

Evolutionary twist on topoisomerases: Conversion of gyrase to topoisomerase IV

Keir C. Neuman¹

Laboratory of Molecular Biophysics, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892

Type II topoisomerases are essential enzymes found in all cells, where they maintain the topology of DNA in a well-defined state (1). The defining activity of type II topoisomerases is an ATP-dependent passage of a segment of dsDNA through a transient double-stranded break in a second segment of dsDNA (Fig. 1) (1, 2). Despite sharing the same core strand passage mechanism, different type II topoisomerases display dramatically different substrate specificities and topological activities. The molecular basis for these differences and their evolutionary origins remains speculative. There is mounting evidence that the differences are encoded in the poorly conserved C-terminal domains (CTDs) (3–6). Similarly, the high degree of homology among type II topoisomerases is clear evidence that they are evolutionarily related, but the evolutionary pathway and the identity of the original type II topoisomerase are unknown (7). In PNAS, the article by Tretter et al. (8) provides biochemical, structural, and evolutionary results showing the conversion of one type II topoisomerase into another. Their results enlarge our understanding of the molecular and structural basis governing the topological specificity and activity of type II topoisomerases, illustrate how these features may have evolved from a common ancestor, and force reconsideration of notions concerning the complement of essential bacterial genes.

Bacteria typically possess two type II topoisomerases: DNA gyrase, which maintains DNA in a slightly unwound (negatively supercoiled) state, and topoisomerase IV (Topo IV), which is primarily responsible for unlinking newly replicated DNA (1). Gyrase is thought to be essential, because some bacteria seem to lack Topo IV (7). These two enzymes are heterotetramers (A_2B_2), share a great deal of sequence homology, and perform the same core strand passage reaction (Fig. 1). However, the substrate selection and activity of the two enzymes are strikingly different. DNA gyrase ($GyrA_2GyrB_2$) introduces a right-handed wrap in the DNA, which, after strand passage, results in the introduction of negative supercoils (Fig. 1). Topo IV ($ParC_2ParE_2$), in contrast, is an efficient decatenase that can also relax supercoiled DNA, although it is more active on posi-

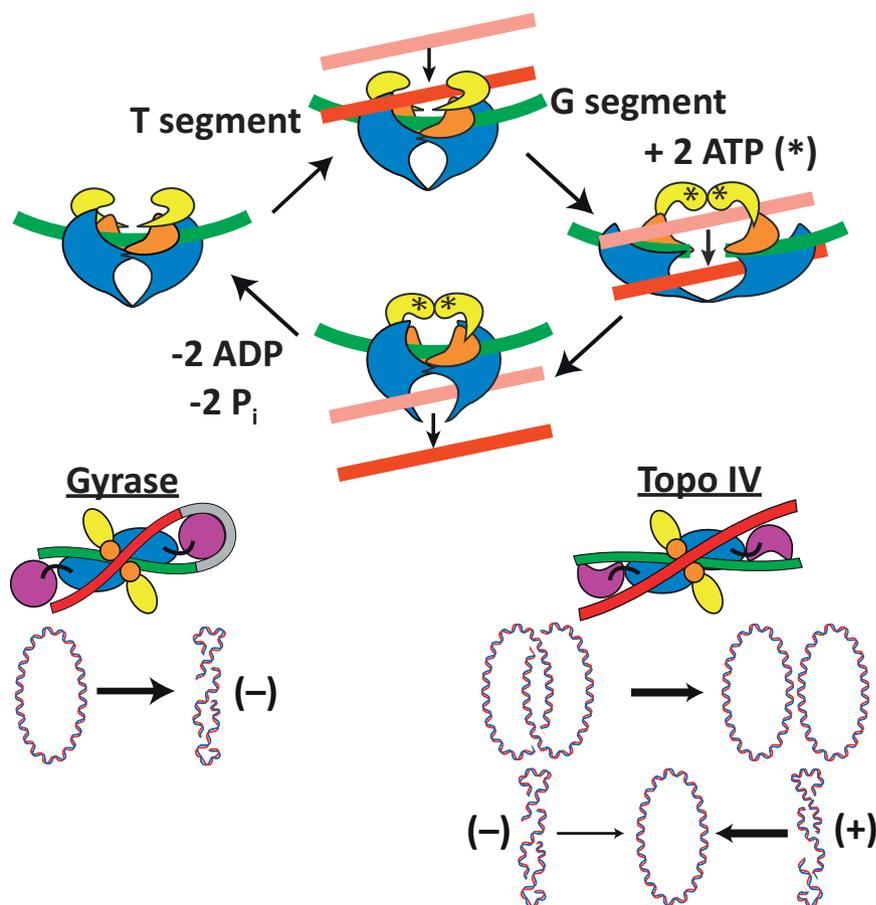


Fig. 1. Core type II topoisomerase strand passage mechanism. (Upper, clockwise from top) Type II topoisomerase (blue, salmon, and yellow) binds gate segment DNA (green) and subsequently captures a transfer segment DNA (pink to red denoting movement of the DNA) followed by the binding of two ATP molecules, which close the N-terminal gate (yellow). This is followed by double-strand cleavage of the gate strand and passage of the transfer strand across the cleaved DNA and through the enzyme. The transfer strand and products of ATP hydrolysis are then released as the enzyme resets for another enzymatic cycle. This core strand passage mechanism is coupled with substrate specificity to achieve different topological activities. Gyrase (Lower Left, blue, salmon, and yellow with purple GyrA CTD) wraps DNA around its GyrA CTD, resulting in the formation of a left-handed DNA crossing, which is converted into a negative supercoil after passage of the transfer segment (red) through the gate segment (green). Topo IV (Lower Right, blue, salmon, and yellow with purple ParC CTD) unlinks catenated DNA molecules and relaxes positive supercoils more efficiently than negative supercoils.

tively than negatively supercoiled DNA (Fig. 1). The determinants of substrate specificity for both topoisomerases have been mapped to the CTDs of their GyrA and ParC domains (4–6). Removal of the CTDs results in type II topoisomerases that indiscriminately relax positively and negatively supercoiled DNA and unlink catenated DNA molecules (4, 5). The GyrA CTD is responsible for the wrap imposed on DNA by gyrase, whereas the ParC CTD slightly bends DNA and provides substrate selection through a de-

bated mechanism (9–11). Also under debate is the evolutionary order: did one of the two type II topoisomerases evolve from the other, and if so, what molecular determinants underlie the change in specificity and topological activity?

Author contributions: K.C.N. wrote the paper.

The author declares no conflict of interest.

See companion article on page 22055 in issue 51 of volume 107.

¹E-mail: neumankc@nhlbi.nih.gov.

Tretter et al. (8) adopt an elegant approach to investigate the structural, biochemical, and evolutionary relationship between bacterial DNA gyrase and topoisomerase IV. They identify an ancient bacteria, *Aquifex aeolicus*, with a single type II topoisomerase classified as a DNA gyrase based on sequence analysis but lacking the GyrA box essential for negative supercoiling (6). They crystallized the *A. aeolicus* GyrA CTD, finding a six-bladed disk similar to other GyrA CTDs, except that the lack of the GyrA box results in an open configuration of the disk resembling a ParC CTD (8). Thus, structurally, the *A. aeolicus* GyrA CTD resembles a ParC CTD rather than a canonical GyrA CTD. The biochemical ramifications were determined by measuring the topological activity of *A. aeolicus* type II topoisomerase. The holoenzyme was unable to negatively supercoil DNA, instead displaying robust decatenation and supercoil relaxation suggestive of Topo IV. The lack of gyrase activity is surprising, because it is a purportedly essential bacterial gene. However, the *A. aeolicus* genome contains many genes from archaea, among which the most hyperthermophilic species lack gyrase (12). Nevertheless, this finding forces reconsideration of the absolute necessity of gyrase among bacteria. In an important extension of their biochemical characterization of the *A. aeolicus* type II topoisomerase, Tretter et al. (8) find that it relaxed positively supercoiled DNA more efficiently than negatively supercoiled DNA, a hallmark of Topo IV. This finding provides a basis to define Topo IV substrate specificity at the molecular level and suggests that, in this instance, Topo IV may have evolved directly from gyrase. This raises an interesting question: is the substrate specificity of Topo IV (i.e., its preference for relaxing positive vs. negative supercoils) an essential feature of the enzyme, or is it an evolutionary holdover from a gyrase predecessor?

Following up on the evolutionary placement of *A. aeolicus* type II topoisomerase, which is near the point of divergence between the ParC/GyrA CTD, Tretter et al. (8) show that the *A. aeolicus* GyrB subunit forms an active gyrase when mixed with *Escherichia coli* GyrA, whereas *E. coli* ParE mixed with *E. coli* GyrA is inactive. Furthermore, substitution of the *A. aeolicus* GyrA CTD with the GyrA CTD containing an intact GyrA box from another

The picture that emerges from this work is that a gyrase lacking the GyrA box acts as a genuine Topo IV.

hyperthermophilic species recapitulated the negative supercoiling activity of gyrase. The authors accomplished a remarkable achievement in converting the activity of a type II topoisomerase between two paralogs (gyrase and Topo IV).

The picture that emerges from this work is that a gyrase lacking the GyrA box acts as a genuine Topo IV. Previously, it was unclear if Topo IV substrate preference and activity were encoded elsewhere in the ParC CTD. Now, a compelling case can be made for Topo IV activity arising from the opening or a distortion of the GyrA CTD. More generally, the chimera experiments revealed how evolutionarily close the *A. aeolicus* enzyme is to gyrase and suggest a pathway through which Topo IV evolved from the gyrase.

This work raises a number of interesting questions and suggests several lines of additional inquiry. From a synthetic biology perspective, probing the generality and reversibility of the findings should prove fruitful. For example, does deletion or modification of the *E. coli* GyrA box

result in a true Topo IV, or are there additional evolutionary modifications of the *E. coli* enzymes rendering a more complicated and subtle functional relationship between gyrase and Topo IV? A similar line of reasoning suggests that insertion of an appropriate GyrA box in the *A. aeolicus* GyrA CTD should restore gyrase activity. Finally, if Topo IV evolved from gyrase, and more generally, an ancestral gyrase was the origin of all type II topoisomerases, the ATP requirement (2) of type II topoisomerases may simply be a vestige of the ancestral gyrase, which required the energy of ATP hydrolysis to perform the energetically costly process of negatively supercoiling DNA.

In summary, the work by Tretter et al. (8) firmly establishes the importance of the CTDs in modulating and controlling the activity of type II topoisomerases. The current findings provide compelling biochemical and evolutionary evidence for the direct conversion of a gyrase into a Topo IV. Moreover, the finding of a bacterial species lacking gyrase questions strict conservation of bacterial gyrase and points to a more sensitive criteria for determining the identity of bacterial type II topoisomerases based solely on their sequence. It is tempting to speculate that the CTDs may harbor additional regulatory functions besides the striking example described here. Indeed, recent work has shown that the condensin MukB physically interacts with the ParC CTD, stimulating the activity of Topo IV and coordinating the condensing and unlinking activities (13, 14). Further work on the modulation of type II topoisomerase activity by the CTDs will likely reveal additional insights and surprises.

ACKNOWLEDGMENTS. I thank Ashley Hardin for preparation of the figure. Work in my laboratory is supported by the intramural research program of the National Institutes of Health, National Heart, Lung, and Blood Institute.

- Wang JC (2002) Cellular roles of DNA topoisomerases: A molecular perspective. *Nat Rev Mol Cell Biol* 3: 430–440.
- Schoeffler AJ, Berger JM (2008) DNA topoisomerases: Harnessing and constraining energy to govern chromosome topology. *Q Rev Biophys* 41:41–101.
- McClendon AK, Rodriguez AC, Osheroff N (2005) Human topoisomerase II α rapidly relaxes positively supercoiled DNA: Implications for enzyme action ahead of replication forks. *J Biol Chem* 280:39337–39345.
- Corbett KD, Schoeffler AJ, Thomsen ND, Berger JM (2005) The structural basis for substrate specificity in DNA topoisomerase IV. *J Mol Biol* 351:545–561.
- Kampranis SC, Maxwell A (1996) Conversion of DNA gyrase into a conventional type II topoisomerase. *Proc Natl Acad Sci USA* 93:14416–14421.
- Kramlinger VM, Hiasa H (2006) The “GyrA-box” is required for the ability of DNA gyrase to wrap DNA and catalyze the supercoiling reaction. *J Biol Chem* 281: 3738–3742.
- Forterre P, Gribaldo S, Gadelle D, Serre MC (2007) Origin and evolution of DNA topoisomerases. *Biochimie* 89:427–446.
- Tretter EM, Lerman JC, Berger JM (2010) A naturally chimeric type IIA topoisomerase in *Aquifex aeolicus* highlights an evolutionary path for the emergence of functional paralogs. *Proc Natl Acad Sci USA* 107: 22055–22059.
- Stone MD, et al. (2003) Chirality sensing by *Escherichia coli* topoisomerase IV and the mechanism of type II topoisomerases. *Proc Natl Acad Sci USA* 100:8654–8659.
- Charvin G, Bensimon D, Croquette V (2003) Single-molecule study of DNA unlinking by eukaryotic and prokaryotic type-II topoisomerases. *Proc Natl Acad Sci USA* 100:9820–9825.
- Neuman KC, Charvin G, Bensimon D, Croquette V (2009) Mechanisms of chiral discrimination by topoisomerase IV. *Proc Natl Acad Sci USA* 106:6986–6991.
- Deckert G, et al. (1998) The complete genome of the hyperthermophilic bacterium *Aquifex aeolicus*. *Nature* 392:353–358.
- Li Y, et al. (2010) *Escherichia coli* condensin MukB stimulates topoisomerase IV activity by a direct physical interaction. *Proc Natl Acad Sci USA* 107: 18832–18837.
- Hayama R, Mariani KJ (2010) Physical and functional interaction between the condensin MukB and the decatenase topoisomerase IV in *Escherichia coli*. *Proc Natl Acad Sci USA* 107:18826–18831.