Methods in ENZYMOLOGY

Volume 591

DNA Repair Enzymes: Cell, Molecular, and Chemical Biology

Edited by

Brandt F. Eichman

VOLUME FIVE HUNDRED AND NINETY ONE

METHODS IN ENZYMOLOGY

DNA Repair Enzymes: Cell, Molecular, and Chemical Biology

Edited by

BRANDT F. EICHMAN
Vanderbilt University
Nashville, TN, United States

ACADEMIC PRESS
An imprint of Elsevier

Academic Press is an imprint of Elsevier
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States
525 B Street, Suite 1800, San Diego, CA 92101–4495, United States
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom
125 London Wall, London, EC2Y 5AS, United Kingdom

First edition 2017

Copyright © 2017 Elsevier Inc. All rights reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Details on how to seek permission, further information about the Publisher's permissions policies and our arrangements with organizations such as the Copyright Clearance Center and the Copyright Licensing Agency, can be found at our website: www.elsevier.com/permissions.

This book and the individual contributions contained in it are protected under copyright by the Publisher (other than as may be noted herein).

Notices
Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

ISBN: 978-0-12-811846-7
ISSN: 0076-6879

For information on all Academic Press publications
visit our website at https://www.elsevier.com/books-and-journals

ELSEVIER Book Aid International
Working together
to grow libraries in
developing countries

www.elsevier.com • www.bookaid.org

Publisher: Zoe Kruze
Acquisition Editor: Zoe Kruze
Editorial Project Manager: Helene Kabes
Production Project Manager: Magesh Kumar Mahalingam
Cover Designer: Greg Harris

Typeset by SPi Global, India

VOLUME FIVE HUNDRED AND NINETY ONE

METHODS IN ENZYMOLOGY

DNA Repair Enzymes: Cell, Molecular, and Chemical Biology

METHODS IN ENZYMOLOGY

Editors-in-Chief

ANNA MARIE PYLE
Departments of Molecular, Cellular and Developmental Biology and Department of Chemistry
Investigator, Howard Hughes Medical Institute
Yale University

DAVID W. CHRISTIANSON
Roy and Diana Vagelos Laboratories
Department of Chemistry
University of Pennsylvania
Philadelphia, PA

Founding Editors

SIDNEY P. COLOWICK and NATHAN O. KAPLAN

此为试读，需要完整PDF请访问：www.ertongbook.com

CONTRIBUTORS

Jennifer Allen
University of California—Santa Cruz, Santa Cruz, CA, United States

Phillip L. Bartels
California Institute of Technology, Pasadena, CA, United States

Jacqueline K. Barton
California Institute of Technology, Pasadena, CA, United States

Stefania Bertora
DNA Metabolism Laboratory, IFOM—The FIRC Institute of Molecular Oncology, Milan, Italy

Cynthia J. Burrows
University of Utah, Salt Lake City, UT, United States

Manel Camps
University of California—Santa Cruz, Santa Cruz, CA, United States

Denisse Carvajal-Maldonado
Saint Louis University School of Medicine, St. Louis, MO, United States

Alejandra Castaño
Stony Brook University, Stony Brook, NY, United States

Layla Cervantes
University of California—Santa Cruz, Santa Cruz, CA, United States

David Cortez
Vanderbilt University School of Medicine, Nashville, TN, United States

Vincenzo Costanzo
DNA Metabolism Laboratory, IFOM—The FIRC Institute of Molecular Oncology, Milan, Italy

James M. Daley
Yale University School of Medicine, New Haven, CT, United States

Anthony J. Davis
University of Texas Southwestern Medical Center, Dallas, TX, United States

Yingxin Deng
California Institute of Technology, Pasadena, CA, United States

Yun Ding
University of Utah, Salt Lake City, UT, United States

Aidan J. Doherty
Genome Damage and Stability Centre, School of Life Sciences, University of Sussex, Brighton, United Kingdom

Julie A. Dragon
The Markey Center for Molecular Genetics, University of Vermont, Burlington, VT, United States

Vitaly Epshtein
New York University School of Medicine, New York, NY, United States

Aaron M. Fleming
University of Utah, Salt Lake City, UT, United States

Thomas G.W. Graham
Harvard Medical School, Boston, MA, United States

Thomas A. Guilliam
Genome Damage and Stability Centre, School of Life Sciences, University of Sussex, Brighton, United Kingdom

Jia Guo
Biodesign Institute & School of Molecular Sciences, Arizona State University, Tempe, AZ, United States

Venu Kamarthapu
New York University School of Medicine; Howard Hughes Medical Institute, New York University School of Medicine, New York, NY, United States

Youngho Kwon
Yale University School of Medicine, New Haven, CT, United States

Delphine Lemacon
Saint Louis University School of Medicine, St. Louis, MO, United States

Joshua Lilly
University of California—Santa Cruz, Santa Cruz, CA, United States

Joseph J. Loparo
Harvard Medical School, Boston, MA, United States

Carolyn G. Marsden
The Markey Center for Molecular Genetics, University of Vermont, Burlington, VT, United States

Joao Matos
Institute of Biochemistry, ETH Zürich, Zürich, Switzerland

Manas Mondal
Biodesign Institute & School of Molecular Sciences, Arizona State University, Tempe, AZ, United States

Evgeny Nudler
New York University School of Medicine; Howard Hughes Medical Institute, New York University School of Medicine, New York, NY, United States

Elizabeth O'Brien
California Institute of Technology, Pasadena, CA, United States

Federica Pezzimenti
DNA Metabolism Laboratory, IFOM—The FIRC Institute of Molecular Oncology, Milan, Italy

Annabel Quinet
Saint Louis University School of Medicine, St. Louis, MO, United States

Upasana Roy
Stony Brook University, Stony Brook, NY, United States

Janapriya Saha
University of Texas Southwestern Medical Center, Dallas, TX, United States

Vincenzo Sannino
DNA Metabolism Laboratory, IFOM—The FIRC Institute of Molecular Oncology, Milan, Italy

Orlando D. Schärer
Stony Brook University, Stony Brook, NY, United States; Center for Genomic Integrity, Institute for Basic Science; Ulsan National Institute of Science and Technology, Ulsan, Korea

Melissa Standley
University of California—Santa Cruz, Santa Cruz, CA, United States

Patrick Sung
Yale University School of Medicine, New Haven, CT, United States

Joann B. Sweasy
The Markey Center for Molecular Genetics, University of Vermont, Burlington, VT; Yale University School of Medicine, New Haven, CT, United States

Alan E. Tomkinson
University of New Mexico School of Medicine, Albuquerque, NM, United States

Alessandro Vindigni
Saint Louis University School of Medicine, St. Louis, MO, United States

Susan S. Wallace
The Markey Center for Molecular Genetics, University of Vermont, Burlington, VT, United States

Johannes C. Walter
Harvard Medical School; Howard Hughes Medical Institute, Boston, MA, United States

Shih-Ya Wang
University of Texas Southwestern Medical Center, Dallas, TX, United States

Stephen C. West
The Francis Crick Institute, London, United Kingdom

Nathaniel E. Wiest
University of New Mexico School of Medicine, Albuquerque, NM, United States

PREFACE

Studies on the effects of radiation on biological organisms in the 1930s and subsequent work on mutagenesis and the discovery of DNA as the genetic material in the 1940s spawned an entire field of science focused on DNA repair. Since that time, the field has discovered a myriad of sources, types, and biological consequences of chemical transformation to DNA (i.e., DNA damage). This led to elucidation of a number of distinct DNA repair pathways that preserve the integrity of the genetic information, some of which function to restore aberrant DNA to an undamaged state, while others help the cell tolerate, or function in spite of, DNA damage. Genetic defects in these repair pathways are often linked to chromosome instability and predisposition of individuals to cancer and other diseases. Importantly, our understanding of the fundamental science behind DNA repair and the mechanisms of the enzymes involved has led to a number of therapeutic strategies to treat these diseases.

In these two volumes of *Methods in Enzymology*, leading investigators in the field of DNA repair present some of the most important, cutting-edge techniques used to probe DNA repair mechanisms across multiple scales—from cells to atoms. Each chapter focuses on a specific type of DNA damage or repair pathway, including base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), double-strand break repair (DSBR), replication-coupled DNA damage response (DDR), homologous recombination (HR), and DNA synthesis by specialized polymerases. Many of the methods described here are not specific to a particular enzyme or pathway, and thus the chapters are organized by a technique rather than by a specific type of DNA repair. Some authors provide detailed protocols and considerations when carrying out a particular experiment, while others present their philosophies for integrating multiple fields of investigation. The first volume (591) focuses largely on cellular, molecular, and chemical biology methods to investigate DNA damage and repair functions in a genomic or cellular context. The second volume (592) is focused on structural, single-molecule, and kinetic methods aimed at elucidating detailed enzymatic and mechanistic information.

Volume 591 ("DNA Repair Enzymes: Cell, Molecular, and Chemical Biology") begins at the DNA replication fork. The first two chapters (Wiest and Tomkinson; Cortez) describe benefits, limitations, and improvements to the use of iPOND (isolation of proteins on nascent DNA) to identify DDR activities at sites of DNA synthesis. This is followed by the uses of DNA fiber analysis to monitor replication fork progression and stalling in the presence of DNA damage, as presented by Quinet et al. In the fourth chapter, Mondal and Guo describe a powerful method to detect strand-specific repair of low levels of DNA damage in cells, followed by a chapter by Saha and colleagues detailing how to monitor DSBR at various stages of the cell cycle. In the sixth chapter, Marsden and coworkers outline an integrated computational and cell biology pipeline to identify and characterize functionally significant mutations in BER genes, followed by a chapter from Standley et al. describing methods to detect bacterial mutations. In the eighth chapter, Fleming and colleagues describe innovative sequencing methods to detect 8-oxoguanine, a principal product of DNA oxidation in the genome. Chapters 9 and 10 provide protocols for the use of cell-free extracts of *Xenopus* oocytes; the first by Sannino et al. describes general aspects of replication-coupled DDR, and the second by Graham et al. outlines ensemble and single-molecule approaches to study DSBR mechanisms. In Chapter 11, Matos and West provide protocols to measure enzymatic activity of structure-selective endonucleases from yeast and human extracts. In vitro and cellular methods to monitor transcription-coupled NER are outlined in Chapter 12 by Epshtein et al., followed in Chapter 13 by an in vitro system by Kwon and colleagues to reconstitute repair DNA synthesis activities at D-loops during HR. In Chapter 14, Guilliam and Doherty provide a historical and practical description of measuring DNA primase activities in vitro. In Chapter 15, Barton and colleagues provide a comprehensive review of their innovative DNA electrochemistry platform that takes advantage of DNA change transport properties to probe binding and catalysis of redox-active proteins, including iron–sulfur cluster-containing DNA repair systems. Volume 591 concludes with useful instructions by Castaño and colleagues for preparation of stable DNA interstrand cross-links that can be used in vitro and in cells to study all aspects of DNA metabolism.

Volume 592 ("DNA Repair Enzymes: Structure, Biophysics, and Mechanism") includes a series of papers on structural techniques, including X-ray crystallography, small-angle X-ray scattering (SAXS), nuclear magnetic resonance (NMR), and electron microscopy, as well as single-molecule and enzyme kinetic approaches to probe mechanistic details of enzymes involved in all types of excision and break repair. The first two chapters (Gradia et al. and Rees et al.) provide state-of-the-art methods for efficient cloning to produce multisubunit complexes in large quantities for structural and biochemical studies. Thompson and colleagues in the third chapter describe SAXS and

NMR methodologies to study conformational states and dynamic properties of multidomain proteins, using DNA primase and replication protein A as examples. In the fourth chapter, Friedhoff and coworkers share their cross-linking approach to trap transient conformational states of proteins involved in MMR. Chapters 5–7 focus on several contemporary challenges to X-ray crystallographic studies. Malaby et al. recount how they overcame challenges in expression and purification of full-length and active deletion constructs of human DNA pol θ, a specialized DNA polymerase involved in DSBR alternative end-joining. Next, Figiel and Nowotny use RNase H2 to summarize crystallographic approaches to protein–nucleic acid complexes, followed by strategies from Chirgadze and colleagues used to push the resolution of the crystal structure of the 4128-residue DNA-dependent protein kinase catalytic subunit important for regulation of nonhomologous end-joining DSBR. The next four chapters (Sawicka et al., LeBlanc et al., Kong et al., Soniat et al.) focus on the examination of physical behaviors of DNA repair machines using single-molecule methods, including electron, atomic force, and fluorescence microscopies coupled to innovative DNA visualization techniques such as DNA tightropes (Kong et al.) and DNA curtains (Soniat et al.). In the 12th chapter, Samara and coworkers illustrate their powerful time-resolved X-ray crystallographic methods that have pushed the boundaries of our understanding of catalysis of nucleic acid synthesis and degradation. Chapters 13–15 beautifully describe different methods and uses of enzyme kinetics—Powers and Washington include a variety of approaches to monitor catalysis and binding activities of translesion DNA polymerases; Coey and Drohat detail the design, execution, and interpretation of single- and multiple-turnover kinetics experiments using DNA glycosylases as an example; Hendershot and O'Brien follow with kinetic strategies to characterize DNA-binding and nucleotide-flipping mechanisms used by DNA glycosylases and many other DNA processing enzymes. The two-volume series concludes with a discussion from Brosey and colleagues, who tie together elements of both volumes by discussing examples of studies that integrate information from structural biochemistry and cell biology to develop a comprehensive understanding of these multifaceted DNA damage responses.

This collection is the culmination of 80 years of ingenuity and discovery in nucleic acid biology and is timely addition to the field, following the 2015 Nobel Prize in Chemistry to Modrich, Lindahl, and Sancar for their mechanistic studies of DNA repair. My hope is that these chapters will help inspire new innovations at the frontier of DNA repair research, while also serving as a guide to scientists engaged in all aspects of molecular biology. It is an

此为试读,需要完整PDF请访问: www.ertongbook.com

exciting time as we address the next challenges focused on how mechanisms, pathways, and regulation of DNA repair intersect with those of human disease, mutagenesis, and evolution. As in other volumes of *Methods in Enzymology*, the approaches described here are applicable across disciplines and therefore have the potential to cross-pollinate and inspire new ideas in other areas of investigation.

Brandt F. Eichman
Vanderbilt University

CONTENTS

此为试读,需要完整PDF请访问: www.ertongbook.com