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RNA Interference, Editing, and Modification

Methods and Protocols

Edited by

Jonatha M. Gott

Case Western Reserve University School of Medicine, Cleveland, OH

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Dedication

This book is dedicated to Eric and Katherine Christian, who provided continual support and joy.

Preface

Two of the more fascinating biological phenomena that have been discovered in recent years are RNA editing and RNA interference. Each of these processes has been found in a cross-section of biological systems, including mammals, viruses, plants, and a range of model organisms (*C. elegans*, *Drosophila*, and various lower eukaryotes). RNA editing, which results in an RNA product different from that predicted by the genome, occurs through a variety of mechanisms. Alterations can occur at either the base level, in which one base is changed to another (substitutional editing/base modification), or via the addition and/or deletion of nucleotides relative to the original template (insertion/deletion editing). RNA interference (RNAi) involves the specific degradation of targeted mRNAs. Although RNA interference, editing, and modification use different enzymes and mechanisms, the targets of each of these reactions are often specified by RNA molecules. Indeed, the discovery of guide RNAs (gRNAs) that direct nucleotide insertion and deletion in trypanosome mitochondria set the precedent for subsequent discoveries of the small nuclear RNAs (snoRNAs) that target pseudouridylation and methylation of stable RNAs and the small double-stranded RNA fragments (siRNAs) that mediate RNAi. Other small RNAs are known to mediate translational regulation during development (small temporal RNAs [stRNAs]) and mRNA stability (microRNAs [miRNAs]), and the recent identification of more than a hundred small “noncoding” RNAs has led to the realization that they may represent only the proverbial “tip of the iceberg.” With the current availability of a large number of complete genomes, this area is one of the fastest growing areas in gene discovery efforts. RNA interference has also proven to be a powerful reverse-genetics tool, and has been used, for example, in the identification of trans-acting factors involved in RNA editing in trypanosomes. More recently there have been some intriguing hints of a possible biological connection between RNA interference and editing, based on both genetic studies in worms and colocalization studies in flies.

RNA Interference, Editing, and Modification is written primarily for those working directly in the fields of gene silencing, RNA interference, editing, and modification, as well as bioinformaticists trying to identify genomic regions that encode RNAs that are not translated into proteins and geneticists and others wanting to use RNA interference as a means of knocking out expression of individual genes and examining associated phenotypes. *RNA*

Interference, Editing, and Modification is split into two parts. Part I describes methods used in transient and stable gene silencing in worms, flies, trypanosomes, mammals, and plants, with an emphasis on parameters that must be considered for each system. Part II includes assays and methods used in studying RNA editing mechanisms in a wide range of organisms, including both systems that involve the conversion of one base to another and insertion/deletion editing. Each topic begins with a brief overview covering both historical background and scientific significance to provide a broader context for readers new to the respective areas. In addition, there are four chapters that focus on methods for the identification and characterization of small RNAs, many of which are involved in RNA interference or modification.

The overall aim of *RNA Interference, Editing, and Modification* is to present, as clearly as possible, methods that represent the current “state-of-the-art” in the fields of RNA interference, editing, and modification. The level of detail provided is such that prior experience with the technique should not be required to replicate the methods described. Since the underlying biological mechanisms usually differ somewhat between species, each section will include multiple protocols representative of the major experimental systems in a given field. It is hoped that by presenting methods developed for a range of organisms, this book may lead to the modification or adaptation of assays and approaches for use in other biological systems.

Finally, I would like to thank the authors for their contributions, which were uniformly excellent, and series editor John Walker for his editorial skills and advice. Their thoroughness and commitment made this book possible.

Jonatha M. Gott

Contents

Dedication	v
Preface	vii
Contributors	xi

PART I. RNA INTERFERENCE AND GENE SILENCING

1 RNA Interference: <i>Historical Overview and Significance</i> Mary K. Montgomery	3
2 Delivery of Double-Stranded RNA into <i>Caenorhabditis elegans</i> Dawn Hull and Lisa Timmons	23
3 Induction and Biochemical Purification of RNA-Induced Silencing Complex From <i>Drosophila</i> S2 Cells Amy A. Caudy and Gregory J. Hannon	59
4 Analysis of Gene Function in <i>Trypanosoma brucei</i> Using RNA Interference Appolinaire Djikeng, Shuiyuan Shen, Christian Tschudi, and Elisabetta Ullu	73
5 Short Hairpin Activated Gene Silencing in Mammalian Cells Patrick J. Paddison, Amy A. Caudy, Ravi Sachidanandam, and Gregory J. Hannon	85
6 Geminivirus Vectors for Transient Gene Silencing in Plants Nooduan Muangsang and Dominique Robertson	101
7 Posttranscriptional Gene Silencing in Plants Susan Varsha Wesley, Chris Helliwell, Ming-Bo Wang, and Peter Waterhouse	117
8 Identification of microRNAs and Other Tiny Noncoding RNAs by cDNA Cloning Victor Ambros and Rosalind C. Lee	131

PART II. RNA EDITING AND MODIFICATION

9 A Historical Perspective on RNA Editing: <i>How the Peculiar and Bizarre Became Mainstream</i> Donna J. Koslowsky	161
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10	Identification of Substrates for Adenosine Deaminases That Act on RNA Daniel P. Morse	199
11	Purification and Assay of Recombinant ADAR Proteins Expressed in the Yeast <i>Pichia pastoris</i> or in <i>Escherichia coli</i> Gillian M. Ring, Mary A. O'Connell, and Liam P. Keegan	219
12	Isolation of an mRNA-Binding Protein Involved in C-to-U Editing Carri A. Gerber, Anne Relich, and Donna M. Driscoll	239
13	In Vitro Assays for Kinetoplastid U Insertion–Deletion Editing and Associated Activities Kenneth Stuart, Reza Salavati, Robert P. Igo, Jr., Nancy Lewis Ernst, Setareh S. Palazzo, and Bingbing Wang	251
14	Identification and Characterization of Trypanosome RNA-Editing Complex Components Kenneth Stuart, Aswini K. Panigrahi, and Achim Schnauffer	273
15	Chimeric Templates and Assays Used to Study <i>Physarum</i> Cotranscriptional Insertional Editing In Vitro Elaine M. Byrne	293
16	Methods for Analysis of Mitochondrial tRNA Editing in <i>Acanthamoeba castellanii</i> Amanda J. Lohan and Michael W. Gray	315
17	In Vitro RNA Editing Systems From Higher Plant Chloroplasts Tetsuro Hirose, Tetsuya Miyamoto, Junichi Obokata, and Masahiro Sugiura	333
18	Studying RNA Editing in Transgenic Chloroplasts of Higher Plants Ralph Bock	345
19	Detection and Quantification of Modified Nucleotides in RNA Using Thin-Layer Chromatography Henri Grosjean, Gérard Keith, and Louis Droogmans	357
20	Functional Characterization of 2'-O-Methylation and Pseudouridylation Guide RNAs Tamás Kiss and Beáta E. Jády	393
21	Experimental RNomics: A <i>Global Approach to Identifying Small Nuclear RNAs and Their Targets in Different Model Organisms</i> Alexander Hüttenhofer, Jérôme Cavallé, and Jean-Pierre Bachellerie	409
	Index	429

Contributors

VICTOR AMBROS • *Department of Genetics, Dartmouth Medical School, Hanover, NH*

JEAN-PIERRE BACHELLERIE • *Laboratoire de Biologie Moléculaire Eucaryote du CNRS, Université Paul Sabatier, Toulouse, France*

RALPH BOCK • *Westfälische Wilhelms-Universität Münster, Institut für Biochimie und Biotechnologie der Pflanzen, Münster, Germany*

ELAINE M. BYRNE • *Centre for Bioengineering, Trinity College Dublin, Dublin, Ireland*

AMY A. CAUDY • *Cold Spring Harbor Laboratory, Watson School of Biological Sciences, Cold Spring Harbor, NY*

JÉROME CAVAILLÉ • *Laboratoire de Biologie Moléculaire Eucaryote du CNRS, Université Paul Sabatier, Toulouse, France*

APPOLINAIRE DIKENG • *Department of Internal Medicine, Yale University School of Medicine, New Haven, CT*

DONNA M. DRISCOLL • *Lerner Research Institute, Department of Cell Biology, The Cleveland Clinic Foundation, Cleveland, OH*

LOUIS DROGMANS • *Laboratoire de Microbiologie, Institut de Recherches, Microbiologiques J. M. Wiame, Université Libre de Bruxelles, Bruxelles, Belgium*

NANCY LEWIS ERNST • *Seattle Biomedical Research Institute, and Department of Pathobiology, University of Washington, Seattle, WA*

CARRI A. GERBER • *Lerner Research Institute, Department of Cell Biology, The Cleveland Clinic Foundation, Cleveland, OH*

MICHAEL W. GRAY • *Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, Nova Scotia, Canada*

HENRI GROSJEAN • *Laboratoire d'Enzymologie et Biochimie Structurales du CNRS, Gif-sur-Yvette, France*

GREGORY J. HANNON • *Cold Spring Harbor Laboratory, Watson School of Biological Sciences, Cold Spring Harbor, NY*

CHRIS HELLIWELL • *CSIRsO Plant Industry, Canberra, Australia*

TETSURO HIROSE • *School of Biomedical Science, Tokyo Medical and Dental University, Tokyo, Japan*

DAWN HULL • *Department of Molecular Biosciences, University of Kansas, Lawrence, KS*

- ALEXANDER HÜTTENHOFER • *Institut für Molekularbiologie, Abt. Funktionelle Genomik, Universität Innsbruck, Innsbruck, Austria*
- ROBERT P. IGO, JR. • *Department of Biostatistics, University of Washington, Seattle, WA*
- BEÁTA E. JÁDY • *Laboratoire de Biologie Moléculaire Eucaryote du CNRS, Université Paul Sabatier, Toulouse, France*
- LIAM P. KEEGAN • *MRC Human Genetics Unit, Western General Hospital, Edinburgh, United Kingdom*
- GÉRARD KEITH • *Institut de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg Cedex, France*
- TAMÁS KISS • *Laboratoire de Biologie Moléculaire Eucaryote du CNRS, Université Paul Sabatier, Toulouse, France*
- DONNA J. KOSLOWSKY • *Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI*
- ROSALIND C. LEE • *Department of Genetics, Dartmouth Medical School, Hanover, NH*
- AMANDA J. LOHAN • *Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, Nova Scotia, Canada*
- TETSUYA MIYAMOTO • *Center for Gene Research, Nagoya University, Nagoya, Japan*
- MARY K. MONTGOMERY • *Biology Department, Macalester College, St. Paul, MN*
- DANIEL P. MORSE • *Chemistry Department, United States Naval Academy, Annapolis, MD*
- NOODUAN MUANGSAN • *Department of Biology, Khon Khaen University, Khon Khaen, Thailand*
- JUNICHI OBOKATA • *Center for Gene Research, Nagoya University, Nagoya, Japan*
- MARY A. O'CONNELL • *MRC Human Genetics Unit, Western General Hospital, Edinburgh, United Kingdom*
- PATRICK J. PADDISON • *Cold Spring Harbor Laboratory, Watson School of Biological Sciences, Cold Spring Harbor, NY*
- SETAREH S. PALAZZO • *Seattle Biomedical Research Institute, and Department of Pathobiology, University of Washington, Seattle, WA*
- ASWINI K. PANIGRAHI • *Seattle Biomedical Research Institute, and Department of Pathobiology, University of Washington, Seattle, WA*
- ANNE RELICH • *Lerner Research Institute, Department of Cell Biology, The Cleveland Clinic Foundation, Cleveland, OH*
- GILLIAN M. RING • *MRC Human Genetics Unit, Western General Hospital, Edinburgh, United Kingdom*

- DOMINIQUE ROBERTSON • *Department of Botany, North Carolina State University, Raleigh, NC*
- RAVI SACHIDANANDAM • *Cold Spring Harbor Laboratory, Watson School of Biological Sciences, Cold Spring Harbor, NY*
- REZA SALAVATI • *Seattle Biomedical Research Institute, and Department of Pathobiology, University of Washington, Seattle, WA*
- ACHIM SCHNAUFER • *Seattle Biomedical Research Institute, and Department of Pathobiology, University of Washington, Seattle, WA*
- SHUIYUAN SHEN • *Department of Internal Medicine, Yale University School of Medicine, New Haven, CT*
- KENNETH STUART • *Seattle Biomedical Research Institute, and Department of Pathobiology, University of Washington, Seattle, WA*
- MASAHIRO SUGIURA • *Graduate School of Natural Sciences, Nagoya City University, Nagoya, Japan*
- LISA TIMMONS • *Department of Molecular Biosciences, University of Kansas, Lawrence, KS*
- CHRISTIAN TSCHUDI • *Departments of Epidemiology and Public Health and Internal Medicine, Yale University School of Medicine, New Haven, CT*
- ELISABETTA ULLU • *Departments of Internal Medicine and Cell Biology, Yale University School of Medicine, New Haven, CT*
- BINGBING WANG • *Seattle Biomedical Research Institute, and Department of Pathobiology, University of Washington, Seattle, WA*
- MING-BO WANG • *CSIRO Plant Industry, Canberra, Australia*
- PETER WATERHOUSE • *CSIRO Plant Industry, Canberra, Australia*
- SUSAN VARSHA WESLEY • *CSIRO Plant Industry, Canberra, Australia*