Mini Review: Revisiting Mobile RNA Silencing In Plants

Xian Zhang [a,1], Tongfei Lai[a,1], Pengcheng Zhang[a,1], Xinlian Zhang[b], Chen Yuan[a], Zhenhui Jin[a], Hongmei Li[a], Zhiming Yu[a], Cheng Qin[a], Mahmut Tör[c], Ping Ma[b], Qi Cheng[d], Yiguo Hong[a,c,e,*]

[a] Research Centre for Plant RNA Signaling, College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 310036, China

[b] Department of Statistics, University of Georgia, Athens, GA 30602, USA.

[c] Worcester-Hangzhou Joint Molecular Plant Health Laboratory, Institute of Science and the Environment, University of Worcester, WR2 6AJ, UK

[d] Nitrogen Fixation Laboratory, Qi Institute, Jiaxing 314000, Zhejiang, China

[e] Warwick-Hangzhou RNA Signaling Joint Laboratory, School of Life Sciences, University of Warwick, Warwick CV4 7AL, UK

[*] Corresponding author.

Emails: yiguo.hong@hznu.edu.cn, yiguo.hong@warwick.ac.uk or y.hong@worc.ac.uk (Y. Hong).

[1] These authors contributed equally to this work.

Keywords:

DICER-LIKEs

Intercellular RNA silencing

Systemic RNA silencing

Mobile silencing signal

Small interfering RNA (siRNA)

Table of Contents

ABSTRACT

Non-cell autonomous RNA silencing can spread from cell to cell and over long-distances in animals and plants. This process is genetically determined and requires mobile RNA signals. Genetic requirement and molecular nature of the mobile signals for non-cell-autonomous RNA silencing were intensively investigated in past few decades. No consensus dogma for mobile silencing can be reached in plants, yet published data are sometimes inconsistent and controversial. Thus, the genetic requirements and molecular signals involved in plant mobile silencing are still poorly understood. This article revisits our present understanding of intercellular and systemic non-cell autonomous RNA silencing, and summarises current debates on RNA signals for mobile silencing. In particular, we discuss new evidence on siRNA mobility, a *DCL2*-dependent genetic network for mobile silencing and its potential biological relevance as well as 22nt siRNA being a mobile signal for non-cell-autonomous silencing in both *Arabidopsis* and *Nicotiana benthamiana*. This sets up a new trend in unravelling genetic components and small RNA signal molecules for mobile silencing in (across) plants and other organisms of different kingdoms. Finally we raise several outstanding questions that need to be addressed in future plant silencing research.

1. Non-Cell Autonomous RNA Silencing

RNA silencing is a regulatory and defence mechanism that controls gene expression and counterattacks pathogenic invasion in fungi, plants, and animals [1,2]. It involves specific-targeting of homologous sequences and can occur at transcriptional and post-transcriptional levels, known as transcriptional and post-transcriptional gene silencing (TGS and PTGS, respectively). TGS modifies related DNA by RNA-directed DNA methylation (RdDM) while PTGS degrades mRNA or blocks translation of homologous RNA transcripts. The genetic requirements and biochemical frameworks for cell-autonomous RNA silencing (CARS) have been well established. CARS can be triggered by double- or single-stranded RNA (ds or

ssRNA) in conjunction with DICER or DICER-LIKE (DCL) ribonuclease III-like enzymes and ARGONAUTE (AGO) proteins [2]. CARS that is directly induced by dsRNA is called primary silencing, in which dsRNA is processed by DCLs into 21–24 nucleotide (nt) small-interfering RNAs (siRNAs), dubbed primary siRNAs [3]. In plants, primary silencing can lead to transitive silencing which is not directly initialed by dsRNA, but indirectly induced by ssRNA. However, ssRNA needs to be converted into dsRNA through the combined activity of DCLs such as DCL2, RNA dependent RNA polymerase 6 (RDR6), and a coiled-coiled domain SGS3 protein. AGOs, siRNAs and other cellular factors form an RNA-induced silencing complex (RISC) that acts on homologous RNA/DNA molecules. Subsequently, transitive CARS can act on sequences that are not initially targeted by primary siRNAs and generate secondary siRNAs, cascading and amplifying the gene-specific silencing effect [3,4]. RNA silencing can travel from cell to cell and over long-distance in animals and plants, or even between organisms such as fungi and plants [1,5]. This phenomenon is called 'non-cell autonomous RNA silencing' (Non-CARS). Non-CARS is determined by mobile signals and various genetic components [6,7]. However, genetic insights into Non-CARS and the nature of the corresponding mobile signals remain two of the least understood, yet the most controversial topics in the field of plant RNA silencing.

2. Cell-to-cell spread of RNA Silencing – Intercellular Non-CARS

2.1. Intercellular spread of RNA silencing

Non-CARS involves two interconnected but distinct processes – cell-to-cell and long-distance spread of intracellular silencing, often referred as intercellular and systemic silencing. Intercellular silencing is a prerequisite for, but not necessarily leads to systemic Non-CARS [8]. Silencing spread from cell to cell has long been implied in agroinfiltration-based local silencing assay. Moreover, through a vascular-specific reporter transgene expression system as well as endogenous target genes together with mutagenesis and genetic analysis, it has

been demonstrated that *DCL4* is required for induction of limited intercellular PTGS in *Arabidopsis* [9]. Interestingly, transgenic over-expression of *DCL2* in the *dcl4 Arabidopsis* increased cell-to-cell spread of PTGS [4]. This phenomenon was thought to be due to that DCL2 may activate 22nt siRNA biogenesis and the latter promotes the production of 21nt siRNA via the RDR6/DCL4 pathway. However, such an explanation contradicts with the fact that *DCL4* was dysfunctional in *dcl4*. Furthermore, an increased cell-to-cell spread of PTGS has also been observed in a different *Arabidopsis dcl4* mutant, indicating that *DCL4* may play a suppressive role in Non-CARS [4]. Thus whether *DCL4* and DCL4-processed 21nt siRNA are indispensable for intercellular PTGS needs further investigation [10]. Nevertheless, several cellular factors including SNF2, a JmjC domain protein JMJ14, and the THO/TREX mRNA export complex are found to be associated with intercellular Non-CARS [11-13]. It should be noted that amplification of signals such as siRNA is also essential for transmission of cell-to-cell RNA silencing in *Arabidopsis* [14].

2.2. Cell-to-cell spread of virus-induced RNA silencing

On the other hand, spread of intracellular CARS from a single cell to neighbouring cells has been definitely demonstrated through a movement-deficient virus-induced gene silencing (VIGS) of a transgenic reporter green fluorescent protein (GFP) gene, a form of PTGS, in *Nicotiana benthamiana*. The coat protein (CP) gene-lacking *Turnip crinkle virus* TCV/GFPΔCP is able to initiate VIGS of GFP expression in a single epidermal cell from which *GFP* silencing spreads to adjacent epidermal and mesophyll cells in a three-dimensional manner [8,15,16]. This process requires RDR6 and two TCV movement proteins [15,17]. Interestingly, the movement protein of *Tobacco mosaic virus* has also been shown to be capable of enhancing Non-CARS of transgenic GFP gene [18]. More recently, Rosas-Diaz et al. [19] reported that a plant receptor-like kinase can promote cell-to-cell spread of RNA silencing and this kinase can be targeted by a geminivirus-encoded silencing suppressor

protein. Furthermore, our recent work demonstrates that *DCL4* inhibits non-cell-autonomous intercellular VIGS, although it plays a major role in cell-autonomous VIGS and intracellular viral siRNA biogenesis. By contrast, *DCL2*, likely along with DCL2-processed/dependent RNA signals such as 22nt siRNA, is required for efficient trafficking of VIGS from epidermal to adjacent cells. The negative regulation of Non-CARS by *DCL4* is probably achieved through DCL4-mediated down-regulation of *DCL2* expression. These discoveries imply that the DCL4-processed 21nt siRNA is an unlike candidate for mobile signals in intercellular Non-CARS targeting at least transgene in *N. benthamina* [20].

3. Long-distance Spread of RNA Silencing – Systemic Non-CARS

3.1. Systemic RNA silencing

Systemic RNA silencing, also known as long distance spread of Non-CARS, is well documented. For instance, silencing induced on local tissues can move to distal tissues through phloem transportation highway, or pass through grafting junction from stock to scion to induce systemic Non-CARS [1,2]. In *Arabidopsis*, essential genes including *RDR2*, *DCL3* and the RNA polymerase IVa gene *NRPD1a* in the TGS pathway are indispensible for, while AGO4 is partially involved in the reception of signals for non-cell-autonomous PTGS [21]. In distal recipient cells, 21 and 22nt siRNAs generated by *DCL4* and *DCL2* respectively lead to PTGS (degradation of target mRNA). Moreover, RDR6 also contributes to signal perception for systemic PTGS in *N. benthamiana* [6,22]. However, neither of the TGS genes nor RDR6, DCL2 or DCL4 *per se* is required for the production of the mobile signals in the incipent cells or for the trafficing of such mobile signals over long distance to induce systemic Non-CARS in *Arabidopsis* [21]. Nevertheless, involvement of the TGS pathway genes in signal perception for systemic PTGS implies the existence of an intriguing cross-talk between TGS and PTGS in plants. By contrast, using a transgene reporter and inverted-repeat dsRNA-mediated PTGS, Taochy et al. [23] performed an elegant genetic screen for *Arabidopsis*

mutants defected in systemic Non-CARS; and this work has shown that *DCL2* plays a crucial role in spreading the RDR6-depenedent PTGS from source root tissue to recipient shoot tissue [23].

3.2. Critical role of DCL2 in Non-CARS

More recently, using a set of newly established *DCL* RNAi lines in *N. benthamiana* along with a transgene reporter and hairpin RNA as intracellular silencing trigger, we have demonstrated that plants may have evolved a coordinated *DCL* genetic pathway in which *DCL2* is critical for systemic Non-CARS whilst both *DCL4* and *DCL3* attenuate long-distance spread of PTGS [24]. *DCL2* is required for the long-distance (leaf-to-leaf) trafficking and short-distance cell-to-cell movement (vascular cells to neighboring cells) of PTGS. This is supported by the facts that (i) suppression of *DCL2* expression can eliminate systemic PTGS and prevent mobile signals exiting from vascular tissues to surrounding mesophyll cells; and (ii) *DCL2* promotes, whilst *DCL4* inhibits, cell-to-cell spread of VIGS [20]. Moreover, *DCL2* is required to produce mobile signals in local source tissues and respond to such signals for non-autonomous PTGS in systemic recipient cells [23,24]. *DCL4* or *DCL3* may have an epistatic effect on *DCL2*, thereby indirectly influencing systemic PTGS in *N. benthamiana*. It should be noted that in contrast to genetic knockout mutants which are complete loss-of-function [21,23], knockdown by RNAi can only lead to partial loss-of-function in these *DCL* lines of *N. benthamiana* plants used for the genetic analysis of spread of RNA silencing [20,24]. Remaining activities of any residual DCLs in the *DCL*-RNAi lines could still affect the overall outcome of non-CARS. It is also worthwhile pointing out that DCL3 and the DCL3 processed 24 nt siRNAs are thought to be essential for systemic TGS in *Arabidopsis* [7]. This would suggest that systemic TGS may be independent of *DCL2*. However, the precise roles of *DCL2* (and other *DCLs*) and mobile siRNAs (and other types of mobile RNAs) in non-cell autonomous TGS have not yet been examined in *N. benthamiana*.

Nevertheless, taken together, these latest reports reveal a previously unknown functionality for *DCL2* in both intercellular and systemic spread of PTGS [20,23,24].

3.3. Root-to-shoot vs shoot-to-root Non-CARS

Non-CARS can occur upward from root to shoot as well as downward from shoot to root. However, the mechanism involved in upward or downward mobile silencing can be different in same or different plant species. For instance, long-distance mobile silencing is phloem-mediated in several different solanaceous species whilst in *A. thaliana*, root-to-shoot silencing travels not in the phloem but by template-dependent reiterated short-distance cell-to-cell spread through the cells of the central stele [1,14]. Seedling-grafting a GFP reporter scion into an hpRNA silencing-initiating rootstock together with a counterpart inducible system produces systemic Non-CARS via reiterating intercellular silencing in *Arabidopsis*, in contrast to phloem mediated long-distance movement of silencing of transgenes, such as GFP in Nicotiana species [1,14]. Such cell-to-cell-facilitated systemic spread of Non-CARS was also affected by auxin and actin transport inhibitors that can alter vesicular transport and cytoskeleton dynamics. These intriguing findings imply that sRNAs, the supposed mobile silencing signals, are transported from cell to cell via plasmodesmata (PD) rather than diffusing from their source in the phloem [14]. On the other hand, it is interesting to note that many studies on systemic silencing have so far focused on upward long-distance trafficking of Non-CARS [1,14,23,24].

3.4. Regulation of mobile RNA silencing by hydrogen peroxide

Following the fascinating work on the cell-to-cell-mediated systemic trafficking of Non-CARS [14], Liang et al. [25] further characterized *RCI3* as a key regulator of silencing mobility in *A. thaliana*. This was achieved by an elegant screen of *Arabidopsis* mutants impaired in the movement of root-to-shoot silencing, but not the production or effectiveness

of the RNA silencing signal. *RCI3* encodes a hydrogen peroxide (H_2O_2) producing type III peroxidase. Intracellular silencing initiated in the roots of *rci3* plants could not spread upward into leaf or floral tissue. However, such mobile silencing deficiency was complemented by exogenous H_2O_2 in *rci3* plants. Moreover, catalase or chemicals that reduce H_2O_2 production can reduce the spread of silencing in wild-type plants. Together with their previous findings [14], Liang et al. [25] suggest that regulation of endogenous H_2O_2 by peroxidases and production of reactive oxygen species (ROS) may control Non-CARS by altering PD permeability through remodeling of local cell wall structure. However, it remains to be elucidated whether the role of ROS in sRNA mobility would involve a *DCL2*-dependent or independent mechanism of intercellular and systemic non-CARS in plants.

4. RNA Signaling In Mobile Non-CARS

4.1. Current debates on RNA signals for mobile Non-CARS in plants

Many plants encode four DCLs for biogenesis of different types of small RNAs, for instance, DCL1 for microRNA (miRNA), while DCL2, DCL3, and DCL4 for 22, 24, and 21nt siRNA, respectively, in *Arabidopsis* and *N. benthamiana* [24,26-28]. It is reported that miRNAs can function as mobile signals in modulation of plant growth and development, although non-cell-autonomous miRNA signalling has not been directly demonstrated [29-31]. However, genetic analysis indicates that *DCL1* is unlikely to be involved in intra-/intercellular and systemic PTGS [20,24]. In *Arabidopsis*, the DCL3-processed 24nt siRNA can move to direct systemic TGS that controls genome-wide RNA-directed DNA methylation (RdDM) in recipient cells [7,26,32,33]. On the other hand, the DCL4-processed 21nt siRNA represents the mobile PTGS signal that moves from leaf companion cells to adjacent cells in *Arabidopsis* [9]. On the contrary, Non-CARS has also been reported to occur in the absence of sRNAs [34] and no specific siRNA produced by any of the four DCLs is required for systemic silencing [21]. Thus, any signal of RNA nature for mobile Non-CARS in plants

remains to be elucidated. By contrast, the dsRNA signal is well-documented to be associated with systemic and even transgenerational RNA interference (RNAi) in *Caenorhabditis elegans* [35-37].

4.2. SiRNA is mobile

Cell-to-cell and long distance movement of different sized siRNAs was first demonstrated in *Arabidopsis* [7]. Using *Arabidopsis* mutants deficient in siRNA biogenesis in either source or recipient tissue, Molnar et al. [7] found that transgene-derived siRNA and endogenous sRNAs can move across the graft union. More recently, we have established a 'siRNA mobility assay' in *N. benthamiana*. PTGS induced by a reporter hairpin dsRNA results in efficient biogenesis of local siRNAs, dubbed L-siRNAs, in incipient cells and local leaf tissues. 21-24nt sense and antisense L-siRNAs were mobile and detected in distal systemic leaves [24]. The systemically mobile L-siRNAs were predominantly 22nt in length although 21nt L-siRNAs were also abundant and only a limited number of 24nt L-siRNAs were present in systemic leaves. These findings differ from the uniform siRNA profiles in recipient tissues of the impaired systemic RNAi *Arabidopsis* mutants [23]. It is important to mention that the 'siRNA mobility assay' avoids any amplification and cascading production of the reporter siRNAs in remote recipient cells, thus unambiguously proving that all sized, sense and antisense L-siRNAs can move from cell to cell and over long-distance in plants. This assay also showed that *RDR6* and *DCLs*, particularly *DCL3* and *DCL4*, may contribute to the long-distance trafficking of L-siRNAs [24].

4.3. 22nt siRNA contributes to mobile signal for Non-CARS

DCL2 is crucial for Non-CARS [20,23,24] and is also required for transitive silencing [3,4]. Collectively these findings suggest that plants require genes involved in the production of dsRNA for transitive silencing in order to respond to mobile signal, thus these works exclude

dsRNA being the mobile signal for Non-CARS in plants. This is in contrast to dsRNA signal required for the systemic RNAi in animals [35]. Moreover, no mRNA or longer fragmented transcripts of the local silencing trigger was detected in systemic young leaves, implying that long ssRNAs cannot contribute to signaling for the mobile Non-CARS. However, detection of L-siRNAs in systemic tissues and the generation of specific systemic siRNAs that were associated with transitive silencing in distal recipient cells indicate that mobile L-siRNAs might represent a component for mobile signals for Non-CARS [24]. On the other hand, DCL4- or DCL3-processed 21 or 24 nt L-siRNAs as well as their RNA precursors unlikely contribute to the mobile silencing signal due to (i) suppression of *DCL4* or *DCL3* enhanced systemic silencing; and (ii) the levels of 21 or 24 nt L-siRNAs in both source and recipient tissues were not correlated with the induction and intensity of systemic PTGS in plants [21,23,24]. However, 21 and 24 nt siRNAs can act primarily as triggers for intracellular CARS including RNA-directed degradation of target mRNA or RdDM [2,6,32,33]. By contract, the abundance of the DCL2-processed 22nt L-siRNA in both local source and systemic recipient was consistent with induction as well as strength of the intercellular and systemic Non-CARS [20,24]. These findings demonstrated that DCL2-processed 22nt L-siRNA at least partially comprises the *bona fide* signals for induction of non-autonomous PTGS in *N. benthamiana*. This idea is consistent with the distinctive role of *DCL2* in efficient biosynthesis of secondary siRNA in systemic recipient cells and tissues [34,38,39].

5. A *DCL2*-Dependend Genetic Network For Mobile Silencing: Potential Biological Significance

The recent discovery of the critical role of *DCL2* and DCL2-dependent genetic network in intercellular and systemic spread of RNA silencing implicates that *DCL2* may have essential functionality, rather than simply acts as a partially *DCL4*-redundant gene in plants. Indeed, unlike *DCL4* acting in the first antiviral defense frontline of the intracellular CARS, *DCL2*

and DCL2-dependent mobile signals are mainly involved in the establishment of the second frontline of the intercellular Non-CARS to counterattack local virus infection in *N. benthamiana* [20]. Considering that *DCL2* promotes intracellular transitive silencing [3,4] and viral suppressors of silencing such as TCV P38 and the *Turnip mosaic virus* HC-Pro proteins can block transitive silencing and secondary siRNA biogenesis [3], *DCL2*-triggered Non-CARS may also have a direct role in plant defense against viruses to establish systemic infection. In addition, the *DCL2*-dependend genetic network for Non-CARS may be of biological relevance to other physiological and developmental processes since DCL2 and its cognate 22nt siRNA are clearly able to affect plant development [20,23,24,38,40-42].

6. Concluding Remarks and Future Perspectives

How cell-autonomous silencing moves from cell to cell and from local source cell and tissue to distal systemic tissue (*e.g.* from one leaf to another leaf) is a long outstanding question in the field of plant RNA silencing. Recent works have revealed that *DCL2* and DCL2-depentdent DCL network along with the DCL2-processed/dependent 22nt siRNA and/or RNA signals are required for intercellular and systemic Non-CARS (Figure 1). These unexpected findings provide a new framework to unravel RNA signaling for mobile silencing in plants, as well as in and across organisms of different kingdoms. In *Arabidopsis*, the DCL3-processed 24nt siRNAs are thought to be the main signals for systemic TGS. However, it is not known whether *DCL2* or *DCL2*-dependent *DCL* pathway necessitates the induction of systemic TGS in *N. benthamiana* and other plant species. Although the 22nt siRNA constitutes a part of the mobile signals for Non-CARS, potential involvements of other types of ss/dsRNAs such as long non-coding RNA in mobile silencing warrantee further investigation.

7. Outstanding Questions

DCL2 or *DCL2*-dependent *DCL* genetic pathway is shown to play an essential role in intercellular and systemic Non-CARS in *Arabidopsis* and *N. benthamiana*. Is the *DCL2*-dependent *DCL* genetic network a common mechanism for mobile Non-CARS in other plant species? This is an immediate question which needs to be addressed.

The positive correlation between the 22nt siRNA and Non-CARS suggests this type of siRNA contributes at least partially to mobile silencing signal. However, a direct proof of this is still lacking. Also whether other types of DCL2-dependent RNA transcripts can function as mobile Non-CARS signals remains to be elucidated.

Does siRNA move from cell to cell and over long-distance in a naked form or in a siRNA-protein complex (sRPC)? Does the size of siRNAs matter in mobile Non-CARS? If not, does a particular size of siRNAs, for instance 22nt siRNA, requires specific modification in order to function as the mobile signal for Non-CARS? Answers to these questions will further our understanding of the molecular mechanism involved in siRNA signaling in plant Non-CARS.

Does CARS spread from cytoplasm to chloroplast, mitochondria and other organelles within a plant cell? This is an overlooked research area; however, intracellular spread of silencing may represent a novel regulatory mode to modulate organelle gene expression by nuclear gene-originated small RNA or *vice versa*.

Funding

This work was supported by grants from the Ministry of Science and Technology of the People's Republic of China (National Key R&D Program of China 2017YFE0110900); the Ministry of Agriculture of the People's Republic of China (2016ZX08009001-004); the National Natural Science Foundation of China (31872636); National Institute of Health (R01 GM113242 and R01GM122080) and Hangzhou Normal University Pandeng Programme (201108). No conflict of interest is declared.

Acknowledgments

X. Zhang, T. Lai, P. Zhang, and Y. Hong discussed the contents and wrote the article in close collaboration with X-l. Zhang, C. Yuan, Z. Jin, H. Li, Z. Yu, C. Qin, M. Tör, P. Ma, and Q. Cheng. Y. Hong is the group leader proof-read and finalized the manuscript.

Reference

[1] Baulcombe, D. (2004) RNA silencing in plants. *Nature* 431, 356-363.

[2] Sarkies, P. and Miska, E. A. (2014) Small RNAs break out: the molecular cell biology of mobile small RNAs. *Nat. Rev. Mol. Cell. Biol.* 15, 525-535.

[3] Mlotshwa, S. *et al.* (2008) DICER-LIKE2 plays a primary role in transitive silencing of transgenes in Arabidopsis. *PLoS One* 3, e1755.

[4] Parent, J-S. *et al.* (2015) Respective contribution of Arabidopsis DCL2 and DCL4 to RNA silencing. *Plant J.* 81, 223-232.

[5] Weiberg, A. *et al.* (2013) Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. *Science* 342,118-123.

[6] Melnyk, C. W. *et al.* (2011) Intercellular and systemic movement of RNA silencing. *EMBO J.* 30, 3553-3563.

[7] Molnar, A. *et al.* (2010) Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. *Science* 328, 872-875.

[8] Ryabov, E. V. *et al.* (2004) Cell-to-Cell, but not long-distance, spread of RNA silencing that is induced in individual epidermal cells. *J. Virol.* 78, 3149-3154.

[9] Dunoyer, P. *et al.* (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, 1356-1360.

[10] Berg, J. M. (2016) Retraction. *Science* 354, 190.

[11] Searle, I. R. *et al.* JMJ14, a JmjC domain protein, is requried for RNA silencing and cell-to-cell movement of an RNA silencing signal in Arabidopsis. *Genes Dev.* 24, 986-991.

[12] Smith, L. M. *et al.* (2007) An SNF2 protein assoicated with nuclear RNA silencing and the spread of a silencing signal between cells in Arabidopsis. *Plant Cell* 19, 1507-1521.

[13] Yelina, N. E. *et al.* (2010) Putative Arabidopsis THO/TREX mRNA export complex is involved in transgene and endogenous siRNA biosynthesis. *Proc. Natl. Acad. Sci. U. S. A.* 107, 13948-13953.

[14] Liang, D., White, R. G. and Waterhouse, P. M. (2012) Gene silencing in Arabidopsis spreads from the root to the shoot, through a gating barrier, by template-dependent, nonvascular, cell-to-cell movement. *Plant Physiol.* 159, 984-1000.

[15] Zhou, Y. *et al.* (2008) Influence of viral genes on the cell-to-cell spread of RNA silencing. *J. Exp. Bot.* 59, 2803-2813.

[16] Shi, Y. *et al.* (2009) Suppression of local RNA silencing is not sufficient to promote cell-to-cell movement of Turnip crinkle virus in Nicotiana benthamiana. *Plant Signal Behav.* 4, 15-22.

[17] Qin, C. *et al.* (2012) Involvement of RDR6 in short-range intercellular RNA silencing in *Nicotiana benthimiana*. *Sci. Rep.* 2, 467.

[18] Vogler, H. *et al.* (2008) Tobacco mosaic virus movement protein enhances the spread of RNA silencing. *PLoS Pathog.* 4, e1000038.

[19] Rosas-Diaz, T. et al. (2018) A virus-targeted plant receptor-like kinase promotes cell-to-cell spread of RNAi. *Proc. Natl. Acad. Sci. U. S. A.* 115, 1388-1393.

[20] Qin, C. *et al.* (2017) Roles of DCL2 and DCL4 in Intra- and Intercellular Antiviral Silencing in *Nicotiana benthmiana*. *Plant Physiol.* 174, 1067-1081.

[21] Brosnan, C. A. *et al.* (2007) Nuclear gene silencing directs reception of long-distance mRNA silencing in Arabidopsis. *Proc. Natl. Acad. Sci. USA.* 104,14741-14746.

[22] Schwach, F. *et al.* (2005) An RNA-dependent RNA polymerase prevents meristem invasion by potato virus X and is required for the activity but not the production of a systemic silencing signal. *Plant Physiol.* 138, 1842-1852.

[23] Taochy, C. *et al.* (2017) A genetic screen for impaired systemic RNAi highlights the crucial role of DICER-LIKE 2. *Plant Physiol.* 175, 1424-1437.

[24] Chen, W. *et al.* (2018) A genetic network for systemic RNA silencing in plants. *Plant Physiol.* 176, 2700-2719.

[25] Liang, D., White, R. G. Waterhouse, P. M. (2014) Mobile gene silencing in Arabidopsis is regulated by hydrogen peroxide. *PeerJ 2*, e701.

[26] Henderson, I. R. *et al.* (2006) Dissecting Arabidopsis thaliana DICER function in small RNA processing, gene silencing and DNA methylation patterning. *Nat. Genet.* 38, 721-725.

[27] Mukherjee, K. *et al.* (2013) Evolution of animal and plant dicers: early parallel duplications and recurrent adaptation of antiviral RNA binding in plants. *Mol. Biol. Evol.* 30, 627-641.

[28] Xie, Z. *et al.* (2004) Genetic and functional diversification of small RNA pathways in plants. *PLoS Biol.* 2, E104.

[29] Carlsbecker, A. et al. (2010) Cell signalling by microRNA 165/6 directs gene dose-dependent root cell fate. *Nature* 465, 316-321.

[30] Pant, B. D. *et al.* (2008) MicroRNA399 is a long-distance signal for the regulation of plant phosphate homeostasis. *Plant J.* 53, 731-738.

[31] Skopelitis, D. S. *et al.* (2017) Boundary formation through a direct threshold-based readout of mobile small RNA gradients. *Dev. Cell* 43, 265-273.

[32] Lewsey, M. G. *et al.* (2016) Mobile small RNAs regulate genome-wide DNA methylation. *Proc. Natl. Acad. Sci. U. S. A.* 113, E801-810.

[33] Melnyk, C. W. *et al.* (2011) Mobile 24 nt small RNAs direct transcriptional gene silencing in the root meristems of *Arabidopsis thaliana*. *Curr. Biol.* 21, 1678-1683.

[34] Mallory, A. C. *et al.* (2001) HC-Pro suppression of transgene silencing eliminates the small RNAs but not transgene methylation or the mobile signal. *Plant Cell* 13, 571-583.

[35] Jose, A. M. *et al.* (2011) Two classes of silencing RNAs move between *Caenorhabditis elegans* tissues. *Nat. Struct. Mol. Biol.* 18, 1184–1188.

[36] Jose, A. M. *et al.* (2009) Export of RNA silencing from C. elegans tissues does not require the RNA channel SID-1. *Proc. Natl. Acad. Sci. U. S. A.* 106, 2283–2288.

[37] Devanapally, S. *et al.* (2015) Double-stranded RNA made in C. elegans neurons can enter the germline and cause transgenerational gene silencing. *Proc. Natl. Acad. Sci. U. S. A.* 112, 2133-2138.

[38] Chen, H. M. *et al.* (2010) 22-nucleotide RNAs trigger secondary siRNA biogenesis in plants. *Proc. Natl. Acad. Sci. U. S. A.* 107, 15269-15274.

[39] Cuperus, J. T. *et al.* (2010) Unique functionality of 22-nt miRNAs in triggering RDR6-dependent siRNA biogenesis from target transcripts in Arabidopsis. *Nat. Struct. Mol. Biol.* 17, 997-1003.

[40] Bouche, N. *et al.* (2006) An antagonistic function for Arabidopsis DCL2 in development and a new function for DCL4 in generating viral siRNAs. *EMBO J.* 25, 3347-3356.

[41] Garcia-Ruiz, H. *et al.* (2010) Arabidopsis RNA-dependent RNA polymerases and Dicer-like proteins in antiviral defense and small interfering RNA biogenesis during Turnip mosaic virus infection. *Plant Cell* 27, 944-945.

[42] Wang, X. B. *et al.* (2011) The 21-nucleotide, but not 22-nucleotide, viral secondary small interfering RNAs direct potent antiviral defence by two cooperative argonautes in Arabidopsis thaliana. *Plant Cell* 23, 1625-1638.

410 **Figure Legend**

411 **Figure 1** Summary of Non-cell-autonomous RNA silencing. Recent development in the field
412 of mobile silencing [14, 20,23-25] is outlined in this figure. Previous findings about
413 intercellular and systemic RNA silencing have been reviewed by others [see references 1,2,6].
414 The sign of T indicates an inhibitory effect whilst red arrows represent a positive influence on
415 the target steps of mobile silencing. A question mark implies an unknown action mode.