REVIEW

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Post-transcriptional gene silencing in plants by RNA

Abstract RNA silencing, which is termed post-transcriptional gene silencing in plants, is an RNA degradation process through sequence-specific nucleotide interactions induced by double-stranded RNA. In plants, RNA silencing not only serves as a component of the defense mechanism, but also participates in the regulation of endogenous gene expression in a variety of developmental processes. This review elaborates the current progress on the understanding of the molecular basis of RNA silencing including a mechanistic link between the regulation of microRNA and RNA silencing. The practical use of RNA silencing as a reverse genetics approach in plant functional genomics is also discussed.

Keywords Double-stranded RNA · MicroRNA · Plant functional genomics · Post-transcriptional gene silencing · RNA silencing


Introduction

Over the last several years much progress has been made in unraveling the mechanism of RNA silencing, a process leading to the degradation of homologous mRNAs, which is also termed RNA interference in animals, post-transcriptional gene silencing (PTGS) in plants, and quelling in fungi (Kooter et al. 1999; Matzke et al. 2001; Vaucheret et al. 2001; Waterhouse et al. 2001; Hannon 2002). Although the phenomenon of RNA inhibition was first described in petunia as ‘cosuppression’ (Napoli et al. 1990), more extensive studies have been carried out in the functional analysis of genes in Caenorhabditis elegans (Fire et al. 1998). The occurrence of apparently similar underlying mechanism for this phenomenon in different species indicates a conserved biological function of RNA silencing during the evolution of organisms.

RNA silencing does not affect the transcription of a gene locus, but only causes sequence-specific degradation of target mRNAs; transcriptional gene silencing (TGS), on the other hand, inhibits transcription by methylation and chromatin remodeling at the DNA level (Fagard and Vaucheret 2000). The common feature shared by RNA silencing and TGS is that both of them are triggered by the presence of double-stranded RNA (dsRNA), which is further cleaved into small RNAs to become functional in a number of epigenetic gene-silencing processes (Waterhouse et al. 2001; Eckardt 2002). Recent studies have suggested that short temporal RNAs (stRNAs), which are a class of noncoding microRNAs (miRNAs) identified originally by their functions in translational repression in animals, also participate in the dsRNA-induced gene silencing machinery (Cerutti 2003). The involvement of the miRNA pathway in the RNA silencing machinery suggests a potential mechanistic link between RNA silencing and miRNA-regulated gene silencing.

In plants, RNA silencing, as an efficient part of gene silencing, not only serves as an essential component of the defense system being targeted against transposable elements and viral infection, but also plays important
roles in the regulation of endogenous gene expression (Voinnet 2002). As demonstrated by the spread of systemic silencing after localized virus infection and grafting experiments, the signals of intracellular RNA silencing can be transmitted systemically from cell to cell over a long distance through the phloem. Short interfering RNAs (siRNAs), aberrant RNAs, and dsRNAs are the suggested candidates for such mobile silencing signals, although the mechanism of their involvement in the process is not clear so far (Palauqui et al. 1997; Voinnet et al. 1998; Ruiz-Medrano et al. 1999; Mlotshwa et al. 2002). Thus, RNA silencing might be coupled with active RNA intercellular trafficking in both plant defense and development. It is noteworthy that some viruses can encode proteins that counteract RNA silencing in plants. In the simple genomes of these viruses, such as potexvirus and cucumovirus, there is one particular open reading frame encoding the protein TGBp1, which is an essential component of the three proteins collectively designated as triple gene block (TGB) proteins (Lough et al. 2000; Vance and Vaucheret 2001). TGBp1 is a viral movement protein, which is not only required for effective cell-to-cell transmission of the virus, but is also associated with an inhibition of the systemic transmission of the PTGS signal (Lough et al. 2000; Voinnet et al. 2000; Vance and Vaucheret 2001). The presence of such a counter-defense strategy in viruses is a paradigm showing the mechanical complexity of RNA silencing when plants are under different biotic stresses.

**dsRNA as an inducer of RNA silencing**

In a broad range of eukaryotic organisms, RNA silencing is triggered by dsRNA, which may be naturally derived from the transcription of inverted-repeat loci or replicating exogenous RNAs by host- or viral-encoded RNA-dependent RNA polymerase (RDRP) (Dalmay et al. 2000; Mourrain et al. 2000; Sijen et al. 2001).

Viruses and transgenes are two main exogenous factors inducing the formation of dsRNAs in plants during RNA silencing. The viral replication by a virus-encoded RDRP in most single-stranded RNA viruses generates a dsRNA intermediate, which contains the sequence-specific information to guide a plant to protect itself by degrading viral RNAs (Sijen et al. 2001). Comparatively, the generation of dsRNA initiated by transgenes may have distinct mechanisms according to the transgene structure and its integration status in the plant genome. RNA silencing caused by the transgene overexpressing an endogenous mRNA requires a putative plant-encoded RDRP, SGS2/SDE1 (*Arabidopsis*) for example, which synthesizes dsRNA intermediates from the template of aberrantly expressed single-stranded RNA (Dalmay et al. 2000; Mourrain et al. 2000; Tang et al. 2003). However, the requirement for RDRP is bypassed if a transgene is designed to create a dsRNA structure, or multiple copies of a transgene are integrated as inverted-repeat insertions in the genome, in which dsRNA is potentially generated by read-through transcription (Waterhouse et al. 1998; Muskens et al. 2000; Wang and Waterhouse 2000). As a corollary to this mechanism, RNA silencing by the design of dsRNA structure in transgenes has now been routinely used as a potent and high-throughput tool of gene silencing in reverse genetics studies in a wide range of plant species.

In contrast to the formation of long dsRNA molecules under stresses imposed by viruses or transgenes, the production of short hairpin dsRNAs within the regions between the annotated protein coding genes, which further leads to the production of miRNAs, appear to be dependent on specific endogenous (developmental) signals (Ambros et al. 2003; Bartel and Bartel 2003). Although the elements controlling the transcription of miRNA precursors are yet to be clarified, the involvement of miRNA function in RNA silencing has at least been shown in plants (Llave et al. 2002a; Rhoades et al. 2002; Tang et al. 2003). In fact, dsRNAs, as inducers of RNA silencing, could be generated in the form of either perfect or imperfect duplexes in response to exogenous or endogenous signals, respectively. The degree of base paring in the dsRNA duplex may contain the potential signal determining the fate of the subsequently generated small RNAs in different regulatory processes.

Recent studies have also revealed that dsRNA could induce sequence-specific hypermethylation of genomic DNA, which suggests an alternative function of dsRNA in TGS, and indicates that RNA silencing and TGS might be mechanistically related in targeted gene silencing (Jones et al. 1999; Wassenegger 2000; Bender 2001).

**Mechanism of RNA silencing in plants**

**Initiation and effector steps**

As shown in Fig. 1, the mechanism of RNA silencing induced by dsRNA can be simplistically summarized as having two major steps, viz., initiation and effector steps (Cerutti 2003). The initiation step involves the cleavage of the triggering dsRNA into siRNAs of 21–26 nucleotides with 2-nucleotide 3' overhangs, which correspond to both sense and antisense strands of a target gene (Hamilton and Baulcombe 1999; Voinnet 2002). In the effector step, the siRNAs are recruited into a multiprotein complex referred to as the RNA-induced silencing complex (RISC), in which the degradation of target mRNAs occurs with the siRNA as a guide (Hammond et al. 2000; Zamore et al. 2000). Each RISC appears to have a single siRNA, an RNase (and may even have two separate RNases—exonucleolytic and endonucleolytic), and an RNA-homology-recognition and binding domain. Further, there are two size classes of siRNA in plants (Hamilton et al. 2002; Mallory et al. 2002; Tang et al. 2003), namely, ‘long siRNAs’ of 24–26 nucleotides that are involved in triggering systemic gene silencing, and the ‘short siRNA’ (21–22 nucleotides) correlated with sequence-specific degradation of target mRNAs. Such cleavage of the target
required for either plant defense or development.

Proteins may mediate the identification and processing of different gene silencing (PTGS) processes, such as translational inhibition, the microRNA ribonucleoprotein complex (miRNPs) that further regulates other post-transcriptional processes. Some might be recruited into the microRNA-ribonucleoprotein complex (miRNP) that further regulates translational inhibition or other PTGS effects, such as mRNA splicing, localization or stability by imperfect complementarity to their targets (Olsen and Ambros 1999; Hutvagner et al. 2001; Voinnet 2002). Accordingly, Dicer processes precursor dsRNAs to generate both siRNAs and miRNAs. While it is clear that siRNAs function in RNA silencing at least some of the miRNAs are also involved in the process.

**Arabidopsis** has Dicer-like proteins

**DICER-LIKE 1** (DCL1, At1g01040), renamed from EMBRYO DEFEKTIVE76 (EMB76)/SUSPENSOR (SUS1)/SHORT INTEGUMENTS1 (SIN1)/CARPEL FACTORY (CAF), is one of the identified *Arabidopsis* orthologs of Dicer (Golden et al. 2002; Schauer et al. 2002). Different mutant alleles of *dcl1* have been independently isolated in genetic studies of embryo, ovule and flower development in *Arabidopsis*, indicating that DCL1 has a broad regulatory function in plant development. Analysis of *dcl1-9* (caf), a T-DNA insertion allele encoding a truncated protein without the second dsRNA-binding domain, has shown a significantly reduced production of miRNA coupled with pleiotropic developmental abnormalities including reduced stature, abnormal leaf shape, loss of axillary buds, and altered floral morphology (Jacobsen et al. 1999; Park et al. 2002; Reinhart et al. 2002). This suggests that the generation of miRNA by DCL1 is involved in a wide range of plant developmental processes. A recent investigation by Finnegan et al. (2003) showed that although miRNA production was clearly blocked in *dcl1-9*, RNA silencing and the production of siRNAs induced by self-complementary hairpin RNAs was not compromised. This finding has raised two different possibilities for the function of DCL1 in plant development. The first explanation proposes that DCL1, like its Dicer homologs in animals, functions in the

**Fig. 1** Working models for RNA silencing in *Arabidopsis*. The RNA silencing pathway can be divided into two steps. In the first (initiation) step, perfect and extensive double-stranded RNA (dsRNA) structures, induced by virus or transgene, are processed by Dicer-like proteins (DCLs) into double-stranded short interfering RNAs (siRNAs). In the second (effector) step, siRNAs are recruited into the RNA-induced silencing complex (RISC) that regulates the endonucleolytic cleavage of target mRNAs by the perfect or near perfect base-pairing between siRNAs and the targeted sequences. The involvement of the microRNA (miRNA) pathway in RNA silencing is a notable feature in plants. In *Arabidopsis*, endogenous developmental signals may trigger the formation of some imperfect dsRNAs, which are subsequently diced by DCL1 and/or other DCLs into double-stranded miRNAs. These miRNAs participate in a variety of regulatory processes: some serve as siRNA molecules in the RNA silencing pathway with perfect or near perfect base complementarity to their mRNA target; some might be recruited into the microRNA ribonucleoprotein complex (miRNP) that further regulates other post-transcriptional gene silencing (PTGS) processes, such as translational inhibition, with imperfect base-pairing interaction with their targets. The interaction between DCL and ARGONAUTE protein (AGO) proteins may mediate the identification and processing of different dsRNA precursors, which produces different types of small RNAs required for either plant defense or development.

mRNA occurs almost at the middle of the siRNA within the relevant RISC (guide RNA), resulting in over 90% inhibition of target gene expression.

The Dicer protein is involved in generating siRNA and miRNA

The processing of a long dsRNA into siRNA is mediated by an RNase-III-like dsRNA-specific ribonuclease, designated Dicer, initially in *Drosophila* (Bernstein et al. 2001). The members of the Dicer protein family, which may be functionally conserved in fungi, plants and animals (Tijsterman et al. 2002), generally contain N-terminal RNA helicase motifs, a PAZ (for ‘Piwi/Argonaute/Zwille’) protein-protein interaction domain, at least one dsRNA-binding domain, and two tandem RNase-III domains that may directly regulate the endonucleolytic cleavage of dsRNA into siRNAs, which is an ATP-dependent process.

Dicer proteins function in two different pathways in gene silencing by recognizing distinct types of precursor dsRNAs (Fig. 1). In the first pathway, exclusively leading to RNA silencing, Dicer cleaves long and perfect dsRNA structures originated mainly from the protein coding region to generate double-stranded siRNAs, which guide the subsequent endonucleolytic cleavage of homologous RNAs with perfect base-pairing interaction. This pathway is closely related to defensive mechanisms in organisms, because almost all siRNAs were identified by sequence homology with transposon or viral genes (Elbashir et al. 2001a; Lau et al. 2001). In the second Dicer-dependent gene silencing pathway, Dicer can dice imperfect RNA duplexes predominantly derived from the regions between protein coding genes into siRNAs, which are subsequently recruited into a microRNA-ribonucleoprotein complex (miRNP) that further regulates translational inhibition or other PTGS effects, such as mRNA splicing, localization or stability by imperfect complementarity to their targets (Olsen and Ambros 1999; Hutvagner et al. 2001; Voinnet 2002).
production of both miRNAs and siRNAs. Thus, the deletion of the second dsRNA-binding domain in dcl1-9 may only cause partial loss of DCL1 activity, which still allows the normal generation of siRNAs, but not miRNAs. In this scenario, DCL1 activity may depend on different concentrations and degree of complementarity of siRNA and miRNA precursors. The second scenario provides a new possibility that distinct Dicer-like enzymes in Arabidopsis may have unique roles in the production of siRNAs and miRNAs. If this is true, DCL1 may only be involved in the generation of miRNAs, while other Arabidopsis members of Dicer-like proteins may function in the production of siRNAs and fulfill the RNase role in RNA silencing (Fig. 1). In fact, there are three additional DCL1-like proteins in Arabidopsis, known as DCL2 (At3g03300), DCL3 (At3g43920), and DCL4 (At5g20320). These four Arabidopsis Dicer-like proteins share 40% to 47% amino acid similarity in the domain with the conserved RNA helicase motif and two tandem RNase-III domains, but not other functional domains. For example, DCL2 has only one dsRNA-binding domain, which is, however, totally lost in DCL3 (Schauer et al. 2002; Finnegan et al. 2003). Such difference in gene structure indicates that DCL proteins may play different roles in the production of small RNA molecules in Arabidopsis.

Interaction of Dicer with other proteins (e.g., ARGONAUTE) may define processing of siRNA and miRNA

It is possible that in certain organisms, encoding only one Dicer, the miRNA and siRNA pathways intersect at the Dicer-mediated step, which also involves other associated effector proteins, such as RNA interference (RNAi) DEFECTIVE (RDE)/ARGONAUTE (AGO) proteins (Cerutti 2003). For instance, C. elegans requires RDE-1 for the generation of functional siRNAs in RNA silencing, while ARGONAUTE-LIKE-1 (ALG-1) and ALG-2 are only necessary for the production of stRNAs (Tabara et al. 1999; Grishok et al. 2001). In several eukaryotes, ARGONAUTE proteins, identified by the presence of a PAZ domain and a C-terminal Piwi domain, have been characterized by their roles in the processing of distinct types of small RNAs via selecting different small RNA precursors (Tabara et al. 1999; Vance and Vaucheret 2001; Hannon 2002; Martinez et al. 2002). Thus, the specificity in the generation of a particular kind of small RNAs (i.e. miRNAs or siRNAs) may be determined by the protein-protein interaction between specific AGO homologs and Dicer proteins, which may be mediated by the PAZ domain present in both proteins (Cerutti et al. 2000). There are more than ten AGO genes identified in Arabidopsis, and at least AGO1 and AGO4 have shown their involvement in both RNA silencing and plant development, which appears to be similar to the combined function of RDE-1, ALG-1, and ALG-2 in C. elegans (Fagard et al. 2000; Morel et al. 2002; Zilberman et al. 2003). It will be interesting to investigate if Arabidopsis has evolved subtle small RNA-processing machinery to regulate the miRNA and siRNA pathways by specific interactions among several DCL proteins and the members of AGO protein family (Fig. 1) at different steps and/or in different cellular compartments, which could be critical to coordinate the response to both plant defense and development.

Function of miRNA in RNA silencing

miRNA-like regions are common in plant and animal genomes

miRNAs, a class of noncoding (untranslated) RNAs of 20–24 nucleotides, are another type of small RNA products processed from dsRNA hairpin precursors by Dicers. So far, more than 200 miRNA genes have been identified in animals and plants, which are mainly derived from the regions between protein coding genes (Lagos-Quintana et al. 2001, 2002; Lau et al. 2001; Lee and Ambros 2001; Llave et al. 2002a; Reinhart et al. 2002; Bartel and Bartel 2003). The loci that encode miRNAs, the MIR genes, can occur in clusters in the genome and may even be transcribed polycistrionically, processed sequentially into pre-miRNA and miRNA (Lee et al. 2002). It is noteworthy that many miRNAs are highly conserved among different species, even across phyla, and some of them have specific expression patterns in different tissues or at different developmental stages, suggesting the important and conserved function of miRNAs in eukaryotic gene regulatory networks (Bartel and Bartel 2003).

The first eukaryotic miRNAs were isolated in C. elegans, where the lin-4 and let-7 genes produce mature 22-nucleotide stRNAs to orchestrate the developmental timing. In particular, these miRNAs downregulate the translation of several developmental genes, such as lin-14 and lin-28, by base pairing to their 3′ untranslated regions (UTR) with imperfect complementarity (Lee et al. 1993; Wightman et al. 1993; Olsen and Ambros 1999; Reinhart et al. 2000). Subsequent efforts to identify more miRNAs in other animal species further revealed that miRNAs have biochemical features similar to siRNAs with 2′ nucleotide-long 3′ overhangs and 5′ phosphate/3′ hydroxyl ends, which are typical signatures produced from Dicer cleavage of stem-loop precursor RNA transcripts (Ebashir et al. 2001a, b; Lagos-Quintana et al. 2001, 2002; Lau et al. 2001; Lee and Ambros 2001). Nonetheless, despite the availability of complete genomic sequence for C. elegans and Drosophila, further elucidation of the function of miRNAs in animal developmental processes remains a major challenge, because the imperfect pairing of animal miRNAs with their target mRNAs hampers computational approaches in analyzing the sequence information of potential downstream genes (Rhoades et al. 2002).
Recent identification of miRNAs in several plant species has provided new insights into the mechanistic implications of miRNA function. In Arabidopsis, nearly 100 miRNAs have been identified, which may only represent a small fraction of miRNAs, since little overlap among the identified miRNAs has been found in different studies (Llave et al. 2002a; Park et al. 2002; Reinhart et al. 2002; Kidner and Martienssen 2003). The most striking feature of these Arabidopsis miRNAs is that many of them show near perfect complementarity to the open reading frames of a wide range of genes, whereas the only proposed animal miRNAs, lin-4 and let-7, have imperfect complementary sites within 3' UTRs of target genes (Lee et al. 1993; Wightman et al. 1993; Moss et al. 1997; Reinhart et al. 2000; Slack et al. 2000). This notable difference was further observed in the computational approaches to predicting miRNA targets in Arabidopsis, C. elegans and Drosophila melanogaster (Rhoades et al. 2002). By allowing for less than four mismatches, a total of 49 potential regulatory targets were identified with near-perfect complementarity to 14 out of the 16 identified Arabidopsis miRNAs, while the same method detected few targets in both C. elegans and D. melanogaster. It has been found that in human cell lines, the fate of miRNAs in either cleaving directly (by exact match) or repressing translation (by imperfect match) of target mRNAs is most likely determined by the degree of complementarity between miRNAs and their target mRNAs (Hutvagner and Zamore 2002; Llave et al. 2002b). Therefore, although it is too early to speculate about the functional difference between plant and animal miRNAs, it is reasonable to propose that at least some of the plant miRNAs function as siRNAs rather than stRNAs as their animal counterparts. Thus, some plant miRNAs are involved in RNA silencing by matching with the target mRNAs with near-perfect complementarity to specify their cleavage, whereas most of the animal miRNAs pair within the 3' UTR region of target mRNAs with imperfect complementarity to attenuate their subsequent translation.

Several plant genes have miRNA target sites

The research by Bartel’s group also revealed two additional significant findings (Rhoades et al. 2002). First, many miRNA complementary sites are conserved in both Arabidopsis and rice, indicating the conserved and important gene regulatory function of miRNAs in flowering plants. Second, of the 49 predicted miRNA targets, 34 encode known or putative transcription factors, which exhibit a high ratio of miRNA targets as regulatory elements, suggesting that many miRNAs may play key roles in gene regulatory networks of plant development.

The idea that plant miRNAs act as siRNAs involved in RNA silencing is strongly supported by two recent studies (Llave et al. 2002b; Tang et al. 2003). Carrington and colleagues showed that one of the Arabidopsis miRNAs, miR171, can direct the endonucleolytic cleavage of its mRNA targets, a family of SCARECROW-LIKE transcription factors, by exact complementarity to these targets. Furthermore, they found that miR171 functions as a single-stranded siRNA in inducing RNA silencing as demonstrated by the action of similar single-stranded siRNAs in animal cells (Martinez et al. 2002; Schwarz et al. 2002). The work from Zamore’s group provided additional significant insights into the involvement of miRNAs in RNA silencing in plants. Using in vitro wheat germ extracts, they demonstrated that another miRNA, miR165, regulates the expression of a target Arabidopsis PHAVOLUTA gene by guiding an endonuclease to cleave its mRNAs, although miR165 lacks perfect complementarity to this target.

Taken together, plant miRNAs seem to resemble their animal counterparts in their biogenesis, in that miRNAs are generated by Dicer proteins from stem-loop precursors originated from specific non-protein-coding genes of the genome. However, the miRNA pathway in plants is at least partially integrated into the RNA silencing process, in that plant miRNAs recognize their targets as siRNAs do in RNA silencing. Consistent with this suggestion, it is interesting to note that dcl1-9 and hen1-1, two Arabidopsis mutants showing defects in the accumulation of miRNAs, have phenotypes similar to the weak ago1 mutant, a mutant being impaired in RNA silencing process (Morel et al. 2002; Park et al. 2002; Reinhart et al. 2002; Kidner and Martienssen 2003). Thus, a series of new paradigms in plants implicate that miRNAs participate in a complex and refined network of RNA silencing, which is essential and important not only for plant defense response, but also for plant development.

Application of RNA silencing in plants

With the completion of Arabidopsis and rice genome sequencing and the expanding crop sequence databases, the practical use of RNA silencing to reduce gene expression in a sequence-specific manner promises to be an essential and routine reverse genetics approach in plant functional genomics. Technologically reliable and high-throughput methods of RNA silencing are being developed by the recent progress on the understanding of the core RNA silencing mechanism. Particularly, the discovery of dsRNA as an inducer of RNA silencing has provided a scheme of dsRNA-mediated interference to direct gene-specific silencing that is more efficient than antisense suppression or cosuppression by overexpression of target genes (Fire et al. 1998; Kennerdell and Carthew 1998; Waterhouse et al. 1998; Sanchez-Alvarado and Newmark 1999).

The dsRNA-mediated silencing was first demonstrated in plants by the simultaneous expression of antisense and sense gene fragments targeted against both an RNA virus and a nuclear transgene (Waterhouse et al. 1998). The methodology of the specific and heritable genetic interference by dsRNAs in Arabidopsis was further established...
in the investigation of several genes involved in floral development (Chuang and Meyerowitz 2000). In this respect, transformation vectors capable of dsRNA formation were constructed by linking the gene-specific sequences in both sense and antisense orientation under the control of a strong viral promoter. These dsRNA-expressing constructs, when delivered into Arabidopsis with Agrobacterium-mediated transformation, created a heritable phenotypic series in the transformants, which corresponded to mutant alleles of different strengths. Thus, the dsRNA interference can generate transformants showing both reduction and loss of function, with gradually reduced expression of a specific gene, which has proved to be an effective tool in studying some dosage-dependent genes involved in plant development and some genes essential for plant viability (Levin et al. 2000; Yu et al. 2002). The application of dsRNA technology for large-scale investigation of gene functions will be facilitated by making the critical experimental procedures fast and efficient (Wesley et al. 2001; Brummell et al. 2003). The so-called “pHELLSGATE” system was developed to convert a polymerase chain reaction product into a dsRNA structure that includes a spacer intron in one simple step by using an in vitro recombinase system (Wesley et al. 2001). This is based on the observation that the inclusion of an intron as a spacer between the sense and antisense arm of a dsRNA construct greatly increases the silencing effect (Wesley et al. 2001). Recently, another simple single-step cloning method of dsRNA interference, termed ‘silencing by heterologous 3’ UTRs’, was shown to operate effectively in both Arabidopsis and Lycopersicon esculentum systems (Brummell et al. 2003). In this method, an inverted repeat of the 3’ UTR of a heterologous gene was placed 3’ to a single-stranded targeting sequence, which induced the effective degradation of endogenous target mRNAs homologous to the target transgene. The successful design of this approach was initiated from the finding of a degradation of RNA 5’ to a dsRNA region (Sijen et al. 2001; Han and Grierson 2002).

Although the dsRNA-mediated RNA silencing is a useful method, the application of this technology in plants has achieved inconsistent effects with some genes. Also, numerous techniques of dsRNA interference are available as suggested by the various references cited in this review and elsewhere. The exact protocol to be used will vary with specific research objectives. Nevertheless, the rapid progress in our understanding of basic RNA silencing mechanism will greatly contribute to the improvement of this approach and other related methods, which will firmly entrench RNA silencing as a potent and handy tool in the study of plant functional genomics in the near future.

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References


