Preface

Recent rapid and unprecedented development of DNA sequencing technology has enabled us to obtain the entire genomic information with extremely low costs within a very short period. As sequence information increases, the need for functional annotation of target genes also increases. Due to the low efficiency of homologous recombination in plants, targeted gene destruction has not been used as a way to knock down gene function.

RNA interference (RNAi) was discovered by Fire, Mello and their colleagues in 1998 and its use has rapidly expanded into the broad area of life science, especially animal and plant cell studies. RNAi, which can ectopically reduce the expression of target genes, is guided by 21–25-nucleotide-long small RNAs (sRNAs). This key molecule was discovered in plants by Hamilton and Baulcombe in 1998. The double-stranded RNA-specific endonuclease, Dicer, processes double-stranded RNA molecules and sRNAs are produced. A single-stranded sRNA is incorporated into Argonaute (AGO) proteins. mRNAs are cleaved at the middle of the complementary region which has been annealed with an sRNA molecule.

Plant cells are resistant to the direct transfer of sRNAs because of their rigid cell walls. Thus in most cases, RNAi is established after stable transformation of plant cells, and an expression cassette which transcribes an inverted repeat sequence is introduced. Its resulting transcripts form a stem–loop structure (the so-called hairpin RNAs). The stem is processed by Dicer, and then sRNAs are produced. Several bypasses without the production of stable transformants are developed in order to assess the effects of RNAi, and some of them are also described in this book. This volume is intended to guide basic RNAi technologies which have been developed in and for plant biology.

A historical overview of RNAi is provided in **Chapter 1**, in which an attractive story about the discovery and early establishment of the RNAi pathway is described and would be useful for increasing readers' interest. Plant science played an important role at the dawn of RNAi research. In **Chapter 2**, the side effect of RNAi technology, an off-target effect, is reviewed. Although RNAi is certainly a powerful tool for molecular biology, understanding regarding the sequence specificity of RNAi is a pre-requisite for the elucidation of RNAi's effects. Construction of an RNAi vector, especially of an inverted repeat structure, is a difficult and time-consuming process. The use of Gateway technology can markedly reduce this laborious work as introduced in **Chapter 3**. Application of RNAi technology to the analysis of essential genes would be difficult because knockdown of essential genes often causes lethality. In **Chapter 4**, an inducible RNAi vector is introduced, which can control gene expression at the spatial and/or temporal level and circumvent viability problems. An example for artificial microRNA (amiRNA) technology in the moss is introduced in **Chapter 5**. AmiRNA technology was developed recently, in which only one effective sRNA molecule is produced. This method has several advantages in comparison with traditional RNAi vectors. In **Chapter 6**, virus-induced gene silencing (VIGS) is introduced. Infection of plant viruses is often associated with the generation of sRNAs targeting viral sequences. When the plant gene sequences are incorporated into the viral genome, sRNAs harbouring these plant gene sequences are generated

after infection of recombinant viruses. In **Chapter 7**, local induction of RNAi by agroinfiltration is introduced. The RNAi vector can be transiently delivered into leaf cells by *Agrobacterium*. A wide-range host of *Agrobacterium* allows us to induce local silencing of target genes in a variety of plants. Direct transfer of sRNAs into protoplasts is also effective for the knockdown of target gene function as is explained in **Chapter 8**. To evaluate the effects of RNAi, researchers often need to detect sRNAs. Detection of sRNAs by northern blot analysis, quantification of sRNAs by qPCR and sequencing of sRNAs at a large scale are introduced in **Chapters 9**, **10** and **11**, respectively. The endogenous sRNAs, especially the so-called microRNAs (miRNAs), are involved in the many aspects of plant development. The prediction of target mRNA is important in functional miRNA analysis. **Chapter 12** provides us with detailed instructions on the computational prediction of miRNA targets. The ensuing four chapters provide instructions on how to evaluate the effects of RNAi. Introduction of RNAi vectors often causes the de novo DNA methylation on sites corresponding to the sRNA sequences (RNA-directed DNA methylation, RdDM). The precise map of methylated cytosine residues can be prepared by bisulphite sequencing of the target genomic regions as described in **Chapter 13**. Induction of DNA methylation at the promoter sequences can reduce transcriptional activity. We can assess the effect of RdDM on transcriptional activity by a nuclear run-on assay as described in **Chapter 14**. Finally, the effects of RNAi can be seen through changes in proteomic profiles. The last two chapters, **Chapters 15** and **16**, provide the instructions for comparative proteomic analysis.

The use of RNAi technology is essential for most plant science researchers. In addition to the functional annotation of unknown genes, RNAi technology has been applied to the genetic engineering of important plant metabolites including starches, oils and storage proteins. RNAi has been used to engineer plants resistant to plant viruses and also to nematodes and insects. This volume will provide many tips on the design of experiments to explore plant gene function to post graduate students and their tutors involved in plant biotechnology and breeding research.

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