Keeping intracellular DNA untangled: A new role for condensin?

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Funding information
Prospects & Overviews; Plan Estatal de Investigación Científica y Técnica of Spain

Abstract
The DNA-passage activity of topoisomerase II accidentally produces DNA knots and interlinks within and between chromatin fibers. Fortunately, these unwanted DNA entanglements are actively removed by some mechanism. Here we present an outline on DNA knot formation and discuss recent studies that have investigated how intracellular DNA knots are removed. First, although topoisomerase II is able to minimize DNA entanglements in vitro to below equilibrium values, it is unclear whether such capacity performs equally in vivo in chromatinized DNA. Second, DNA supercooling could bias topoisomerase II to untangle the DNA. However, experimental evidence indicates that transcriptional supercooling of intracellular DNA boosts knot formation. Last, cohesin and condensin could tighten DNA entanglements via DNA loop extrusion (LE) and force their dissolution by topoisomerase II. Recent observations indicate that condensin activity promotes the removal of DNA knots during interphase and mitosis. This activity might facilitate the spatial organization and dynamics of chromatin.

KEYWORDS
chromatin, cohesin, condensin, DNA knot, DNA supercoiling, loop extrusion, topoisomerase II

INTRODUCTION

One fascinating enzyme found in nature is the type-2 DNA topoisomerase, because it converts DNA molecules into phantom chains that can pass through each other. To do this, type-2 enzymes, such as eukaryotic topoisomerase II (topo II), transiently cleave the DNA double helix to open a gate, which is then crossed by another double-stranded DNA segment in an ATP dependent manner¹ (Figure 1A). When the gated DNA (G-segment) and the transported DNA (T-segment) are in the same DNA molecule, topo II can remove DNA supercoiling crossings and consequently relax the DNA. Intramolecular DNA passages can also lead to the removal or formation of DNA knots.¹ Conversely, when the G- and the T-segment belong to different DNA molecules, intermolecular passages lead to DNA concatenation or decatenation (Figure 1B). Type-2 DNA topoisomerases are essential and highly conserved through evolution because they remove the interlinks produced between newly replicated DNA molecules and modulate the supercooling of DNA during gene transcription and chromosome replication.²

The activity of type-2 topoisomerases, however, carries also some dangers. First, the DNA cleaving step can be a source of chromosomal damage when the enzyme fails to religate the gated DNA.³⁻⁴ Many antibiotics and antitumoral agents target precisely such DNA cleavage-religation activity.⁵⁻⁶ Second, although the DNA-passage activity allows to relax supercoils and remove knots and catenates, it can also produce the opposite effect. This happens because the topological conversions catalyzed by topo II rely mainly on the juxtaposition probability of DNA segments or, in other words, on the probability...
FIGURE 1  Mechanism and DNA topology conversions catalyzed by topoisomerase II. (A) Topo II uses a three-gate mechanism to pass one segment of DNA (T-segment) through another (G-segment) in an ATP dependent manner. Upon topo II binding to the G-segment, the T-segment is captured by closing the entrance gate (N-gate) of the enzyme. The T-segment is then passed through the transiently cleaved G-segment (DNA-gate) and it is released outside the enzyme through the exit gate (C-gate). (B) Intramolecular DNA passage allows relaxation of (+) and (−) supercoils, as well as removal of knots. Intermolecular DNA passage allows decatenation of linked DNA molecules. (C) DNA bridging proteins (orange) and other DNA condensing agents, that increase the juxtaposition probability of DNA segments, can promote formation of knots and catenates via topo II activity. (D) Long range analyses of nuclear architecture show little intermingling of chromosomal territories and very few entangles in the path of high-order chromatin fibers. However, in short-range scales such as nucleosomal fibers, topo II can accidentally entangle DNA by conducting strand passage of two juxtaposed DNA segments (depicted in green and yellow in the example).

of the enzyme to find a DNA crossover. Such probability is high when DNA molecules are supercoiled, highly knotted, or catenated. But DNA juxtaposition probability is also elevated when DNA molecules are highly concentrated (> 10 mg/mL) or when they are kinked and condensed by proteins (Figure 1C). In these cases, random inversion of DNA crossovers brings the DNA into a steady-state topological equilibrium, in which the DNA might end up highly entangled. This outcome was reported in numerous in vitro studies, in which proteins or agents that promote DNA condensation invariably led to the knotting and concatenation of DNA plasmids.[6–8]

Considering the high concentration of DNA in a cell nucleus (10 mg/mL) and the plethora of ensembles that fold chromatin by bringing distant DNA segments in close proximity, one could expect to find many entanglements produced by random topo II activity in vivo. Indeed, computer simulations have demonstrated that DNA confined in biological systems would be massively entangled if topo II could freely equilibrate its global topology.[9–12] Fortunately, this prospect does not occur because the hierarchical folding of DNA into chromatin fibers and domains drastically reduces the topological complexity of intracellular DNA. In this respect, it has been proposed that chromatin architecture has a scaling behavior similar to a fractal globule,[13,14] such that any segment of the chromatin fiber is prevented from passing around another one.[15,16] Accordingly, analyses of nuclear architecture show little intermingling of chromosomal territories and chromatin domains.[17,18] Moreover, the 3D path of chromatin fibers is rarely found entangled at the megabase scale.[19–22] However, this might not be the case when chromatinized DNA tries to topologically equilibrate in short-range scales. For instance, since the DNA path is often tortuous within a bunch of nucleosomes, topo II activity might accidentally entangle the DNA (Figure 1D). Likewise, when two chromatin fibers come in contact, topo II might accidentally interlink their interface DNA. Recent studies have uncovered that such local DNA entanglements frequently occur in vivo, but that there are also mechanisms able to minimize them.[23–25] Here, after a brief description of how DNA knots are formed and analyzed, we present an overview of these studies.
FORMATION AND ANALYSIS OF DNA KNOTS

What is a DNA knot and how is it formed?

A closed polymer chain, such as a circular DNA molecule, is said to be knotted when it has irreducible crossovers. These crossovers are ones that cannot be eliminated by deforming the DNA ring in any way such as stretching, bending or twisting it. Therefore, in a circular DNA molecule or in a DNA loop, the only possible way to create or eliminate a knot is by cutting and resealing the DNA, a process that is typically performed by type-2 topoisomerases. Yet, other DNA cutting-religation enzymes, such as DNA recombinases, can also produce DNA knots. Likewise, a DNA knot can be formed within a linear DNA molecule and then be captured by circularizing the DNA with a ligase (Figure 2A).

Knots have many degrees of complexity and are classified by their number of irreducible crossings. The first possible knot has 3 irreducible intersections (trefoil or 31), and it is followed by the knot with 4 intersections (41), two possible knot forms with 5 intersections (51 and 52), and so on (Figure 2B). The above-mentioned prime knots are also classified by their overall geometry (e.g., toroidal, twisted) and by their unknotting number, which refers to the number of crossing inversions needed to dissolve the knot. Knots can also be regarded as composite when they can be decomposed into more than one prime knot. Finally, most knots are chiral, this means that they cannot be deformed into their mirror image, which is a different knot.

How are DNA knots analyzed?

To identify a knot in a DNA molecule, it is essential to distinguish the supercoiling crossings (consequent to DNA helical tension) from the irreducible crossings of a knot. To this end, the DNA is enzymatically nicked to relax its helical tension. Afterwards, DNA knots can be visualized via electron microscopy (EM) or atomic force microscopy (AFM). However, these techniques are semiquantitative and require DNA samples enriched in knotted forms. A more suitable method to characterize DNA knotting is by agarose gel electrophoresis. Upon
nicking circular DNA to remove supercoils, knotted molecules remain more compact than the unknotted ones and, consequently, move faster in a gel (Figure 2B). Suitably, the electrophoretic velocity of a DNA knot is roughly proportional to its number of irreducible crossings but on high resolution gels it is possible to separate different types of knots that have the same number of irreducible crossings.[26] Since in one-dimensional (1D) gels, the knotted DNA molecules overlap with linear DNA fragments, they are better resolved in two dimensional (2D) gels, in which the first dimension resolves the different populations of knotted molecules and the second dimension separates them from linear DNA (Figure 2B).[27] Gel-blot quantification of knotted molecules allows the calculation of the DNA's knotting probability in a given experimental condition; and the identification of different knot types, which can suggest how these knots have been generated.

Note that quantitative analyses of DNA knotting via microscopy or electrophoresis is feasible in circular DNA molecules of modest size (up to about 20 kb). The characterization of entanglements in larger DNA molecules or in genomic DNA remains challenging. However, recent methods for serial fluorescent in situ hybridization have made it possible to trace the trajectory of consecutive genomic loci in vivo and disclose whether such trajectories are knotted or not.[22]

**Why are DNA knots informative?**

The knotting probability ($P_{kn}$) of any chain, such as DNA or a chromatin fiber, depends on its length, its stiffness, and its thickness. It is easy to imagine that the longer a chain is, the higher its $P_{kn}$ will be (just recall your wired headphones). Likewise, the chain stiffness or flexibility largely affects the $P_{kn}$. The stiffness of a chain is measured in terms of its persistence length ($P_L$), which relates to the length over which the correlation between the directions of the tangents originating from two points of the chain is lost.[28,29] Polymers with a large $P_L$ are more rigid and thereby have lower $P_{kn}$. Thus, a chain length and its flexibility can be modeled as a number of connected segments (N), where each individual length correlates to the $P_L$. Finally, the $P_{kn}$ inversely correlates with the effective diameter ($d_E$) of the chain, which not only takes into account the geometric thickness, but also the possible repulsion or attraction between chain segments. Therefore, the $d_E$ can be zero or even negative. Computer simulations of random walks or worm-like chains allow predicting how the length, stiffness, and thickness of a polymer will affect its knotting probability. Comparison of such theoretical $P_{kn}$ values with those obtained experimentally with DNA in free solution have been useful to measure the DNA’s persistence length ($P_L = 50$ nm or about 150 bp) and its effective diameter ($d_E = 5$ nm in physiological buffers).[30–32] Since DNA is often condensed within biological ensembles, computer simulations also predicted the effect of condensation and confinement on the $P_{kn}$. A classic example is the highly knotted DNA molecules found in some bacteriophages.[19,33,34] Computer analyses validated that such knots result from the accidental circularization of linear DNA inside the phage capsid, in which the DNA is found extremely condensed.[9,35] (Figure 2C).

Finally, knots are informative because they are non-invasive footprints of the spatial trajectory of DNA. In other words, a knot is a topological invariant that has captured the DNA’s 3D path. In this regard, computer simulations demonstrate that, whereas a random path produces random knots, a non-random path will produce specific knots. Consequently, it is possible to infer how DNA was folded in a biological ensemble by analyzing the knot spectrum of such DNA. For instance, the knots formed inside phage capsids were highly enriched in chiral and toroidal knot forms, which allowed computer simulations to model the DNA’s folding like a wool ball.[36] (Figure 2C).

Note that, since the $P_{kn}$ depends on the average biophysical properties and constraints of long contours of DNA (i.e., several kb), it is unlikely that short DNA base pair sequences have per se any significant impact on the $P_{kn}$ of an entire DNA molecule. However, in biological systems, specific DNA sequences could favor knot formation or resolution if such sequences were involved in the regulation of DNA looping, DNA condensation or motor activities that can alter the topology and spatial trajectory of DNA.

**DNA KNOTS ARE COMMON IN EUKARYOTIC CHROMATIN**

A few years after the observation of highly knotted DNA in bacteriophages,[33,34] abundant DNA knots were found in bacterial plasmids with altered topoisomerase activities[37,38] and in plasmids with stalled DNA replication forks.[39,40] In the case of eukaryotic cells, the existence of DNA knots had not been documented until recently. DNA knots were found in numerous circular minichromosomes of budding yeast (Saccharomyces cerevisiae).[22] Similar to the DNA of cellular chromosomes, the DNA of circular minichromosomes is chromatinized with nucleosomes and replicates autonomously during S-phase. As in the case of bacterial plasmids, distinct transcription units, replication origins, centromeres, and other regulatory DNA sequences can be inserted into circular minichromosomes to study the structure and function of chromatin elements and their impact on DNA topology.

As explained below, the analysis of DNA knots found in yeast circular minichromosomes has now provided new insights about chromatin conformation and dynamics (Figure 3A).

Firstly, DNA knots occur in a great diversity of circular minichromosomes regardless of their DNA sequences, structural and functional elements. Moreover, DNA knots are present throughout the cell cycle, irrespective of DNA replication and cell proliferation. Their abundance was only transiently altered, in a topo II dependent manner, during DNA transcription. These observations strongly suggested that steady state fractions of DNA knots produced by topo II might be common along the nucleosomal fibers of eukaryotic chromatin.[23]

Secondly, the $P_{kn}$ of the minichromosomes increases linearly with DNA length up to a size of about 5 kb (i.e., about 25 nucleosomes) (Figure 3B). For this size, $P_{kn}$ is around 0.025 and the types of knots formed are similar to those obtained via computer simulations of polymer chains that follow a random path.[28,29] These findings indicated...
FIGURE 3  Knotting probability of in vivo chromatin. (A) Circular minichromosomes in yeast cells present steady state fractions of knotted and unknotted forms produced by the DNA passage activity of topo II. Upon DNA extraction and deproteinization, the DNA of the minichromosomes is found negatively supercoiled because each nucleosome was restraining about one negative supercoil. Such negative supercoils produce a typical arch of linking number topoisomers (Lk) in a 2D-gel. Upon nicking the DNA, the supercoils are eliminated but not the knots. The DNA molecules containing a knot (Kn) can be then revealed in a 2D-gel, as previously described in Figure 2B. Unknotted nicked circles (N) and linear DNA fragments (L) are indicated. (B) $P_{kn}$ of yeast circular minichromosomes of increasing size (kb). $P_{kn}$ increases linearly with DNA length up to a size of about 5 kb (about 25 nucleosomes). However, there is an inflection in the $P_{kn}$ slope when the DNA length surpasses about 5 kb.

that the nucleosomal fibers of the minichromosomes behave in vivo like a polymer chain with a $P_t$ of 10±2 nm and $d_E$ zero. A larger $P_t$ value would imply negative $d_E$ values, whereas positive $d_E$ values approaching the geometrical diameter of the DNA (2 nm) would imply nucleosomal fibers with very small $P_t$ (<3 nm). These values inferred from the $P_{kn}$ demonstrate that nucleosomal fibers are exceptionally flexible in vivo.[23]

Thirdly, although the $P_{kn}$ of the minichromosomes of sizes up to 5 kb increased proportionally to DNA length, the $P_{kn}$ barely increased in larger minichromosomes (6–13 kb)[23] (Figure 3B). This inflection was striking because $P_{kn}$ was expected to keep increasing proportionally to DNA length. Such correlation is observed in computer simulations of polymer knotting[26–31] and when naked DNA molecules with different lengths are knotted in vitro.[31,32] Therefore, while the high flexibility of nucleosomal fibers favored DNA entanglement, some chromatin feature or activity was attenuating a continuous scaling of DNA knot formation and/or promoting the removal of DNA knots beyond a critical length.

Three hypotheses have been experimentally tested to unravel the mechanism that minimizes the entanglement of intracellular DNA. The first hypothesis was based on the potential capacity of topo II to read and alter the equilibrium topology of DNA towards the removal of supercoils and knots.[41] The second hypothesis was that DNA supercoiling could bias topo II activity to untangle the DNA.[42,43] The third hypothesis relied on the notion that active extrusion of DNA loops conducted by cohesin and/or condensin could constrain DNA entanglements and favor their removal by topo II.[44–46] Testing these hypotheses have led to striking findings.

Topoisomerase II activity alone might not suffice to minimize intracellular DNA knots

Topo II not only removes DNA tangles, but it is also able to produce steady-state fractions of DNA catenates, knots and supercoils that are many times lower than the corresponding topological equilibrium fractions[41] (Figure 4A). Although this activity was only observed in vitro with naked DNA, it has been assumed that it also minimizes the entangling of intracellular DNA. The mechanism by which topo II simplifies the DNA’s equilibrium topology remains poorly understood. Several mechanisms have been proposed, such as the sliding model,[41] the G-segment hairpin model,[47] or the dual T-segment interaction models.[48,49] Yet, these models either lack biochemical support or cannot explain the extent of the observed effects.[50,51] However, subsequent studies found that the topo II capacity to simplify the DNA’s equilibrium topology was abolished when the entrance gate (N-gate) was locked after T-segment entry[52] (Figure 4B). Moreover, topo II was also unable to simplify the DNA equilibrium topology when the exit gate (C-gate) was removed[52] (Figure 4C). Remarkably, type-2B topoisomerases, which innately lack a C-gate, also lack the capacity to simplify the DNA equilibrium topology.[53]

The above in vitro observations allowed to test whether impairing the DNA topology simplification activity of topo II had some effect on the knotting probability of intracellular DNA. To this end, yeast cells were treated with the drug ICRF-193, which locks N-gate after T-segment entry.[54] The other approach was inactivating intracellular topo II and then express a truncated topo II, in which the C-gate was removed.[52] Remarkably, both experiments did not produce any
Topo II capacity to simplify the DNA equilibrium topology (A) When topo II removes DNA supercoils, knots and catenanes from DNA molecules in vitro, the enzyme is able to reduce them to below the equilibrium fractions. However, topo II loses its capacity to simplify the DNA equilibrium topology when (B) the N-gate is irreversibly locked after the passage of the T-segment; and when (C) the C-gate of topo II is removed to allow quick release of the passed T-segment. (D) $p_{kn}$ of in vivo chromatin in presence of wild-type, C-gate less, and N-gate locked topo II enzymes.

significant change on the $p_{kn}$ of intracellular chromatin$^{[25]}$ (Figure 4D). These results suggested that the capacity of topo II to simplify the equilibrium topology of free DNA in vitro might not perform in the same way in chromatinized DNA in vivo. However, these negative results cannot exclude that topo II might indeed contribute to simplify the equilibrium topology of intracellular DNA to some extent.

**DNA supercoiling does not reduce but markedly increases DNA knotting in chromatin**

In vitro experiments have evidenced that type2-topoisomerases produce more knots in supercoiled DNA plasmids than in relaxed ones$^{[6,7]}$ (Figure 5A). The capacity of supercoiling to promote knot formation has been reproduced by computer simulations.$^{[55]}$ However, other theoretical studies also proposed that supercoiling would tighten preformed DNA knots and facilitate their removal by topo II.$^{[43,56,57]}$ (Figure 5B). To clarify this matter, the effect of supercoiling on knot formation has been recently tested in native chromatin.$^{[24]}$ In this study, both negative ($-$)S or positive ($+$)S DNA supercoiling were accumulated in yeast cells$^{[58,59]}$ (Figure 5C). Interestingly, whereas $-$S did not alter the $p_{kn}$ of in vivo chromatin, $+$S produced a 25-fold increase in the $p_{kn}$. This burst in knot formation suggested that $+$S was markedly compacting the nucleosomal fibers.$^{[24]}$ This possibility was validated by computer simulations, which showed that a 5-fold volume compaction (i.e., reducing the radius of gyration of the nucleosomal fibers to 60%) would suffice to increase the $p_{kn}$ by 25-fold.$^{[24]}$ Conversely, $-$S did not increase $p_{kn}$, given that it undergoes a distinct level of compaction. In this respect, single-molecule experiments had already indicated that $-$S does not compact nucleosomal fibers to the same extent as $+$S.$^{[60-62]}$ Thus, the analyses of DNA knotting corroborated the differential response of in vivo chromatin conformation to $+$S and $-$S, and provided novel information on the level of chromatin compaction induced by S($+$) (Figure 5C).

**DNA knots generated during transcriptional supercoiling of DNA can stall RNA polymerases**

The ($+$)S that boosted $p_{kn}$ in circular minichromosomes was generated during DNA transcription. RNA polymerases transcribe DNA at rates of around 100 bp/s, which means that the DNA in front of the polymerases is over-twisted at rates of around 10 turns/s.$^{[62]}$ Since minichromosomes are circular, such ($+$)S is neutralized (cancelled) by the ($-$)S generated behind the RNA polymerases (Figure 5C). However, when DNA is transcribed within the large cellular chromosomes, ($+$)S and ($-$)S cannot be cancelled. Moreover, high levels of ($+$)S are likely to occur either when RNA polymerases run into barriers that block DNA twist diffusion, or when the advance of one polymerase converges with another one or with a replication fork. In all these scenarios, RNA polymerases are still able to elongate until ($+$)S reaches supercoiling densities (greater than +0.05) comparable to those experimentally generated in the circular minichromosomes.$^{[58]}$ After reaching this point, the DNA ahead of the transcribing complex is likely to become knotted by topo II concomitantly to the accumulation of ($+$)S (Figure 5D). However, the occurrence of such knots must be ephemeral since, in normal conditions, ($+$)S is rapidly relaxed by topo I or topo II itself. Then, the progression of RNA polymerases might not only depend on how quick the DNA’s ($+$)S is relaxed but also untangled. Otherwise, RNA polymerases would be stalled by DNA knots as it had been demonstrated by in vitro studies$^{[63]}$. Remarkably, this scenario explains why the inactivation of topo II during the transcription of long genes stops the
FIGURE 5  Interplay of DNA supercoiling and knotting in intracellular chromatin

(A) Illustration of how DNA supercoiling increases the juxtaposition of DNA segments and thereby favors topo II-mediated knotting of DNA. (B) Illustration of how DNA supercoiling can tighten existing knots and favor their removal by topo II. (C) DNA transcription generates (+)S and (-)S domains, which are normally relaxed by cellular topoisomerases. However, either the (-)S or (+)S can accumulate when DNA relaxation activities are insufficient or unbalanced. 2D-gels of Lk topoisomers reveal such accumulation of (-)S and (+)S in yeast minichromosomes. Upon nicking the DNA, Pkn is found to be 25-fold higher in the (+)S minichromosomes than in the (-)S ones. (D) When (+)S increases during genomic DNA transcription, DNA might become knotted to the same extent as observed in circular minichromosomes. The progression of the RNA polymerases might then require not only the relaxation of (+)S, but also the removal of concomitant DNA knots

of cohesin is thought to generate the topological associated domains (TADs) that organize chromatin during interphase. However, cohesin was initially discovered for its role in physically connecting sister chromatids following DNA replication. Thereby, cohesin is able to connect DNA regions in cis to produce loops or TADs and also bridge DNA regions in trans to keep sister chromatids cohered until anaphase.

An interesting outcome of forming DNA loops via LE activity, rather than by stochastic DNA interactions, is that LE displaces and constricts any DNA entanglement towards the outside domains of the extruded loop. Once an entanglement is tightened, it can be quickly removed by topo II (Figure 6B). Accordingly, computer simulations have demonstrated that LE activities can drive the removal of equilibrium DNA interlinks and achieve the spatial individualization of mixed chromosomes. Analogous simulations also illustrated that LE activities can drive the reduction of knots generated during the DNA's

progression of RNA polymerases in vivo (Figure 5D). The stalled polymerases were only rescued by topo II, but not by relaxing the DNA with topo I. Since only topo II is able to remove DNA knots, RNA polymerases might often be temporarily stalled by knots generated during the transcriptional supercoiling of DNA (Figure 5D).

Removal of DNA entanglements via loop extrusion (LE)

Structural maintenance of chromosome (SMC) complexes, such as cohesin and condensin, dictate the long-range architecture of chromatin during interphase and mitosis. This function apparently relies on the capacity of SMC complexes to extrude DNA loops (Figure 6A). The LE activity of condensin is likely driving the condensation of mitotic chromosomes whereas the LE activity of cohesin is thought to generate the topological associated domains (TADs) that organize chromatin during interphase. However, cohesin was initially discovered for its role in physically connecting sister chromatids following DNA replication. Thereby, cohesin is able to connect DNA regions in cis to produce loops or TADs and also bridge DNA regions in trans to keep sister chromatids cohered until anaphase.

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Effects of cohesin and condensin on the $P_{kn}$

(A) Illustration of DNA loop extrusion (LE) activity of SMC complexes. (B) LE activity can constrict DNA knots outside the extruded loop and tighten the DNA knot crossovers to promote unknotting by topo II. (C) Effects of condensin and cohesin on the $P_{kn}$ of chromatin. Inactivation of condensin increases $P_{kn}$ to levels that restore a linear correlation with DNA length, whereas inactivation of cohesin slightly reduces $P_{kn}$. (D) Stable DNA loops generated by LE within specific boundaries can favor topo II-mediated knotting of the DNA. (E) Plausible general role of condensin in minimizing DNA entanglements both in mitotic and interphase chromatin. Topo II accidentally produces DNA knots as well as interlinks between nearby DNA loops and chromatin domains that are in close proximity. LE activity of condensin tightens these entanglements to enforce their removal by topo II.

Codensin, not cohesin, is required to minimize DNA knotting in chromatin

The inactivation of cohesin and condensin was found to produce opposite effects in the knotting probability of yeast minichromosomes. Inactivation of cohesin produced a slight decrease of the $P_{kn}$ of large minichromosomes, whereas inactivation of condensin increased knot formation to levels that restored the expected linear correlation between $P_{kn}$ and DNA length (Figure 6C). In other words, condensin activity was required to produce the inflection that minimizes DNA knotting in large minichromosomes. These effects of cohesin and condensin occurred irrespectively of the DNA sequences present in the minichromosomes and were observed throughout the cell cycle.

The above findings support the idea that the LE activity of condensin might perform in vivo to promote the removal of DNA entanglements (Figure 6A). However, the opposite effect of cohesin on the $P_{kn}$ is puzzling since cohesin is also able to extrude DNA loops. These opposite effects could rely on distinct LE turnovers. Namely, cohesin is likely to perform single rounds of LE in order to generate long-lasting loops within specific boundaries (TADs). Such stable DNA loops would then favor the intramolecular entanglement of the DNA, as it has been demonstrated by computer simulations (Figure 6D). Conversely, condensin might perform repetitive rounds of LE without stopping at specific boundaries. This reiteration would allow condensin to continuously scan the presence of DNA entanglements genome-wide and tighten them to promote their removal by topo II (Figure 6B). Yet, it cannot be discarded that condensin and/or cohesin might be using other mechanisms to modulate DNA entanglements. For instance, cohesin...
and condensin might functionally interact with topo II in different ways to drive either the knotting or unknotting of DNA. In this respect, some studies indicated that condensin could physically interact with topo II;[80,81] and that cohesin colocalizes with topo II at DNA loop boundaries.[82,83] Lastly, since the effects condensin and cohesion on DNA knotting are mostly observed in large minichromosomes, they could also be related to distinct binding capacities of these SMC complexes to short and long stretches of chromatin.

Condensin activity and resolution of sister chromatid interlinks (SCIs)

When DNA replication is completed, the newly born DNA molecules are interlinked (catenated) and bridged by cohesin. However, although sister chromatids remain cohered from G2 phase until anaphase, SCIs are almost completely removed by topo II at the end of prophase.[84] This is surprising because topological equilibration of the cohered chromatids should favor their entanglement, not the opposite. In this regard, a crucial observation was that, if condensin is inactivated during metaphase, newly formed SCIs appear.[78,85] Therefore, condensin is promoting the disentangling of sister chromatids despite their close proximity, which favors their interlinking. Former studies, had proposed that condensin could direct topo II toward the removal of SCIs by means of generating (+)S.[86] However, subsequent in vitro studies indicated that condensin compacts DNA by extruding loops rather than by supercoiling.[87] Moreover, since (+)S increases knot formation[24] and Pkn does not increase in mitotic chromatin,[22,25] it is unlikely that condensin minimizes DNA entanglements by inducing (+)S. Condensin might then be using the same mechanism, most likely LE, to reduce both DNA knots and SCIs (Figure 6E). Remarkably, in both cases condensin counteracts the effect of cohesin, which favors DNA knot formation within TADs during interphase and the persistence of SCIs up to metaphase.

Disentangling chromatin during interphase: A new role of condensin?

When cells exit mitosis, chromosomes decondense and recover their territorial organization.[88] Inside each territory, chromosomes are compartmentalized into active and inactive regions; and chromatin fibers are folded or looped into a series of domains or TADs.[89] Whereas this long-range organization drastically reduces the topological complexity of intracellular DNA, the occurrence of knots in short-length scales indicates that topo II can still randomly pass nearby DNA segments through each other and entangle them.[23] It could be then expected that, when DNA segments that belong to different chromosome territories, chromatin domains or loops are in close proximity, topo II might accidentally entangle them (Figure 6E). Such interlinks, however, are rarely observed.[13,20] Chromatin domains from one chromosome can locally invade each other to some extent, without apparently becoming topologically linked.[89] The trajectory of chromatin fibers at the megabase scale is frequently found unknotted.[22] It is then tempting to propose that, in the same way that condensin promotes the removal of SCIs during mitotic prophase, it operates similarly to minimize intra- and inter-domain entanglements of DNA during interphase (Figure 6E).

The plausible new role of condensin to minimize DNA entanglements could explain its presence in interphase chromatin,[81,90–92] although its best known function is the compaction of mitotic chromosomes.[93,94] Whereas yeast cells have one form of condensin, higher eukaryotes contain two condensin complexes, condensins I and II,[95] which associate with mitotic chromosomes at different stages.[96,97] However, condensin inactivation during interphase provokes genome decompaction in budding yeast.[98] In mammals, depletion of condensin II produces hyper-clustering of pericentric heterochromatin and an increase of inter-chromosome associations.[99] In Drosophila, inactivation of condensin II causes stronger interactions between chromosome territories.[100,101] These nuclear rearrangements often associate to alterations of transcriptional regulation and DNA repair.[102–104] Moreover, a comparison of the nuclear architecture across the tree of life revealed that the species having condensin I and II share a similar architecture type during interphase.[105] Therefore, condensin seems to play some role in maintaining chromosome folding patterns during interphase, which are unusually stable across different cell types.[100] This mysterious role could arise from the condensin capacity to promote the elimination of DNA entanglements.

CONCLUSIONS AND PROSPECTS

The problem of DNA entanglement has likely been present from the early life evolution as soon as DNA genomes increased in size and packaging complexity. Remarkably, type-2 topoisomerases and SMCs complexes are invariably found in Archaea, Bacteria, and Eukarya.[104,106] The formation of DNA knots and interlinks probably emerged early on, as an inevitable side effect of type-2 topoisomerases and other genome transactions. However, in the same way that cells eliminate all the SCIs generated during DNA replication, cells found how to counteract the topological equilibration of their genomes by actively removing DNA entanglements throughout the cell cycle. This crucial task appears to rely mainly on the concerted action of topo II and condensin.

Future research will elucidate whether condensin exploits its LE activity or other mechanisms to promote the removal of DNA knots and interlinks; and whether the multiple dysfunctions of chromatin architecture observed upon condensin inactivation are consequent to global failure in minimizing such DNA entanglements. This role of condensin raises additional questions, such as how the activities of condensin and cohesion are coordinated; and how they interplay with topo II both in interphase and in mitotic chromatin. Lastly, in the same way that cells strive to minimize DNA entanglements, we should not discard other activities or variables that might modulate knot formation or dissolution at specific loci. For instance, active knotting (or knitting) of DNA could be exploited to shape and stabilize specific chromatin architectures.
AUTHOR CONTRIBUTIONS

All the authors contributed extensive scientific knowledge and participated in the preparation of the manuscript. Joaquim Roca wrote the manuscript.

DATA AVAILABILITY STATEMENT

Data sharing not applicable – no new data generated.

CONFLICT OF INTEREST

The author declares no conflict of interests.

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**How to cite this article:** Roca, J., Dyson, S., Segura, J., Valdés, A., & Martínez-García, B. (2022). Keeping intracellular DNA untangled: A new role for condensin?. *BioEssays*, 44, e2100187. https://doi.org/10.1002/bies.202100187