
Preface

Little over a decade ago, Andrew Fire, Craig Mello, and colleagues demonstrated that double-stranded (ds)RNA induces sequence-specific gene silencing in the nematode *Caenorhabditis elegans* (RNA interference, RNAi). This work converged with research in plants, in which related RNA-based silencing processes were known to exist. Ever since, research in the field has progressed at an astonishing rate, resulting in our appreciation of small silencing RNAs as central regulators of gene expression, as guards of genome integrity, and as essential mediators of antiviral defense. The discovery that synthetic small interfering RNA (siRNA) induces gene silencing in mammals, by Thomas Tuschl and colleagues in 2001, has further boosted the development of novel therapeutics and experimental tools based on RNAi technology.

Viruses and RNAi share an intricate relationship at many levels. Early work in plants indicated that viruses can be both inducers and targets of RNA-based post-transcriptional gene silencing (which we now know as RNAi or RNA silencing). The concept of RNAi as an antiviral defense mechanism is now well-established in plants and other organisms, including insects. In vertebrates, viruses also interact with a related RNA silencing mechanism, the microRNA (miRNA) pathway. Many nuclear DNA viruses encode their own set of miRNAs, by which they regulate viral or host gene expression and modify, for example, the transition from latent to lytic infection and the recognition of infected cells by the host immune system. Furthermore, cellular miRNAs likely regulate expression of many genes that are important for virus biology, but they have been suggested to directly target viral RNA as well.

The therapeutic potential of RNAi-based antiviral drugs was recognized early on. It is now clear that replication of many, if not all, mammalian viruses can be suppressed by RNAi in cell culture. While these results have raised considerable optimism about the potential of RNAi-based drugs, important hurdles remain, including issues related to the delivery and stability of siRNAs and the risk of viral escape.

From this brief overview it will be apparent that a great — and increasing — number of tools and techniques are available for those interested in the interface of viruses and RNAi. *Antiviral RNAi: concepts, methods, and applications* provides a collection of protocols for the analysis of natural antiviral RNAi responses and viral miRNAs, as well as for the development and optimization of RNAi-based antiviral drugs. As RNAi is a central regulatory mechanism in the cell, the methods in this volume can also be applied out of the context of a virus infection. In the established tradition of the *Methods in Molecular Biology* series, *Antiviral RNAi: concepts, methods, and applications* provides detailed step-by-step protocols and extra tools and tricks that should be useful to those new to the field and experienced scientists alike.

This volume consists of five parts. Part 1 reviews important basic concepts in the field of antiviral RNAi. Part 2 provides experimental and bio-informatic tools for the analysis of small silencing RNAs. Part 3 covers methods to biochemically dissect RNAi-based antiviral defense and viral counter-defense mechanisms. Part 4 describes methods for the design, expression, and delivery of therapeutic antiviral siRNAs. Part 5 presents genome-wide

RNAi approaches for the identification of factors involved in virus replication, which may represent novel targets for antiviral therapy.

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Concepts, Methods, and Applications

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