METHODS IN MOLECULAR BIOLOGY

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Argonaute Proteins

Methods and Protocols

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Humana Press

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ISSN 1064-3745 ISSN 1940-6029 (electronic)
Methods in Molecular Biology
ISBN 978-1-4939-7338-5 ISBN 978-1-4939-7339-2 (eBook)
DOI 10.1007/978-1-4939-7339-2

Library of Congress Control Number: 2017952703

Cover image: This image is the crystal structure of human Argonaute2 in complex with a guide and a target (PDB ID: 4W5O)

Printed on acid-free paper

This Humana Press imprint is published by Springer Nature
The registered company is Springer Science+Business Media, LLC
The registered company address is: 233 Spring Street, New York, NY 10013, U.S.A.

Preface

Since the discovery that Argonautes play essential roles in RNA silencing in a variety of eukaryotes, biochemical properties and biological functions of Argonautes have been extensively studied using an assortment of techniques [1, 3]. Biochemical and structural analyses in the past decade clarified their detailed molecular architectures along with the mechanisms by which Argonautes bind guide and target nucleic acids and cleave targets [4, 5]. Despite the similarity of the overall domain structures, the roles of eukaryotic and prokaryotic Argonautes are quite diverse. Most of them seem to serve mainly as defense systems against foreign nucleic acids, such as viruses/bacteriophages and transposons, while some others are involved in gene regulation [6, 7].

Although the functionality of endogenous small regulatory RNAs was already described in developmental biology studies using *C. elegans* in the 1990s [8, 9], the importance of the underlying molecular mechanism was not recognized until the early 2000s. However, once the deep conservation of the small RNA processing enzymes, Dicers, and the effector proteins, Argonautes, was discovered, the scientific community quickly developed a large body of knowledge regarding biological functions of small regulatory RNA pathways. The development was further accelerated by advances in deep sequencing technologies that allowed massive identification of small RNA species in biological systems. Thanks to recent studies, we now have a seemingly complete catalog of Argonaute-bound small RNAs in a few model organisms as well as humans [10].

However, we are only beginning to understand biological roles for individual small RNAs. Furthermore, latest studies revealed that functions of small RNA-Argonaute complexes have been highly diversified during evolution ranging from DNA/chromatin modifications to mRNA cleavage/destabilization, presumably due to the specialized ability of Argonautes to recruit various downstream effectors [11]. Needless to say, a full understanding of such non-conserved functions of Argonautes is important to elucidate the molecular mechanisms behind biological phenomena in natural contexts. Meanwhile, studies of diverse Argonaute-mediated mechanisms are urged further in terms of the potential of Argonautes for applications as reprogrammable gene regulatory modules. Some of them may become important additions of artificial gene silencing/editing techniques to the current list of various RNAi- and CRISPR-mediated methods.

On the other hand, the vast diversity of Argonaute-mediated mechanisms makes it a difficult task to fully understand the biology of Argonautes in various organisms. The important steps include (1) Identification and expression analysis of guide nucleic acids and their targets. (2) Analysis of biochemical properties of Argonautes. (3) Biological functions of Argonautes. Furthermore, for all these studies, a common question is (4) How do we obtain materials and set up analysis platforms? This volume provides a comprehensive set of protocols that would help to address some of the important questions in these four areas.

1. *Identification and expression analysis of guide nucleic acids and their targets*
   The key step toward a full understanding of Argonaute functions is to comprehensively catalog the guide nucleic acids. This includes efficient cloning/sequencing of small RNAs bound to Argonautes (Chapter 1), precisely quantifying the absolute amounts of

Argonaute-bound small RNAs (Chapter 2), identifying intrinsic sequence preferences of Argonaute proteins (Chapter 3), and quantifying individual small RNA sequences in an isoform-specific manner (Chapter 4). For multicellular organisms, it is important to analyze the expression patterns of small RNA genes. In situ hybridization using whole-mount worm samples provides insights into biological functions of individual small RNA genes (Chapter 5). It is also critical to fully catalog targets of guide RNA-Argonaute complexes in tissues using genome-wide methods such as CLIP (cross-linking and immunoprecipitation: Chapter 6).

2. *Analysis of biochemical properties of Argonautes.*
   Thanks to the successful establishment of protocols to purify individual components of small RNA processing pathways as recombinant proteins, their enzymatic activities and biochemical properties can now be quantitatively measured with unprecedented accuracy. This volume includes protocols for quantitative analysis of substrate processing by Dicer enzymes (Chapter 7), binding kinetics between guide nucleic acids and effector Argonaute complexes in prokaryotes (Chapter 8) and eukaryotes (Chapters 9 and 10). In particular, these protocols offer a wide range of quantification methods from traditional enzymatic kinetics analyses to measurements of binding affinities based on fluorescence polarization and single-molecule measurements.

3. *Biological functions of Argonautes*
   To understand biological roles for Argonautes, it is inevitable to study their functions at cellular or organismal levels. The challenge is that Argonautes may play an extremely wide range of roles in various biological aspects. This volume provides protocols that allow us to study several aspects of Argonaute functions, including regulation of the high-order chromatin structure (Chapter 11), and in vivo miRNA functions in fish embryos (Chapter 12) and mouse embryonic brains (Chapter 13).

4. *Preparation of materials and analysis platforms.*
   To answer the above-mentioned questions, important steps are to prepare reliable materials and streamline their analytical pipelines. A protocol is described for an establishment of embryonic fibroblast cultures from Argonaute2 knockout mice (Chapter 14). Procedures are introduced for epitope tagging of the endogenous Argonaute1 locus in a *Drosophila* cell line using the CRISPR/Cas9 system (Chapter 15). For genome-wide analysis of small RNA sequencing data, a major challenge is to correctly identify the genomic origins of reads that are derived from repetitive sequences, such as tRNA genes. A streamlined bioinformatics resource provides analysis platform for small RNAs derived from tRNA genes and related sequences, some of which are known to bind Argonaute proteins (Chapter 16).

This collection of protocols covers methods to analyze various Argonaute proteins from a wide range of organisms to understand their properties at different levels, from the molecular to the organismal level. The editors hope that this volume will serve as a valuable resource. Lastly, we would like to sincerely thank all the authors who generously contributed their latest protocols to this volume of Methods in Molecular Biology *Argonaute Proteins.*

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