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

Plant Epigenetics

Methods and Protocols

Edited by

Igor Kovalchuk

Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta, Canada

Franz J. Zemp

Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta, Canada

Humana Press

Editors
Igor Kovalchuk, MD, Ph.D.
Department of Biological Sciences
University of Lethbridge
Lethbridge, Alberta
Canada
igor.kovalchuk@uleth.ca

Franz J. Zemp
Department of Biological Sciences
University of Lethbridge
Lethbridge, Alberta
Canada
franz.zemp@uleth.ca

ISSN 1064-3745 e-ISSN 1940-6029
ISBN 978-1-60761-645-0 e-ISBN 978-1-60761-646-7
DOI 10.1007/978-1-60761-646-7
Springer New York Dordrecht Heidelberg London

Library of Congress Control Number: 2010922364

© Springer Science+Business Media, LLC 2010
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.
While the advice and information in this book are believed to be true and accurate at the date of going to press, neither the authors nor the editors nor the publisher can accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

Printed on acid-free paper

Humana Press is a part of Springer Science+Business Media (www.springer.com)

Preface

The discovery of DNA as the genetic material brought great hope to scientists all over the world. It was believed that many of the lingering questions in genetics and the mechanisms of heredity would finally be answered. However, as often is the case in science, more questions arose out of this discovery. What defines a gene? What are the mechanisms of gene regulation? Further discovery and technological innovations brought about sequencing techniques that allowed the study of complete genomes from many organisms, including Arabidopsis and humans. Despite all the excitement surrounding these technologies, many features of the genome remained unclear. Peculiar characteristics in genome composition such as significant redundancy consisting of many repetitive elements and noncoding sequences, active transcriptional units with no protein product, and unusual sequences in promoter regions added to the mysteries of genetic make-up and gene regulation. Indeed, the more we discovered about the genome, the more difficult it became to understand the complexity of cellular function and regulation.

Out of the study of the intricacies of the genome and gene regulation, arose a new science that was independent of actual DNA changes, but critical in maintaining gene regulation and genetic stability. Epigenetics, literally translated as "above genetics," is the science that describes the mechanisms of heritable changes in gene regulation that does not involve modifications of DNA sequence. These changes may last through somatic cell division and, in some cases, throughout multiple generations.

Epigenetics is perhaps one of the most popular and quickly evolving fields of modern science. Despite the fact that the ideas behind epigenetics had already been developing in the late nineteenth and early twentieth centuries, major advances have only occurred within the last 10–15 years as the mechanisms surrounding epigenetic regulation began to be uncovered. It was hoped by many that the mysteries of gene regulation and inheritance that remained unanswered would finally be elucidated with the help of this new science. Since, the understanding of the contribution of epigenetic regulation to cell function has helped scientists from many distinct fields of research such as molecular biology, population genetics, microbiology, ecology, developmental biology, and evolution.

Gene silencing as an epigenetic mechanism to control gene expression was first described in plants. This occurred with the beginning of the era of plant transgenesis, and almost undermined the new paradigm of improvement of plant performance via transgenic techniques. Silencing was a serendipitous discovery, as this finding revitalized the field of epigenetics. Phenomena such as plant acclimation and adaptation to stress, hybrid and heterozygote vigor (heterosis), plant tolerance to viral infection, transgenerational changes in genome stability, paramutations, among others, are now considered excellent candidates for regulation via epigenetic mechanisms. Future studies involving various protocols for the analysis of methylation patterns, histone modifications, chromatin structure, and small RNA expression, the hallmarks of epigenetic regulation, will undoubtedly help to explain these phenomena. It will be exciting to discover how plants utilize these mechanisms to adapt to stress, and how we can manipulate these characters for the generation of better and hardier crops.

In this book we have collected a variety of protocols for the study of the function of small noncoding RNAs, DNA methylation, and histone modifications in plants. Where possible and appropriate, we presented several protocols with different degrees of complexity. We also include protocols for plant transgenesis and the analysis of genome stability, with a discussion for their applications to epigenetic studies. It was our aim to put together a single manual that researchers in the field of plant epigenetics can turn to in hopes to answer the many yet undiscovered and unexplained phenomena in plant biology.

Lethbridge, AB, Canada

Igor Kovalchuk
Franz J. Zemp

Contents

Contributors

David Baulcombe • *The Sainsbury Laboratory, John Innes Centre, Norwich, UK*
Bernard Billoud • *Atelier de bioinformatique, Université Pierre et Marie Curie, Paris, France*
Todd Blevins • *Pikaard Laboratory, Biology Department, Washington University, MO, USA*
Martine Boccara • *Unité de Recherche en Génomique Végétale (URGV), INRA/CNRS/UEVE, Evry cedex, France*
Alex Boyko • *Department of Biological Sciences, University of Lethbridge, Lethbridge, AB, Canada*
Agnes Bulski • *CNRS UMR8186, Département de biologie, Ecole Normale Supérieure, Paris cedex, France*
Jose Antonio Cabezas • *Departamento de Investigación Agroalimentaria, Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario, Alcalá de Henares, Spain*
María-Teresa Cervera • *Departamento de Sistemas y Recursos Forestales, CIFOR, Madrid, Spain*
Louise Chapell • *The Sainsbury Laboratory, John Innes Centre, Norwich, UK*
Vincent Colot • *Unité de Recherche en Génomique Végétale (URGV), INRA/CNRS/UEVE, Evry cedex, France*
Nuria de María • *Departamento de Sistemas y Recursos Forestales, CIFOR, Madrid, Spain*
Julia Engel*horn* • *Max Planck Institute for Plant Breeding Research, Köln, Germany*
Andrea M. Foerster • *Gregor Mendel Institute of Molecular Plant Biology, Austrian Academy of Sciences, Vienna, Austria*
Ulrike Göbel • *Max Planck Institute for Plant Breeding Research, Köln, Germany*
Ueli Grossniklaus • *Institute of Plant Biology & Zürich-Basel Plant Science Center, University of Zürich, Zürich, Switzerland*
Roger P. Hellens • *HortResearch, Mt Albert Research Centre, Auckland, New Zealand*
Jennifer Hetzl • *Institute for Computer Graphics and Algorithms, Vienna University of Technology, Vienna, Austria*
Naoko Inaba • *Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Tokyo, Japan*
Palak Kathiria • *Department of Biological Sciences, University of Lethbridge, Lethbridge, AB, Canada*
Igor Kovalchuk • *Department of Biological Sciences, University of Lethbridge, Lethbridge, AB, Canada*
Enwu Liu • *NRC Plant Biotechnology Institute, Saskatoon, SK, Canada*

Ortrun Mittelsten Scheid • *Gregor Mendel Institute of Molecular Plant Biology, Austrian Academy of Sciences, Vienna, Austria*
Christoph Müllner • *Institute for Computer Graphics and Algorithms, Vienna University of Technology, Vienna, Austria*
Jonathan E. Page • *NRC Plant Biotechnology Institute, Saskatoon, SK, Canada*
Stéphane Pien • *Institute of Plant Biology & Zürich-Basel Plant Science Center, University of Zürich, Zürich, Switzerland*
Julia J. Reimer • *Max Planck Institute for Plant Breeding Research, Köln, Germany*
Leonor Ruiz-García • *Departamento de Biotecnología y Protección de Cultivos, Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA), Murcia, Spain*
Alexis Sarazin • *CNRS UMR8186, Département de biologie, Ecole Normale Supérieure, Paris cedex 05, France*
Yuko Tagami • *Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Tokyo, Japan*
Andrea T. Todd • *NRC Plant Biotechnology Institute, Saskatoon, SK, Canada*
Franziska Turck • *Max Planck Institute for Plant Breeding Research, Köln, Germany*
Erika Varkonyi-Gasic • *HortResearch, Mt Albert Research Centre, Auckland, New Zealand*
Yuichiro Watanabe • *Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Tokyo, Japan*
Alicja Ziemienowicz • *Department of Biological Sciences, University of Lethbridge, Lethbridge, AB, Canada*