MOLECULAR BIOLOGY

Of Topo and Maxwell's Dream

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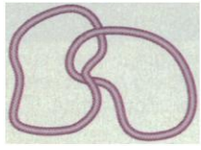
Discovery of unexpected greatness in nature is always delightful. We are treated on page 690 of this issue to a report by Rybenkov *et al.* (*1*) of grandiose behavior among the DNA topoisomerases, enzymes that change the topology of DNA. They show that the most abundant eukaryotic topoisomerase II (topo II) (*2*) and the closely related *Escherichia coli* topoisomerase IV (topo IV) (*3*) can each—almost without error—recognize and undo a knot in a DNA circle many times larger than themselves, forcing a population of coiled, catenated, and knotted DNAs to an untangled state far from equilibrium.

The ability of topoisomerases to alter the linked states of DNA is essential for cell survival. These enzymes fall into two broad categories: the type I and the type II enzymes, which are distinguished by the number of DNA single strands (one or two) cleaved during each reaction cycle. Although both types of topoisomerase are required for chromosome replication, recombination, transcription, and segregation, the individual enzymes make different contributions to each of these (*4*).

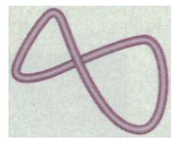


Catenated DNA

The subjects of the new work, topo II/IVs, are proficient in DNA decatenation and unknotting. In these reactions, a DNA double helix is transiently cleaved to allow passage of another, and the break is then resealed (*5*, *6*). Although gyrase, the first of the type II class to be discovered, can catalyze these reactions (*7*), its activity is not sufficient to meet the needs of *E. coli* in segregating its chromosomes during cell division. In this organism, topo IV is needed to complete the job (*6*).

Topoisomerization reactions have no intrinsic energy requirement. The chemical energy of the phosphodiester bond (or bonds) is retained within the DNA-enzyme complex through covalent attachment of the phosphates from cleaved DNA strands to tyrosine residues in the enzyme and is thus available for driving reattachment after strand passage. Nevertheless some topoisomerases have adenosine triphosphatase (ATPase) activities that are related to their functions. Gyrase uses ATP hydrolysis to forcibly unwind its substrate DNA molecule (*8*). Reverse gyrase, a special type I topo from thermophilic organisms, uses ATP for the opposite process of overwinding DNA (*9*).

Topo II/IVs are homologous to gyrase and require ATP. They do not supercoil DNA and so, until Rybenkov *et al.*'s new report, had no obvious reason to require ATP's assistance. Indeed, an unusual type II topoisomerase isolated from trypanosomes does not require ATP (*10*), and yeast topo II can complete a single decatenation reaction in the presence of a noncleavable ATP analog (*11*).

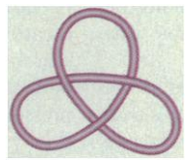


Supercoiled DNA

Previous measurements of topo II/IV's prowess have not addressed the question of whether the product population of DNA molecules represents an equilibrium state under the conditions of the reaction (*12*). In a common assay for topo II/IV, catenated forms (chain-linked circles) are made in the presence of a DNA-condensing agent that effectively raises the DNA concentration to that of moist, solid DNA. At these concentrations catenation is so strongly favored that it is not possible to determine whether equilibrium is ever reached.

In three separate measurements Rybenkov and colleagues have now answered this question in the negative. In two of these, the products were shown to contain as few as 1/80th as many knotted or catenated DNA molecules as are created by ligation of linear molecules and circles under equivalent conditions. In effect, topo II/IVs act as specific DNA "anti-kitten" to undo the entropically favored tangled states that arise at thermal equilibrium. We now know why topo II/IV requires ATP—to drive the population away from equilibrium.

In their third measurement Rybenkov *et al.* addressed a peculiar but very revealing question. When a circular DNA sample is closed by a ligation reaction, the molecules are trapped in a series of topological states (topoisomers) that result from fixing of the number of twists (linking number) of the intertwined strands by the closing event. The relative abundance of each of these topological states is determined by thermal motion and the elastic modulus of the DNA (*13*, *14*). This variation in linking number results from an equilibration process: An identical family of topoisomers was generated when a single topoisomer from the distribution was allowed to equilibrate with the other family members in the presence of a eukaryotic topoisomerase I (*14*), an enzyme that transiently nicks one strand, allows rotation, and then reseals the break (*15*).



Knotted DNA

Rybenkov *et al.* show that if a topoisomer population previously equilibrated by topoisomerase I is subsequently treated with topo II/IV—without altering the conditions—a new topoisomer population is generated that has the same mean linking number as the first, but that has fewer topoisomers with linking numbers that diverge from the mean. It is as if the enzyme were able to cool the DNA to a very low temperature, without causing any change in mean linking number. This is a truly unexpected outcome. Were it not for the fact that these enzymes are ATPases, their anti-kitten activity would double as a very superior Maxwell's demon who, recognizing a hot molecule on its way out of his box, prevents its return by slamming the door. Topo II/IV is indeed dexterous.

Despite the thermodynamic sense now made of the ATP requirement for topo II/IV, the fun has just begun. Unlike ATP hydrolysis by gyrase and reverse gyrase, topo II/IV does not impose a unique direction on the strand passage reaction. Instead, the direction of the strand passage is (on average) determined by the linkage state of the strands being passed. It is easy to imagine how a large object may inspect a smaller one, but it is difficult to see how an enzyme such as topo II/IV could inspect an enormously larger DNA molecule to determine its state of catenation or knotting. Our anti-kitten has the relative body mass of a flea. As Rybenkov *et al.* point out, the most obvious possibility—that many enzyme molecules work together to perform a function that one could not—is ruled out by lack of cooperativity in the action of topo II (*11*).

Instead they propose that the enzyme expands its view of the DNA by first binding to two distantly located sites and then sliding along the DNA to the point where the two come together. In the event that another DNA segment is trapped between the enzyme and the DNA molecule to

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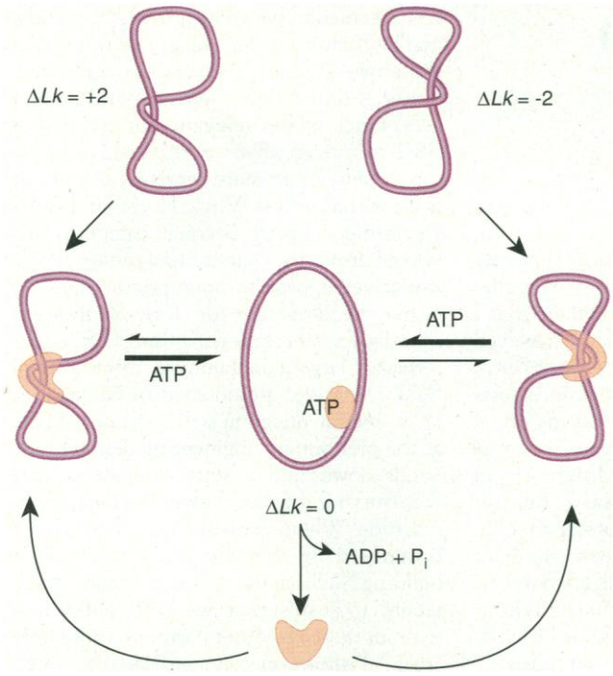

Proposed mechanism for Topo II–induced change in topoisomer distribution. ATP-directed unkinking associated with strand transfer reduces the relative concentration of topoisomers with either right- or left-handed superhelical turns relative to the concentration of a topoisomer having no supercoils. P; inorganic phosphate.

which it initially bound, a strand passage reaction either decatenates [see figure 4A in (*1*)] or unknots [figure 4B in (*1*)] the molecules as the situation demands. This idea addresses the kinetics of locating topological links. But how can we address the thermodynamic question of how ATP hydrolysis is coupled to give direction to the reaction?

The model can be modified to give direction to the sliding process by ATP hydrolysis, in effect committing the sliding clamps to converge. Catenated or knotted molecules would then be efficiently trapped so that they could be unlinked. If each clamp inadvertently associated with a different, unlinked molecule, trapping would never occur. Decatenation and unknotting would be selective, and the hydrolysis of ATP would have a defined function. Even with this amendment, the model appears to have an (acknowledged) difficulty [see figure 4C in (*1*)] in accounting for the increased homogeneity in linking number upon reaction with unknotted circular molecules, because in this case there is no trapped segment of DNA.

The action of topo II/IV on supercoiled DNA [figure 4C in (*1*)] provides a test that may discriminate between the two models. A topoisomer family interacting with topoisomerase I loses its equilibrium character as soon as the enzyme is inactivated. When the structures of molecules having fewer or more helical turns in the backbone are time averaged, negative or positive superhelical turns will be present. The nonzero writhe component of these superhelical turns can be detected by its effect on the electrophoretic mobilities of the topoisomers. A single kink in a DNA molecule cannot constrain writhe, whereas a pair of kinks can (see the figure). Although an enzyme molecule that binds to an isolated kink cannot by itself discriminate between a positive and a negative superhelical turn, it will reduce the total number of superhelical turns if in the course of strand passage, it unbends the kink that is initially detected. The proposed kinkase activity of our anti-kitten, therefore, provides a simple explanation for a reduced topoisomer dispersion in a family after this enzyme has operated.

To the best of my knowledge this is the first time it has been suggested that an enzyme can use a rare and transient conformational state in its substrate to direct an outcome. A quantitative treatment of each model will be difficult but necessary before the system can be fully understood. There are subtleties here that will entertain us for some time to come.

References

1. V. V. Rybenkov, C. Ullsperger, A. V. Vologodskii, N. R. Cozzarelli, *Science* **277**, 690 (1997).
2. T. Hsieh and D. Brutlag, *Cell* **21**, 115 (1980).
3. J. Kato *et al.*, *ibid.* **63**, 393 (1990).
4. J. C. Wang, *Annu. Rev. Biochem.* **65**, 635 (1996).
5. L. F. Liu, C. C. Liu, B. M. Alberts, *Cell* **19**, 697 (1980); T. Hsieh and D. Brutlag, *ibid.* **21**, 115 (1980).
6. J. Kato *et al.*, *ibid.* **63**, 393 (1990).
7. K. Mizuuchi, L. M. Fisher, M. H. O'Dea, M. Gellert, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1847 (1980); K. N. Kreuzer and N. R. Cozzarelli, *Cell* **20**, 245 (1980).
8. M. Gellert, K. Mizuuchi, M. H. O'Dea, H. A. Nash, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3872 (1976).
9. A. Kikuchi and K. Asai, *Nature* **309**, 677 (1984).
10. S. Douc-Rasy, A. Kayser, J. F. Riou, G. Riou, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7152 (1986).
11. J. Roca and J. C. Wang, *Cell* **77**, 609 (1994).
12. L. F. Liu, J. L. Davis, R. Calendar, *Nucleic Acids Res.* **9**, 3979 (1981).
13. D. E. Depew and J. C. Wang, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4275 (1975).
14. D. E. Pulleyblank, M. Shure, D. Tang, J. Vinograd, H. P. Vosberg, *ibid.*, p. 4280.
15. J. Champoux and R. Dulbecco, *ibid.* **69**, 143 (1972).

WEBWATCH

Virtual telescope

http://skyview.gsfc.nasa.gov/skyview.html

SkyView is NASA's virtual observatory on the Web. Both professional and amateur astronomers will find this cleanly designed Web site useful for calling up images of any portion of the sky at wavelengths from radio to gamma rays. Several interfaces are available, from basic to advanced, including a Java application for interactively searching a large number of survey databases. With its extensive definitions and glossary, the site is very helpful to nonastronomers interested in learning more about the field.

Viruses A to Z

http://www.tulane.edu/~dmsander/garryfavweb.html

Virology information is replicating rapidly on the Web, and this site—ambitiously entitled "All the Virology on the WWW"—is one of the better-designed and more comprehensive sources. Hosted by R. F. Garry's laboratory at Tulane University, ATVOTW contains many links to virology labs around the world, online tutorials, and virology dictionaries. Not to be missed is the Big Picture Book of Viruses, which tabulates a large number of DNA- and RNA-containing viruses along with related images and links.

Small, smaller, smallest

http://www.pbrc.hawaii.edu/~kunkel/

D. Kunkel's Microscopy Web site at the University of Hawaii is a striking collection of photomicrographs of microbes, insects, and crystals of many kinds. In addition to the aesthetically compelling pictures, there is much of scientific interest, including discussions of different kinds of microscopy and links to microscopy sites. The site's "Zoom In" feature allows the user to enlarge selected portions of several organisms.

Edited by David Voss

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