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DNA Disentangling by Type-2 Topoisomerases

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A type-2 topoisomerase cleaves a DNA strand, passes another through the break, and then rejoins the severed ends. Because it appears that this action is as likely to increase as to decrease entanglements, the question is: how are entanglements removed? We argue that type-2 topoisomerases have evolved to act at "hooked" juxtapositions of strands (where the strands are curved toward each other). This type of juxtaposition is a natural consequence of entangled long strands. Our model accounts for the observed preference for unlinking and unknotting of short DNA plasmids by type-2 topoisomerases and well explains experimental observations.

Keywords: DNA crossovers; helix–helix juxtaposition; catenane; knot; DNA supercoil

Type-2 topoisomerases lower the probability of DNA linking and knotting of small plasmid DNAs by a factor of 16 to 90-fold relative to that level achieved by random DNA strand passage.[1] This finding was interpreted to mean that these enzymes prefer to disentangle, which is the required biological activity, rather than tangle DNA. Several models for how type-2 topoisomerases achieve this have been offered. Rybenkov *et al.* proposed that the topoisomerase tracked along two DNA helices to pinch a third.[1] However, Yan *et al.* calculated that the probability that three DNA strands coincide in space was too infrequent to account for this type-2 topoisomerase activity.[2,3] Instead, they proposed a "kinetic proofreading model" in which the enzyme, bound to one DNA helix, required two consecutive additional helix collisions to effect DNA strand passage. Vologodskii and co-workers proposed that a short region of localized high curvature, a hairpin, is created by the topoisomerase and that the DNA strand passage occurs when another DNA helix is enclosed by the hairpin.[4,5] These models are locally "blind" in the sense that they do not let topoisomerase use local information already present on the substrate to determine good places for action. The hairpin, in particular, is placed by the topoisomerase at random points along the strand so that the enzyme must wait until strands are brought into juxtaposition to then act.

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Local structure of juxtapositions

Type-2 topoisomerases bind helix–helix juxtapositions.[6–11] Here, we propose that type-2 topoisomerases use the local information at the juxtaposition to distinguish an entanglement from two helices that are juxtaposed, but not entangled. To illustrate this, we depict in Figure 1 double-stranded DNA as ropes. The position of the ropes in space is determined by their center curves, K_1 and K_2. Let P_1, P_2 denote the points on the curves K_1, K_2, respectively, such that $|P_1 - P_2|$ is minimized. Let p_{12} denote $P_1 - P_2$, and $p_{21} = P_2 - P_1$ denote the separation vectors for P_1 and P_2. The juxtaposition exists when $|p_{12}| < c$, for some predetermined constant c, let us say roughly equal the diameter of a type-2 topoisomerase, ~100 Å. Let T_1, T_2 denote unit tangent vectors to K_1, K_2 at P_1, P_2, respectively. Let r_1, r_2 denote the curvature vectors at P_1 and P_2.

We could classify the juxtapositions by the triple of vectors T_1 T_2 and the separation vector, which would allow us to distinguish between right and left hand. The categories could be further subdivided by considering the magnitudes of the curvatures and subdivisions of the angles between the tangents. Because DNA contains directional readout, that information is also present at a juxtaposition. We can also consider the time derivatives of the vector sets shown in Figure 1E and denote

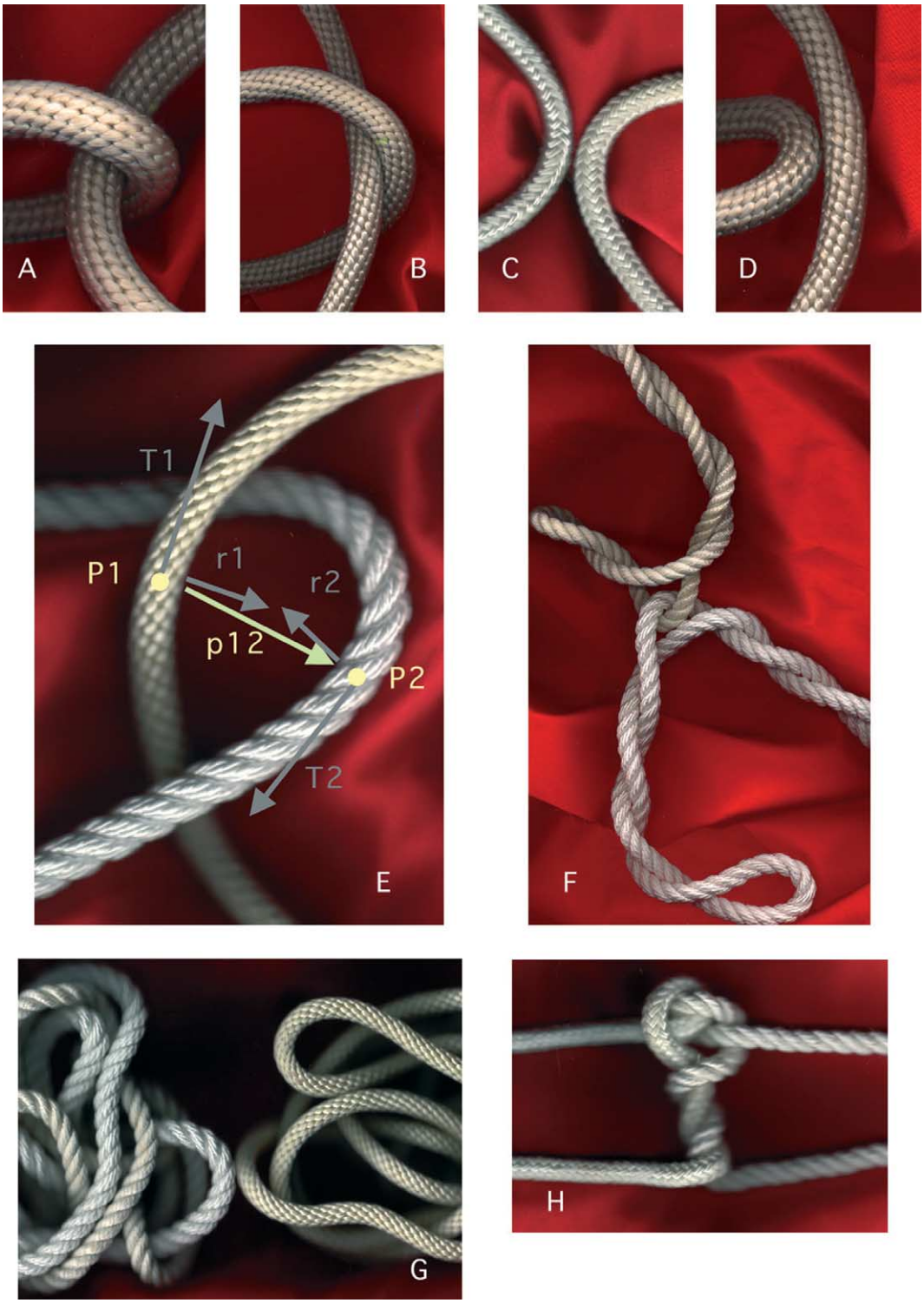

Figure 1. How strands juxtapose. We show four cases. Hooked juxtapositions are cases A and E, where the curves are curved toward each other ($r_1 \cdot p_{21} > 0$ and $r_2 \cdot p_{12} > 0$) and B, where one curve encloses the other ($r_1 \cdot p_{21} < 0$ and $r_2 \cdot p_{12} > 0$ and $r_1 > r_2$). Free juxtapositions are case C, where the curves curve away ($r_1 \cdot p_{21} < 0$ and $r_2 \cdot p_{12} < 0$), and case D, where the inner curve has the greater curvature ($r_1 \cdot p_{21} > 0$ and $r_2 \cdot p_{12} < 0$ and $r_1 > r_2$). Through most of this work we are concerned with cases A and C. F, Supercoiled links tend to have hooked juxtapositions. G, The most exposed sections of loops tend to be those that curve away from an arbitrary path of approach, so unlinked loops tend to have free juxtapositions. H, Two tangled loops being pulled apart, creating hooked juxtapositions.

them P_1t, P_2t, $p_{12}t$, etc. Each of these has geometrical interpretation. $p_{12}t$, for example, can measure the rate at which the closest points tend to come together or separate. If $p_{12}t$ is small, the juxtaposition is persistent. If P_1t is large, but P_2t and $p_{12}t$ are small, we could imagine that K_1 is locally sliding past K_2, an axial motion. Some sort of pulling force or random motion could cause $r_1t > 0$ and $r_2t > 0$.

Methods for recognizing local structure

One way that type-2 topoisomerases might distinguish juxtapositions would be if, built in to the enzyme, was a template into which the juxtaposition fit or not. Alternatively, curvature could be read from the bending strain on the helical structure. Right and left-handed juxtapositions lie upon each other differently,[10] which provides a way for topoisomerases to distinguish handedness.[12,13] Because type-2 topoisomerases have preferred DNA cleavage sequences, it is likely that DNA read-out influences how these enzymes distinguish juxtapositions. Time may come into play. If a type-2 topoisomerase acts with some likelihood on any juxtaposition, then those juxtapositions with greater persistence may be preferentially recognized. With time, fluctuations could place the DNA strands in correct position for the topoisomerase to fit a template. This would happen quickly for juxtapositions that are already near the template shape, slowly for ones that are not. Curvature and persistence are connected through two routes. First, hooked juxtapositions have greater local constraints than free juxtapositions. A simplistic analysis: assume that the two strands are relatively stiff and consider the local rigid motions of K_1, holding K_2 fixed. A basis for these motions is the positive and negative translations in the p_{12}, T_1 and B_1 directions (where B_1 is the binormal at P_1), and the three rotations about these vectors (assume the origin is at P_1). For the hooked juxtaposition in Figure 1A, the motion is constrained in each translational direction except the negative p_{12}, and in two of the three rotations. In the free juxtaposition case in Figure 1C, the motion is constrained only in the negative p_{12} direction of the six translations and in only one of the rotations, and even that is a large angle constraint. Similar considerations show that the hooked in Figure 1B is more constrained than the free in Figure 1D, but the difference is less than for A and C (Figure 1).

Second, it is generally held that two DNA helices must overcome electrostatic repulsion to create a juxtaposition. Consider the potential energy of two like charged strands, spreading the charge out uniformly along the strands (assume unit charge density). We calculate only the effect of each strand upon the other, and not the effect of the strand upon itself (the self-potential of a charged string is well known to have a logarithmic singularity.[14] The assumption is that the bonds along the strand locally counteract the self-potential. Here, strands are bent at a $\pi/2$ angle, and moved toward one another by rigid translation (Figure 2A). The maximum energy takes place when $p > 0$, and decreases through $p = 0$, so that once the strands are close enough, bringing them even closer is energetically favored (Figure 2B). Therefore, hooked juxtapositions are locally attracting (or at least not repelling), and free juxtapositions are repelling.

Similar considerations hold for the angle, α,

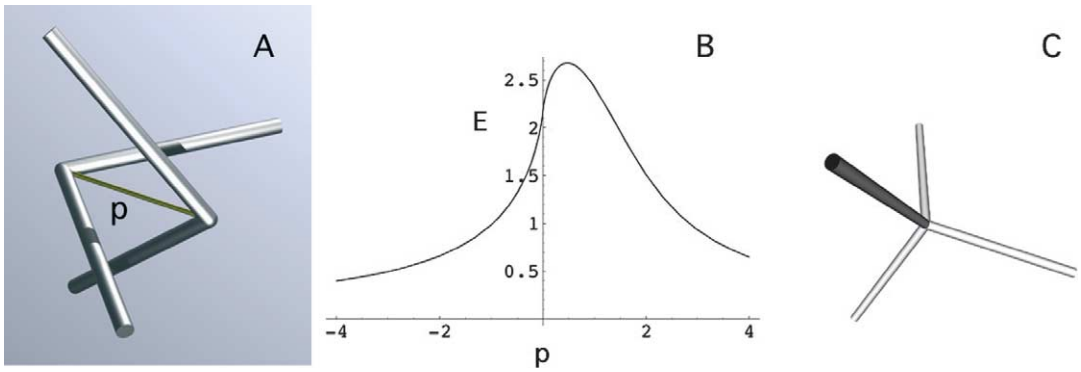

Figure 2. Effect of charge on strand juxtapositions. In B, the x-axis is the separation, p, depicted in A, the y-axis is the electrostatic potential E of the strands upon each other. For this computation, the edges are of length square-root 2, the charge density along the edges is constant 1, and the graph is of the integral of the inverse distance between pairs of points on the edges, where the distance p is the distance between the central vertices of the two angled filaments. The graph was computed by the software Mathematica, the complete code is: $x1[t\_] := t$; $y1[t\_] := t$; $z1[t\_] := 0.0$; $K1[t\_] := \{x1[t], y1[t], z1[t]\}$; $x2[s\_] := 0.0$; $y2[s\_] := -s + m$; $z2[s\_] := s$; $K2[s\_] := \{x2[s], y2[s], z2[s]\}$; CHPL[$v\_$]:= NIntegrate[2/(((K1[t] − K2[s])·(K1[t] − K2[s]))^.5),{t,0,1},{s,0,1}]; Plot[CHPL[v],{m, − 4,4}]. Note that this is the contribution from the interaction of one edge with both edges of the other filament. For the full cross-potential the numerator of the integrand should be 4, which of course does not change the shape of the curve. A, was rendered with Pov-Ray. For C, discrete charges were uniformly spread along the filaments and all interactions between charges were calculated. The endpoints of the strands form a regular tetrahedron and $\alpha = \pi/2$, where α is the angle between the tangents at the juxtaposition. The software Knotplot was employed for both the calculation and the rendering.

between the tangents. In Figure 2C is depicted the result of a numerical computation of the minimum electrostatic energy conformation of two flexible strands that are joined at their center points. If $p = 0$, hooked and free juxtapositions are indistinguishable. The conformation is non-planar and so has non-zero curvature at the midpoint of the strands, an additional connection between curvature and juxtapositions.

How global topology is expressed in local structure

A thought experiment illustrates how type-2 topoisomerases may act. Imagine N (large number) exact (perfectly round) circles moving by random continuous motion within a finite volume. Employ a rule, rule H: if the juxtaposition is hooked, then the strands are passed through one another; otherwise they are not. Because we have exact circles, rule H will not link any pair and, as long as the circles are free to move, it will eventually unlink all linked pairs. So the long-run behavior is N unlinked circles: trivial topology. In contrast, rule F passes DNA strands only in free juxtapositions. There is no unlinking, so in the long run every circle is linked. Rules H and F use local juxtaposition information to arrive at opposite topological outcomes. This result is dependent on the length and rigidity of the circles. That is, as long as the loops are perfect geometric circles, the local geometry completely determines the topology. However, DNA is an elastic filament with bending energy. So increasing length adds geometric flexibility, the loops move further and further away from perfect circle conformations. We can imagine the opposite end of the spectrum from the perfect circle case: very long loops packed at a high density and mixed about one another, think of a bowl of spaghetti. Because the loops are very long, and intertwine with one another, some rule H crossings will now create links. Either rules H or F, applied to a system of arbitrarily long chains in constant density, would tend to the same order of topological complexity proportional to $L^{4/3}$, where L is the total length of the system.[15] Consider an intermediate case: random closed loops of a fixed length, sparsely distributed. Isolated closed loops have fewer rule H than rule F accessibility, meaning that when two isolated loops come into contact, the juxtapositions are more likely to be free than hooked. Rule H will, in this case, decrease linking, though not to the extent it does for perfect circles. In general, the effectiveness of a rule that depends upon local information will depend upon both the length and the density of the DNA strands.

Let $S1$ be the smallest sphere containing loop K_1, $S2$ the smallest sphere containing K_2. Generically, $S1(S2)$ is determined by four points along K_1. At these points the curvature vector points inside $S1(S2)$. Assume K_1 and K_2 are not linked, and brought near one another. The points most likely to be brought into juxtaposition are near these external points (see Figure 1F). However, such juxtapositions cannot be hooked. More generally, place two unit spheres randomly in a large box and compute the conditional probability that, if they intersect, how far apart are their centers. Shallow intersections (distance between centers between 1/2 and 1) are roughly sevenfold more likely than deep (distance between 0 and 1/2) ones. However, shallow intersections tend to give free juxtapositions of unlinked loops. In general, if we approach the loop from an arbitrary direction, then at the points most likely to hit first, the strand is curved away from the direction of approach.

Therefore, we define rule H (in)accessibility. A tightly wound ball of string is perfectly rule H inaccessible: from all directions of approach the string curves away at the point of first incidence. Two compact globules are very unlikely to have hooked juxtapositions when they first meet. On the other hand, the external loops of any conformation provide opportunities for rule F crossing change. In contrast, let K_1 and K_2 be linked. Under random motion, their centers drift apart. This eventually provides a kind of "pulling apart" force, which results in hooked juxtapositions. A rule H move, or a series of rule H moves, will unlink them.

The analysis for untying knots is similar to that for unlinking catenanes. The principal difference is that knots are built out of several partial loops. A typical example is pictured in Figure 3. If the DNA were a phantom chain (allowing every strand to pass through), then the labeled crossing change would create a trefoil, the DNA strand behind must pass through the loop. The loop has a *de facto* inside and outside. Getting from the outside to the inside cannot be a rule H move. Thus, by rule H, knots are not likely to be tied, especially for the short lengths of a plasmid. On the other hand, if we start with a trefoil knot, then a bend in the strand passing through the loop, even a slight one, creates a hooked juxtaposition, so rule H unties the knot. This effect is more pronounced in

Figure 3. Random strand passage would generate a knot. A strand crossing change at the indicated position would create a trefoil, but this is unlikely to happen by rule H.

knots than in catenanes of the same length, because the subarcs of the knot are the loops and these loops are even shorter in length than the catenane loops. As with links, the proportion of hooked to free juxtapositions in a knot depends upon strand length and density.

In numerical work, it has been observed that long, random chains tend to localize a good portion of their knotting.[16,17] By inspection, we observe that these conformations usually have outer arcs bent around inner lengths of lesser curvature. These are hooked juxtapositions by our definition.[15] These conformations are not generic in our sense because they have continuous families of closest pairs of points, but they help illustrate the role of the length constraints.

Additional experimental results and our interpretations

Type-2 topoisomerases preferentially recognize and remove the biologically problematic DNA topological obstructions, knots and catenanes, over biologically required DNA supercoils.[18–21] DNA supercoiling increases the effectiveness of type-2 topoisomerases in unlinking catenanes, and has a lesser or no effect on knots.[20–23] These results are not reconciled by any existing model. Our postulate that type-2 topoisomerase follow rule H explains these data. For a supercoiled closed loop, an approach from any direction first hits a subarc curving away from the direction of approach, so that the probability of a hooked juxtaposition occurring between two loops is nearly 0. If the supercoiled loops are linked already, a juxtaposition is likely to be hooked (see Figure 1F). If a minimum curvature threshold exists for rule H action, then supercoiling makes this more likely to be reached. The case of a supercoiled knot is different. Because the strands in a knot node are already interacting with each other, supercoiling would have a lesser effect because these juxtapositions are already rule H friendly. Thus, rule H will unlink supercoiled catenanes more efficiently than it will supercoiled knots. Because the curvature in the juxtaposition between the loops shown in Figure 1F is greater than that in the juxtapositions created by supercoiling, a type-2 topoisomerase would unlink or unknot before it would relax supercoils.

To model a system such as that in the Rybenkov *et al*. experiment,[1] we reason dynamically, and assume that the system will equilibrate at $M = L/U$, where L is the rate of linking, and U is the rate of unlinking. For example, for rule H on perfect circles, $L = 0$ and $U > 0$, so the limit is 0. For rule F on perfect circles, $L > 0$, $U = 0$, so the limit is $M =$ infinity (all loops are linked). Above we observed that in the catenane case supercoiling decreases L and increases U. The experimental system used in which DNA loops were opened and closed with some frequency[1] should be roughly equivalent to a rule where some fixed percentage of all juxtapositions allows DNA strand passage (so rule F pass throughs are allowed). When a type-2 topoisomerase was introduced, the equilibrium was lowered. A type-2 topoisomerase has a lower ratio of L to U than a "blind" system that cannot distinguish between types of juxtapositions. Let L_b and U_b be the constants for this blind system, L_{to} and U_{to} be the constants for a system governed by rule H. Then the equilibrium is $M = (L_b + L_{to})/(U_b + U_{to})$. Many factors determine L and U. At least two can create a difference in the speeds of the reactions. The loops may open and close at speeds differing from type-2 topoisomerase reaction speed. Also, the opening and closing of one of the loops in this system occurs at only one locus along the DNA strand, but topoisomerase should be free to act anywhere along the loop. Therefore, even if the gate opened and closed with speed comparable to enzyme action, the effective speed would be reduced by the proportion of the size of the gate to the arclength of the loop. Hence, the natural assumption that the equilibrium of the combined system is close to L_{to}/U_{to}. For knots, we let K denote the knotting constant and U the unknotting constant. Our analysis gives a prediction for the dependencies of $(L_b/U_b)/(L_{to}/U_{to})$ and $(K_b/U_b)/(K_{to}/U_{to})$ on loop length, which is depicted schematically in Figure 4A.

We can relate Figure 4A to the experimental evidence:[1] a 16-fold reduction (relative to what was termed "topological equilibrium") in linking in loops of length 10 kb (thus 20 kb total length), a 40-fold reduction in knotting in loops of length 10 kb, and an 800-fold reduction in knotting in loops of length 7 kb (Figure 4B). As illustrated in Figure 4A, there is a vertical asymptote at length = C. Our discussion of rule H inaccessibility gave a rough estimate of $D = 8$ for longer loops in low-density situations. The limiting value D depends on density, but we can estimate that under similar experimental conditions, at 50 kb or 100 kb, the ratio would be nearly D.

Rybenkov *et al*. reported that type-2 topoisomerase reduces the variance of the linking number distribution for a plasmid DNA, meaning that the enzyme creates a distribution of supercoiling more tightly clustered about 0 than a random generation method.[1] We interpret these findings to mean that greater supercoiling, on average, creates more hooked juxtapositions with higher curvature (because length is fixed), so the loops with greater supercoiling are more likely to be acted upon by rule H.

In the Rybenkov experiments,[1] in the measurement of entanglement as a function of enzyme concentration, it was seen that entanglement declines with increasing concentration, reaches a minimum, then increases with increasing concentration. We can explain this as follows. In the experiments, the loops are opening and closing at some rate. When the enzyme concentration is very low, the gates, on average, create entanglements faster than the

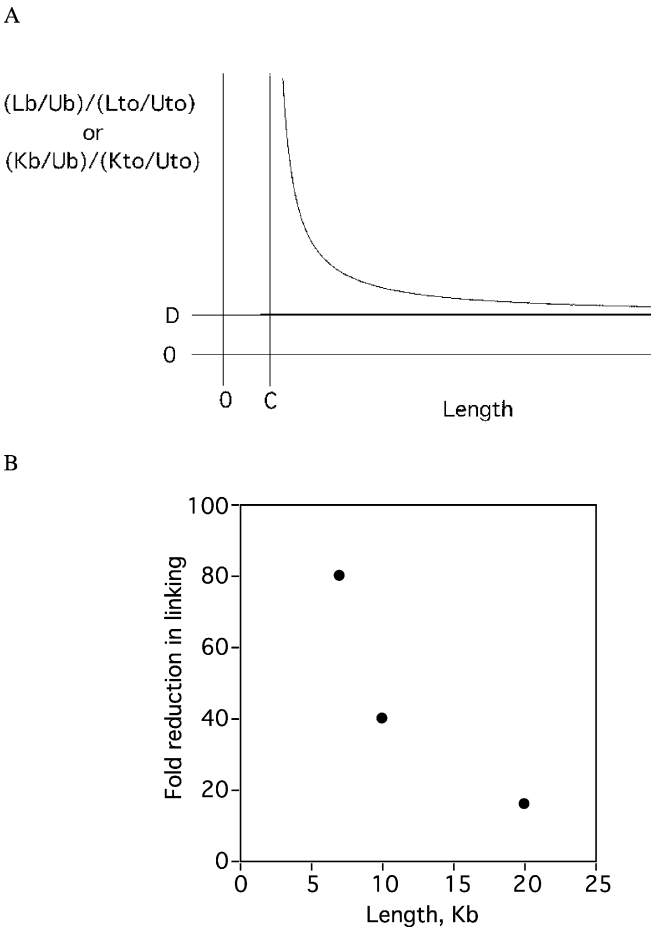

Figure 4. Length dependence of knotting and linking. A, The length is the length of an individual loop. We assume that the strands have some elastic resistance to bending, some thickness, and that the overall density is low. For linking, the asymptote at length = C: as the circles get shorter, they must become more like perfect circles because of the bending constraints. However, rule H is perfect on perfect circles, so $L_{to}/U_{to} = 0$, but $L_b/U_b > 0$, hence the vertical asymptote. The horizontal asymptote at $(L_b/U_b)/(L_{to}/U_{to}) = D$ as length tends to infinity. Given rule H inaccessibility, we have that even very long loops, if density is sufficiently low, are less likely to be linked under rule H than by arbitrary passage, hence the limit at $D > 1$. If instead we allow the density to increase as we increase length, so the loops become both very long and completely intermingled, then we conjecture that $D = 1$ and topoisomerase cannot lower the equilibrium. We expect different values for C and D for knots. The asymptote at length = C: as the length gets small, the ratio of free to hooked juxtapositions in knots goes to zero, knots are formed by subarcs of the circle that themselves, because of the constraints, behave like perfect circles in that they are rule H inaccessible. (In tight conformations the curvature of one strand is greater than that of the strand passing through it). The horizontal asymptote at $(K_b/U_b)/(K_{to}/U_{to}) = D$ as length tends to infinity: this case is similar to the linking analysis, in that the density is an important factor. The mathematical theory here is incomplete, but models based on random walks in unconstrained spaces show that chains tend to localize at least some of their knotting and tangling.[16,17] The curvature is likely to be high at the localization, ripe for rule H action. In a localized knot component, the effective length of that part of the strand is small, so we effectively are close to the value length = C, so we expect that type-2 topoisomerase would be much more likely to remove a localized knot that to create one. On the other hand, it has been shown that in unconstrained random walks there is at least some global knotting, so the determination of D in this model is an open question. An unconstrained random walk is only a model for DNA, and clearly has some deficiencies as a model for DNA in the cell, where the packing is not random and there are severe volumetric constraints. Again, the mathematical theory is lacking, but it is plausible that a volume constraint would give a greater percentage of global knotting, thus reducing D for random chains. B, Data are from Rybenkov *et al.*[1] The fold reduction is relative to the amount of linking (or knotting) seen in equilibrium without type-2 topoisomerase.

enzyme can act, thus near the equilibrium value of entanglement. As the concentration increases, the enzyme can, on average, act faster, and so detangle the system. To see what happens when concentration increases beyond biological levels, we consider further the connection between hooked juxtapositions and persistence. If the loops are long enough, then two unlinked loops may meet in a hooked juxtaposition. Our argument is not that hooked juxtapositions are impossible for unlinked loops, just that they are less likely than free ones. Because they are unlinked, this juxtaposition is not likely to be as persistent as a hooked juxtaposition of linked loops. The salient point is that there is information in the length of time juxtaposition persists. If the concentration of the enzyme is very high, then all hooked juxtapositions, both those that would have been transient and those that would have been persistent, are acted on immediately. So this persistence information is lost, as a result of which the ratio of persistent to transient hooked juxtapositions acted upon is given by the ratio of the rates at which the respective juxtapositions are formed. If, on the other hand, the average time it takes the enzyme to act is long (lower concentration), then the proportion of transient to persistent acted upon now depends on the length of time the juxtapositions persist. We imagine that this phenomenon also happens in the cell, that for this reason too much enzyme could actually slow disentanglement and therefore cell division.

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