Front. Biol. 2011, 6(2): 140–146
DOI 10.1007/s11515-011-1145-3

REVIEW

Mobile silencing in plants: what is the signal and what defines the target

Dacheng LIANG[1], E. Jean FINNEGAN[1], Elizebeth S. DENNIS[1], Peter M. WATERHOUSE[2], Ming-Bo WANG (✉)[1]

[1] CSIRO Plant Industry, Clunies Ross Street, Canberra, ACT 2601, Australia
[2] School of Molecular Bioscience, University of Sydney, Sydney NSW 2006, Australia

© Higher Education Press and Springer-Verlag Berlin Heidelberg 2011

Abstract **RNA-mediated silencing in plants can spread from cell to cell and over a long distance, and such mobile silencing has been extensively studied in the past decade. However, major questions remain as to what is the exact nature of the mobile silencing signals, how the components of the RNA-directed DNA methylation pathway are involved, and why systemic spread of silencing has only been observed for transgenes but not endogenous genes. In this review, we provide an overview of the current knowledge on mobile gene silencing in plants and present a model where systemic silencing involves long nuclear RNA transcripts that serve as a template to amplify primary siRNA signals.**

Introduction

RNA-mediated silencing plays important roles in regulating growth and development as well as in responses to environmental stress (Zhang et al., 2006; Chinnusamy and Zhu, 2009). Plants have evolved three different types of RNA-mediated silencing pathways namely, the microRNA pathway, the small interfering RNA (siRNA)-mediated post-transcriptional gene silencing (PTGS) pathway, and the siRNA-mediated transcriptional gene silencing (TGS) pathway known as RNA-directed DNA methylation (RdDM). One of the most intriguing aspects of RNA silencing is its distinctive non-cell-autonomous character, where localized introduction or expression of silence-inducing RNAs can cause silencing at short or long distances from the initial induction site (Voinnet, 2005). This phenomenon was first demonstrated in the pioneering work by two groups using different methods: 1) infiltrating green fluorescent protein (GFP)-expressing *Nicotiana benthamiana* with *Agrobacterium* carrying a GFP construct (Voinnet and Baulcombe, 1997) and 2) grafting a tobacco scion expressing a nitrite reductase transgene (35S-*Nia*) on a tobacco rootstock containing a *Nia*-silenced transgene (Palauqui et al., 1997). In both cases strong systemic silencing of the target genes, *GFP* or *Nia*, was observed. Subsequent studies provided further support for the existence of mobile silencing in plants (Mlotshwa et al., 2002). Mobile silencing has attracted extensive research effort in the past few years because of its biological significance as well as its potential application in biotechnology and agriculture. However, the underlying mechanism for the spread of silencing has yet to be fully elucidated, and the exact nature of mobile silencing signals is still a subject of debate. In this review, we give an overview of the current understanding of mobile silencing in plants, and present a model concerning short and long-distance mobile silencing.

Received February 15, 2011; accepted March 1, 2011
Correspondence: Ming-Bo WANG
E-mail: Ming-Bo.Wang@csiro.au

Short and long-distance silencing

Mobile silencing involves both cell-to-cell short-range movement and systemic long-distance spread (Kalantidis et al., 2008). Systemic silencing, also called long-distance mobile silencing, refers to the phenomenon whereby gene silencing is initiated in a few cells or specific tissues before spreading systemically throughout the plant (Voinnet, 2005; Kalantidis et al., 2008). Systemic silencing has so far been mainly observed in solanaceous plants, such as tobacco and tomato (Palauqui et al., 1997; Voinnet et al., 1998; Crete et al., 2001; Shaharuddin et al., 2006) and has also been reported in *Arabidopsis* (Brosnan et al., 2007). In experiments using these solanaceous plant models, the pattern of silencing spread is similar to that of dye movement which occurs mainly through the phloem (Roberts et al., 1997), leading to the suggestion that systemic silencing signals move through the long-distance transport system, the phloem, and are subsequently loaded into surrounding cells through plasmodesmata.

However, RNA-mediated silencing is not always associated with systemic spread, and in some cases, it can exert only a short-range silencing effect. When Himber et al. (2003) first used phloem-restricted PVX (potato virus X) to target the endogenous rubisco small subunit (*RbcS*) mRNA, they found that *RbcS* silencing, manifested by the phenotype of leaf chlorosis, was confined to the region around the vascular system. This differs from the systemic leaf chlorosis observed when a non-phloem restricted PVX vector was used. Subsequent studies, using a phloem-specific promoter AtSuc2-directed hairpin RNA (hpRNA) transgenes targeting either the endogenous *RbcS* or sulfur desaturase (*Sul*) mRNA, showed that the spread of endogenous gene silencing is restricted to 10–15 cells surrounding the vasculature.

Apart from endogenous genes, cell-to-cell short range silencing has also been observed for transgenes when some of the genetic factors involved in the siRNA-mediated PTGS pathway are mutated. When introduced into wild type plants, a phloem-specific hpRNA silencing inducer containing the first 400 bp of the *GFP* gene (referred to as "hpGF") can induce systemic silencing of *GFP* expression. However, when RDR6/SDE1 is mutated, silencing is restricted to the vascular system and adjacent cells (Himber et al., 2003). This silencing pattern is similar to that of the endogenous gene *Sul* induced by the phloem-specific hpRNA transgene AtSuc2-hp*Sul*, although silencing of *Sul* is not affected in the *rdr6*/*sde1* mutant. RDR6/SDE1 is required for transitivity of gene silencing, the spread of silencing beyond the region targeted by the inducer construct (Vaistij et al., 2002), by catalyzing the production of secondary siRNAs up- or down-stream of the primary target region (Wassenegger and Krczal, 2006; Voinnet, 2008). Three independent studies, using tissue-specific transgenic approaches (Himber et al., 2003), VIGS (Vaistij et al., 2002) and grafting (Brosnan et al., 2007), have shown that long-distance silencing is dependent on secondary siRNA production, and that the extent of systemic silencing is proportional to the abundance of secondary siRNAs (Himber et al., 2003). These observations support a model where systemic silencing requires amplification of primary silencing signals, which has been described as an amplifying relay process occurring between tissues that produce the silencing signal and those that receive it (Himber et al., 2003).

Small RNAs are mobile

Recent experiments have demonstrated that systemic mobile silencing can occur in different plant species (Voinnet, 2005; Kalantidis et al., 2008). However, the nature of the mobile silencing signal(s) has remained elusive. With the discovery of the role of small RNAs in PTGS or RNA interference (RNAi) pathways in plants and animals (Hamilton and Baulcombe, 1999; Waterhouse et al., 2001; Bagasra and Prilliman, 2004; Baulcombe, 2004; Herr and Baulcombe, 2004; Tijsterman and Plasterk, 2004), recent studies have focused on determining whether small RNAs are the mobile signal.

In one of these studies, Klahre et al. (2002) bombarded synthetic siRNAs into plant cells, and showed that double-stranded small RNAs can induce systemic post-transcriptional gene silencing, suggesting that small RNAs are the mobile silencing signal. However, in the same study the authors demonstrated that longer RNA species can also induce systemic silencing, and therefore the possibility that precursors of small RNAs act as a mobile signal could not be excluded.

Another recent study combined *Arabidopsis* mutants impaired in RNA silencing pathways with micro-grafting (Brosnan et al., 2007). In *Arabidopsis*, 20-22-nt microRNAs are processed by Dicer-like 1 (DCL1) and are involved in the control of plant development, while 21-24-nt siRNAs are produced by DCL2, DCL3 and DCL4 and direct silencing in the PTGS or RdDM pathways (Baulcombe, 2004; Fusaro et al., 2006). Brosnan et al. (2007) showed that expression of a hpGF transgene that targets the 5′ half of GFP in an *Arabidopsis* rootstock defective for *dcl1* or *dcl2*, *dcl3* and *dcl4* still triggered systemic GFP silencing in the scion. This result appears to suggest that small RNAs may not be the long-distance silencing signal.

Results from an earlier study also appear to argue against small RNAs being the mobile silencing signal (Mallory et al., 2001). A tobacco rootstock expressing the viral silencing suppressor P1/HC-Pro was able to send mobile silencing signals targeting a β-glucuronidase (GUS) transgene in the scion despite the fact that P1/Hc-Pro stopped GUS silencing and prevented the accumulation of GUS siRNAs at detectable levels in the rootstock.

However, neither of these experiments can exclude the role of small RNAs in mobile silencing. The four DCLs in *Arabidopsis* have partial functional redundancy (Xie et al., 2004) and small RNAs may not be completely eliminated in the *dcl2 dcl3 dcl4* triple mutant used by Brosnan et al.. Use of a *dcl1 dcl2 dcl3 dcl4* quadruple mutant could help to resolve the issue, but it is difficult to obtain such a quadruple mutant since a complete loss of function in DCL1 would make the plant unviable. Similarly, in the P1/Hc-Pro rootstock (Mallory et al., 2001), low levels of siRNA could still exist as recent studies have suggested that P1/Hc-Pro functions by sequestering siRNAs or blocking siRNA-mediated cleavage rather than by preventing siRNA biogenesis (Lakatos et al., 2006).

Several recent studies have lent support for a direct role of small RNAs in the signaling of mobile silencing in plants. By mutagenising the phloem-specific Atsuc2-hp*Sul* transgenic system, Dunoyer et al. (2005) have shown that DCL4 is required for the cell-to-cell short range spread of silencing. They found that 21-nt siRNAs, the product of DCL4, are associated with the spread of silencing but the 24-nt siRNAs, product of DCL3, are dispensable. A similar conclusion was made by Smith et al. (2007), who showed that 21-nt siRNAs, rather than 24-nt siRNAs, are required for the short-distance

silencing of the endogenous phytoene desaturase (PDS) gene. This was reinforced by the recent experiments showing that re-introduction of *DCL4* into the phloem companion cells in a *dcl4* mutant background restored the cell-to-cell movement of *Sul* silencing (Dunoyer et al., 2010a). Furthermore, companion cell-specific expression of the viral silencing suppressor P19, which specifically binds and sequesters 21-nt siRNAs (but not 24-nt siRNAs), blocked the short distance spread of silencing (Dunoyer et al., 2010a).

Arabidopsis micro-grafting and next-generation RNA sequencing, provided evidence suggesting that the 24-nt siRNAs are the long-distance mobile silencing signal (Molnar et al., 2010). Sequencing of the small RNA populations isolated from both scion and rootstock, 5 weeks after grafting, showed that scion-specific siRNAs, predominantly 23 and 24 nt in length, were present in the rootstock. When the *pol iv* and *dcl2 dcl3 dcl4* mutants were used as the scion, the 24-nt scion-specific siRNAs were no longer detected in the rootstock. More importantly, 24-nt scion-specific siRNAs still accumulated in the *pol iv* and *dcl2 dcl3 dcl4* rootstocks that are defective in 24-nt siRNA biogenesis, suggesting that the siRNAs, but not their longer precursor molecules, move from the scion to the root. Further analysis indicated that the majority of endogenous mobile siRNAs detected in this study are derived from transposons. The authors examined three such loci and showed that the mobile siRNAs can direct DNA methylation in the grafted rootstock, which suggests a functional significance of the 24-nt mobile siRNAs. In agreement with this finding, Dunoyer et al. (2010b) showed that *IR71*, an endogenous locus, which resembles an inverted-repeat or hpRNA transgene, also gave rise to long-distance mobile siRNAs that were detected in the rootstock, and the majority of the mobile siRNAs are of the 24-nt class although 21- and 22-nt siRNAs were also found. Taken together, these studies suggest that 24-nt siRNAs are the signal for long-distance silencing.

However, evidence from the early study by Brosnan et al. (2007) contradicts the findings of Molnar et al. (2010) and Dunoyer et al. (2010b). Brosnan et al. observed that GFP silencing in the scion, induced by the hpGF transgene in the rootstock, was associated mainly with 21-nt siRNAs corresponding to the 3′ region (P region) of the GFP transgene that is not targeted by the primary hpGF-derived siRNAs. GFP silencing was observed even when the rootstock was a *dcl3* mutant that is defective in 24-nt siRNA biogenesis. Thus, GFP silencing in the scion does not appear to require 24-nt siRNAs in the rootstock, which argues against 24-nt siRNAs being the long-distance mobile silencing signal.

Involvement of RdDM components in mobile silencing

A surprising observation by Brosnan et al. (2007) was that components of the RdDM pathway are also involved in systemic silencing. While the key RdDM factors Pol IV, RDR2, DCL3 and AGO4 are not required for transmission of the GFP silencing signal from the hpGF rootstock, they are essential for GFP silencing in the scion, i.e. for the perception of the silencing signals. Pol IV, RDR2 and DCL3 are involved in the biogenesis of 24-nt siRNAs, which bind AGO4 to direct the methylation of homologous DNA in the nucleus (Wang and Dennis, 2009; Law and Jacobsen, 2010). However, systemic GFP silencing is regarded as being post-transcriptional, as there was only a subtle reduction in transgene transcription, and no significant cytosine methylation or histone modification was detected in the GFP transgene in the silenced scion (Brosnan et al., 2007).

Some of the RdDM components have also been shown to be required for the short-distance spread of silencing. Silencing of the endogenous genes *PDS* and *Sul* induced by the phloem-specific AtSuc2-hp*PDS* or AtSuc2-hp*Sul* transgenes was diminished in the *pol iv* and *rdr2* mutants (Smith et al., 2007; Dunoyer et al., 2007), suggesting a role for these RdDM factors in short-distance silencing. However, while DCL3 and AGO4 are required for the perception of long-distance silencing signals (Brosnan et al. 2007), mutations of these two proteins either did not affect (in the case of *Sul* silencing; Dunoyer et al., 2007), or enhanced (in the case of *PDS* silencing; Smith et al., 2007), the spread of the short-distance silencing. These observations suggest that the RdDM components contribute in a different way to short-distance and long-distance mobile silencing.

A model for mobile silencing

Current knowledge on mobile silencing, as discussed above, can be summarized as follows: 1) mobile silencing can be characterized into two types, short-distance cell-to-cell spread and long-distance systemic movement; 2) 21-nt siRNAs are the predominant mobile signal for the short-distance spread of silencing; 3) long-distance silencing is dependent on secondary siRNA production; 4) while 24-nt siRNAs are mobile and can move over long-distance through the phloem, their role in long-distance silencing remains unclear; and 5) components of the RdDM pathway are required for systemic silencing, but the silencing appears post-transcriptional and is not associated with significant DNA methylation or histone modification in the target gene sequence.

So what is the long-distance silencing signal and how is the signal perceived in the target tissue to induce the silencing effect? A plausible model was proposed by Brosnan et al. (2007) where long-distance signals of unknown identity trigger the production of 24-nt siRNAs by Pol IV, RDR2 and DCL3 in the nucleus of the recipient cells; these 24-nt siRNAs bind to AGO4 and direct cleavage of target transcripts to trigger the production of secondary siRNAs downstream of the initial target region. These secondary siRNAs in turn induce PTGS of the target mRNA. However,

this model does not explain why long-distance mobile silencing has only been found to occur for transgenes but not for endogenous genes, a fact often overlooked in the discussions about mobile silencing in plants. For instance, Palauqui et al. (1997) showed that the endogenous nitrate reductase gene *Nia* in a wild-type tobacco scion could not be silenced by grafting with the *Nia*-silenced rootstock; *Nia* silencing only occurs when a 35S-*Nia* transgene locus is present in the scion. Similarly, Shaharuddin et al. (2006) demonstrated that the expression of an inverted repeat or hpRNA transgene targeting the 5′ UTR of the *ACC oxidase* gene (*ACO1*) did not trigger the silencing of the endogenous *ACO1* gene in the grafted scion. However, a highly expressed 35S-*ACO1* transgene was efficiently silenced when grafted to the same inverted repeat transgenic line; *ACO1* transgenic loci with low-level expression were not silenced by the same silencer rootstock (Shaharuddin et al., 2006). These observations suggest that transgenes, particularly those expressed at high levels, are susceptible to long-distance silencing, whereas endogenous genes appear to be resistant to systemic silencing.

To explain both short- and long-distance mobile silencing, and to address the questions regarding the identity of long-distance signals, the involvement of RdDM components in the perception of these signals, and the difference between transgenes and endogenous genes, we present a revised model invoking cell-to-cell and long-distance movement of siRNA signals, amplification of the siRNAs signals and cell-autonomous silencing of the target genes (Fig. 1). The key prerequisites in this model are a strict requirement for a long nuclear RNA transcript in the production of secondary siRNAs and a clear separation of nuclear siRNA amplification from mRNA degradation. Briefly, all siRNAs can move over long distances or from cell to cell. However, 21-nt siRNAs, and to some extent 22-nt siRNAs, function exclusively to direct the degradation of homologous mRNA in the recipient cells resulting in post-transcriptional silencing, as observed. However, these siRNAs do not trigger secondary siRNA production. In contrast, 24-nt siRNA signals, whether acquired from long distance or from adjacent cells, enter the nucleus of the recipient cells and target a nuclear transcript to 1) induce further production of 24-nt siRNAs from the direct target sequence by the RdDM machinery, and 2) trigger the production of 21-nt secondary siRNAs through a mechanism similar to that of trans-acting siRNA (ta-siRNA) biogenesis (Allen et al., 2005; Yoshikawa et al., 2005). When the recipient cells do not have the nuclear transcript, or no 24-nt siRNA signals are produced in the sender cells, the spread of silencing depends entirely on the concentration of the 21-22-nt siRNAs, which can move to adjacent cells and induce silencing through a concentration gradient. This scenario accounts for the short-range spread of silencing observed for the endogenous *Sul* gene in the AtSuc2-hp*Sul* system or *PDS* gene in the AtSuc2-hp*PDS* system.

But why are RdDM factors required for the short-distance spread of silencing and how are they involved? We favor a scenario where 21- and 22-nt siRNAs in the sender cells are derived from two pathways: the processing of primary dsRNA or hpRNA precursor transcribed from the silence-inducing transgene, and the biogenesis of secondary siRNAs that requires the RdDM factors Pol IV and RDR2 in the same sender cells. The combined amount of 21-22-nt siRNAs from both pathways determines the degree of short-distance silencing. Indeed, Smith et al. (2007) showed that the diminished short-distance spread of *PDS* silencing in the *pol iv* or *rdr2* mutant was associated with reduced siRNA accumulation, and proposed that Pol IV and RDR2 are involved in the production of dsRNA. How Pol IV and RDR2 are involved in dsRNA production remains unclear, but it is possible that Pol IV uses the primary hairpin dsRNA as template to synthesize single-stranded RNA in the nucleus that is then converted to dsRNA by RDR2. Smith et al. (2007) also provided a plausible explanation for the enhanced *PDS* silencing in the *dcl3* and *ago4* mutant backgrounds. They proposed that the AtSuc2-hp*PDS* transgene is subject to self-silencing that is catalyzed by DCL3 and AGO4. Mutations in these two proteins release the silencing, which results in enhanced levels of the primary hpRNA and hence siRNAs. Consistent with this proposition, they demonstrated that both the primary hp*PDS* transcript and the *PDS* siRNAs were upregulated in the *dcl3* and *ago4* mutants. It remains unclear how DCL3 and AGO4 cause self silencing, but the self silencing in this system does not appear to be transcriptional as mutation in the key downstream component of the RdDM pathway, Pol V, did not affect the short-distance spread of silencing. One possibility is that the DCL3-derived 24-nt siRNAs bind AGO4 to direct cleavage of the primary hpRNA transcript in the nucleus. This would reduce the amount of dsRNA template available for DCL4 or DCL2 to produce 21-22-nt siRNAs.

The model in Fig. 1 predicts that 24-nt siRNAs entering the nucleus have two fates: 1) there is no nuclear target transcript to which they can bind and so no silencing occurs, or 2) they direct binding of a protein complex to a nuclear target transcript and induce the production of 24-nt siRNAs that can move from cell-to-cell or over long distance to perpetuate the long-distance signaling, and initiate the biogenesis of 21-nt secondary siRNAs. These then direct mRNA degradation both cell-autonomously or in adjacent cells to cause the full silencing effect. In this model, transgenes are different from endogenous genes in that transgenes are associated with nuclear non-coding transcripts while most endogenous genes lack such nuclear transcripts, and this difference accounts for the observation that only transgenes have so far been shown to be susceptible to systemic silencing.

Consistent with this nuclear siRNA amplification model, most of the RNA silencing factors, including RDR6 and DCL4 that are required for ta-siRNA biogenesis, are localized in the nucleus. Also, early studies have shown that transgene silencing has frequently been associated with the accumulation of nuclear RNA transcripts (Metzlaff et al., 1997; Wang

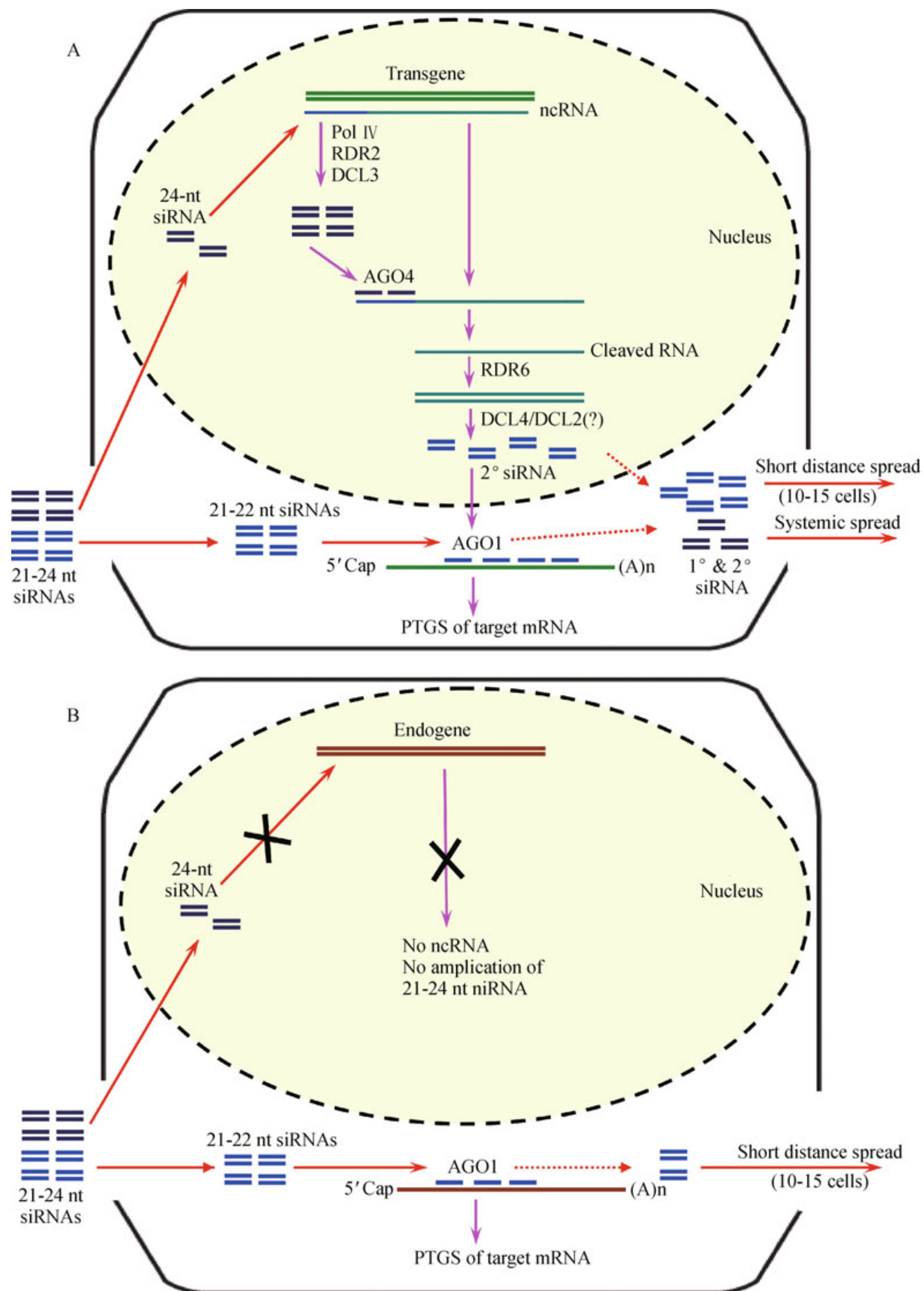

Figure 1 A model for mobile RNA silencing in plants. All size classes of small RNAs can move from cell to cell or over a long distance through the phloem and enter cells to induce systemic or short-distance mobile silencing. The 21-22 nt siRNAs direct mRNA degradation and are responsible for the observed silencing effects in both short and long-distance silencing. These 21-22-nt siRNAs can move through 10–15 cells to cause short-distance spread of silencing but they do not induce further amplification of the siRNA signal (A and B). The 24-nt siRNAs enter the nucleus and interact with a long nuclear transcript to induce the production of secondary (2°) 24-nt siRNAs by PolIV and RDR2 from the homologous target region (A). Both the primary (1°) and 2° 24-nt siRNAs can bind to AGO4 and direct cleavage of the nuclear transcript at the homologous region, generating an RNA cleavage fragment. This fragment is used as template by RDR6 to synthesize dsRNA which is processed by DCL4 (and perhaps DCL2) to generate 2° 21-22-nt siRNAs, and these siRNAs in turn direct cleavage of target mRNA leading to the effect of systemic silencing (A). Excess 1° and 2° nt siRNAs (indicated by dashed arrowheads) can move further from cell to cell or over a long distance to perpetuate the silencing. Only transgenes or endogenous loci that produce such nuclear transcript are susceptible to the 24-nt siRNA-induced systemic silencing (A). Most endogenous genes do not have such transcript and therefore are resistant to systemic silencing (B).

and Waterhouse, 2000; Wang and Metzlaff, 2005). The idea that RdDM components are required for noncoding RNA and secondary siRNA production is consistent with a recent study, which showed that secondary siRNAs are generated downstream of a hpRNA-targeted region and that the production of these secondary siRNAs is associated with a noncoding transcript and requires the RdDM factors Pol IV and RDR2 (Daxinger et al., 2009).

Conclusion and perspective

While the model in Fig. 1 provides a possible explanation for many of the observations concerning mobile gene silencing in plants, several important questions remain. For instance, why are *pol iv* or *dcl2 dcl3 dcl4* mutants, which are deficient in 24-nt siRNAs, still able to send long-distance signals to the grafted scion? Is it possible that unprocessed hpRNA can move through a few cells in the graft junction into the adjacent scion cells to be processed by DCL3, generating the initial 24-nt siRNA signals? Another question that is not addressed in this model is how the siRNA signals move in plants. For instance, is the movement a passive process or an active process assisted by RNA transport proteins? Knowledge from studies on viral RNA movement in plants could provide some clues on this. More importantly, the model requires a critical experimental test to confirm the existence of the nuclear RNA transcript and the involvement of this transcript in secondary siRNA production.

The model in Fig. 1, if proven, has practical implications. For instance, effective systemic silencing against endogenous genes would be highly desirable for knocking down gene expression in plant species that are recalcitrant to genetic transformation or have a long juvenile period. The model predicts that endogenous genes without the nuclear transcripts would not be susceptible to systemic induction of silencing. A genome-wide search for such nuclear transcripts could identify endogenous genes that can be silenced systemically. In some transgenic applications, such as overexpression of transgenes to improve crop traits, the induction of systemic transgene silencing would be detrimental. As discussed earlier, not all transgene loci are susceptible to systemic silencing, suggesting that some transgene loci do not have the nuclear transcript. Screening primary transgenic populations for the existence of such nuclear transcripts could help identify lines that lack the transcript and therefore have durable transgene expression. Methods to minimize the production of the nuclear RNA transcript, or to “endogenize” the transgene, would also be valuable in maintaining long-term transgene stability.

References

Allen E, Xie Z, Gustafson A M, Carrington J C (2005). microRNA-directed phasing during trans-acting siRNA biogenesis in plants. Cell, 121(2): 207–221

Bagasra O, Prilliman K R (2004). RNA interference: the molecular immune system. J Mol Histol, 35(6): 545–553

Baulcombe D (2004). RNA silencing in plants. Nature, 431(7006): 356–363

Brosnan C A, Mitter N, Christie M, Smith N A, Waterhouse P M, Carroll B J (2007). Nuclear gene silencing directs reception of long-distance mRNA silencing in *Arabidopsis*. Proc Natl Acad Sci USA, 104(37): 14741–14746

Chinnusamy V, Zhu J K (2009). Epigenetic regulation of stress responses in plants. Curr Opin Plant Biol, 12(2): 133–139

Crete P, Leuenberger S, Iglesias V A, Suarez V, Schöb H, Holtorf H, van Eeden S, Meins F Jr (2001). Graft transmission of induced and spontaneous post-transcriptional silencing of chitinase genes. Plant J, 28(5): 493–501

Daxinger L, Kanno T, Bucher E, van der Winden J, Naumann U, Matzke A J, Matzke M (2009). A stepwise pathway for biogenesis of 24-nt secondary siRNAs and spreading of DNA methylation. EMBO J, 28 (1): 48–57

Dunoyer P, Brosnan C A, Schott G, Wang Y, Jay F, Alioua A, Himber C, Voinnet O (2010b). An endogenous, systemic RNAi pathway in plants. EMBO J, 29(10): 1699–1712

Dunoyer P, Himber C, Ruiz-Ferrer V, Alioua A, Voinnet O (2007). Intra- and intercellular RNA interference in *Arabidopsis thaliana* requires component of the microRNA and heterochromatic silencing pathways. Nat Genet, 39(7): 848–856

Dunoyer P, Himber C, Voinnet O (2005). DICER-LIKE 4 is required for RNA interference and produces the 21-nucleotide small interfering RNA component of the plant cell-to-cell silencing signal. Nat Genet, 37(12): 1356–1360

Dunoyer P, Schott G, Himber C, Meyer D, Takeda A, Carrington J C, Voinnet O (2010a). Small RNA duplexes function as mobile silencing signals between plant cells. Science, 328(5980): 912–916

Fusaro A F, Matthew L, Smith N A, Curtin S J, Dedic-Hagan J, Ellacott G A, Watson J M, Wang M B, Brosnan C, Carroll B J, Waterhouse P M (2006). RNA interference-inducing hairpin RNAs in plants act through the viral defence pathway. EMBO Rep, 7(11): 1168–1175

Hamilton A J, Baulcombe D C (1999). A species of small antisense RNA in posttranscriptional gene silencing in plants. Science, 286(5441): 950–952

Herr A J, Baulcombe D C (2004). RNA silencing pathways in plants. Cold Spring Harb Symp Quant Biol, 69(1): 363–370

Himber C, Dunoyer P, Moissiard G, Ritzenthaler C, Voinnet O (2003). Transitivity-dependent and -independent cell-to-cell movement of RNA silencing. EMBO J, 22(17): 4523–4533

Kalantidis K, Schumacher H T, Alexiadis T, Helm J M (2008). RNA silencing movement in plants. Biol Cell, 100(1): 13–26

Klahre U, Crété P, Leuenberger S A, Iglesias V A, Meins F Jr (2002). High molecular weight RNAs and small interfering RNAs induce systemic posttranscriptional gene silencing in plants. Proc Natl Acad Sci USA, 99(18): 11981–11986

Lakatos L, Csorba T, Pantaleo V, Chapman E J, Carrington J C, Liu Y P, Dolja V V, Calvino L F, López-Moya J J, Burgyán J (2006). Small RNA binding is a common strategy to suppress RNA silencing by several viral suppressors. EMBO J, 25(12): 2768–2780

Law J A, Jacobsen S E (2010). Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nat Rev Genet, 11

(3): 204–220

Mallory A C, Ely L, Smith T H, Marathe R, Anandalakshmi R, Fagard M, Vaucheret H, Pruss G, Bowman L, Vance V B (2001). HC-Pro suppression of transgene silencing eliminates the small RNAs but not transgene methylation or the mobile signal. Plant Cell, 13(3): 571–583

Metzlaff M, O'Dell M, Cluster P D, Flavell R B (1997). RNA-mediated RNA degradation and chalcone synthase A silencing in petunia. Cell, 88(6): 845–854

Mlotshwa S, Voinnet O, Mette M F, Matzke M, Vaucheret H, Ding S W, Pruss G, Vance V B (2002). RNA silencing and the mobile silencing signal. Plant Cell, 14(Suppl): S289–S301

Molnar A, Melnyk C W, Bassett A, Hardcastle T J, Dunn R, Baulcombe D C (2010). Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. Science, 328(5980): 872–875

Palauqui J C, Elmayan T, Pollien J M, Vaucheret H (1997). Systemic acquired silencing: transgene-specific post-transcriptional silencing is transmitted by grafting from silenced stocks to non-silenced scions. EMBO J, 16(15): 4738–4745

Roberts A G, Cruz S S, Roberts I M, Prior D, Turgeon R, Oparka K J (1997). Phloem unloading in sink leaves of *Nicotiana benthamiana*: Comparison of a fluorescent solute with a fluorescent virus. Plant Cell, 9(8): 1381–1396

Shaharuddin N A, Han Y, Li H, Grierson D (2006). The mechanism of graft transmission of sense and antisense gene silencing in tomato plants. FEBS Lett, 580(28–29): 6579–6586

Smith L M, Pontes O, Searle I, Yelina N, Yousafzai F K, Herr A J, Pikaard C S, Baulcombe D C (2007). An SNF2 protein associated with nuclear RNA silencing and the spread of a silencing signal between cells in *Arabidopsis*. Plant Cell, 19(5): 1507–1521

Tijsterman M, Plasterk R H (2004). Dicers at RISC; the mechanism of RNAi. Cell, 117(1): 1–3

Vaistij F E, Jones L, Baulcombe D C (2002). Spreading of RNA targeting and DNA methylation in RNA silencing requires transcription of the target gene and a putative RNA-dependent RNA polymerase. Plant Cell, 14(4): 857–867

Voinnet O (2005). Non-cell autonomous RNA silencing. FEBS Lett, 579 (26): 5858–5871

Voinnet O (2008). Use, tolerance and avoidance of amplified RNA silencing by plants. Trends Plant Sci, 13(7): 317–328

Voinnet O, Baulcombe D C (1997). Systemic signalling in gene silencing. Nature, 389(6651): 553

Voinnet O, Vain P, Angell S, Baulcombe D C (1998). Systemic spread of sequence-specific transgene RNA degradation in plants is initiated by localized introduction of ectopic promoterless DNA. Cell, 95(2): 177–187

Wang M B, Dennis E S (2009). SPT5-like, a new component in plant RdDM. EMBO Rep, 10(6): 573–575

Wang M B, Metzlaff M (2005). RNA silencing and antiviral defense in plants. Curr Opin Plant Biol, 8(2): 216–222

Wang M B, Waterhouse P M (2000). High-efficiency silencing of a β-glucuronidase gene in rice is correlated with repetitive transgene structure but is independent of DNA methylation. Plant Mol Biol, 43 (1): 67–82

Wassenegger M, Krczal G (2006). Nomenclature and functions of RNA-directed RNA polymerases. Trends Plant Sci, 11(3): 142–151

Waterhouse P M, Wang M B, Finnegan E J (2001). Role of short RNAs in gene silencing. Trends Plant Sci, 6(7): 297–301

Xie Z, Johansen L K, Gustafson A M, Kasschau K D, Lellis A D, Zilberman D, Jacobsen S E, Carrington J C (2004). Genetic and functional diversification of small RNA pathways in plants. PLoS Biol, 2(5): E104

Yoshikawa M, Peragine A, Park M Y, Poethig R S (2005). A pathway for the biogenesis of trans-acting siRNAs in *Arabidopsis*. Genes Dev, 19 (18): 2164–2175

Zhang B, Pan X, Cobb G P, Anderson T A (2006). Plant microRNA: a small regulatory molecule with big impact. Dev Biol, 289(1): 3–16