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# Preface

The past decade has witnessed a true revolution in our understanding of how RNA molecules can act as regulators of gene expression. Central to this new understanding is a growing appreciation that short duplex RNAs can trigger potent and highly specific inhibitory effects, most in the cytoplasm but some also in the nucleus. The underlying regulatory pathway is the ancient and conserved RNA interference (RNAi) pathway utilizing small duplex RNAs. RNAi is carried out in two steps. In the first, long dsRNAs are processed into short 21–23 nt-long effector dsRNAs (siRNAs, small interfering RNAs; 21–25 nt in plants) by the action of the enzyme Dicer. In the second step, the siRNAs are assembled into protein-RNA complexes (RNA-induced silencing complexes, RISC) that direct the specific cleavage of target mRNAs. In these complexes, the short dsRNA duplex is unwound, generating active RISC complexes containing single siRNA strands.

In addition to siRNAs, there is another abundant and important class of small ~22 nucleotide noncoding RNA species in cells, the microRNAs (miRNAs). These RNAs are involved in many processes, including regulation of gene expression during development and defense against viruses. In the past several years there has been great progress in the biochemical and computational identification of novel miRNA species, and there are now hundreds of these small RNAs known. Most current models envision miRNAs acting as negative regulators of translation by acting in a still unknown way on translating ribosomes.

Though it is important to understand and appreciate the concepts and mechanisms of RNAi and miRNA action, it is also critical to be able to apply these emerging technologies in a rational and effective manner. *RNA Silencing: Methods and Protocols* is intended to facilitate the translation of gene silencing concepts into practical applications, and includes a broad but useful set of RNA silencing protocols.

The first three chapters deal with biochemical aspects of the silencing machinery. Siomi and Siomi describe a powerful biochemical method for the purification and identification of RNAi components. Pham and Sontheimer then describe electrophoretic methods to separate distinct silencing complexes. Then Lee and Kim describe the purification of the Drosha protein, which is a central player in the generation of miRNAs.

Chapters 4–6 describe detailed methods for RNA silencing in nonmammalian organisms. Dudley and Goldstein detail methods for RNAi in the nematode *C. elegans*. Clayton et al. then describe how to carry out RNAi-mediated gene

silencing in *Trypanosoma brucei*. Finally, Mette et al. describe methods for the study of transcriptional gene silencing in the nuclei of plant cells.

Then, there follow a series of chapters detailing ways to design, prepare, and use RNAs to silence gene expression. These include the use of small RNA duplexes (siRNAs). In Chapter 7, Sioud and Walchli describe methods for the design of siRNA libraries. In Chapter 8 Myers and Ferrell offer an incredibly valuable and detailed description of how to design, prepare, and use pools of siRNAs generated by *in vitro* Dicer cleavage. In Chapter 9 Chae and Hla describe a similar approach. Chapters 10 and 11 (Sui and Shi, and Harper and Davidson) describe methods for the design and use of DNA vectors that express short RNA hairpins that can lead to RNAi silencing *in vivo*.

RNA interference cannot be effective in cells unless the active RNAs are efficiently introduced into them, or expressed in them. Therefore, Chapters 12–14 describe several current methods and strategies for the *in vivo* delivery of siRNAs and silencing vectors. Sioud (Chapter 12) describes some approaches for the delivery of siRNAs, and Simeoni et al. (Chapter 13) describe a novel peptide-based strategy for this purpose. In Chapter 14, Li and Rossi describe how lentiviral vectors can be used for the very efficient delivery and expression of active siRNAs into cultured and primary hematopoietic cells.

Chapter 15 describes how RNAi technology can be used to fine-tune the regulation of some gene expression by targeting specific isoforms of a given gene.

Chapters 16–18 describe methods for the study and use of microRNAs. Maniataki et al. describe the isolation of the ribonucleoprotein particles that contain miRNAs, and methods for miRNA cloning. Kiriakidou et al. then describe how to detect miRNAs as well as assays for miRNA function. Sioud and Rosok detail a high-throughput analysis of miRNA expression.

Finally, I think that it is important to keep in mind that the RNAi and miRNA pathways are not the only ways that cells can use small RNAs to regulate gene expression using natural and endogenous mechanisms. Small RNAs are important in a number of biological processes, including pre-mRNA splicing. It has been shown recently that one of the key components of the splicing machinery, U1 snRNA, can be redirected to nuclear RNA targets in a way that results in a striking downregulation of gene expression. In contrast to siRNAs and miRNAs, which act primarily in the cytoplasm of mammalian cells, U1 snRNA acts only in the nucleus. Thus, Chapter 19 details a U1-based approach that should be considered as an alternative to RNAi, and which is associated with distinct strengths and weaknesses.

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RNA Silencing

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