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## Preface

The study of small noncoding RNAs was accelerated by the discovery of RNA interference (RNAi) in the nematode *Caenorhabditis elegans* in 1998, by two Nobel Prize laureates Andrew Fire and Craig Mello. Researchers have subsequently focused on understanding how short interfering RNAs (siRNAs) and microRNAs (miRNAs), which represent two major RNAi-triggering small noncoding RNAs, actually induce gene silencing and what mechanisms are responsible for producing them from double-stranded RNA precursors. Studies revealed that RNAi is highly conserved in a wide variety of animals and plants, as well as in unicellular organisms such as fission yeast. They further demonstrated that in addition to *C. elegans*, other model species such as *Drosophila melanogaster*, *Arabidopsis thaliana*, and mice, along with cultured cell lines such as HeLa and *Drosophila* Schneider 2 (S2) could serve as useful tools for investigating the molecular nature of RNAi. Indeed, these organisms have been used to address the fundamental questions of the biogenesis and functions of RNAi-triggering small noncoding RNAs. It is clear that the ubiquity of RNAi has a significant impact on our understanding of the RNAi world.

PIWI-interacting RNAs (piRNAs) are the third and most-recently discovered group of silencing-inducible small RNAs in animals. The delay in their discovery was mainly the result of their expression predominantly in the gonads. Cultured HeLa and S2 cells, which are otherwise excellent tools for studying ubiquitous RNAi, do not express piRNAs to a detectable level. Although the limited expression of these piRNAs has meant they have been less well studied than other small RNAs, it has conversely stimulated researchers' curiosities. Comprehensive high-throughput sequencing analysis of piRNAs in embryos, testes, and ovaries of *D. melanogaster*, as well as in mouse and rat testes, has raised the profile of piRNAs and thus further accelerated piRNA studies.

piRNAs associate with specific members of the Argonaute family of proteins to form RNA-induced silencing complexes (RISCs), as with siRNAs and miRNAs, and piRNA-containing RISCs (piRISCs) implement RNA silencing similarly to siRISCs and miRISCs, though their mechanisms may vary. This indicates the equivalence of the various small RNAs. However, piRNA precursors are thought to be single-stranded because piRNA processing occurs independently of Dicer. piRNAs are several nucleotides longer than miRNAs and siRNAs. Most piRNAs are complementary to transcripts of transposable elements and represses the mobile genomic elements to maintain the integrity of the genome in the germline. These piRNA-specific features further highlight the uniqueness of piRNAs, prompting studies aimed at developing a comprehensive understanding of the small RNA world. However, the process whereby piRNAs mature from their precursors remains unknown. We still do not understand how piRNA precursors are selectively chosen or the mechanisms responsible for silencing of target elements. Given these unanswered basic questions, it is clear that the piRNA studies are still a long way from their final goal. In order to further progress towards this goal, this is an ideal time to gather and share our

expertise and knowledge on the experimental methods used to unveil the piRNA world. This book “PIWI-interacting RNAs: Methods and Protocols” provides the most recent methods and protocols for studying piRNAs in the gonads of a wide range of species, as well as in any other organs where piRNAs may be detected. This book will help both established researchers and newcomers to the field to progress towards the ultimate goal of understanding the mechanisms and actions of piRNAs.

*Tokyo, Japan*

*Mikiko C. Siomi*

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