METHODS IN MOLECULAR BIOLOGY™

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

For other titles published in this series, go to
www.springer.com/series/7651

METHODS IN MOLECULAR BIOLOGY™

Chromatin Protocols

Second Edition

Edited by

Srikumar P. Chellappan

H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

Editor
Srikumar P. Chellappan
H. Lee Moffitt Cancer Center
and Research Institute
Tampa, FL, USA
Srikumar.Chellappan@moffitt.org

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

ISSN 1064-3745 e-ISSN 1940-6029
ISBN 978-1-58829-873-7 e-ISBN 978-1-59745-190-1
DOI 10.1007/978-1-59745-190-1

Library of Congress Control Number: 2008941241

© Humana Press, a part of Springer Science+Business Media, LLC 2009
All rights reserved. This work may not be translated or copied in whole or in part without the written permission of the publisher (Humana Press, c/o Springer Science+Business Media, LLC, 233 Spring Street, New York, NY 10013, USA), except for brief excerpts in connection with reviews or scholarly analysis. Use in connection with any form of information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed is forbidden.
The use in this publication of trade names, trademarks, service marks, and similar terms, even if they are not identified as such, is not to be taken as an expression of opinion as to whether or not they are subject to proprietary rights.

Printed on acid-free paper

springer.com

Preface

Significant advancements have been made in the study of chromatin structure and function over the past 50 years, but few as spectacular as those made in the last decade. Development of novel techniques and the ability to sequence large stretches of DNA has facilitated in-depth analysis of DNA structure and modifications as well as proteins associated with it. It has been well established that histones and non-histone proteins associated with DNA in chromatin modulate many vital cellular functions, including transcription, replication, repair and gene silencing. Moreover, the modifications of the proteins associated with chromatin have been correlated with many of these functions. Development of novel molecular techniques in the past decade has provided immense momentum to detailed analysis of chromatin, which can now be carried out with remarkable precision. The second edition of *Chromatin Protocols* compiles many of these techniques that facilitated the explosion of information on chromatin structure and function.

The first edition of *Chromatin Protocols* was published in 1999 and had become the staple of laboratories studying chromatin structure and function. Since its publication, the landscape of chromatin biology has changed immensely. The concept of 'histone code' was proposed during the earlier part of this decade and has found universal acceptance. Now it is possible not only to analyze specific histone modifications on each specific nucleosome but such analysis can also be carried out on a genome-wide scale. To a great extent, these advances in the analysis of histone modifications in relation to transcriptional regulation and gene expression were facilitated by the development of chromatin immunoprecipitation (ChIP) assays. Development and refinement of the ChIP assay have led to an understanding of molecular changes associated with histone modifications, transcription factor binding and gene expression in the cell at a level that could only be imagined a decade ago; the progress this technique and its variants have brought to the field of chromatin biology is similar to the progress electrophoretic mobility shift assays brought to transcription factor biology in the early 1980s. These are examples of techniques developed and fine-tuned in a handful of labs changing the landscape of an entire area of biology.

The second edition of *Chromatin Protocols* encompasses a wide array of techniques spanning from isolation of nucleosomes, assembly of nucleosomes and study of the basic chromatin structure to detailed analysis of histone modifications and chromatin function. Techniques to prepare chromatin assembly extracts, analyze chromatin decondensation and drug-induced premature chromatin condensation, study of reconstituted chromatin, assess DNA topology in chromatin, analyze unique mononculeosomes and a variety of other protocols to study chromatin structure form the initial part of this volume.

This section is followed by techniques to study DNA damage and repair in the context of chromatin; techniques for monitoring DNA breaks, detailed methods to study transcription-coupled repair in chromatin, techniques for analysis of H2AX phosphorylation to assess DNA damage as well as monitoring DNA breaks in chromatin are included here. A technique to study DNA damage induced by various drugs as well as radiation is included, in addition to a detailed protocol to study genomic aberrations by comparative genomic hybridization. These techniques to study DNA damage in chromatin are followed by an assay to study DNA replication in vitro using mammalian cell extracts. This technique and its variations allow the analysis of the role of different histone and non-histone proteins involved in the replication of DNA.

The above sections are followed by detailed protocols to study DNA methylation as well as histone modifications including methylation, acetylation, phosphorylation and ubiquitination. In addition to the analysis of histone modifications, protocols are also provided to study the activities of many of the enzymes involved, including histone demethylases and deacetylases. An elegant technique to study histone phosphorylation by immunostaining is provided, which can be modified to study other histone modifications and chromatin-associated proteins as well. Further, detailed methods to successfully carry out chromatin immunoprecipitation assays are presented. A protocol is presented which describes how genome-wide ChIP analysis can be conducted, using a ChIP-on-chip technique; success of such studies depends to a great extent on the analysis of the data generated and methods to analyze ChIP-on-chip tiling arrays are presented. Imaginative utilization of these ChIP based techniques has immense potential to generate information on various aspects of chromatin biology.

The role of telomeres in maintaining chromatin integrity and how alterations in telomerase activities lead to oncogenesis as well as other proliferative disorders have gained substantial interest in recent times. Two protocols are given in this broad context, one describing techniques to study telomerase activity and telomere length and one to study ATM-dependent chromatin modifications. These techniques, we believe, would be very useful for those working in these areas in the context of normal biology of chromatin as well as proliferative disorders associated with telomere function.

We believe that these protocols, as well as their creative modifications, will facilitate in-depth molecular analysis of various aspects of chromatin structure and function including transcriptional regulation, DNA repair and replication. As can be imagined, this volume would not have been made possible without valuable contributions from a truly international panel of authors, who are all experts in their fields. My sincere thanks to them for taking the time and effort to pen down the intricate details of their favorite techniques and for their willingness to share them with the readers. In addition, my thanks to Dr. John Walker, the series editor, without whose helpful suggestions and persistence this volume would not have been completed in its present form; it was a real pleasure working with John. It is our genuine belief that the second edition of Chromatin Protocols will be a valuable tool for studying various aspects of chromatin function and would contribute significantly to the development of new techniques as well as ideas in the field of chromatin biology.

Contents

Preface v
Contributors ix
Color Plates xiii

1 Preparation of Chromatin Assembly Extracts from Preblastoderm *Drosophila* Embryos 1
Edgar Bonte and Peter B. Becker

2 Analysis of Reconstituted Chromatin Using a Solid-Phase Approach 11
Raphael Sandaltzopoulos and Peter B. Becker

3 In Vivo Chromatin Decondensation Assays: Molecular Genetic Analysis of Chromatin Unfolding Characteristics of Selected Proteins 27
Sherry L. Winter, Philip Wong, and Mark G. Alexandrow

4 DNA Methyltransferase Probing of Chromatin Structure Within Populations and on Single Molecules 41
Carolina Pardo, Scott A. Hoose, Santhi Pondugula, and Michael P. Kladde

5 Visualization of the Expression of HMGN Nucleosomal Binding Proteins in the Developing Mouse Embryo and in Adult Mouse Tissues 67
Takashi Furusawa and Michael Bustin

6 Drug-Induced Premature Chromosome Condensation (PCC) Protocols: Cytogenetic Approaches in Mitotic Chromosome and Interphase Chromatin 83
Eisuke Gotoh

7 Analysis of DNA Topology in Yeast Chromatin 93
Randall H. Morse

8 Preparation and Analysis of Uniquely Positioned Mononucleosomes 109
Daria A. Gaykalova, Olga I. Kulaeva, Vladimir A. Bondarenko, and Vasily M. Studitsky

9 Monitoring DNA Breaks in Optically Highlighted Chromatin in Living Cells by Laser Scanning Confocal Microscopy 125
Michael J. Kruhlak, Arkady Celeste, and André Nussenzweig

10 Methods to Study Transcription-Coupled Repair in Chromatin 141
Hélène Gaillard, Ralf Erik Wellinger, and Andrés Aguilera

11 Cytometric Analysis of DNA Damage: Phosphorylation of Histone H2AX as a Marker of DNA Double-Strand Breaks (DSBs) 161
Toshiki Tanaka, Dorota Halicka, Frank Traganos, and Zbigniew Darzynkiewicz

12 Detection of DNA Damage Induced by Topoisomerase II Inhibitors, Gamma Radiation and Crosslinking Agents Using the Comet Assay 169
Lori A. Hazlehurst

13 Analysis of Genomic Aberrations Using Comparative Genomic Hybridization of Metaphase Chromosomes 177
Melanie Carless

14 In Vitro Replication Assay with Mammalian Cell Extracts 203
Wasia Rizwani and Srikumar P. Chellappan

15 Investigation of Genomic Methylation Status Using Methylation-Specific and Bisulfite Sequencing Polymerase Chain Reaction 217
Melanie Carless

16 Biochemical Analysis of Arginine Methylation in Transcription 235
Marc Tini, Hina Naeem, and Joseph Torchia

17 In Vitro Histone Demethylase Assays 249
Kenji Kokura and Jia Fang

18 Histone Chaperone as Coactivator of Chromatin Transcription: Role of Acetylation 263
Shrikanth S. Gadad, Jayasha Shandilya, Venkatesh Swaminathan, and Tapas K. Kundu

19 Histone Deacetylase Activity Assay 279
Zhigang Yuan, Natalie Rezai-Zadeh, Xiaohong Zhang, and Edward Seto

20 In Vitro and In Vivo Assays for Studying Histone Ubiquitination and Deubiquitination 295
Ling Zhai, Heui-Yun Joo, and Hengbin Wang

21 Detection of Histone H3 Phosphorylation in Cultured Cells and Tissue Sections by Immunostaining 311
Jaya Padmanabhan

22 Chromatin Immunoprecipitation Assays: Analyzing Transcription Factor Binding and Histone Modifications In Vivo 323
Smitha Pillai, Piyali Dasgupta, and Srikumar P. Chellappan

23 ChIP on Chip Assays: Genome-Wide Analysis of Transcription Factor Binding and Histone Modifications 341
Smitha Pillai and Srikumar P. Chellappan

24 ChIP-on-Chip Analysis Methods for Affymetrix Tiling Arrays 367
Sean J. Yoder and Steven A. Enkemann

25 Non-Radioactive Assay Methods for the Assessment of Telomerase Activity and Telomere Length 383
Partha P. Banerjee and Shankar Jagadeesh

26 Detecting ATM-Dependent Chromatin Modification in DNA Damage and Heat Shock Response 395
Sandeep Misri, Shruti Pandita, and Tej K. Pandita

Index 411

Contributors

ANDRÉS AGUILERA • *CABIMER, Universidad de Sevilla-CSIC, Sevilla, Spain*
MARK G. ALEXANDROW • *Molecular Oncology Program, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*
PARTHA P. BANERJEE • *Department of Biochemistry and Molecular & Cellular Biology, Georgetown University Medical Center, Washington, DC, USA*
PETER B. BECKER • *Molekularbiologie, Adolf-Butenandt-Institut, München, Germany*
VLADIMIR A. BONDARENKO • *Department of Pharmacology, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ, USA*
EDGAR BONTE • *Molekularbiologie, Adolf-Butenandt-Institut, München, Germany*
MICHAEL BUSTIN • *Protein Section, Laboratory of Metabolism, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA*
MELANIE CARLESS • *Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX, USA*
ARKADY CELESTE • *Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA*
SRIKUMAR P. CHELLAPPAN • *Drug Discovery Program, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*
ZBIGNIEW DARZYNKIEWICZ • *Brander Cancer Research Institute and Department of Pathology, New York Medical College, Valhalla, NY, USA*
PIYALI DASGUPTA • *Drug Discovery Program, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*
STEVEN A. ENKEMANN • *Microarray Core Facility, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*
JIA FANG • *Molecular Oncology Program, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*
TAKASHI FURUSAWA • *Protein Section, Laboratory of Metabolism, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA*
SHRIKANTH S. GADAD • *Transcription and Disease Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bangalore, India*
HÉLÈNE GAILLARD • *CABIMER, Universidad de Sevilla-CSIC, Sevilla, Spain*
DARIA A. GAYKALOVA • *Department of Pharmacology, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ, USA*
EISUKE GOTOH • *Division of Genetic Resources, National Institute of Infectious Diseases, Tokyo, Japan; Department of Radiology, Jikei University School of Medicine, Tokyo, Japan*

DOROTA HALICKA • *Brander Cancer Research Institute and Department of Pathology, New York Medical College, Valhalla, NY, USA*
LORI A. HAZLEHURST • *Department of Interdisciplinary Oncology, Experimental Therapeutics and Drug Discovery Programs, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*
SCOTT A. HOOSE • *Department of Biochemistry and Molecular Biology and UF Shands Cancer Center Program in Cancer Genetics, Epigenetics and Tumor Virology, University of Florida College of Medicine, Gainesville, FL, USA*
SHANKAR JAGADEESH • *Department of Biochemistry and Molecular & Cellular Biology, Georgetown University Medical Center, Washington, DC, USA*
HEUI-YUN JOO • *Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL, USA*
MICHAEL P. KLADDE • *Department of Biochemistry and Molecular Biology and UF Shands Cancer Center Program in Cancer Genetics, Epigenetics and Tumor Virology, University of Florida College of Medicine, Gainesville, FL, USA*
KENJI KOKURA • *Molecular Oncology Program, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*
MICHAEL J. KRUHLAK • *Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA*
OLGA I. KULAEVA • *Department of Pharmacology, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ, USA*
TAPAS K. KUNDU • *Transcription and Disease Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bangalore, India*
SANDEEP MISRI • *Washington University School of Medicine, St Louis, MO, USA*
RANDALL H. MORSE • *Wadsworth Center, NY State Department of Health and SUNY School of Public Health, Albany, NY , USA*
HINA NAEEM • *Department of Oncology, London Regional Cancer Program, Department of Biochemistry, The University of Western Ontario, London, Ontario, Canada*
ANDRÉ NUSSENZWEIG • *Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA*
JAYA PADMANABHAN • *Johnnie B. Byrd, Sr. Alzheimer's Center and Research Institute, Tampa, FL, USA*
SHRUTI PANDITA • *Washington University School of Medicine, St Louis, MO, USA*
TEJ K. PANDITA • *Department of Radiation Oncology, Washington University School of Medicine, St Louis, MO, USA*
CAROLINA PARDO • *Department of Biochemistry and Molecular Biology and UF Shands Cancer Center Program in Cancer Genetics, Epigenetics and Tumor Virology, University of Florida College of Medicine, Gainesville, FL, USA*
SMITHA PILLAI • *Drug Discovery Program, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*
SANTHI PONDUGULA • *Department of Biochemistry and Molecular Biology and UF Shands Cancer Center Program in Cancer Genetics, Epigenetics and Tumor Virology, University of Florida College of Medicine, Gainesville, FL, USA*

Natalie Rezai-Zadeh • *H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*
Wasia Rizwani • *H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*
Raphael Sandaltzopoulos • *Department of Molecular Biology and Genetics, Democritus University of Thrace, University Campus, Alexandroupolis, Greece*
Edward Seto • *H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*
Jayasha Shandilya • *Transcription and Disease Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bangalore, India*
Vasily M. Studitsky • *Department of Pharmacology, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ, USA*
Venkatesh Swaminathan • *Workman Lab, Stowers Institute for Medical Research, Kansas City, MO, USA*
Toshiki Tanaka • *Department of Surgery and Clinical Science, Division of Chest Surgery, Graduate School of Medicine, Yamaguchi University, Yamaguchi, Japan; Brander Cancer Research Institute and Department of Pathology, New York Medical College, Valhalla, NY, USA*
Marc Tini • *Department of Physiology and Pharmacology, The University of Western Ontario, London, Ontario, Canada*
Joseph Torchia • *Department of Oncology, London Regional Cancer Program, Department of Biochemistry, The University of Western Ontario, London, Ontario, Canada*
Frank Traganos • *Brander Cancer Research Institute and Department of Pathology, New York Medical College, Valhalla, NY, USA*
Hengbin Wang • *Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL, USA*
Ralf Erik Wellinger • *CABIMER, Universidad de Sevilla-CSIC, Sevilla, Spain*
Sherry L. Winter • *Molecular Oncology Program, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*
Philip Wong • *Molecular Oncology Program, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*
Sean J. Yoder • *Microarray Core Facility, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*
Zhigang Yuan • *H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*
Ling Zhai • *Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL, USA*
Xiaohong Zhang • *Department of Pathology and Cell Biology, USF College of Medicine, Tampa, FL, USA*

Color Plates

Color Plates following p. 322

Color Plate 1 Coimmunostaining analysis of H4 cells using P-histone H3 (serine 10) and alpha-tubulin antibodies: The cells are immunostained using (**A**) P-histone H3 (serine 10) antibody and (**B**) alpha-tubulin antibody. Staining was visualized using Alexa 594 goat anti-rabbit or Alexa 488 goat anti-mouse fluorophores, respectively. (**C**) Represents staining of the same cells using Hoechst to analyze nuclei, and **D** is the composite image showing all the three stainings. The *arrows* indicate P-histone H3 positive cells in different phases of the cell cycle. (*See* discussion on p. 316)

Color Plate 2 Immunohistochemical analysis of brain sections using P-histone H3 (serine 10) and NeuN antibodies: Brain sections are coimmunostained using (**A**) P-histone H3 (serine 10) and (**B**) neuron-specific NeuN antibody. Staining was visualized using Alexa 594 goat anti-rabbit or Alexa 488 goat anti-mouse fluorophores, respectively. (**C**) Represents the nuclear staining (in pyramidal layer of hippocampus) in sections using Hoechst and (**D**) is a composite image of all the three stainings. The *arrows* indicate P-histone H3 positive cells in the brain section. (*See* discussion on p. 319)