent with regulatory rewiring for recombination initiation rather than classical strand passage [16].

4.4. Topoisomerase Evolution in Eukarya

Eukaryotes, with their large, linear chromosomes and complex cellular processes, exhibit a sophisticated topoisomerase repertoire, including Type IB (TOP1), Type IA (TOP3A/B), and Type IIA (TOP2A/B), reflecting adaptations to chromatin, mitosis, and meiosis.

4.5. Complexity and Specialization

TOP1 (Type IB) is a cornerstone of eukaryotic DNA management, relaxing both positive and negative supercoils in chromatin-rich nuclei [11]. Its "controlled rotation" mechanism, cleaving one strand and allowing it to swivel, suits the transcriptional demands of eukaryotes, where RNA polymerase generates supercoils around histone-bound DNA. TOP2A (Type IIA), conversely, untangles mitotic chromosomes, using ATP to pass DNA segments through double-strand breaks, a necessity for segregating linear chromosomes during cell division [11]. TOP2B, a related isoform, supports transcription in non-dividing cells, highlighting functional specialization [12]. TOP3A and TOP3B (Type IA) resolve recombination intermediates like Holliday junctions, critical for DNA repair and meiotic recombination [11].

This complexity reflects eukaryotic genomic evolution, where linear chromosomes and nuclear compartmentalization demanded versatile topological solutions. Unlike bacteria's circular DNA, eukaryotic chromosomes require extensive untangling during mitosis and meiosis, driving the specialization of TOP2A/B [12]. TOP1's role in chromatin dynamics, meanwhile, addresses the supercoiling generated by transcription within nucleosomes, a challenge absent in prokaryotes [11].

4.6. Gene Duplication and Isoform Development

The diversity of eukaryotic topoisomerases, particularly TOP2A and TOP2B, stems from gene duplication events, a hallmark of eukaryotic evolution. Phylogenetic studies suggest an ancestral Type IIA gene duplicated in early eukaryotes, producing isoforms with distinct roles. TOP2A for proliferation and TOP2B for differentiated cells [12]. Similarly, TOP3A and TOP3B arose from a Type IA ancestor, adapting to recombination and repair rather than general supercoiling [11]. These duplications enabled tissue-specific expression and functional redundancy, enhancing eukaryotic adaptability to complex cellular processes like mitosis and meiosis [10].

TOP2A is a cell-cycle regulated protein with peaks in late S/G2–M phase. It is essential for chromosome condensation and segregation. TOP2B functions in differentiated cells to modulate gene programs through localized cleavage events in promoters and regulatory domains. This demonstrates post-duplication specialization within a conserved IIA framework [11]. Subcellular localization is partitioned among TOP1, TOP2, TOP1MT, TOP3A, and TOP3B isoforms. TOP1 and TOP2 isoforms are nuclear, while TOP1MT and TOP3A include mitochondrial-targeted variants. TOP3B is nucleo-cytosolic, aligning with its roles in RNA metabolism and cytosolic translation contexts [11].

4.7. Horizontal Gene Transfer and Evolutionary Fluidity

HGT has significantly influenced topoisomerase diversification, particularly in archaea and eukaryotes. Archaeal Type IB and eukaryotic variants like TOP1 may have been acquired from viral sources, such as poxviruses, which encode similar enzymes [1]. This lateral exchange is evident in the patchy distribution of Type IB, absent in most bacteria but present in eukaryotes and some archaea, suggesting viral or endosymbiotic origins [2]. In

archaea, the presence of Type IC and IIB further supports HGT, possibly from bacteria or eukaryotes, facilitated by shared extreme environments [13]. This complex pattern challenges a linear, three-domain model, revealing an evolutionary fluidity where topoisomerases were shuffled and refined across lineages [1].

5. Molecular Mechanisms and Evolutionary Adaptations of Topoisomerases

Topoisomerases are molecular marvels, their mechanisms finely tuned through billions of years of evolution to address the diverse topological challenges posed by DNA across cellular contexts. From the simplicity of Type IA's magnesium-dependent cleavage to the ATP-driven complexity of Type IIA, each subfamily reflects adaptations to specific genomic and ecological demands. These mechanisms whether relaxing supercoils, resolving catenanes, or managing chromatin dynamics evolved in response to cellular needs, from the rapid replication of bacteria to the intricate chromosome segregation of eukaryotes. Additionally, an evolutionary arms race with natural inhibitors has further refined their flexibility, shaping their active sites and catalytic efficiency.

5.1. Type IA: A Magnesium-Dependent Relic of Early Life

Type IA topoisomerases, found in bacteria (e.g., E. coli Topo I), archaea, and eukaryotes (e.g., human TOP3A and TOP3B), embody a mechanism rooted in simplicity and efficiency, likely reflecting the metabolic constraints of early life [18]. These enzymes relax negative supercoils through a magnesium-dependent, ATP-independent process, a hallmark of their evolutionary antiquity. The mechanism begins with a conserved tyrosine residue in the active site attacking the DNA phosphodiester backbone, forming a covalent 5'-phosphotyrosine intermediate with one strand [10]. This single-strand break allows the intact strand to rotate around the cleavage site, unwinding negative supercoils generated by replication or transcription, after which magnesium ions stabilize the transition state, facilitating religation to restore DNA integrity [18].

The reliance on magnesium as a cofactor, rather than ATP, underscores an adaptation to early cellular environments where energy resources were scarce [1]. Magnesium, abundant in primordial cells, coordinates with the Toprim domain a conserved catalytic motif shared with Type IIA enzymes to position the DNA for cleavage and stabilize the negatively charged phosphate during the reaction [9]. This domain, structurally characterized by a β-α-β-α-β fold, is a testament to Type IA's deep evolutionary roots, likely present in the LUCA [2,19].

In bacteria, Topo I counteracts the positive supercoils introduced by gyrase, maintaining a balanced topology essential for replication fork progression [6]. In eukaryotes, TOP3A and TOP3B have evolved to resolve recombination intermediates, such as Holliday junctions, adapting the ancestral mechanism to specialized repair functions [11]. This conservation across domains highlights Type IA's evolutionary stability, its magnesium-dependent cleavage a relic of early metabolic simplicity adapted for supercoil relaxation. The lack of ATP dependency allowed Type IA to thrive in resource-limited early cells, a trait retained in modern organisms where rapid, localized relaxation remains critical [11]. Its mechanism's elegance lies in its minimalism, a foundational adaptation that paved the way for more complex topoisomerases as genomes grew and cellular demands diversified.

5.2. Type IB: A Swivel Mechanism for Eukaryotic Complexity

Type IB topoisomerases, prevalent in eukaryotes (e.g., human TOP1), some archaea, and viruses, represent a mechanistic leap tailored to the needs of large, chromatin-packed genomes [11]. Unlike Type IA, Type IB employs a "controlled rotation" mechanism, relaxing both positive and negative supercoils without requiring ATP or metal ions. The process involves a tyrosine residue cleaving one DNA strand at the 3'-end, forming a covalent phosphotyrosine intermediate, while the enzyme's protein clamp encircles the DNA, allowing the intact strand to swivel around the break in a controlled manner [10]. Religation follows swiftly, restoring the double helix without external energy input [11].

This swivel mechanism is a profound adaptation to eukaryotic needs, where rapid, localized relaxation is essential within the dense, histone-bound chromatin of large genomes. In human cells, TOP1 operates ahead of RNA polymerase, relieving supercoils generated during transcription, a task critical in nuclei where DNA is wound around nucleosomes [11]. The absence of magnesium or ATP dependency distinguishes Type IB from Type IA, reflecting an evolutionary shift toward efficiency in complex cellular environments. Structurally, Type IB lacks the Toprim domain, instead featuring a unique cap domain that stabilizes the DNA during rotation, a divergence suggesting a later origin, possibly via HGT from viral ancestors like poxviruses [1]. The evolutionary adaptation of Type IB lies in its versatility and speed, suited to the dynamic topological changes in eukaryotic nuclei. Unlike Type IA's focus on negative supercoils, Type IB's ability to handle both supercoil types addresses the bidirectional stress of transcription and replication in chromatin, a refinement co-evolving with eukaryotic genome complexity [12]. This mechanism's precision and autonomy highlight an evolutionary solution optimized for the spatial and temporal demands of higher organisms.

5.3. Type IIA: ATP-Driven Power for Catenation and Supercoiling

Type IIA topoisomerases, ubiquitous in bacteria (e.g., gyrase, Topo IV) and eukaryotes (e.g., TOP2A, TOP2B), harness ATP to perform energy-intensive tasks like catenation resolution and supercoiling, reflecting an adaptation to more complex genomic challenges [12]. Their mechanism involves cleaving both DNA strands with two tyrosine residues—one per subunit—forming a transient DNA-enzyme gate [10]. A second DNA segment (T-segment) passes through this gate, resolving catenanes, knots, or supercoils, with ATP hydrolysis driving conformational changes, including the opening and closing of a C-gate, before religation.

In bacteria, gyrase's unique ability to introduce negative supercoils co-evolved with rapid replication, compacting circular chromosomes and aiding fork progression [6]. This process requires ATP to power the strand-passage cycle, with the GyrB subunit's ATPase domain providing energy to underwind DNA, a function critical for bacteria like E. coli. Topo IV, by contrast, decatenates chromosomes post-replication, using a similar mechanism but targeting catenanes rather than supercoiling [6]. In eukaryotes, TOP2A resolves mitotic catenanes, ensuring chromosome segregation, while TOP2B supports transcription, adaptations reflecting the demands of linear chromosomes and cell division [11].

The ATP dependency of Type IIA marks a significant evolutionary advancement over Type I, enabling strand passage to address catenation—a problem absent in small, early genomes but prevalent in larger, replicating DNA [12]. The Toprim domain, shared with Type IA, suggests a common catalytic origin, but Type IIA's multi-subunit structure—GyrA/GyrB or TOP2 dimers—adds complexity suited to robust topological resolution [9]. Gyrase's negative supercoiling likely emerged through gene duplication and specialization from a Topo IV-like ancestor, tailoring it to bacterial replication speed [6]. This ATP-driven power reflects an evolutionary response to increased genome size and cellular complexity, bridging prokaryotic and eukaryotic adaptations.

5.4. Type IIB: A Streamlined Solution for Archaea and Plants

Type IIB topoisomerases, such as Topo VI in archaea and plants, share Type IIA's double-strand cleavage and strand-passage mechanism but feature a simpler design, lacking the C-gate. Comprising an A subunit for cleavage and a B subunit for ATP hydrolysis, Type IIB resolves catenanes and supercoils with a streamlined architecture [2]. In archaea, Topo VI dominates in extremophiles, managing topology in high-temperature environments, while in plants, it supports meiotic chromosome segregation [12]. The absence of a C-gate, a protein clamp in Type IIA, suggests an evolutionary divergence, possibly an independent origin or simplification from a Type IIA-like ancestor [1]. This adaptation suits archaeal needs for efficient decatenation in compact genomes under stress, while in plants, it complements Type IIA's mitotic roles. The reliance on ATP aligns with Type IIA, but Type IIB's reduced complexity reflects a tailored solution for specific lineages, highlighting evolutionary plasticity in addressing catenation across domains [2].

5.5. Evolutionary Arms Race with Inhibitors

The evolution of topoisomerase mechanisms has been shaped by an arms race with natural inhibitors, driving resistance mutations that enhance enzyme flexibility Bacterial gyrase and Topo IV face quinolones (e.g., ciprofloxacin), which trap the DNA-enzyme complex, halting replication. Mutations in the GyrA subunit's quinolone resistance-determining region (QRDR) alter the active site, reducing inhibitor binding while preserving function [6]. In eukaryotes, TOP1 is targeted by camptothecin, stabilizing the cleavage complex, with resistance emerging via mutations in the catalytic domain [11]. This arms race, spanning natural toxins and modern drugs, has refined topoisomerase active sites, balancing catalytic efficiency with inhibitor evasion. In bacteria, gyrase's adaptability to quinolones reflects ongoing evolution, while eukaryotic TOP1's resistance to camptothecin underscores its flexibility in complex genomes [11]. This dynamic interplay highlights how environmental pressures have sculpted topoisomerase mechanisms, enhancing their evolutionary resilience.

5.6. Genomic and Proteomic Insights into Topoisomerase Evolution

Genomic and proteomic data serve as indispensable lenses through which we can decipher the evolutionary saga of topoisomerases, enzymes critical for managing DNA topology across all domains of life. These molecular archives reveal the intricate interplay of conserved structural domains, gene duplication events, HGT, and regulatory frameworks that have sculpted topoisomerase diversity over billions of years. From the ancient Toprim domain shared by Type IA and IIA enzymes to the sophisticated regulatory divergence in eukaryotes, these insights illuminate a dynamic evolutionary narrative shaped by genomic innovation, ecological adaptation, and cellular complexity.

5.7. The Toprim Domain as a symbol of Catalytic Evolution

The Toprim domain stands as a genomic hallmark of topoisomerase evolution, a conserved catalytic module shared by Type IA and Type IIA enzymes that bridges their mechanistic and phylogenetic origins [9]. Structurally, the Toprim domain is a compact, evolutionarily stable fold comprising a β-α-β-α-β topology, featuring a cluster of acidic residues typically aspartates and glutamates that form a magnesium-binding pocket [9]. This pocket coordinates divalent magnesium ions, which are essential for stabilizing the negatively charged phosphate backbone during DNA cleavage, a process mediated by a nearby tyrosine residue [9,11,13]. In Type IA enzymes, such as E. coli Topo I and human TOP3A, the Toprim domain facilitates single-strand cleavage to relax negative supercoils, a magnesium-dependent reaction that requires no ATP.

In Type IIA enzymes, like bacterial DNA gyrase and eukaryotic TOP2A, it supports double-strand cleavage and strand passage, integrating with ATP hydrolysis to resolve catenanes and supercoils Genomic sequencing across diverse organisms ranging from thermophilic bacteria like Thermotoga maritima to mammals reveals the Toprim domain's deep conservation, encoded in genes such as topA (Type IA) and gyrA (Type IIA), suggesting its presence in the LUCA [2,10,12]. Proteomic analyses further highlight its structural integrity, with X-ray crystallography showing a consistent magnesium-binding site across species, from archaeal Type IA variants to eukaryotic TOP2 dimers [2]. This conservation implies strong selective pressure to retain its

catalytic function, likely due to its efficiency in early DNA-based life, where magnesium was abundant and ATP scarce [1]. The domain's absence in Type IB and IIB enzymes, however, suggests either independent recruitment or early divergence, with Type IA and IIA evolving from a shared progenitor that predates the ATP-dependent mechanisms of Type II [9].

The evolutionary significance of the Toprim domain lies in its dual role across topoisomerase families. In Type IA, its magnesium dependency reflects an adaptation to resource-limited early cells, enabling supercoil relaxation with minimal energy input, a trait retained in modern bacteria for replication efficiency. In Type IIA, its integration with ATPase domains marks an elaboration, supporting the energy-intensive strand-passage mechanism required for larger genomes and complex topological tasks [12]. Genomic alignments reveal subtle variations e.g., additional loops in eukaryotic TOP2A enhancing DNA binding indicating adaptive refinements, yet the core Toprim structure remains a genomic signature of catalytic continuity, bridging prokaryotic simplicity with eukaryotic sophistication [10].

5.8. Gene Duplication as a driver of Eukaryotic Specialization

Gene duplication emerges as a transformative force in eukaryotic topoisomerase evolution, enabling the specialization of isoforms to address the complexities of large, linear genomes and intricate cellular processes like mitosis and meiosis [12]. Genomic evidence pinpoints the duplication of an ancestral Type IIA gene in early eukaryotes, giving rise to TOP2A and TOP2B, paralogs with distinct yet complementary roles. TOP2A, expressed predominantly in proliferative cells, resolves catenanes during mitosis, ensuring the faithful segregation of linear chromosomes a task critical as eukaryotic genomes expanded and nuclear division became more elaborate [11]. TOP2B, conversely, operates in non-dividing cells, relaxing supercoils around transcriptionally active genes, a function vital in differentiated tissues like neurons and cardiac muscle [12].

Proteomic studies reveal these isoforms share a conserved Type IIA architecture comprising ATPase, Toprim, and DNA-cleavage domains—but diverge in regulatory regions and protein interactions [10]. Genomic sequencing in organisms like Saccharomyces cerevisiae (with a single TOP2) versus mammals (with TOP2A and TOP2B) traces this duplication to the rise of multicellularity, when increased cellular specialization demanded tailored topological solutions [12]. For instance, human TOP2A and TOP2B genes, located on chromosomes 17 and 3 respectively, show syntenic conservation with orthologs in mice and fish, with phylogenetic reconstructions suggesting a duplication event over 500 million years ago [11]. Post-duplication, regulatory elements like promoters diverged, with TOP2A under cell-cycle control and TOP2B responsive to developmental signals, reflecting evolutionary fine-tuning [14,20].

Similarly, Type IA enzymes TOP3A and TOP3B in eukaryotes arose from duplication, adapting the ancestral supercoil relaxation mechanism to specialized roles in DNA repair and recombination [12]. Genomic data show TOP3A and TOP3B on human chromosomes 17 and 22, with distinct expression patterns TOP3A in proliferating cells, TOP3B in the brainindicative of functional divergence [11]. Proteomic analyses reveal TOP3B's interaction with RNA-binding proteins, suggesting a novel role in RNA topology, an adaptation absent in bacteria. This duplication-driven specialization provided eukaryotes with functional redundancy and flexibility, allowing topoisomerases to meet the diverse demands of chromatin management, cell division, and tissue-specific gene expression, a genomic hallmark of eukaryotic evolution [10].

5.9. Horizontal Gene Transfer via Viral and Endosymbiotic Contributions

HGT has profoundly shaped topoisomerase evolution, particularly for Type IB, whose genomic distribution points to viral or endosymbiotic origins [2]. Unlike the ubiquitous Type IA and IIA, Type IB exemplified by eukaryotic TOP1 is conspicuously absent in most bacteria but prevalent in eukaryotes, some archaea, and viruses [11]. Genomic sequencing reveals Type IB's structural divergence, lacking the Toprim domain and featuring a unique cap domain that stabilizes DNA during its "controlled rotation" mechanism, a profile distinct from other families [11]. This patchy distribution defies a simple vertical descent model, suggesting HGT as a key mechanism, with viruses like poxviruses as potential donors [1].

Phylogenetic trees constructed from TOP1 sequences cluster eukaryotic and archaeal Type IB with viral homologs, rather than bacterial Type IA or IIA, supporting a viral origin [2]. Poxviruses encode a Type IB-like enzyme with a tyrosine-based cleavage site and cap domain, mirroring TOP1's structure, suggesting ancient viral infections introduced this gene to eukaryotic ancestors, possibly during endosymbiosis events like mitochondrial integration [13]. Genomic evidence bolsters this, with TOP1 loci in humans flanked by sequences resembling mobile genetic elements, hinting at past lateral transfer [11]. In archaea, Type IB's presence in extremophiles like Methanococcus j. further supports HGT, potentially from eukaryotic or viral sources in shared ecological niches [13].

Proteomic data reinforce this narrative, showing TOP1's active site and cap domain align more closely with viral topoisomerases than bacterial counterparts, with subtle amino acid variations enhancing its swivel mechanism [11]. This HGT event likely provided eukaryotes with a versatile tool for chromatin relaxation, unmet by Type IA's negative-specific mechanism, reflecting an evolutionary adaptation to nuclear complexity [12]. The genomic imprint of HGT underscores topoisomerase evolution as a mosaic, integrating external innovations to expand functional repertoires across domains.

5.10. Regulatory Complexity: Bacterial Operons vs. Eukaryotic Divergence

Genomic and proteomic insights highlight a striking regulatory divergence between bacteria and eukaryotes, reflecting their evolutionary trajectories. In bacteria, topoisomerase genes are tightly co-regulated with replication machinery, often organized in operons for synchronized expression [14]. In E. coli, the topA gene (Type IA) and gyrA/gyrB operon (Type IIA) are transcribed under promoters responsive to supercoiling levels or replication stress, ensuring Topo I, gyrase, and Topo IV act in concert with DNA polymerase and helicase at the replication fork [6]. Proteomic studies reveal these enzymes form dynamic complexes with replication proteins, optimizing supercoiling and decatenation in real-time, a system honed for the rapid division of circular genomes. This operon-based regulation reflects bacterial genomic simplicity, prioritizing efficiency and coordination in compact systems.

In eukaryotes, topoisomerase regulation diverges into a complex, context-specific framework suited to large, linear genomes and nuclear organization [14]. Genes like TOP1, TOP2A, and TOP3A are dispersed across chromosomes, each governed by distinct regulatory elements-promoters, enhancers, and silencers responsive to cell cycle phases, developmental cues, or tissue-specific signals [11]. Genomic sequencing reveals introns and alternative splicing in TOP2B, producing isoforms with unique C-terminal domains tailored to neuronal or cardiac functions, a sophistication absent in bacteria [12]. Proteomic analyses show eukaryotic topoisomerases interacting with chromatin components—e.g., TOP1 with histones, TOP2A with condensin-embedding them in nuclear architecture and transcriptional regulation [11,21]. This divergence from bacterial operons to dispersed, nuanced control reflects an evolutionary shift, adapting topoisomerases to the multifaceted demands of chromatin dynamics, mitosis, and multicellularity.

6. Modern Implications

Topoisomerases, ancient enzymes honed by billions of years of evolution, have transcended their foundational roles in DNA topology to become pivotal players in modern medicine and biotechnology. Their universal presence across bacteria, archaea, and eukaryotes, coupled with their mechanistic diversity, has positioned them as critical targets in combating diseases like cancer and bacterial infections, as well as indispensable tools in genetic engineering and molecular biology. In cancer therapy, inhibitors exploit topoisomerase overexpression to halt tumor growth; in antibiotics, they target bacterial replication, though resistance reflects ongoing evolutionary dynamics; and in biotechnology, they resolve topological challenges in recombinant DNA technologies.

6.1. Topoisomerases in Cancer Therapy

The role of topoisomerases in cancer therapy underscores their significance as both biomarkers and drug targets, leveraging their essential functions in DNA replication and repair within rapidly dividing cells. Type IIA topoisomerases, particularly TOP2A, are overexpressed in many tumors breast, lung, and leukemias—due to their critical role in resolving catenanes during mitosis, a process amplified in cancer cells with uncontrolled proliferation [11]. Genomic analyses reveal TOP2A amplification in up to 20% of breast cancers, correlating with aggressive phenotypes and poor prognosis, making it a prime therapeutic target [12]. Proteomic studies further show TOP2A's elevated expression in S-phase cells, where it disentangles replicated chromosomes, a dependency exploited by chemotherapeutic agents [11].

Etoposide, a TOP2 inhibitor, exemplifies this strategy, stabilizing the TOP2A-DNA cleavage complex by binding at the enzyme-DNA interface, preventing religation of the double-strand break [11]. This trapped complex triggers DNA damage responses double-strand breaks detected by ATM and ATR kinases leading to cell cycle arrest or apoptosis, selectively killing cancer cells with high TOP2A activity [12]. Structurally, etoposide intercalates between DNA base pairs near the cleavage site, locking TOP2A in a covalent intermediate, a mechanism elucidated through crystallographic studies of human TOP2A [11].

TOP1, a Type IB enzyme, is another cancer target, with camptothecin and its derivatives (e.g., topotecan) inhibiting its "controlled rotation" mechanism [11]. In ovarian and colorectal cancers, TOP1 overexpression facilitates transcription in rapidly growing tumors, making it vulnerable to camptothecin, which traps the enzyme in a single-strand cleavage complex, inducing replication fork collapse and cell death. Genomic profiling shows TOP1 mutations in resistant tumors, altering the active site to evade drug binding, a testament to evolutionary adaptability even in disease contexts [11]. These inhibitors highlight topoisomerases' evolutionary legacy—mechanisms refined over eons repurposed as Achilles' heels in cancer, bridging ancient biology with modern therapeutics.

6.2. Antibiotics and the Evolutionary Arms Race

In the realm of infectious disease, bacterial topoisomerases particularly Type IIA enzymes gyrase and Topo IV are linchpins of antibiotic action, yet their evolution drives resistance, reflecting an ongoing arms race rooted in their ancient origins [6]. Gyrase, unique for introducing negative supercoils, and Topo IV, adept at decatenation, are essential for bacterial replication and chromosome segregation, making them ideal targets for quinolones like ciprofloxacin. Quinolones bind the GyrA subunit near the DNA cleavage site, stabilizing the enzyme-DNA complex and blocking religation, halting replication and triggering bacterial cell death [6].

Crystallographic data reveal ciprofloxacin's interaction with gyrase's quinolone resistance-determining region (QRDR), disrupting the ATP-driven strand-passage cycle. The efficacy of quinolones—used against pathogens like E. coli, Staphylococcus aureus, and Mycobacterium tuberculosis—stems from gyrase's evolutionary conservation across bacteria, a legacy of its role in

compacting circular genomes [6]. However, resistance emerges rapidly, driven by mutations in gyrA and parC (Topo IV), altering the QRDR to reduce quinolone affinity while preserving catalytic function [6]. Genomic sequencing of resistant strains-e.g., S. aureus with GyrA Ser84Leu-shows these mutations cluster near the active site, a pattern echoing natural selection pressures from ancient inhibitors like coumarins. Proteomic studies reveal resistant gyrase retains strand-passage efficiency, illustrating evolutionary flexibility honed by environmental challenges over millennia [6].

This arms race extends beyond quinolones, with aminocoumarins (e.g., novobiocin) targeting gyrase's GyrB ATPase domain, disrupting energy supply for supercoiling [14]. Resistance via gyrB mutations further underscores topoisomerases' adaptability, a trait likely refined in early bacteria facing natural toxins [6]. The clinical challenge e.g., multidrug-resistant Mycobacterium tuberculosis -mirrors this evolutionary dynamic, necessitating new inhibitors to outpace resistance, a modern echo of topoisomerases' ancient survival strategies In biotechnology, topoisomerases' ability to resolve DNA supercoiling has revolutionized recombinant DNA technologies, leveraging their evolutionary mechanisms for practical applications [10].

During molecular cloning, plasmid replication in bacterial hosts generates supercoils, complicating ligation, transformation, and sequencing. Type IA enzymes, like E. coli Topo I, are employed to relax these supercoils in vitro, enhancing plasmid yield and stability [19]. Purified Topo I, commercially available, cleaves one strand to unwind DNA, a mechanism mirroring its natural role in bacterial replication, adapted for lab efficiency [10]. Type IIA enzymes, such as gyrase and eukaryotic TOP2, offer complementary tools, introducing negative supercoils to mimic bacterial plasmid topology or resolving catenanes in concatenated constructs [19]. In synthetic biology, TOP2A resolves topological stress in large DNA assemblies, like yeast artificial chromosomes (YACs), ensuring stable propagation [12]. Genomic engineering benefits from these enzymes, with CRISPR-Cas9 workflows using Topo I to relax supercoiled templates, improving guide RNA access and editing precision . Proteomic assays confirm these enzymes' specificity, with TOP2A's strand-passage activity optimized for high-throughput cloning platforms [19].

Beyond cloning, topoisomerases facilitate structural biology and drug discovery. Crystallographic studies of TOP1 and gyrase with inhibitors rely on their ability to form stable DNA complexes, elucidating binding sites for rational drug design [11]. In gene therapy, viral vectors like adeno-associated viruses (AAVs) use topoisomerase activity to manage supercoiling during packaging, enhancing vector stability [14,19]. These applications harness topoisomerases' evolutionary legacy—mechanisms refined for replication and repair repurposing them as molecular tools to advance biotechnology, from basic research to therapeutic innovation [10].

6.3. Evolutionary Context and Future Directions

The modern implications of topoisomerases are deeply rooted in their evolutionary history. TOP2A's role in cancer reflects its adaptation to eukaryotic mitosis, a complexity absent in prokaryotes [11]. Gyrase's antibiotic vulnerability stems from its bacterial-specific supercoiling, an ancient innovation now a liability [6]. Biotechnological uses leverage mechanisms conserved across domains, from Type IA's simplicity to Type IIA's power [19]. This interplay of evolution and application highlights topoisomerases' dual nature—biological relics and modern assets.

Future directions hinge on overcoming resistance and expanding utility. In cancer, combination therapies pairing TOP2 inhibitors with DNA repair blockers (e.g., PARP inhibitors) aim to enhance efficacy, countering resistance mutations [11]. Antibiotic development targets novel gyrase sites, like the GyrB ATPase domain, to bypass QRDR resistance [6]. In biotechnology, engineered topoisomerases with enhanced specificity or thermostability—drawing from archaeal variants like reverse gyrase—promise improved cloning and gene editing [1]. These advancements build on topoisomerases' evolutionary foundation, adapting their ancient mechanisms to meet contemporary challenges.

7. Conclusion

DNA topoisomerases, vital enzymes for managing DNA topology, have undergone a remarkable evolutionary journey shaped by vertical inheritance, HGT, and functional adaptation. Found across bacteria, archaea, and eukaryotes, their universal presence points to origins tracing back to the last universal common ancestor. The diversity of topoisomerase types—Type I (single-strand breaks) and Type II (double-strand breaks)—and their subfamilies (e.g., Type IA in bacteria and archaea, Type IB in eukaryotes, Type IIA across domains, and Type IIB in archaea and some eukaryotes) reflects adaptations to distinct genomic and ecological pressures. Conserved features like the Toprim domain in Type IA and IIA topoisomerases provide molecular evidence of shared ancestry, while HGT, notably via viruses, has enriched their distribution and capabilities. Evolutionary pressures from inhibitors, such as antibiotics targeting bacterial topoisomerases, have driven resistance mechanisms, underscoring ongoing adaptation. Today, their significance extends to medicine (e.g., cancer therapies targeting TOP1 and TOP2A) and biotechnology (e.g., resolving supercoiling in cloning), highlighting how understanding their evolution informs both the history of life and practical innovations [22].

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