Comment

Topology simplification: important biological phenomenon or evolutionary relic?

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The review, *Disentangling DNA molecules* [1], gives an excellent technical description of the phenomenon of topology simplification (TS) by type IIA DNA topoisomerases (topos). In the 20 years since its discovery [2], this effect has attracted a good deal of attention, probably because of its apparently magical nature, and because it seemed to offer a solution to the conundrum that all type II topos rely on ATP hydrolysis, but only bacterial DNA gyrases were known to transduce the free energy of hydrolysis into torsion (supercoiling) in the DNA. It made good sense to think that the other enzymes are using the energy to reduce the level of supercoiling, knotting, and particularly decatenation (unlinking), below equilibrium, particularly as the key activity of the non-supercoiling topos is the removal of links between daughter chromosomes [3]. As Vologodskii discusses [1], there have been a number of theoretical models developed to explain how the local effect of a type II topo can influence the global level of knotting and catenation in large DNA molecules, and he

explains how features of two of the most successful models (bent G segment and hooked juxtapositions) may be combined to explain the magnitude of the effect and overcome a kinetic problem with the hooked juxtaposition model.

However, there are a number of points that argue against TS as a significant biological effect. The reduction in the steady-state fraction of catenanes in the presence of even the most effective type IIA enzyme (*Escherichia coli* topo IV), described as 'dramatic' by Vologodskii [1] (actually a not all that dramatic 15-fold), corresponds to a trivial steady-state increase in the free energy of the DNA of under 10 J mol^{-1}, less than 1 % of *kT* [4]. This is in contrast to the related DNA gyrases, which can introduce supercoiling to a steady-state level corresponding to >500 kJ mol^{-1}, more than 10,000 times greater.

As far as we know, all organisms rely on type II topos to unlink chromosomes at the end of replication; however, it is not clear that TS is a necessary part of that mechanism. The different type IIA topos tested [2, 4] vary significantly in their efficiency of TS, while the archaeal type IIB enzyme topo VI (also present in plants and some single-celled eukaryotes) apparently does not do TS at all, but merely equilibrates topological forms [4, 5], although the reaction still requires ATP hydrolysis. This implies that TS is not required for chromosome segregation and partition in all organisms. Further, it has recently been shown [6] that topo VI from *Plasmodium falciparum* can functionally complement a yeast (*Saccharomyces cerevisiae*) null mutation in the single topo II (type IIA) gene, suggesting that TS is not required for replication and cell division in *S. cerevisiae*. (However, it should be noted that *P. falciparum* topo VI has not explicitly been shown to lack TS.)

There are fundamental differences in the overall structure of the related type IIA and IIB enzymes that may account for the absence of TS in the latter; we have hypothesised that the IIB enzymes may not carry out directional strand passage, since they contain only two protein-protein gates, rather than the three in type IIA [7]. Indeed, experiments in which one of the protein gates (the exit gate) was deleted from a type IIA enzyme, essentially converting it into a type IIB, showed loss of TS [8], although the resultant enzyme had severely compromised activity. Although it has been widely suggested that TS requires G-segment bending [1], it has been shown that type IIB enzymes also bend DNA [9], confirming that although G-segment bending may be necessary for TS, it is not sufficient. ATP-dependent directional strand-passage is also needed, which may require the presence of the exit gate in type IIA topos.

There are a number of features of DNA in real organisms that may help to drive decatenation of DNA to completion, even in the absence of TS. Chief amongst these are the supercoiling and condensation of DNA referred to by Vologodskii [1]. DNA supercoiling straightforwardly reduces the equilibrium fraction of catenanes, by reducing the area of the surface a strand from a linked DNA must cross; the self-condensation of eukaryotic chromosomes by SMC proteins has the same effect. As long as type II topos are active during the condensation phase, as daughter DNA molecules separate to form discrete mitotic chromosomes, then the number of inter-chromosome links will be driven essentially to zero even by an equilibrating enzyme. Additionally, Stasiak and co-workers [10] have recently proposed a specific mechanism for decatenation in bacterial supercoiled DNA. The dynamic formation of supercoiled domains in DNA by gyrase can drive catenated strands towards a

nicked DNA site, at which a type I topoisomerase can carry out decatenation. This uses the well-attested energy transduction effect of gyrase to drive decatenation; the same process might be driven by transient transcription-driven supercoiling in eukaryotes [11].

While it may be seductive to argue that the existence of ATP-dependent TS in type IIA topos means that it must have an important function in cells, there are sufficient exceptions and alternative mechanisms to suggest that complete decatenation can occur without it. We have instead suggested [7] that ATP hydrolysis is required by all type II topos (IIA and IIB) for a more fundamental reason: to avoid formation of dangerous permanent double-strand breaks in DNA. The type II topos break both strands of the DNA helix as a necessary part of their mechanism, and rely on very strong protein-protein interactions between the symmetrical subunits of the enzymes to avoid the two broken ends becoming separated. However, strand-passage between the broken ends requires that that this strong interface is transiently broken. The energy cycle of ATP hydrolysis is needed to mediate the sequential breakage and formation of at least two protein-protein gates to maintain the integrity of the complex as the strand-passage reaction takes place. The gyrase class of type IIA topos evolved specifically to introduce negative supercoils into DNA; this involves the bending and wrapping of the DNA around the enzyme to strongly select a second (T) segment of DNA with the appropriate geometry, and requires a unidirectional strand-passage reaction [12]. The topology simplification reaction may simply be an evolutionary relic of that mechanism retained in enzymes that have lost the ability to carry out supercoiling.

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