METHODS IN MOLECULAR BIOLOGY

Series Editor
John M. Walker
School of Life and Medical Sciences
University of Hertfordshire
Hatfield, Hertfordshire, AL10 9AB, UK

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DNA Topoisomerases

Methods and Protocols

Edited by

Marc Drolet

Département de microbiologie, infectiologie et immunologie
Université de Montréal, Montréal, QC, Canada

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Editor
Marc Drolet
Département de microbiologie, infectiologie et immunologie
Université de Montréal
Montréal, QC, Canada

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Preface

Topological problems such as underwinding, overwinding, knotting, and tangling are inherent to the double-helical structure of the DNA. Every time the DNA is transcribed, replicated, or repaired, such problems may arise and have to be resolved to allow the normal progression and completion of DNA transactions. DNA topoisomerases (topos), which are nicking-closing enzymes with strand passage activity, are ubiquitous, unique, and essential enzymes that solve these topological problems. Over the last years, it has become more and more evident that these enzymes play major roles in the maintenance of genomic stability. However, because they cleave DNA they also have the potential to fragment the genome and trigger genomic instability and cell death. In fact, this DNA cleavage property of topoisomerases is used by some of the most potent anticancer and antimicrobial drugs.

More recently, an RNA topoisomerase activity has been described for type 1A topos from bacteria, yeast, and higher eukaryotes. This suggests the occurrence of topological problems that need to be resolved during cellular processes involving RNA. What is the nature of these topological problems is currently unknown. In the field of DNA topology, a hot topic is R-loop formation during transcription. Both type 1A and IB topos, by relaxing DNA, can inhibit their formation. Whether or not the RNA topo activity of type 1A enzymes is also implicated in the inhibition of R-loop formation remains to be seen. Bacterial enzymes of the type 1A family were the first topos to be discovered, but only recently that small molecules were shown to inhibit their activity.

The present book, I believe, very well reflects the evolution of the topo field over the last years and the fact that DNA topoisomerases are directly or indirectly involved in a myriad of cellular processes. Over the last years the topo field has largely benefited from genome-wide technologies (e.g., next-generation sequencing), single-molecule approaches as well as more sophisticated cell biology approaches. This book is intended to provide specialists and nonspecialists with an overview of selected hot topics in the field, and with state-of-the-art protocols to study DNA topology, DNA topoisomerase functions and activity as well as their inhibition by various compounds.

The first three chapters of the book are review papers on selected hot topics in the topo field, including type 1A topos with an evolutionary perspective (Chap. 1), the beneficial and detrimental effects of type 1B topos on genomic stability (Chap. 2), and an update about antimicrobial agents targeting topos (Chap. 3). The following six chapters present experimental protocols related to DNA topology including a basic technique to measure DNA supercoiling in bacteria (Chap. 4), the use of two-dimensional agarose gel electrophoresis to reveal the dynamic of DNA topology during replication (Chap. 5), the use of genome-wide technologies to map topo IV binding and activity sites (Chap. 6), the application of psoralen photobinding to study transcription-induced supercoiling genome-wide in eukaryotic cells (Chap. 7), the use of immunoprecipitation to map sites of R-loop formation genome-wide in yeast cells (Chap. 8), and finally the application of EdU (5-ethynyl-2'-deoxyuridine) labeling to detect replication from R-loops in bacteria by flow cytometry (Chap. 9). The next four chapters describe protocols for in vitro studies including the preparation of substrates for the use of magnetic tweezer for single-molecule analysis of topoisomerases (Chap. 10), the synthesis of hemicatenanes to study type 1A topo activity (Chap. 11), an assay to detect RNA topo activity (Chap. 12), and two assays for Tyrosyl-DNA

phosphodiesterase 1 (TDP1) activity, an enzyme removing topo I-associated DNA breaks (Chap. 13). The following four chapters are related to in vivo studies of topos and include a protocol for chromatin immunoprecipitation of topo II for the genome-wide mapping of their binding sites (Chap. 14), two additional protocols related to topo II with one about mitotic chromosomal defects of topo II mutants (Chap. 15) and the next one about the monitoring of topo II checkpoint in yeast (Chap. 16), and protocols describing assays to identify mutations and other forms of DNA damage, resulting from Top1-cleavage at unrepaired genomic ribonucleotides (Chap. 17). Finally, the last five chapters concern topos inhibitors and include a protocol for a fluorescence-based assay to identify bacterial topo I inhibitors (Chap. 18), one for the detection of fluoroquinolone-gyrase cleaved complex (Chap. 19), assays for the immunodetection of topo-DNA covalent complexes in extracted genomic DNA (Chap. 20) or in agarose-embedded unfixed cells (Chap. 21), and the last one about the study of plasmid-mediated quinolone resistance in bacteria (Chap. 22).

I am grateful to the authors for their contributions and patience, as well as to John Walker for his continuous support and hope that this book will be well received in the topo field.

Montréal, QC, Canada *Marc Drolet*

Contents

Contributors

Muzammil Ahmad • *Genome Instability and Chromatin Remodeling Section, Lab of Genetics and Genomics, National Institute on Aging, National Institute on Health, Baltimore, MD, USA*

Jay Anand • *Biopharmaceutical Sciences Department, University of Illinois College of Pharmacy, Rockford, IL, USA*

Thirunavukkarasu Annamalai • *Department of Chemistry and Biochemistry, Florida International University, Miami, FL, USA; Biomolecular Sciences Institute, Florida International University, Miami, FL, USA*

Caroline A. Austin • *Institute for Cell and Molecular Biosciences, University of Newcastle upon Tyne, Newcastle upon Tyne, UK*

Aurélien Balleydier • *Département de microbiologie, infectiologie et immunologie, Université de Montréal, Montréal, QC, Canada*

Laura Baranello • *Laboratory of Pathology, NCI/NIH, Bethesda, MD, USA*

Julien Brochu • *Département de microbiologie, infectiologie et immunologie, Université de Montréal, Montréal, QC, Canada*

Alicia Castán • *Department of Cellular and Molecular Biology, Centro de Investigaciones Biológicas (CSIC), Madrid, Spain*

Bokun Cheng • *Department of Pediatrics, Albert Einstein College of Medicine, Bronx, NY, USA*

Shih-Chieh Chiang • *Department of Molecular Biology and Biotechnology, Krebs Institute, University of Sheffield, Sheffield, UK*

Jang-Eun Cho • *Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC, USA*

Duncan J. Clarke • *Department of Genetics, Cell Biology & Development, University of Minnesota, Minneapolis, MN, USA*

Ian G. Cowell • *Institute for Cell and Molecular Biosciences, University of Newcastle upon Tyne, Newcastle upon Tyne, UK*

Hélène Debat • *Université Versailles St-Quentin, Institut Jacques Monod, UMR 7592 CNRS-Univ. Paris Diderot, Paris, France*

Karl Drlica • *Department of Microbiology, Biochemistry & Molecular Genetics, Public Health Research Institute, New Jersey Medical School, Rutgers Biomedical and Health Sciences, Rutgers University, Newark, NJ, USA*

Marc Drolet • *Département de microbiologie, infectiologie et immunologie, Université de Montréal, Montréal, Québec, Canada*

Olivier Espéli • *Center for Interdisciplinary Research in Biology (CIRB), College de France, CNRS/UMR 7241 – INSERM U1050, PSL Research University, Paris Cedex, France*

Katherine Furniss • *Department of Genetics, Cell Biology & Development, University of Minnesota, Minneapolis, MN, USA; St. John's University, Collegeville, MN, USA*

Florence Garnier • *Université Versailles St-Quentin, Institut Jacques Monod, UMR 7592 CNRS-Univ. Paris Diderot, Paris, France*

Juan F. Giménez-Abián • *Department of Cell and Molecular Biology, Synthetic Microbial Macromolecular Assemblies, CIB, CSIC, Madrid, Spain*

Kathryn H. Gunn • *Department of Molecular Biosciences, Northwestern University, Evanston, IL, USA*
Aziz El Hage • *Wellcome Centre for Cell Biology, University of Edinburgh, Edinburgh, UK*
Sherif F. El-Khamisy • *Department of Molecular Biology and Biotechnology, Krebs Institute, University of Sheffield, Sheffield, UK*
Pablo Hernández • *Department of Cellular and Molecular Biology, Centro de Investigaciones Biológicas (CSIC), Madrid, Spain*
Hiroshi Hiasa • *Department of Pharmacology, University of Minnesota Medical School, Minneapolis, MN, USA*
Tao-shih Hsieh • *Institute of Cellular and Organismic Biology, Academia Sinica, Taipei, Taiwan*
Zhi-Chun Hua • *School of Life Sciences, Nanjing University, Nanjing, Jiangsu, People's Republic of China; Changzhou High-Tech Research Institute of Nanjing University and Jiangsu TargetPharma Laboratories Inc., Changzhou, Jiangsu, People's Republic of China*
George A. Jacoby • *Lahey Hospital and Medical Center, Burlington, MA, USA*
Sue Jinks-Robertson • *Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC, USA*
Neelam Keswani • *Department of Chemistry and Biochemistry, Florida International University, Miami, FL, USA; Biomolecular Sciences Institute, Florida International University, Miami, FL, USA*
Fedor Kouzine • *Laboratory of Pathology, NCI/NIH, Bethesda, MD, USA*
Dora B. Krimer • *Department of Cellular and Molecular Biology, Centro de Investigaciones Biológicas (CSIC), Madrid, Spain*
Thomas A. Kunkel • *Genome Integrity and Structural Biology Laboratory, National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH), Research Triangle Park, NC, USA*
Andrew B. Lane • *Department of Genetics, Cell Biology & Development, University of Minnesota, Minneapolis, MN, USA*
Shun-Hsiao Lee • *Department of Structural Cell Biology, Molecular Mechanisms of DNA Repair, Max Planck Institute of Biochemistry, Martinsried, Germany*
Fenfei Leng • *Biomolecular Sciences Institute, Florida International University, Miami, FL, USA; Department of Chemistry & Biochemistry, Florida International University, Miami, FL, USA*
David Levens • *Laboratory of Pathology, NCI/NIH, Bethesda, MD, USA*
Yingting Liu • *Biomolecular Sciences Institute, Florida International University, Miami, FL, USA; Department of Chemistry & Biochemistry, Florida International University, Miami, FL, USA; School of Life Sciences, Nanjing University, Nanjing, Jiangsu, People's Republic of China*
Kirsty Liversidge • *Department of Molecular Biology and Biotechnology, Krebs Institute, University of Sheffield, Sheffield, UK*
Gan Luan • *Department of Microbiology, Biochemistry & Molecular Genetics, Public Health Research Institute, New Jersey Medical School, Rutgers Biomedical and Health Sciences, Rutgers University, Newark, NJ, USA*
John F. Marko • *Department of Molecular Biosciences, Northwestern University, Evanston, IL, USA; Department of Physics and Astronomy, Northwestern University, Evanston, IL, USA*
Makisha Martel • *Département de microbiologie, infectiologie et immunologie, Université de Montréal, Montréal, QC, Canada*

Alfonso Mondragón • *Department of Molecular Biosciences, Northwestern University, Evanston, IL, USA*

Marc Nadal • *Institut Jacques Monod, UMR 7592 CNRS-Université Paris Diderot, Paris, France*

Karin C. Nitiss • *Biopharmaceutical Sciences Department, University of Illinois College of Pharmacy, Rockford, IL, USA; Biomedical Science Department, University of Illinois College of Medicine, Rockford, IL, USA*

John L. Nitiss • *Biopharmaceutical Sciences Department, University of Illinois College of Pharmacy, Rockford, IL, USA*

Hafez El Sayyed • *Center for Interdisciplinary Research in Biology (CIRB), College de France, CNRS/UMR 7241 – INSERM U1050, PSL Research University, Paris Cedex, France*

Jorge B. Schvartzman • *Department of Cellular and Molecular Biology, Centro de Investigaciones Biológicas (CSIC), Madrid, Spain*

Grace Ee-Lu Siaw • *Institute of Cellular and Organismic Biology, Academia Sinica, Taipei, Taiwan*

Kayleigh A. Smith • *Institute for Cell and Molecular Biosciences, University of Newcastle upon Tyne, Newcastle upon Tyne, UK*

Yilun Sun • *Biopharmaceutical Sciences Department, University of Illinois College of Pharmacy, Rockford, IL, USA*

David Tollervey • *Wellcome Centre for Cell Biology, University of Edinburgh, Edinburgh, UK*

Yuk-Ching Tse-Dinh • *Department of Chemistry and Biochemistry, Florida International University, Miami, FL, USA; Biomolecular Sciences Institute, Florida International University, Miami, FL, USA*

Amit C. J. Vas • *Department of Genetics, Cell Biology & Development, University of Minnesota, Minneapolis, MN, USA; Biotechnology R&D, Cargill, Inc., Plymouth, MN, USA*

Weidong Wang • *Genome Instability and Chromatin Remodeling Section, Lab of Genetics and Genomics, National Institute on Aging, National Institute on Health, Baltimore, MD, USA*

Jessica S. Williams • *Genome Integrity and Structural Biology Laboratory, National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH), Research Triangle Park, NC, USA*

Dongyi Xu • *Genome Instability and Chromatin Remodeling Section, Lab of Genetics and Genomics, National Institute on Aging, National Institute on Health, Baltimore, MD, USA; State Key Laboratory of Protein and Plant Gene Research, School of Life Sciences, Peking University, Beijing, China*

Yang Zhao • *Biopharmaceutical Sciences Department, University of Illinois College of Pharmacy, Rockford, IL, USA; Biomedical Science Department, University of Illinois College of Medicine, Rockford, IL, USA*