METHODS IN MOLECULAR BIOLOGY

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Animal Endo-siRNAs

Methods and Protocols

Edited by

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Humana Press

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Preface

Short interfering RNAs (siRNAs) are widely used in biomedical research to modulate gene expression in a wide spectrum of cell types. The fact that *exogenous* siRNAs elicit a predictable effect when transfected into a cell, usually the knock down of a specific target mRNA, suggests that endogenous pathways utilizing *endogenous* siRNAs are in place. These short RNA oligonucleotides, now called endo-siRNAs, however, proved a rather elusive species in mammalian cells. Only the recently developed RNAseq technologies provided sufficient sequencing depth to comprehensively demonstrate and map endo-siRNAs in vertebrate animal systems. Yet, their biological role is still controversial. In non-vertebrates such as *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* the molecular basics of endo-siRNAs are well established, both with regard to their molecular mechanism of synthesis and their biological function(s). As a consequence, researchers from separate research communities may define the term "endo-siRNA" in different ways.

The generally low expression level of endo-siRNAs in specific tissues and their close resemblance to breakdown products of endogenous cellular RNAs represent formidable challenges in this field. Moreover, detection of endo-siRNAs often involves reverse transcription, a step that produces low levels of false positive signals when strand specificity is of experimental importance—which is the case in studies focusing on endo-siRNAs. These difficulties emphasize the importance of established highly sensitive protocols to monitor small RNAs, specifically strategies for high-throughput sequencing, array technologies, and northern blotting (*see* Chapters 8–11 in this book). A further important consideration in the research of endo-siRNAs is the choice of model systems. As indicated above, *C. elegans* is by far the best established model system to study endo-siRNAs: Here, they have distinct structural features (length and defined first base, i.e., 26G siRNAs) and their regulatory role in gene expression is well documented (*see* Chapters 6 and 7 in this book). In the fruit fly *Drosophila* the synthesis of endo-siRNAs involves a specific Dicer molecule (Dicer2) and the pathway is essential to ensure normal fly development under perturbed environmental conditions (*see* Chapters 4 and 5 in this book). However, in vertebrates endo-siRNAs have only recently been detected and their biological role is unclear.

The aim of this book is to present a variety of approaches to investigate endo-siRNAs. Many of the protocols are generally applicable to study short RNAs expressed at a low level. Others highlight model systems that are particularly suitable to investigate specific aspects of endo-siRNAs, their synthesis, their genomics, or regulatory role. Unfortunately, there are no "one fits for all" methods when dealing with endo-siRNAs, especially in vertebrates. Consequently, this book suggests a compilation of applicable strategies for researchers entering the field of endo-siRNAs. Importantly, the book also contains many practical tips that are absent in standard lab manuals. In that sense, I hope the different contributions stimulate research and progress in the field of endo-siRNAs.

To conclude, I would like to sincerely thank all the contributors who shared their knowledge and experience and took their time and effort to realize the book on endo-siRNAs. Moreover, special thanks go to all the helpful individuals who assisted the preparation of this book.

Newcastle, UK *Andreas Werner*

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