Twist to disentangle

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Intertwined DNA molecules frequently result in the formation of 'ultra-fine bridges' between sister chromatids that need to be resolved during their segregation into daughter cells. Although it has been established that these DNA bridges are coated by a helicase named PICH, it had remained unknown how PICH assists in their resolution. A study now reveals that PICH directs the formation of positive DNA supercoiling in the presence of type-I topoisomerases to promote the subsequent disentanglement of these DNA helices by type-II topoisomerases. Remarkably, PICH might be able to reconfigure DNA topology by extruding loops of DNA while it moves along the double helix.

Due to their long helical nature, sister DNA molecules emerge from the replication machinery highly entangled [1]. These DNA intertwines are typically resolved by type-II topoisomerases (topo II), which are enzymes that temporarily create a double-strand break in one DNA helix to allow the passage of a second DNA helix through the gap in the first one. Failure to untangle the bulk of DNA catenation before the onset of cell division, for example as a result of topoisomerase inhibition, gives rise to so-called 'anaphase bridges'; DNA that is stretched between the segregating sister chromatids. Yet, even in unperturbed cells, interlinks between sister chromatids persist for at least a short time into anaphase. Such 'ultrafine bridges' (UFBs) have been reported to consist of nucleosome-free DNA coated by several distinct proteins, including the Plk1-interacting checkpoint helicase (PICH)[2], Bloom (BLM) helicase[3] and a multi-subunit complex of type-IA topoisomerase IIIα (Top3a), Rmi1 and Rmi2 (TRR)[4].

How PICH, BLM and TRR help to resolve UFBs has remained a major unanswered question. It has been suggested that PICH and topo II cooperate in the resolution of UFBs that are caused by persistent DNA catenation, possibly by PICH-mediated recruitment and catalytic stimulation of topoisomerase IIα (Top2A)[5]. However, topo II can equally well decatenate DNAs as it can introduce new catenation. Formation of intra-chromatid loops by the condensin protein complex (see below) presumably provides one option to shift the balance towards decatenation, since condensin removal from metaphase chromosomes results in a topo II-dependent re-entanglement of sister chromatids[6]. Another option to promote decatenation seems to lie in the superhelical nature of the DNA helix: Positive supercoiling of circular yeast chromosomes has been found to increase in a condensin-dependent manner as cells enter mitosis and this change in superhelicity enhanced their decatenation by topo II *in vitro*[7]. Whether a similar mechanism applies to the resolution of intertwined linear chromosomes and if so, how positive supercoiling could be introduced into eukaryotic DNA, has remained unknown.

Bizard *et al.* now provide intriguing insights into this puzzle. The authors report that addition of a mixture of purified PICH and TRR, but neither PICH nor TRR alone, introduces a high density of positive supercoils (1 supercoil every 100 base pairs (bp)) into circular plasmid DNA in the presence of adenosine triphosphate (ATP). PICH therefore cooperates with TRR to overwind the DNA double helix — an activity that has hitherto only been associated with enzymes found exclusively in hyperthermophilic archaea and eubacteria, termed reverse gyrases. Reverse gyrases are thought to catalyze the cleavage of one of the two strands of the DNA helix, directional strand passage and subsequent re-sealing of the nick at their topoisomerase IA domain through ATP-driven conformational transitions within their helicase-like domain[8]. Might PICH and TRR employ a homologous strategy?

In single-molecule experiments, the retention time of PICH on double-stranded DNA substrates increases as the tension applied to the DNA increases [9]. This behavior explains how PICH specifically associates with UFBs that are stretched by mitotic spindle forces. Once bound to DNA, PICH not only promotes the recruitment of BLM and the TRR complex [10], but also translocates along the DNA helix in a manner that depends on its ATPase activity [9]. Bizard *et al.* found that the ATPase activity of PICH is essential for the formation of positive supercoils. Surprisingly, TRR can be replaced by other type-IA topoisomerases (which can relax only negative supercoils) but not by type-IB topoisomerases (which can relax both, positive and negative supercoils). This finding is consistent with the suggestion that positive supercoils accumulate in front and negative supercoils accumulate in the wake of translocating PICH, the latter of which are then selectively removed by the action of type-IA topoisomerases, like the Top3A subunit of TRR (Fig. 1). Notably, Top3A requires the DNA double helix to be partially unwound for effective strand passage, which is facilitated by a high degree of negative supercoiling. Such a mechanism would, however, only work if PICH could create a topological barrier that isolates a domain of negative supercoils in its wake from being compensated by the positive supercoils created in front of the enzyme. Indeed, atomic force microscopy images of PICH-bound plasmid DNA are consistent with the formation of such topological DNA domains.

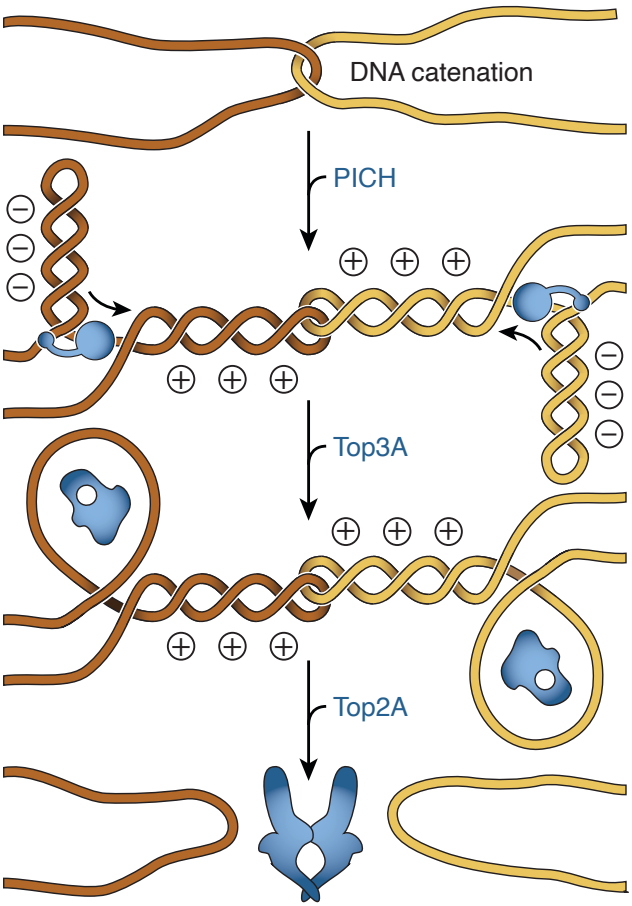

Figure 1 | Model for the resolution of DNA catenations by PICH and topoisomerases. By extruding DNA loops, PICH creates topological domains of positive and negative supercoiling. Negative supercoils are then removed by type-IA topoisomerase Top3A, resulting in net positive supercoils that promote the disentanglement of DNA catenation by type-II topoisomerase Top2A.

Like PICH, condensin can stimulate the formation of positive supercoils in circular plasmid DNA in the presence of ATP and type-I topoisomerases[11]. Whereas it had initially been suggested that DNA over-winding might be achieved by wrapping DNA around condensin's ATPase domains[12], the recent discoveries that condensin is able to extrude large loops of DNA as it translocates along DNA[13,14] provide an alternative mechanistic explanation. If PICH held on to its original DNA binding site while translocating, then it would inevitably extrude a DNA loop in a similar fashion as condensin does. Although PICH and condensin might hence extrude DNA loops in a similar manner, their DNA motor activities differ in speed, resistance to tension, processivity and the extent of superhelicity they generate. Whereas condensin can achieve surprisingly high velocities (>1,000 bp s^{-1}) over distances of serval kilobase pairs (kbp) of slack DNA without stopping or turning around, it readily stalls as soon as the DNA is stretched at low forces. PICH, in contrast, translocates at moderate speeds (30–80 bp s^{-1}) over distances of less than 1 kbp and frequently changes direction[9]. PICH is, however, able to shorten stretched DNA against much higher forces (8 pN or more) than condensin can (up to ~1.5 pN)[15]. In contrast to condensin, it also introduces a high degree of positive superhelicity (1 supercoil for every 17 bp translocated); a density of supercoiling that is even higher than what can be achieved by reverse gyrases[16]. PICH hence seems to be a highly robust motor that very efficiently changes DNA topology during its movement.

The emerging principle that a variety of chromosome transactions — ranging from gene regulation to DNA recombination, sister chromatid resolution and mitotic chromosome condensation — are controlled by protein complexes that fold DNA into loop structures[17,18]

emphasizes the necessity to understand the mechanisms by which molecular machines can initiate and expand such loops. Although the lower translocation processivity of PICH [9] might make it more challenging to directly reveal its proposed DNA loop extrusion activity in single-molecule experiments at the same detail as it has recently been achieved for condensin[14] or some restriction endonucleases[19,20], future experiments will need to vadiate the function of PICH as a DNA-loop extruder. The mechanistic insights into the activities of PICH uncovered in the work by Bizard *et al.* paves the way towards understanding how DNA motor proteins harness torsion and superhelicity of DNA to drive cellular processes. Analysis of DNA superhelicity in living cells, as exemplified by the use of psoralen as a supercoiling-sensitive interchelator[21], will be important to establish the physiological significance of DNA supercoiling by PICH/TRR and condensin.

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