Mathematical validation of a biological model for unlinking replication catenanes by recombination

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The price for simplicity of the linear genetic code is the inevitably large size of genomes and the attendant DNA entanglements. Circular chromosomes, or closed circular chromosome domains, are prone to knotting and catenation by a variety of DNA transactions. Knots in DNA pose road blocks to replication and transcription and increase the frequency of genetic rearrangements (1). Topoisomerases, enzymes that remove obstructive DNA crossings, play a crucial role in the duplication, decoding, and dissemination of biological information. An intriguing problem is how proteins that are much smaller than the large DNA molecules on which they act manage to resolve topological exigencies rather than exacerbate them. In their recent work, Shimokawa et al. address a particular aspect of this problem (2).

Perhaps no other life process better illustrates the intricacies and pageantry of DNA topology than replication. The intertwined nature of the double helix, the inherent

negative supercoiling of DNA in vivo, the build-up of counterbalancing negatively and positively supercoiled domains on either side of the replisome, the propensity for knotting within replication bubbles, and the formation of “precatenanes” (interwound daughter duplexes) by diffusion of supercoils across the replication fork contribute to a veritable topological extravaganza (3). The net result is that the products of replication of a circular chromosome are interlinked circles (catenanes) that need to be unlinked for faithful segregation (Fig. 1). In *Escherichia coli*, this task is accomplished by the type II topoisomerase Topo IV by breaking and joining double strands, and transporting one double-stranded DNA segment through another in the process (Fig. 1).

Unlinking of a different sort, the resolution of a dimer of the *E. coli* chromosome formed by homologous recombination back into monomers, is performed by the XerCD site-specific recombinase assisted by FtsK,

an ATP hydrolyzing motor protein that translocates along DNA. Quite surprisingly, the FtsK–XerCD system is able to mimic Topo IV in unlinking two catenated DNA circles in vitro or catenated sister chromosomes in vivo (4, 5). A plausible scheme, consistent with experimental results, is the simplification of topology in steps of one by the repeated action of the recombinase (Fig. 1). This explanation, based on the in vitro results, relies on the implicit faith that the electrophoretic migration of individual DNA bands reveals their true topological character. In reality, this need not be so. Furthermore, alternative—if somewhat less elegant—routes for unlinking cannot be ruled out.

Shimokawa et al. (2) have now provided a mathematical validation of the biologists’ intuitive model for unlinking catalyzed by FtsK–XerCD. The method the authors use is tangle analysis, which has been successfully applied to deduce strand exchange mechanisms catalyzed by site-specific recombinases (6). In a simplified view, a tangle is a 3D ball containing two strings, representing segments of a DNA molecule, connected to four points on the sphere (NE, NW, SE, and SW in a geographical sense, in Fig. 2). The strings may cross each other in a variety of ways. Three negative supercoil crossings from a circular plasmid substrate trapped by the interaction between a recombinase and its target sites are shown in Fig. 2. This “topological filter” will then dictate the topology of the recombinant product resulting from strand exchange. In their present work (2), Shimokawa et al. extend the tangle method to the unlinking reaction, under the assumption that each recombination step reduces the topological complexity of the substrate. Their analysis reveals a unique shortest pathway for accomplishing this end. Based on tangle analysis and theorems from classic knot theory, the authors propose a specific 3D representation of the FtsK–XerCD recombination synapse. Rigid body rotation of the synapse provides three possible tangle solutions, signifying a

Fig. 1. Resolution of a (4)-torus catenane by Topo IV or by FtsK–XerCD. A (4)-torus catenane formed by the replication of an imaginary circular DNA with two helical turns is shown. The thin red and blue lines indicate the two strands of the double helix. The thick red and blue lines represent double helical daughter DNA molecules. The catenane is unlinked by Topo IV in two steps (the straight path) or by FtsK–XerCD in four steps (the zig-zag path). The reduction of crossing number, by two for each step of Topo IV action and by one for each step of FtsK–XerCD action, is shown in parentheses.

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See companion article on page 20906.

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Fig. 2. The tangle representation of recombination. The DNA crossings (three in this example) trapped by a recombinase enzyme to form the recombination synapse are contained in the O_b tangle: they dictate the product topology (three-noded knot) when the parental target sites are recombined (P tangle $\rightarrow$ R tangle).

unique topological mechanism for the strand-exchange reaction that is at the heart of the unlinking process.

The mathematical reasoning behind the conclusions reached by Shimokawa et al. (2) is posited in two theorems. According to theorem 1, the shortest pathway for the unlinking of a $(2m)$ -torus catenane by FtsK–XerCD comprises exactly $2m$ steps. In other words, unlinking a six-noded catenane would take a minimum of six steps, that of a four-noded catenane four steps (Fig. 1), and so on. Theorem 2, which embodies the principal outcome of the present analyses, speaks to the topology of the intermediates formed during unlinking. If the recombinase reduces the number of crossings at each round of its action, every intermediate will have precisely one less crossing than its precursor, and its topological type will be known. Unlinking of a $(2m)$ -torus catenane will proceed along a unique pathway via a $(2m - 1)$ -torus knot, a $(2m - 2)$ -torus catenane, and so forth to finally yield unlinked circles. For a (4)-catenane, the sequential intermediates are a trefoil, a (2)-catenane and an unknot before unlinking occurs in a total of four steps, in agreement with theorem 1 (Fig. 1).

The proof of theorem 2 is based on a classic invariant called the “signature,” which for a link L is the difference between the number of positive and negative eigenvalues of a matrix associated to the surface bounded by L . Shimokawa et al. (2) show that the absolute value of the signature for any link L , $|\sigma(L)|$, is at most $c(L) - 1$, where $c(L)$ is the crossing number of L . The authors further show that $(2m)$ -torus links and $(2m - 1)$ -torus knots are special in that they realize the equality, $|\sigma(L)| = c(L) - 1$. Finally, by combining their tangle model for XerCD action with a theorem from knot theory (7), the authors establish that the signature of the product goes down by, at most, one for a single round of enzyme action. Collectively, these facts imply that, when FtsK–XerCD acts on a $(2m)$ -torus catenane to reduce its crossing number, the signature as well as the crossing number must decrease by exactly

one in the immediate product, a $(2m - 1)$ -torus knot. Repeated action of the enzyme will thus simplify topology one crossing at a time until unlinking is completed.

The mechanism of unlinking by recombination addressed by Shimokawa et al. (2) has broader implications for the general problem of topology simplification in DNA. How does Topo IV faithfully unlink replicated chromosomes during each *E. coli* cell cycle, and do so more efficiently than FtsK–XerCD? Prokaryotic and eukaryotic type II topoisomerases are remarkable in their capacity to reduce the fraction of knots or catenanes 10- to 100-fold lower than that expected at thermodynamic equilibrium (8). The topoisomerases do use the energy of ATP hydrolysis, but how that energy drives unknotting and unlinking below equilibrium is a thorny problem. Potential solutions suggested include topoisomerase-induced oriented DNA bends (one-way gates), special local geometry of juxtaposed DNA segments (hooks and twisted-hooks), and a two-step “activating”/“strand passing” pair of collisions (kinetic proof-reading) (9–11). Although these models invoke their own visions of Maxwell’s demon, his or her true identity remains shrouded.

Although the mathematics of unlinking torus catenanes (2) and the resolution of replication catenanes by repeated rounds of recombination (5) are mutually consistent, this

solution, at least in the biological sense, is not yet unequivocal. XerCD recombination in a supercoiled circular plasmid stimulated by the isolated γ domain of FtsK, which lacks motor activity, yields topologically complex products (12). Similarly, recombination by Flp, which is mechanistically related to XerCD, can generate a four-noded knot and a five-noded knot by acting on a trefoil and a four-noded catenane, respectively, thus increasing topological complexity (13). Perhaps the increased slithering of supercoils or the dissolution of plectonemic branches promoted by the translocating $\alpha\beta$ motor of FtsK and the simultaneous interaction of γ with the recombinase act collaboratively to simplify the recombination synapse, and hence product topology. It is not obvious that such a mechanism is sufficient to account for directed stepwise unlinking of replication catenanes.

The ensemble of catenanes with a range of crossing numbers used in the in vitro experiments that gave rise to the unlinking model precludes precise precursor-product assignments. A (4)-torus catenane with appropriately oriented XerCD target sites may be easily constructed using topological tricks familiar to recombination biochemists. Now that Shimokawa et al. (2) have provided the mathematical expectations for the action of FtsK–XerCD on this topologically unique substrate (Fig. 1), biologists would be remiss not to put them to an experimental test.

The results of Shimokawa et al. (2) must please biologists by providing mathematical backing for their intuitive interpretations of not so clear-cut experimental results. The elegant arguments that Shimokawa et al. apply to biologically important catenanes must satisfy mathematicians, especially knot theorists, for their implications in crossing number conversions among torus links and knots.

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