
Preface

Enzymes that alter the topology of DNA, collectively DNA topoisomerases and gyrases, arose early in the evolution of the DNA-based cells of archaea, bacteria, and eukaryotes. Together, they perform essential functions that are required for the basic processes of cell division: DNA replication, transcription, recombination, chromosome condensation, and chromosome segregation. Previous volumes in the *Methods in Molecular Biology* series have covered in great depth the methodologies needed to study topoisomerases from a biochemical perspective, including the topoisomerase–drug interactions relevant to clinical applications, and have provided indispensable protocols to topoisomerase researchers that cover almost every aspect of topoisomerase enzymology. However, the past decade has seen an expansion in topoisomerase research at the molecular and cellular levels, and this activity will doubtless continue to increase. With a greater understanding of the cellular functions performed by topoisomerases, there now is a clear need for a compendium of protocols that extends into the new areas of research that have been recently uncovered. Thus, this volume is not an updated republication of methods, nor does it aim to be all-inclusive, but rather the methods provided here add new approaches to the study of topoisomerase functions that are relevant in what is a rapidly changing field of research.

First in this volume is a series of chapters that describe methods to analyze DNA topologies and the interaction of topoisomerases with DNA as tools to measure topoisomerase functions (Chapters 2, 3, 4, 5, 6, and 7). Although the topoisomerase reactions performed by different topoisomerases have been defined, little is understood about their regulation by co-factors or post-translational modifications. These assays will be useful in assessing factors that influence topoisomerase activity. A novel example of a DNA topological change performed by a topoisomerase has recently become known. Holliday junction *dissolution* functions to allow the repair of recombination intermediates without sister chromatid exchange. An assay to monitor this reaction is presented in Chapter 8.

Chapters 9, 10, and 11 pertain to the binding of topoisomerases to specific sites in the genomes of eukaryotes, including origins of DNA replication. The interaction of topoisomerases with DNA can now be measured by ChIP-on-chip analyses, revealing their specific locations within whole chromosomes and within the cell cycle context. Chapter 9 describes methods to detect topoisomerase binding sites across the genome, and Chapters 10 and 11 explain how the binding of topoisomerases to DNA at specific sites can be measured, even to single-nucleotide resolution. Using these methods, the association of topoisomerases with functional units such as replication origins can be determined.

The molecular approaches described above are followed by a series of chapters that delve into the consequences of perturbed topoisomerase function. It has long been known that DNA topology must be fashioned to allow the dramatic changes in chromatin packing that take place during mitosis, but methods for assessing the effects

of topoisomerase inhibition in vivo on chromosome structure have not been compiled in a single volume. Methods to examine chromosome structural aberrations and measure DNA damage after topoisomerase inhibition are included in Chapters 12 and 15. Because DNA replication produces catenated sister chromatids, topoisomerases have key functions in the segregation of the genome. Perturbed topoisomerase II function delays progression into and through mitosis to prevent failed attempts to segregate the genome. Methods of measuring cell cycle progression into mitosis and the associated changes in MPF and Plk1 kinase activity are described in Chapter 13. These methods provide reliable ways to assay the integrity of the G2 topoisomerase II checkpoint. Chapter 14 contains protocols that can be used to analyze similar topoisomerase II checkpoints in yeast cells, which offer the advantage of being genetically tractable.

Finally, the last series of chapters in this volume include methods specific to the study of topoisomerase II and its regulation in vivo (Chapters 16, 17, 18, and 19). Recent studies have begun to reveal the importance of the post-translational modifications that befall topoisomerases, such as sumoylation, which can affect the localization of topoisomerases in cells. Cell biology methods are also needed to put into context the modifications with the cell cycle. The dynamics of topoisomerases in live cells can also now be measured. Thus, methods designed to study sumo modification (Chapters 16 and 17) as well as the dynamics of topoisomerase II in the nucleus (Chapter 18) are included, which ought to aid further research in this important area.

With the expanded study of topoisomerases in relation to cell cycle position, there has been a need to devise and optimize protocols that achieve depletion of topoisomerases from mammalian cells, as well as methods that combine depletion with cell cycle synchrony. This volume concludes with Chapter 19 that discusses the different approaches that can be employed to deplete topoisomerase II from mammalian cells.

In all, this collection of protocols describes the methodology needed to study topoisomerases in the molecular and cellular context, reflecting an expanded understanding of the functions of these essential enzymes. The studies described above provide evidence that research on the functions of topoisomerases is in a growth phase and that key discoveries will be made in the coming years. It is the hope that the methods provided here will aid in those discoveries.

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DNA Topoisomerases

Methods and Protocols

Clarke, D.J. (Ed.)

2009, X, 268 p. 59 illus., Hardcover

ISBN: 978-1-60761-339-8

A product of Humana Press