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Comment

Model of DNA topology simplification has come full (supercoiled) circle after two decades of research Comment on "Disentangling DNA molecules" by Alexander Vologodskii

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Being a geek of DNA topology, I remember very well the stir caused by 1997 Science paper showing that DNA topoisomerases have the ability to simplify DNA topology below the topological equilibrium values [1]. In their seminal experiments Rybenkov et al. [1] started with linear double-stranded DNA molecules with cohesive ends. The mutual cohesiveness of DNA ends was due to mutual complementarity of single-stranded extensions at both ends of linear double-stranded DNA molecules. When such DNA molecules were heated up and then slowly cooled down the single-stranded ends eventually annealed with each other causing DNA circularization. This experimental protocol permitted the authors to establish topological/thermodynamic equilibrium within samples of circularized DNA molecules. Among simple unknotted circles one also observed knotted and catenated DNA molecules. The fraction of knotted molecules in DNA samples at topological equilibrium was increasing with the length of DNA molecules undergoing slow circularization. The fraction of catenated molecules was increasing with the length and the concentration of the molecules undergoing slow circularization. Rybenkov et al. incubated then such equilibrated DNA samples with type II DNA topoisomerases, which pass DNA duplex regions through each other, and observed that as the result of it the fraction of knotted and catenated DNA molecules was dramatically decreased (up to 80-fold). This elegant experiment indicated for the first time that type II DNA topoisomerases acting on knotted or catenated DNA molecules have the ability to select among many potential sites of DNA–DNA passages these that result in DNA unknotting or decatenation. Without such a selection topoisomerases could only maintain the original topological equilibrium obtained during the slow cyclization. The big question was how DNA topoisomerases can be directed to do DNA–DNA passages that preferentially result in DNA unknotting and decatenation.

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The authors of the 1997 Science paper proposed a "toothpaste squeezing model" where an individual topoisomerase lands on a random point of contact involving two regions of the same DNA molecule and then actively translocates along the DNA. During this translocation the topoisomerase maintains the contacts with two unrelated duplex regions but progressively slides in one direction with respect to the bound DNA. Such an active translocation, which in principle could be driven by ATP hydrolysis (type II DNA topoisomerases are ATPases), would provide the possibility to concentrate the knotted or catenated region within a small loop, eventually squeezing out the entrapped entanglements through topoisomerase-mediated transient opening in the DNA. DNA–DNA passages resulting from such a process would almost always result in DNA unknotting or decatenation. The proposed model was very attractive and for several years I was entertaining students of DNA topology classes by showing that by applying that model, even with closed eyes I was able to localize a knot in long knotted silicon tubes and pinpoint a juxtaposition where a passage would result in unknotting.

There was though a serious problem with the proposed model. The postulated DNA translocation activity of DNA topoisomerases, needed to confine knotted or catenated regions within small DNA loops, was already doubtful at the time when the model was proposed and subsequent research only strengthened this doubt. For this reason Vologodskii in his current review [2] states: "We will not discuss models which have evident flaws, like the one suggested in the original study . . . ". Such a statement is very cryptic and most of the readers of the review would not know what the author considered as evident flaws in his own early model.

When the DNA topology community realized the problems with the original model, different other models were proposed and they are very well presented in the review [2]. It is accepted now, thanks to research of many groups, including important contributions of Vologodskii, that semiflexible character of DNA molecules can guide DNA topoisomerases to preferentially perform intersegmental passages leading to unknotting or decatenation. Since efficient DNA unknotting and especially postreplicative DNA decatenation are necessary for cells' survival there was strong evolutionary pressure that permitted selection of such characteristics of DNA topoisomerases that made them very efficient in unknotting and decatenation of DNA molecules. It was proposed in 2004 that semiflexibility of relatively short DNA molecules makes that when in knotted or catenated DNA molecules one encounters a hooked juxtaposition, where two independent DNA regions are hooked with each other (such as shown in Fig. 7 of the review by Vologodskii), the action of topoisomerase permitting the hooks to pass through each other is very likely to result in unknotting or decatenation [3]. It was therefore proposed that type II DNA topoisomerases selectively recognize and act on hooked juxtapositions [3]. The hooked juxtaposition model is in part supported by the structural data [4] and by numerical simulations showing very efficient reduction of knotting or catenation level below the level of topological equilibrium [5–7].

A potential problem with the hooked juxtaposition model was that if topoisomerases need to wait for the spontaneous formation of a juxtaposition where two strongly bent DNA regions are hooked with each other, this waiting time may be very long making the unknotting/decatenation process very slow. Earlier modelling studies of hooked-juxtaposition model were mainly concerned with the attained steady state level of topological simplification rather than with the speed of the reaction. In his current review Vologodskii formally analyses different steps of the reaction and shows that the hooked juxtaposition model can in fact result in sufficiently fast kinetics especially when the binding of the T segment proceeds in two steps (T segment is the one that during the reaction is Transferred through a transient opening in the other DNA segment bound by the topoisomerase). Thus we seem to have now a structural and kinetic model of topology simplification that can explain the results of experiments performed by Rybenkov et al. [1]. However, there is still a big problem. The experiments by Rybenkov et al. showed that in the studied by them reactions the DNA topology simplification abilities of DNA topoisomerases were decreasing with the size of DNA molecules. This also concerns the computational models of these reactions and Vologodskii clearly states in his review: "As one can expect, the effect of topology simplification gradually disappears with increase of DNA length". If DNA topology simplification would not work for long DNA molecules its entire biological utility could be compared to an umbrella that opens only if it does not rain. Rybenkov et al., used relatively short, nonsupercoiled DNA molecules for their experiments as this permitted good electrophoretic separation of knotted, catenated and unknotted forms. However, DNA molecules in living cells are very long and the equilibrium level of knotting and catenation strongly increases with the size of DNA molecules. So something is missing here.

This missing element is most likely DNA supercoiling. Studies of knotted and catenated DNA molecules that were in addition supercoiled revealed that entanglements resulting from knotting or catenation are strongly confined within small portions of the molecules [8,9]. Once the region of knotting or catenation is confined the size of the

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