Cell Cycle 10:9, 1339-1340; May 1, 2011; © 2011 Landes Bioscience

DNA knots and DNA supercoiling

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Key words: DNA topoisomerases, DNA topology, DNA structure

Type II DNA topoisomerases permit passages of double stranded DNA segments through each other and this is achieved via a complex mechanism involving a transient cleavage of one duplex, a passage of the second duplex through the topoisomerase-spanned cleavage site and finally resealing of the cut duplex.[1] Type II DNA topoisomerases facilitate many DNA transactions requiring manipulation of long DNA molecules and are necessary for the completion of such vital processes as DNA replication.[1] Type II DNA topoisomerases are, however, doubly edged swords: whereas most of the catalysed passages are beneficial, some can be deleterious to living cells. When passages lead to formation of knots these impede transcription and replication and need to be eliminated quickly.[2,3] Elimination of knots cannot be simply done by random passages since in long DNA molecules crammed within a small volume, such as a bacterial nucleoid, random passages would only result in formation of highly complicated Gordian knots.

In 1997, Rybenkov et al. provided a partial explanation to the DNA knotting problem by demonstrating that type IIA DNA topoisomerases acting in vitro on relaxed DNA plasmids maintain the knotting level up to 50-fold below the level that would be obtained after random passages. However, the same reactions also demonstrated that this unknotting ability of type IIA DNA topoisomerases sharply decreases with DNA length.[4] Since then, two puzzling questions were disputed by the topoisomerase community: (1) How can type IIA DNA topoisomerases preferentially accomplish intersegmental passages leading to unknotting while avoiding passages leading to knotting? (2) How to reconcile the fact that in in vitro reactions the efficiency of type IIA DNA topoisomerases sharply decreases with the DNA length, with the proposal that one of the biological functions of type IIA DNA topoisomerases is to protect long genomic DNA from knotting.

Considering the first question, one has to realize that finding a knot in a crammed DNA molecule seems a priori as difficult as finding the proverbial needle in a haystack (**Fig. 1A**). Topoisomerases are much smaller than the overall dimensions of DNA molecules and they can only recognize some local features that could distinguish crossings resulting from knotting from those resulting from accidental juxtapositions. Several groups proposed various models that could explain how type IIA DNA topoisomerases achieve their feat. A hairpin model was proposed where type II DNA topoisomerase was bending the bound DNA segment and accepting for a passage only a DNA segment approaching from the inside of the bend.[5] The simulations testing that model showed a significant reduction of the steady state knotting level as compared to random passages.[5] A hooked juxtaposition model was proposed where type IIA DNA topoisomerases were specifically binding and acting on DNA juxtapositions where two regions of the DNA were bent over each other.[6,7] Simulations testing that model exceeded the knotting reduction level obtained with hairpin model and reached the level observed experimentally.[7,8] According to both of those models, type IIA DNA topoisomerases should bind and act preferably on DNA with high curvature. More recent crystallographic studies indeed showed that DNA is highly bent while bound by type IIA DNA topoisomerase.[9]

Both the hairpin and hooked juxtaposition models seemed to explain the principle how type IIA DNA topoisomerases may decrease the knotting level below the topological equilibrium. However, unexplained remained the question why a mechanism protecting from knotting is apparently not effective when acting on long DNA molecules. If such a protection is biologically important then it should be still efficient in the biologically relevant range of long DNA molecules that are very likely to be knotted by random intersegmental passages. While pondering that latter question, we realized that Rybenkov et al. tested unknotting activity on relaxed DNA, whereas supercoiled DNA represents a more natural state of DNA both in prokaryotes and eukaryotes.[10,11] This prompted us to use Brownian dynamics simulation to model knotted supercoiled DNA molecules. The simulations showed that DNA knots in supercoiled DNA molecules adopt a rather tight form (**Fig. 1B**). In that form, a locally acting protein such as a DNA topoisomerase could easily distinguish knotted regions from others just by showing preferential binding to strongly bent DNA. Since DNA in a complex with type IIA DNA topoisomerases is highly bent,[9] DNA topoisomerases will preferably bind the DNA that is already prebent as binding there will not be opposed by the energetic costs of DNA bending. Our simulation results showed that knot's tightening is largely independent of the length of DNA molecules,[12] and therefore the unknotting activity of type IIA DNA topoisomerases should not decrease with the length of DNA molecules. Knots'

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Submitted: 02/25/11; Accepted: 02/27/11
DOI: 10.4161/cc.10.9.15293
Comment on: Witz G, et al. Proc Natl Acad Sci USA 2011; 108:3608–11.

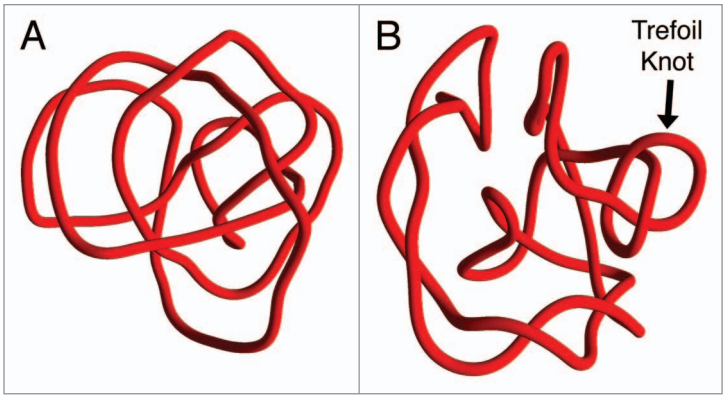

Figure 1. Recognition and elimination of knots on crammed, long DNA molecules become easy when the DNA is supercoiled. (A) Schematic presentation of torsionally relaxed, knotted DNA molecule that forms a trefoil knot and is confined to a small volume. Specific unknotting of such a molecule requires topoisomerase IIA action on these crossings where a passage would lead to unknotting. However, these crossings are hardly different from accidental overlaps and topoisomerases IIA action on those crossings would rather introduce new knots than unknot the existing one. (B) In supercoiled DNA molecules, the knotted portion becomes tightened as this decreases their free energy.[12] Tightened knots have a higher curvature than the rest of the DNA, allowing type IIA DNA topoisomerases to specifically bind and act on them as these enzymes have high affinity to bent DNA.[9]

tightening by DNA supercoiling provides an efficient way to differentiate knotted portions from the rest of the molecules. Finding the needle in the haystack is easy if one has a strong magnet. Similarly type II DNA topoisomerases could use their affinity to bent DNA as a knot magnet, provided that the DNA is supercoiled. The proposal that type IIA DNA topoisomerases preferentially recognize knots due to their increased curvature is consistent with the hairpin[5] and hooked juxtaposition models[6,7] since tightened knots can be seen as composed of hairpins or of hooked juxtapositions. In addition, our proposal would explain why the unknotting ability of type IIA DNA topoisomerases sharply decreases with the size of relaxed DNA molecules. Indeed, studies of the size of the knotted domains in torsionally relaxed polymers revealed that the size of the knotted domain increases with the size of the molecules,[13] and therefore the curvature in the knotted regions decreases.

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