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METHODS IN MOLECULAR BIOLOGY™

RNAi

Design and Application

Edited by

Sailen Barik

Department of Biochemistry and Molecular Biology,
University of South Alabama, College of Medicine,
Mobile, Alabama

Humana Press

Editor
Sailen Barik
Department of Biochemistry
and Molecular Biology
College of Medicine
University of South Alabama
Mobile, Alabama

Series Editor
John. M. Walker
School of Life Sciences
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Cover illustration: Figure 3, Chapter 16, "Temporal Control of Gene Silencing by *in ovo* Electroporation" by Thomas Baeriswyl, Olivier Mauti, and Esther T. Stoeckli. Figure 3, Chapter 17, "Altering Flower Color in Transgenic Plants by RNAi-Mediated Engineering of Flavonoid Biosynthetic Pathway," by Yoshikazu Tanaka, Noriko Nakamura, and Junichi Togami.

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In memory of my Mother, Promilla Barik (1932–2006)

Preface

RNA interference (RNAi), in which RNA silences RNA, is the most recent discovery to revolutionize biology and to be recognized by a Nobel Prize (in 2006, to Andrew Fire and Craig Mello). It is a story that began with historic observations in plants and fungi and eventually worked its way up to humans. If one were to describe the major steps of RNAi very briefly, it would read as follows: RNAi is triggered by double-stranded RNA (dsRNA), produced endogenously or introduced by scientists –> Long dsRNA is trimmed into short interfering RNA or microRNA (siRNA or miRNA) by Dicer –> The individual strands of the si/miRNA then guide the assembly of a multiprotein complex, known as RISC, the key constituent of which is Argonaute –> Depending on the extent of homology of the guide RNA to the target, RISC either destroys the target RNA or suppresses its translation, leading to gene silencing.

The chapters in *RNAi: Design and Application*, contributed by leaders in the field, sum up the state-of-the-art methods on practical, everyday use of RNAi in biological research. Although multiple books and monographs have been published on RNAi, there is a noticeable dearth of bench protocols that can be used quickly and easily by beginners aspiring to enter this new field. This volume aims to fill that void.

RNAi: Design and Application is divided into two parts. The first and smaller part (chapters 1–4) covers the fundamentals including designs of RNAi, biochemical assay protocols for the major components of RNAi, and study of potential off-target effects. The larger second part (chapters 5–18) covers various applications of RNAi in diverse model organisms and systems, from antiviral and anticancer applications to altering flower color in plants. Armed with this volume, a researcher with standard molecular biological training should be able to perform today's major RNAi-related experiments and carry out gene knock-down analyses in virtually any cell line or species of interest. In the established tradition of the *Methods in Molecular Biology*™ series, each chapter contains step-by-step protocols, extra notes, and problem-solving tips, which are usually not found in original research papers. As the horizon of RNAi application is rapidly broadening, we have strived to offer the most recent protocols in each area so that they remain useful for years to come.

My sincere thanks go to all the authors and the Humana staff for bringing it all together, and to Professor John M. Walker for his guidance. I remain indebted to my wife, Kumkum, and my children, Titus and Tiasha, for their immeasurable support and encouragement.

Sailen Barik

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Contributors

Mohammed Amarzguioui • *The Biotechnology Centre of Oslo, Oslo, Norway*

Emily Anderson • *Dharmacon, ThermoFisher Scientific, Lafayette, CO*

Alfred Ayala • *Division of Surgical Research, Department of Surgery, Rhode Island Hospital/Brown University School of Medicine, Providence, RI*

Thomas Baeriswyl • *Institute of Zoology, University of Zürich, Zürich, Switzerland*

Sailen Barik • *Department of Biochemistry and Molecular Biology, University of South Alabama, College of Medicine, Mobile, AL*

Vivian Bellofatto • *Department of Microbiology and Molecular Genetics, UMDNJ-NJ Medical School, International Center for Public Health, Newark, NJ*

Vira Bitko • *Department of Biochemistry and Molecular Biology, University of South Alabama, College of Medicine, Mobile, AL*

Queta Boese • *Dharmacon, ThermoFisher Scientific, Lafayette, CO*

Chun-Shiang Chung • *Division of Surgical Research, Department of Surgery, Rhode Island Hospital/Brown University School of Medicine, Providence, RI*

Toshihiko Eki • *Department of Ecological Engineering, Toyohashi University of Technology, Toyohashi, Japan*

Pedro Gonzalez-Alegre • *Department of Neurology, Carver College of Medicine at The University of Iowa, Iowa City, IA*

Wenyi Gu • *Cancer Biology Program, Centre for Immunology and Cancer Research, Princess Alexandra Hospital, University of Queensland, Brisbane, Australia*

Nancy Guillén • *Unité de Biologie Cellulaire du Parasitisme, Institut Pasteur, Paris, France*

Fumio Hanaoka • *Graduate School of Frontier Biosciences, Osaka University, and SORST, Japan Science and Technology Agency, Osaka, Japan*

Scott Q. Harper • *Center for Gene Therapy, Department of Pediatrics, The Ohio State University, Columbus, OH*
Hiroshi Imai • *Laboratory of Reproductive Biology, Department of Agriculture, Kyoto University, Kyoto, Japan*
Mohammad Nurul Islam • *International Center for Genetic Engineering and Biotechnology, PMB Lab, New Delhi, India*
Sumona Karjee • *International Center for Genetic Engineering and Biotechnology, PMB Lab, New Delhi, India*
Jon Karpilow • *Dharmacon, ThermoFisher Scientific, Lafayette, CO*
Anastasia Khvorova • *Dharmacon, ThermoFisher Scientific, Lafayette, CO*
Baojian Li • *Top Genomics, Ltd., and College of Life Sciences, Sun Yat-sen University, Guangzhou, China*
Qinghua Liu • *Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX*
Joanne Lomas-Neira • *Division of Surgical Research, Department of Surgery, Rhode Island Hospital/Brown University School of Medicine, Providence, RI*
Patrick Y. Lu • *Sirnaomics, Inc., Rockville, MD*
Chikahide Masutani • *Graduate School of Frontier Biosciences, Osaka University; and SORST, Japan Science and Technology Agency, Osaka, Japan*
Olivier Mauti • *Institute of Zoology, University of Zürich, Zürich, Switzerland*
Nigel McMillan • *Cancer Biology Program, Centre for Immunology and Cancer Research, Princess Alexandra Hospital, University of Queensland, Brisbane, Australia*
Naojiro Minami • *Laboratory of Reproductive Biology, Department of Agriculture, Kyoto University, Kyoto, Japan*
Keita Miyoshi • *Institute for Genome Research, University of Tokushima, Tokushima, Japan*
Sunil K. Mukherjee • *International Center for Genetic Engineering and Biotechnology, PMB Lab, New Delhi, India*
Tomoko Nagami-Okada • *Institute for Genome Research, University of Tokushima, Tokushima, Japan*
Noriko Nakamura • *Institute for Advanced Core Technology, Suntory Ltd., Osaka, Japan*
Tsuyoshi Ohkumo • *Graduate School of Frontier Biosciences, Osaka University; and SORST, Japan Science and Technology Agency, Osaka, Japan*

Jennifer B. Palenchar • *Department of Chemistry, Villanova University, Villanova, PA*

Mario Perl • *Universitätsklinikum Ulm, Zentrum für Chirurgie, Klinik für Unfallchirurgie, Hand-, Plastische- und Wiederherstellungschirurgie, Ulm, Germany*

Lisa Putral • *Cancer Biology Program, Centre for Immunology and Cancer Research, Princess Alexandra Hospital, University of Queensland, Brisbane, Australia*

John J. Rossi • *Division of Molecular Biology, Beckman Research Institute of the City of Hope, Duarte, CA*

Michael E. Scharf • *Molecular and Applied Insect Toxicology, Entomology and Nematology Department, University of Florida, Gainesville, FL*

Margaret A. Schwinghammer • *Department of Entomology, Purdue University, West Lafayette, IN*

Haruhiko Siomi • *Keio University School of Medicine, Tokyo, Japan*

Mikiko C. Siomi • *Institute for Genome Research, University of Tokushima, JST, CREST, Tokushima, Japan*

Carlos F. Solis • *Unité de Biologie Cellulaire du Parasitisme, Institut Pasteur, Paris, France*

Esther T. Stoeckli • *Institute of Zoology, University of Zürich, Zürich, Switzerland*

Yoshikazu Tanaka • *Institute for Advanced Core Technology, Suntory Ltd., Osaka, Japan*

Qingquan Tang • *OriGene Technologies, Inc., Rockville, MD*

Junichi Togami • *Institute for Advanced Core Technology, Suntory Ltd., Osaka, Japan*

Hiroshi Uejima • *Institute for Genome Research, University of Tokushima, Tokushima, Japan*

Toshiaki Watanabe • *Division of Human Genetics, Department of Integrated Genetics, National Institute of Genetics, Research Organization of Information and Systems; and Department of Genetics, School of Life Science, The Graduate University for Advanced Studies (SOKENDAI), Mishima, Japan*

Doreen E. Wesche-Soldato • *Division of Surgical Research, Department of Surgery, Rhode Island Hospital/Brown University School of Medicine, Providence, RI*

Martin Woodle • *Nanotides Pharmaceuticals, Inc., Rockville, MD*

Xuecheng Ye • *Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX*

Pumin Zhang • *Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX*

Xuguo Zhou • *Molecular and Applied Insect Toxicology, Entomology and Nematology Department, University of Florida, Gainesville, FL*