

**Title: “The benefit of DNA supercoiling during replication”**

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## Abstract

**DNA topology changes dynamically during DNA replication. Supercoiling, precatenation, catenation and knotting interplay throughout the process that is finely regulated by DNA topoisomerases. Here we present an overview of theoretical and experimental approaches to understand the interplay between various manifestations of topological constraints acting on replicating DNA molecules. Discussed data reveal that DNA entanglements (supercoils and catenanes) play an active role in preventing the formation of deleterious knots.**

## Introduction

DNA molecules need to dynamically change their shape to accomplish most of their normal functions. DNA is transcribed, replicates, is damaged and subsequently repaired, must condense to segregate properly during cell division and rapidly expand again thereafter. In addition, to accomplish all these tasks DNA interact with numerous proteins and this interaction changes the shape of both: DNA and proteins [1].

Many of functional changes of DNA shape are facilitated by actions of type I and type II DNA topoisomerases that mediate inter-strand and inter-duplex passages, respectively. The study of these changes belongs to the domain of DNA topology. As in all living cells, despite its shape and length, genomic DNA is organized in macrodomains or compartments (loops) that behave as closed topological domains [2, 3]. Most of the studies on DNA topology are usually performed in small circular molecules that in prokaryotes are called plasmids. These circular plasmids are closed topological domains, too. Hence, the observations made are subsequently extrapolated to the big loops of eukaryotic chromosomes. These extrapolations, though, should be made with care. Bacterial plasmids are tiny topological domains that may not accurately reflect the physical conditions that occur in the big loops or compartments of eukaryotic chromosomes [4].

## DNA topology

In torsionally relaxed B-DNA it takes about 10.5 bp for a complete turn of the double-helix. The Linking number (Lk) is probably one of the best known topological descriptors. In closed topological domains, Lk is a constant that measures the winding of the two strands of the double helix around each other. Although Lk cannot vary unless the molecule is broken,

it is determined by two geometrical variables: twist (Tw) and writhe (Wr) according to the equation:  $Lk = Tw + Wr$  [5, 6]. For this reason any change in twist must be accompanied by a corresponding change in writhe and vice-versa. Another very useful descriptor of DNA topology is  $\Delta Lk$ , the difference between  $Lk$  and  $Lk^0$ , where  $Lk^0$  corresponds to the equilibrium twist for a given molecule when it is torsionally relaxed as it would be the case when the molecule contained a single-stranded break. Finally, supercoiling density ( $\sigma$ ) also describes the deviation from the relaxed state but in a length-independent manner.  $\sigma$  is obtained dividing  $\Delta Lk$  by  $Lk^0$  [5-7].

### **Experimental identification and analysis of DNA topology**

The first experimental evidence that DNA molecules can be supercoiled was obtained in electron micrographs of the circular genome of polyoma virus [8]. Identification and analysis of these molecules were achieved also by agarose gel electrophoresis [9] and ten years later Keller used ethidium bromide intercalation and agarose gel electrophoresis to determine for the first time the number of superhelical turns in the circular genome of simian virus 40 [10]. Two-dimensional agarose gel electrophoresis was subsequently used in many laboratories to analyse the circular DNA molecules isolated from many organisms (reviewed in [11, 12]). In this way supercoiled, knotted and catenated molecules formed *in vivo* are readily identified [13-15]. Finally, nowadays agarose gel electrophoresis is also used as a preparative method to enrich DNA samples for specific molecules that can be subsequently analyzed by electron or atomic force microscopy [14, 16, 17].

### **The topology of DNA during replication**

Probably the most dramatic set of changes that DNA experiences takes place during replication. For the genetic information to be accurately replicated, the two complementary strands of the double-helix must be physically separated. A DNA helicase leads the replisome and is primarily responsible for this task. The bacterial DnaB helicase is a ring-shaped hexameric protein that encircles the lagging strand and unwinds DNA with a 5' to 3' polarity. Due to the intertwined nature of the DNA molecule, as the helicase advances the parental duplex becomes overwound ahead of the replication fork. As in all closed topological domains  $Lk$  cannot change, overwinding of the parental duplex ahead of the replicating fork must be accompanied by a corresponding change in writhe. For this reason, positive supercoiling ( $Wr$ ) forms in the unreplicated region as the replisome

advances [18]. The progressive accumulation of this torsional tension would eventually preclude progression of the fork. It was repeatedly shown that negative supercoiling favors unwinding of the duplex whereas positive supercoiling prevents it [19, 20]. Moreover, the accumulation of positive supercoiling causes regression of the replication forks and formation of the so-called “chicken-foot” structures [16, 21-23]. How do cells deal with this potential problem? Bacterial cells evolved three different mechanisms to cope with it: DNA gyrase introduces negative supercoiling directly ahead of the replicating fork to partially compensate it [24]. In addition, Topoisomerase IV (Topo IV) actually eliminates some of the positive supercoiling that forms ahead of the fork [25]. But the processivity of DNA helicases unwinding the duplex exceeds the capacity of DNA gyrase and Topo IV acting together to remove all the positive supercoiling that rises ahead. Therefore, despite these two mechanisms, positive supercoiling still accumulates ahead of the replicating fork. This circumstantial and localized accumulation of left-handed positive supercoiling directly ahead of the progressing fork causes it to rotate and this swiveling promotes its diffusion from the unreplicated to the replicated region (Figure 1). In the replicated region, though, this positive supercoiling adopts the form of right-handed precatenanes [7, 18, 26, 27]. In this dynamic way, throughout replication, intermediates remain negatively supercoiled in the unreplicated region while in the replicated one, the two sister duplexes become progressively intertwined in a right-handed manner (Figure 2). Here Topo IV, the bacterial decatenase [28], eliminates these precatenanes. Formation of precatenanes throughout the replication process is still a matter of debate [29] despite robust although circumstantial experimental evidence supporting it [7, 30-33]. In any case, it was repeatedly shown that as soon as replication is over, the two sister duplexes are highly catenated [15] and the most likely source for this high degree of catenation is precatenation. For fully replicated bacterial plasmids, as Topo IV decatenates sister duplexes [34], DNA gyrase progressively introduces negative supercoiling [15]. This interplay of negative supercoiling and decatenation is thought to play an active role all along the decatenation process [35] but how do supercoiling and decatenation interact is still poorly understood. Surprisingly, it was recently found that in *Saccharomyces cerevisiae*, chromosomal DNA becomes positively supercoiled during mitosis [13]. This transient accumulation of positive supercoiling is thought to facilitate decatenation by topoisomerase II in eukaryotes [36].

### **Interplay of supercoiling with catenation and the Topo IV decatenation paradox**

How can Topo IV decatenate precatenanes and catenanes that wind around

each other in a right-handed manner but spare from relaxation negatively supercoiled molecules in which the opposing segments also wind around each other in a right-handed manner? Several theoretical models were proposed to answer this question but none was experimentally confirmed *in vivo* [35, 37-43]. The solution of this paradox is not yet complete but we now know several important key elements. It was first shown that Topo IV acts much more efficiently on left-handed crossing, such as those present in positively supercoiled DNA than on the right-handed crossings present in negatively supercoiled DNA [44]. This observation explains why Topo IV can efficiently remove the positive supercoils that accumulate ahead of a replicating fork and at the same time is practically inactive with respect to the negative supercoiling needed to support progression of the fork. However, these observations made even more mysterious the question as to how right-handed interwinding of postreplicative duplexes can be efficiently removed. A partial solution to this problem was provided by single-molecule studies where braids composed of two DNA molecules were strongly wound around each other in a right-handed sense, just like it is the case for precatenanes and catenanes. When kept under high extension force these braids were resistant to the action of Topo IV [35]. However, when the extension force decreased to levels comparable to those that occur in living cells, these braids decreased their elastic energy by forming a higher order coiling with left-handed crossings. These left-handed crossings were then efficiently recognized and served as a substrate for interdplex passages leading to progressive unbraiding, which mimicks progressive decatenation.

Therefore, in freshly replicated molecules with high levels of right-handed catenation, decatenation is thought to progress first via passages between higher order coils and this could progress to the point where catenation would not induce higher order coiling anymore. Starting from this point the winding of catenanes becomes loose and two catenated rings can approach each other forming left-handed crossing that could be recognized by Topo IV and serve again for interdplex passages leading to complete decatenation.

### **Formation of replication knots**

Replication knots affect both sister duplexes and should be distinguished from the regular knots that occur in non-replicating circular molecules (Figure 3). The latter were originally visualized in electron micrographs of bacterial plasmids isolated from *E. coli* strains carrying deletion of the DNA topoisomerase I gene ( $\Delta topA$ ) with a compensatory mutation of the DNA gyrase gene (*gyrA* or *gyrB*). Comparison with plasmids isolated from

isogenic wild-type strains led the authors to propose that the increased production of knotted plasmids observed in the mutants was closely related to the compensatory mutations of the DNA gyrase genes [45-47].

Replication knots, on the other hand, were originally identified in the replication intermediates of bacterial plasmids containing stalled forks [22, 48, 49] but can be observed also in the intermediates of plasmids that replicate apparently unconstrained [31]. It was soon realized that even subtle changes in the degree of precatenation affects the abundance of this type of knots. Specifically, head-on collision of transcription and replication and the slow-down of replication fork progression lead to increasing numbers and complexity of replication knots [50, 51]. We propose that the torsional tension of closed topological domains is tightly regulated as replication progresses. The rate of progression of replication forks determines the degree of positive supercoiling that accumulates ahead and this in turn regulates their swiveling and the rate of formation of precatenanes.

When replication forks stall, no positive supercoiling forms ahead and DNA gyrase together with Topo IV suffice to maintain the unreplicated region negatively supercoiled. Under these extreme conditions, replication forks do not swivel and no or very few precatenanes form in the replicated region (Figure 2). This lack of intertwining of the sister duplexes prompts Topo IV to inadvertently make the strand passages that lead to the formation of replication knots [14]. In addition, it was also suggested that supercoiling and catenation could favor Topo IV to recognize and eliminate DNA knots more efficiently [52-54]. As previously mentioned, replication knots can form also in the absence of fork stalling [31]. It was repeatedly shown that even during apparently unconstrained replication the rate of progression of replication forks is not uniform [50, 55, 56]. In *Saccharomyces cerevisiae*, rates of replication fork movement vary greatly from region to region in the genome and specialized helicases, such as Rrm3p and Pif1p, regulate progression of the replication forks to traverse protein-DNA complexes [57, 58]. All this data strongly suggest that even if replication is not impaired, replication forks do not progress smoothly but in an intermittent manner. This behavior affects the rate of formation of positive supercoiling ahead of the advancing forks, their removal and the swiveling of the forks to generate precatenanes in the replicated region. In other words, the degree of intertwining of sister duplexes is also expected to vary significantly along the replication process. In those transient periods when precatenation lessens, Topo IV again could inadvertently make the strand passages that lead to the formation of replication knots.

As to the formation of knots in non-replicating plasmids, they could form *de novo* or may derive from catenanes where at least one of the rings is knotted. These knotted catenanes were readily observed *in vivo* [15, 59]. Here we propose that even when formed *de novo*, Topo IV could be responsible for their formation in poorly supercoiled molecules. The frequency of non-replicating knotted plasmids increases significantly when they are isolated from *E. coli* cells carrying deletion of the topoisomerase I gene with a compensatory mutation of DNA gyrase [45-47]. In these cells plasmid's supercoiling density ( $\sigma$ ) is significantly lower than for those isolated from wild-type cells [60-63]. Therefore, it is plausible that in poorly supercoiled non-replicating plasmids, Topo IV could inadvertently make the strand passages that lead to the formation of these knots, too.

In summary, here we propose that DNA entanglements (supercoiling and catenation) prevent the formation of knots that if not removed efficiently could lead to cell death [64].

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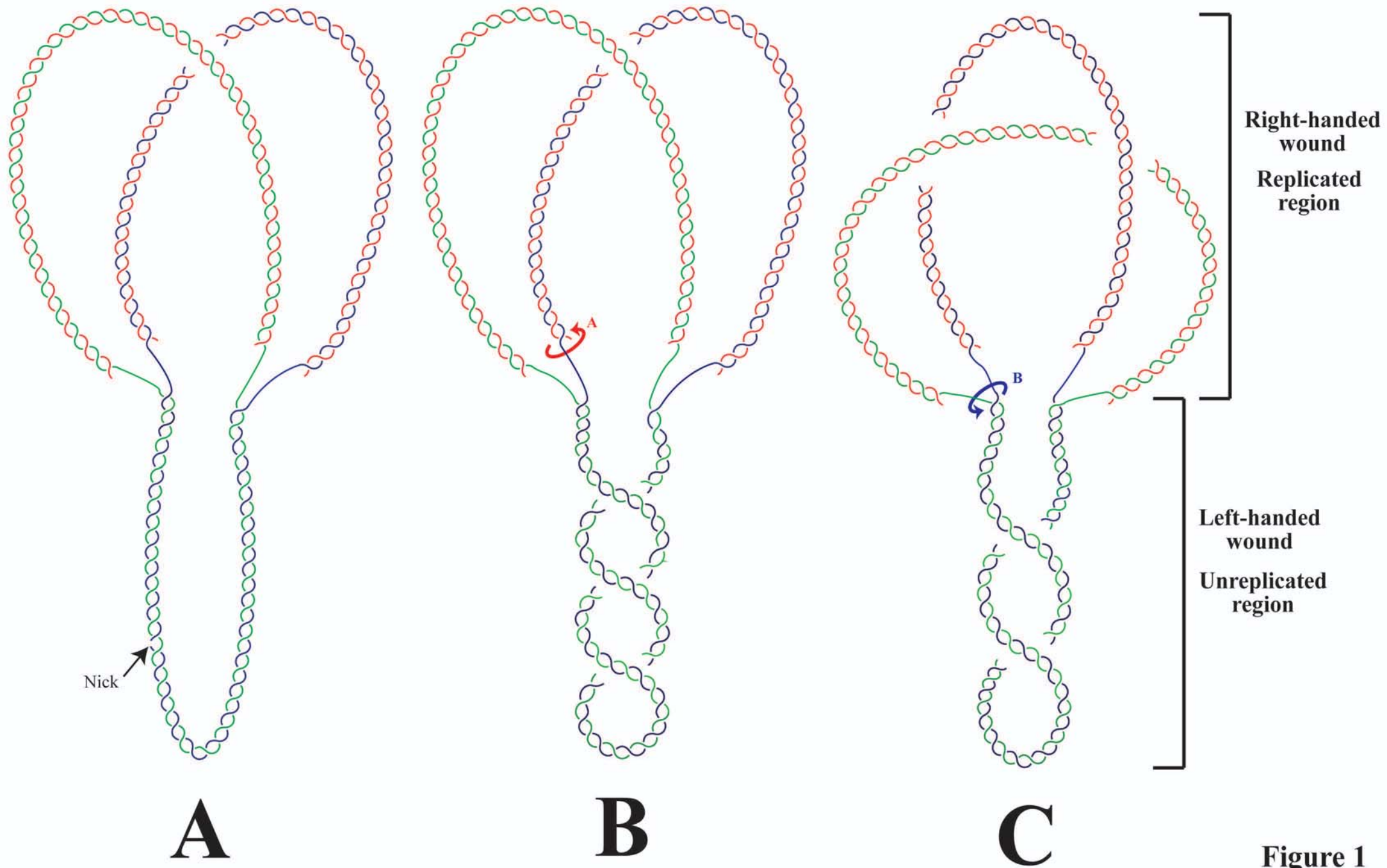
### **LEGEND TO FIGURES**

**Figure 1: Cartoon illustrating the topology of different DNA replication intermediates.** (A) Totally relaxed replication intermediate (RI) with a nick in the unreplicated region. (B) Partially replicated covalently closed molecule (CCRI) displaying positive supercoiling in the unreplicated region. As the nascent strands (in red) have free ends, they can rotate unrestricted around their corresponding parental strands (red arrow marked as “A”). Therefore, the sister duplexes cannot support supercoiling. (C) Swiveling of the forks (blue arrow marked as “B”) allows one of the left-handed positive supercoils to migrate to the replicated region where it adopts the form of a right-handed precatenane. Note that on a plane, each supercoil crossing, after migration to the replicated region, derives into two precatenane crossings. All native negative supercoiling was omitted for clarity. Parental-duplexes are indicated in blue and green while nascent strands are depicted in red.

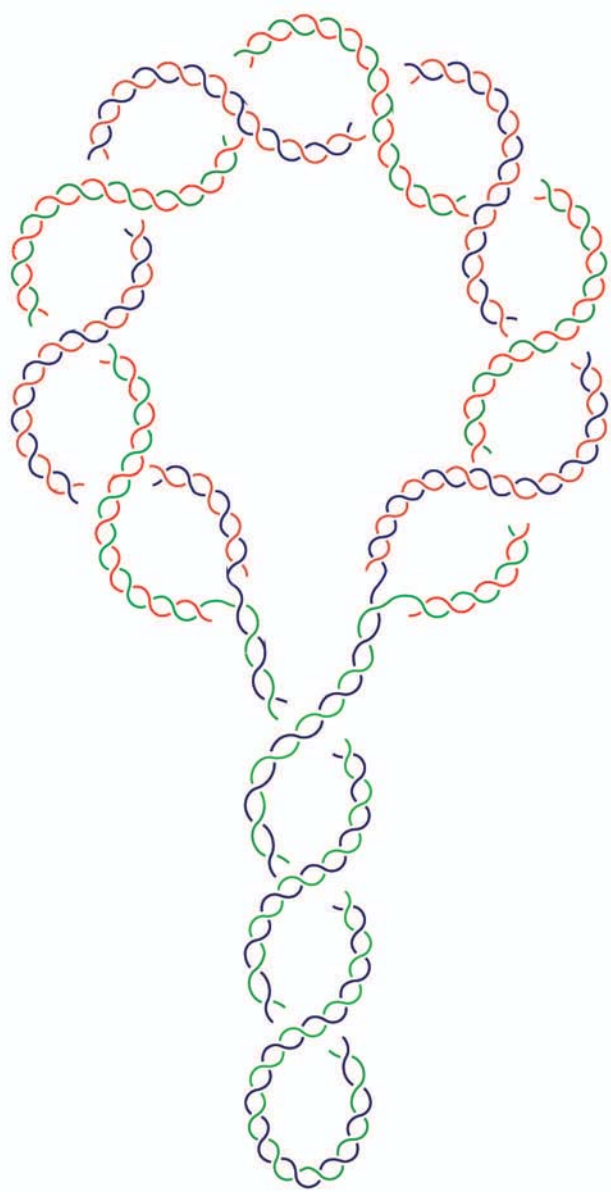
**Figure 2: Cartoon illustrating replication intermediates with different precatenation numbers.** (A) CCRI displaying negative supercoiling in the unreplicated region that is heavily precatenated in the replicated one. (B) CCRI displaying negative supercoiling in the unreplicated region that is poorly precatenated in the replicated one. This poor intertwining of sister duplexes would assist Topo IV to inadvertently make the strand passages

that lead to the formation of replication knots. Note that here, contrary to the situation for the cartoons shown in Figure 1, the unreplicated regions are shown negatively supercoiled. For this reason, crossings occur in a right-handed manner in the unreplicated as well as in the replicated regions. Parental-duplexes are indicated in blue and green while nascent strands are depicted in red.

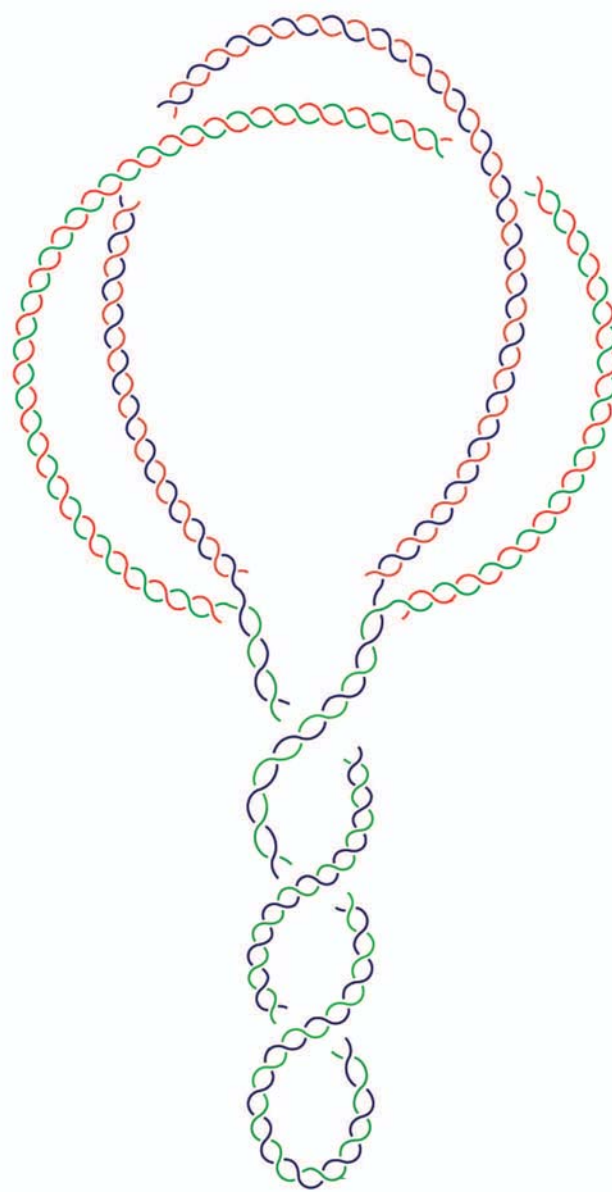
**Figure 3: Cartoons illustrating two different knotted molecules.** (A) Unreplicated circular nicked molecule displaying an intra-molecular trefoil knot. (B) Partially replicated molecule with a nick in the unreplicated region containing an inter-chromatid trefoil knot. Parental-duplexes are indicated in blue and green while nascent strands are depicted in red.



**Figure 1**

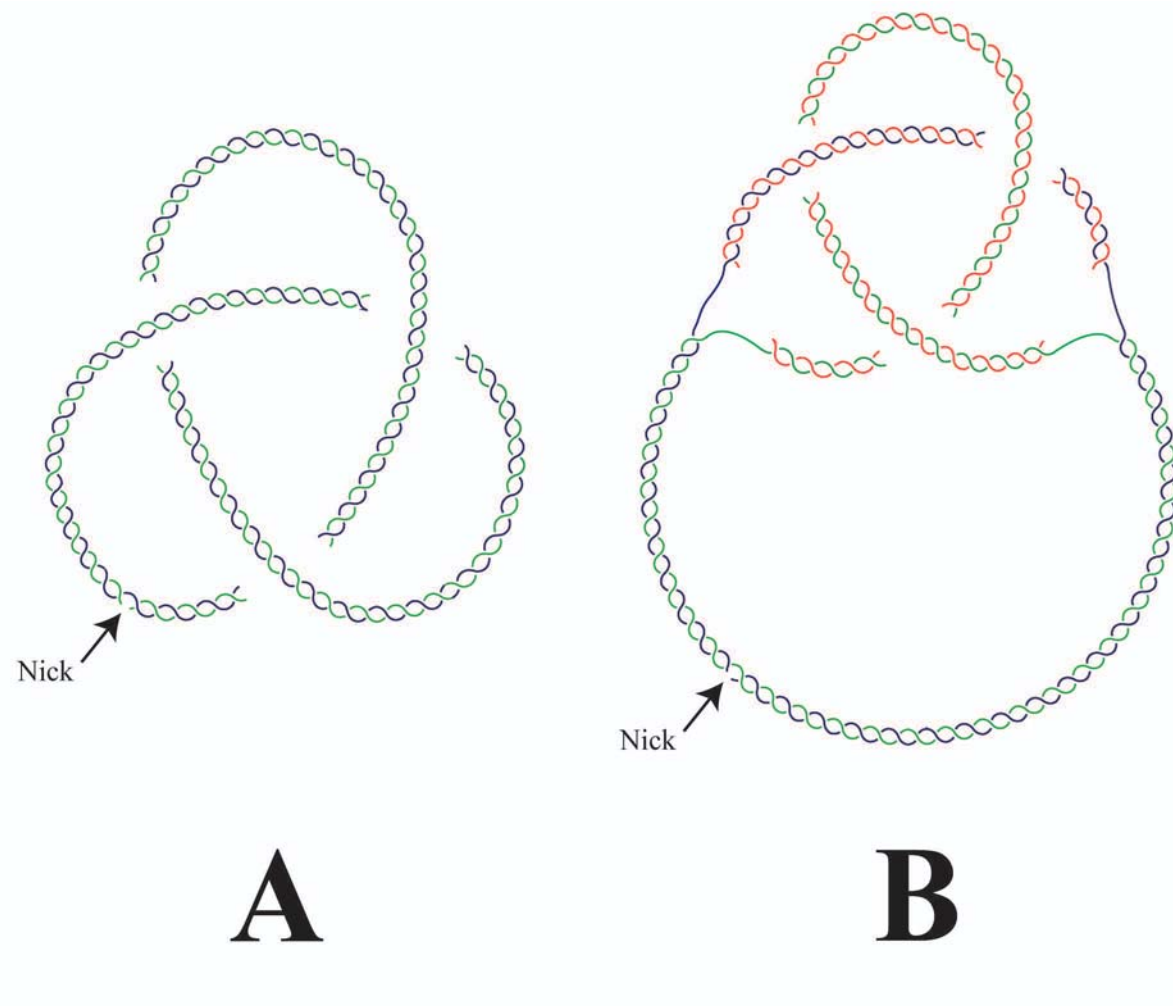


**A**



**B**

**Figure 2**



**A**

**B**