Published in final edited form as:

J Mol Biol. 2013 September 9; 425(17): 3025–3028. doi:10.1016/j.jmb.2013.07.004.

The tail that wags the dog: Topoisomerase IV ParC C-Terminal domain controls strand passage activity through multipartite topology-dependent interactions with DNA

Keir C. Neuman

Laboratory of Molecular Biophysics, National Heart, Lung, and Blood Institute, National Institutes of Health. Bethesda, MD 20892. neumankc@mail.nih.gov

Type IIA Topoisomerases are essential enzymes that contribute to chromosomal integrity by controlling the degree of supercoiling and segregating newly replicated chromosomes¹. They share a conserved core mechanism in which a double-stranded segment of DNA is passed through a transient double-stranded break in a second segment of DNA (Figure 1). Remarkably, this conserved strand passage reaction results in a range of distinct activities catalyzed by different but related type IIA topoisomerases². At one extreme is prokaryotic DNA gyrase that negatively supercoils DNA, but is a poor decatenase. At the other extreme is the closely related prokaryotic topoisomerase IV that is an efficient decatenase and relaxes positive supercoils more efficiently than negative supercoils¹. Eukaryotic type IIA topoisomerases display similar, though less extreme, topologically-dependent differences in activity³. For example, human topoisomerase II α preferentially relaxes positively supercoiled DNA, whereas the other human isoform, topoisomerase II β , relaxes positively and negatively supercoiled DNA with equal efficiency. The differences in activity among these enzymes can largely be attributed to differences in the poorly conserved C-terminal domains (CTDs), which contain a highly positively charged DNA binding surface⁴. Deletion of the gyrase GyrA CTD or topoisomerase IV ParC CTD results in a type IIA topoisomerase lacking chiral preference in relaxation and which no longer introduces negative supercoils^{4, 5}. Indeed, chiral discrimination by topoisomerase IIA enzymes appears to be entirely dictated by the CTD domain. The chirality-dependent activity of chimeras between the human topoisomerase II α and β core enzymes with the alternative CTDs is almost entirely dictated by the CTDs rather than the core enzyme⁶. In addition, subtle mutations in a gyrase GyrA CTD result in an enzyme that exhibits topoisomerase IV-like chirality-dependent strand passage activity⁷. Whereas the domain responsible for chiral discrimination has been identified, the mechanistic basis for chiral discrimination by type IIA topoisomerases remains under debate. Proposed mechanisms include discrimination based on DNA crossing geometry and chirality-dependent differences in processivity^{4, 8; 9; 10; 11}. Crystal structures of bacterial GyrA CTDs and ParC CTDs have not provided a great deal of additional insight. The GyrA and ParC CTDs are multi-bladed

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

structures that contain a highly positively charged region that binds DNA¹². The similarity of the five blades found in *E. coli* ParC CTD suggests that it may simply provide an extended high-affinity DNA binding surface^{4; 12}. Thus, despite determining the structure of the domain responsible for chiral discrimination by topoisomerase IV, the relationship between the structure and mechanism was not immediately clear.

In this issue, Vos, Lee, and Berger present the results of a systematic dissection of the role of key residues in each of the five blades of the topoisomerase IV Par-C CTD. By measuring the chirality-dependent relaxation and unlinking activity of point mutations of highly conserved basic residues in each blade, complemented by binding and bending assays of the intact enzyme as well as the isolated CTD, the authors made a number of striking discoveries. Rather than being a monolithic DNA binding element, each blade in the CTD appears to affect different aspects of topoisomerase IV activity in dramatically different manners. These results provide an important connection between the structure of the CTD and chirality-dependent modulation of strand passage activity. Remarkably, interactions between the DNA and the Par-C CTD appear to both stimulate and inhibit specific topology and substrate-dependent activities of topoisomerase IV.

Perhaps the most interesting finding is that residues in blade 1, which is proximal to the N-terminal portion of the ParC domain, contribute to bending of the G-segment DNA bound by the core enzyme. Disruption of this residue leads to a severe decrease in activity across all measurements, consistent with the growing body of evidence establishing the importance of DNA bending for topoisomerase IIA activity^{13; 14}. However, this is the first evidence that the ParC CTD participates in G-segment DNA interactions and it explains the dramatic decrease in activity observed in the topoisomerase IV ParC CTD deletion mutant⁴.

The remaining blades modulate the activity of topoisomerase IV in distinct DNA substrate-dependent manners. Conserved basic residues in blades 2–4 modulate the activity, rate, and processivity of positive supercoil relaxation. Mutations of these residues decrease the overall activity of topoisomerase IV in relaxing positive supercoils. Paradoxically, these mutations slightly increase the relaxation rate while decreasing the processivity of positive supercoil relaxation. Mutations in blade 5 have little overall effect on the relaxation of positive supercoils.

Remarkably, blade 5 appears to specifically inhibit negative supercoil relaxation as both the overall activity and rate increase when interactions between this blade and the DNA are disrupted. Negative supercoil relaxation appears to be governed by blades 2 and 3 as mutations in these blades decrease the overall activity, processivity, and rate of topoisomerase IV in relaxing negative supercoils. Mutations in blade 4 have virtually no effect on negative supercoil relaxation.

Similar to negative supercoil relaxation, decatenation appears to be inhibited through the interaction of DNA with blade 5, but only blade 3 appears to be important for decatenation. Mutations in blades 2 and 4 have essentially no effect on decatenation activity or rate.

The final surprising discovery by Vos and co-workers is that the effects of disrupting DNA binding at each blade did not uniformly decrease the overall binding affinity of the isolated

CTD. Blades 1 and 2 appear to have little effect on DNA binding, whereas blades 3, 4, and 5 appear to play important roles in DNA binding, with mutations in blade 4 showing the strongest effect.

Conclusions

Specific and topologically distinct interactions between individual blades of topoisomerase IV ParC CTD with T-segment DNA result in modulation of the core relaxation activity, rate and processivity. These complex topology-dependent interactions suggest that chiral discrimination arises from inhibition of relaxation activity on negative supercoils and to some degree on catenated DNA. Furthermore, the proximal blade of topoisomerase IV ParC CTD interacts with, and promotes the bending of, G-segment DNA bound by the core enzyme. Rather than playing a passive role in binding and guiding transfer segment DNA, the topoisomerase ParC CTD appears to be a sophisticated DNA topology and geometry sensor that controls the overall strand-passage activity of the enzyme.

Outlook

This systematic study of the effects of mutations in conserved residues in the blades of the topoisomerase IV ParC- CTD suggest an intriguing mechanism of chirality discrimination based on inhibition of negative supercoil relaxation and DNA decatenation. This regulation may be associated with, and enhanced by, different topologically-dependent conformations of the CTDs relative to the remainder of the enzyme. As the authors suggest, elucidating the details of this subtle process will require further exploration, but this elegant work points the way forward and provides the structural and contextual basis to determine the detailed conformations and dynamics leading to chiral discrimination by topoisomerase IV.

Acknowledgments

I thank Tamara Litwin and Yeonee Seol for critical reading of the manuscript and Yeonee Seol for preparing the figure. Research in the author's laboratory is supported by the intramural research program of the National Heart, Lung, and Blood Institute in the National Institutes of Health.

References

1. Vos SM, Tretter EM, Schmidt BH, Berger JM. All tangled up: how cells direct, manage and exploit topoisomerase function. *Nature Reviews Molecular & Cellular Biology*. 2011; 12:827–841.
2. Champoux JJ. DNA topoisomerases: structure, function, and mechanism. *Annual Review of Biochemistry*. 2001; 70:369–413.
3. McClendon AK, Rodriguez AC, Osheroff N. Human topoisomerase II α rapidly relaxes positively supercoiled DNA: implications for enzyme action ahead of replication forks. *Journal of Biological Chemistry*. 2005; 280:39337–39345. [PubMed: 16188892]
4. Corbett KD, Schoeffler AJ, Thomsen ND, Berger JM. The structural basis for substrate specificity in DNA topoisomerase IV. *Journal of Molecular Biology*. 2005; 351:545–561.
5. Kampranis SC, Maxwell A. Conversion of DNA gyrase into a conventional type II topoisomerase. *Proc Natl Acad Sci U S A*. 1996; 93:14416–14421. [PubMed: 8962066]
6. McClendon AK, Gentry AC, Dickey JS, Brinch M, Bendsen S, Andersen AH, Osheroff N. Bimodal Recognition of DNA Geometry by Human Topoisomerase II α : Preferential Relaxation of Positively Supercoiled DNA Requires Elements in the C-Terminal Domain†. *Biochemistry*. 2008; 47:13169–13178. [PubMed: 19053267]

7. Tretter EM, Lerman JC, Berger JM. A naturally chimeric type IIA topoisomerase in *Aquifex aeolicus* highlights an evolutionary path for the emergence of functional paralogs. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:22055–22059. [PubMed: 21076033]
8. Charvin G, Bensimon D, Croquette V. Single-molecule study of DNA unlinking by eukaryotic and prokaryotic type-II topoisomerases. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100:9820. [PubMed: 12902541]
9. Neuman KC, Charvin G, Bensimon D, Croquette V. Mechanisms of chiral discrimination by topoisomerase IV. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:6986–6991. [PubMed: 19359479]
10. Stone MD, Bryant Z, Crisona NJ, Smith SB, Vologodskii A, Bustamante C, Cozzarelli NR. Chirality sensing by *Escherichia coli* topoisomerase IV and the mechanism of type II topoisomerases. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100:8654. [PubMed: 12857958]
11. Seol Y, Gentry AC, Osheroff N, Neuman KC. Chiral discrimination and writhe dependent relaxation mechanism of human topoisomerase II α . *J Biol Chem*. 2013
12. Corbett KD, Shultzaberger RK, Berger JM. The C-terminal domain of DNA gyrase A adopts a DNA-bending β -pinwheel fold. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101:7293–7298. [PubMed: 15123801]
13. Lee I, Dong KC, Berger JM. The role of DNA bending in type IIA topoisomerase function. *Nucleic Acids Res*. 2013; 41:5444–5456. [PubMed: 23580548]
14. Hardin AH, Sarkar SK, Seol Y, Liou GF, Osheroff N, Neuman KC. Direct measurement of DNA bending by type IIA topoisomerases: implications for non-equilibrium topology simplification. *Nucleic Acids Research*. 2011; 39:5729–5743. [PubMed: 21421557]

Figure 1.

Topoisomerase IIA strand passage reaction. Topoisomerase binds gate segment DNA (blue) followed by binding of transfer segment DNA (yellow) and ATP that closes the N-gate (light blue). The gate segment is cleaved and the C-gate (light green) opens allowing the transfer segment to pass through the gate segment and escape. Release of ADP closes the C-gate and opens the N gate, resetting the enzyme for another strand transfer reaction. This conserved strand passage mechanism is modulated in a DNA topology-dependent manner by the C-terminal domains of different type IIA topoisomerases to achieve different effects. *E.*

coli DNA gyrase negatively supercoils DNA (left). Eukaryotic type IIA topoisomerases, with the exception of human topoisomerase II α , relax positively and negatively supercoiled DNA with equal efficiency (Center), whereas *E. coli* topoisomerase IV relaxes positively supercoiled DNA much more efficiently than negatively supercoiled DNA (right).