J. Plant Biol. (2009) 52:10–18
DOI 10.1007/s12374-008-9001-y

REVIEW

Non-cell Autonomous RNA Trafficking and Long-Distance Signaling

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Received: 15 November 2008 / Accepted: 22 November 2008 / Published online: 28 January 2009

Abstract A wide range of proteins and RNA molecules in plants have been recently identified as non-cell autonomous, phloem-mobile molecules and suggested to play important roles in physiological and developmental processes. Systemic movement of both protein-coding mRNAs and non-coding small RNAs is shown to correlate with the epigenetic changes brought about across grafting junctions, supporting their potential roles as long-distance signaling molecules. Plants appear to have evolved this unique RNA-based signaling mechanism to control systemic regulation of various responses to environmental stimuli and challenges such as photoperiods, nutrient availabilities, and pathogen attacks. This mechanism may have been exploited by viroids, non-coding RNA pathogens, to spread infection cell to cell and through phloem. A model describing potential molecular mechanisms by which the systemic RNA trafficking occurs will be presented.

Keywords Long-distance signaling · Non-cell autonomous · RNA trafficking · Phloem · Plasmodesmata · Small RNAs

Introduction

To survive in an ecosystem as sedentary organisms, plants have evolved unique communication networks at the cellular, tissue, and whole organism levels. Various environmental signals perceived by specific tissues or organs are delivered to other distantly located organs where target cells or tissues process the signals to bring about appropriate changes in physiological and/or developmental condition. This inter-organ or long-distance communication in plants is facilitated by their intricate vascular systems comprised of the xylem and phloem. The xylem provides essential conduits for mineral and water transport, while the phloem conducts photosynthates.

The phloem-mobile proteins and protein/RNA complexes rely on symplasmic connections formed by plasmodesmata (PD) for their cell-to-cell transport and the entry into and exit from the phloem [30, 34]. Plasmodesmata establish a local communication network that allows for selective trafficking of macromolecules in addition to diffusion of ions, nutrients, and hormones [29, 38, 46]. A wide range of proteins and RNA molecules have been recently identified as systemic signaling molecules that play important roles in physiological and developmental processes [30, 32]. Notably, endogenous mRNAs have been shown not only to move systemically across graft junctions but also to function as information molecules [28, 40]. It was also found that small RNAs produced by transgene silencing, virus-induced gene silencing, or endogenous processing of non-coding RNAs can systemically move from rootstocks through phloem and accumulate in scions [23, 43, 44]. Silencing of the target genes in a scion was shown to correlate with the accumulation of a specific type of small RNAs. RNA viruses and viroids have exploited the PD and phloem-based endogenous trafficking system to spread infectious materials locally and systemically, respectively.

Here, we will provide an up-to-date overview on exciting recent discoveries that highlight the role of systemic RNA trafficking in plant growth and development and defense responses against pathogens. Potential molecular mechanisms by which this systemic RNA trafficking occurs will be discussed.

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Long-Distance Signaling Mediated by Non-cell Autonomous mRNAs via Phloem

To deliver water and nutrients throughout their whole bodies, plants have developed vascular systems composed of water-conducting xylem and photosynthate-conducting phloem [30, 33, 40]. Functional xylem and phloem systems consist of non-living and living cells, respectively. Phloem contains sieve elements (SEs) and companion cells (CCs) that are derived from the same sieve mother cells by asymmetric cell division but have undergone distinct cell fates. SEs are conducting cells which rely on tightly associated CCs for their metabolic support because they lose nuclei and other selected organelles as they mature. CCs are connected to SEs though apparently specialized, structurally distinct PD that are specific to the CC–SE junction [33]. It is assumed that macromolecules needed for SE function are produced in CCs and transported into the coupled SEs through PD. PD also play an essential role in mass flow of phloem sap contents along the connecting sieve tubes; as SEs mature, the PD at the cell wall junctions between SEs undergo structural modifications to form sieve plate pores. These pores become much greater in diameter to support the mass flow of the phloem stream.

SUT1 mRNA Traffics from Companion Cells to Sieve Elements through PD

The potential for plants to transport endogenous mRNAs by allowing them to pass through the PD from CCs to functional SEs has been demonstrated by the detection of mRNA encoding solanaceous sucrose transporter *SUT1* within SEs around the PD connecting CC and SE [25]. The SUT1 protein was shown to exclusively localize at the plasma membrane of SEs even though the *SUT1* gene expression is only active in CCs [26], suggesting that the protein trafficked from CC to SE. To confirm that the protein is produced within the CC and moved to the SE through PD, the authors examined the localization of *SUT1* mRNA by *in situ* hybridization. Surprisingly, *SUT1* mRNA was found to accumulate within mature SEs at a higher level than detected in CCs. These data raised the question as to whether the protein might have been synthesized from *SUT1* mRNA accumulated within the SEs by an unknown mechanism. The presence of *SUT1* mRNA within SEs was confirmed by independent studies on phloem sap analyses from other plant species [12, 25, 39]. A recent study by Schmitt et al. [41], however, denoted that the former immunolocalization datum may have been an artifact resulted from a non-specific binding of the antibodies that were employed. By using affinity-purified SUT1-specific antibodies in immunolocalization tests, they concluded that SUT1 localizes to companion cells and xylem parenchyma but not in SEs [41]. This result now eliminates the possibility that *SUT1* mRNA accumulated in SE may be used as template to synthesize SUT1 protein. The biological function of this non-cell autonomous mRNA and the underlying mechanism by which the transcript traffics through PD remain to be elucidated.

Phloem-Mobile mRNAs Can Regulate Leaf Development

Currently, several lines of evidence strongly support the idea that non-cell autonomous RNAs (hereafter termed NCARs) may act as long-distance information molecules that play a key role in the physiological and developmental transitions of plants [22, 30]. The transcript of the dominant mutant *Me* of tomato was shown to be graft transmissible, altering the leaf morphology of wild-type scion leaves to that of the *Me* mutant [24]. Similarly, a study performed on the pumpkin homologue of an *Arabidopsis* GRAS family member of transcription factors *GIBBERELLIC ACID INSENSITIVE* (*GAI*) was found in phloem sap and shown to move through phloem. Accumulation of *gai* mRNAs in wild type scions, which are translocated from transgenic rootstocks overexpressing the semidominant mutant transcripts, was also shown to correlate with an altered leaf morphology of the wild type scions [20]. Although these studies were based on gain-of-function mutant transcripts and the non-cell autonomous roles of wild type transcripts remain to be elucidated, the data are consistent with the notion that phloem-mobile mRNAs may play roles in developmental regulation.

The *Me* mutant transcript is a chimeric mRNA produced by a fusion between *PYROPHOSPHATE-DEPENDENT PHOSPHOFRUCTOKINASE* (*PFP*) and *LeT6* which encodes a member of KNOX gene family transcription factors that play vital roles in shoot meristem development [42]. The mutation results in an overexpression of the chimeric gene due to the strong promoter activity of *PFP*, which controls the expression of the chimeric transcript. When grafted to a wild-type scion, the *Me* transcript expressed in the mutant rootstock was shown to specifically accumulate in the shoot apex and leaf primordia of the scion and to alter the leaf morphology of the wild type scion to that of the mutant (Fig. 1a). This localization pattern was similar to that in ungrafted *Me* mutant, which suggests that the accumulation of *Me* transcript within the wild type scion correlates with the resulting mutant phenotype of the scion. The hypothesis that the *Me* transcript was transported through phloem was supported by the detection of the *Me* mRNA in phloem cells by *in situ* reverse-transcriptase-coupled PCR. The specific *Me* localization pattern in scion indicates also that its phloem entry and exit followed by cell-to-cell trafficking into the target tissue may be an intrinsic characteristic of the NCAR.

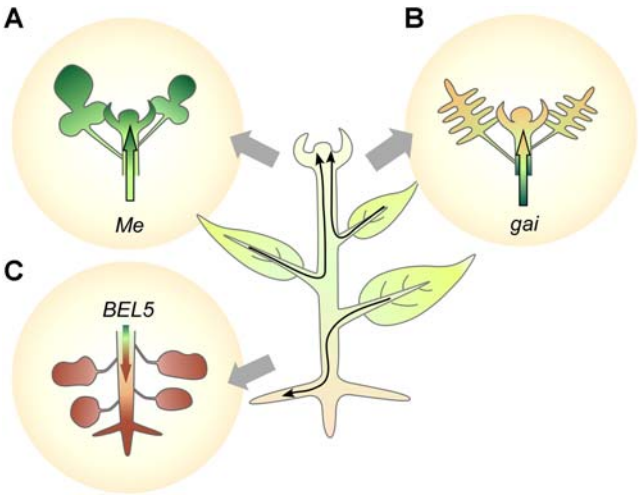

Fig. 1 A schematic diagram illustrating that specific phloem-mobile mRNAs can regulate leaf morphology and tuber development. Phloem-mobile *Me* (**a**) and *gai* (**b**) transcripts translocate from rootstocks to shoot apexes and alter leaf morphology of wild-type scions. *BEL5* expressed in shoots moves to stolon tips in roots and induces tuberization (**c**)

The *CmGAIP* mRNA has been shown as one of the graft-transmissible transcripts that were found in pumpkin phloem sap and specifically accumulated in developing shoot apex of heterograft scion [39]. The semi-dominant *gai-1* mutant in *Arabidopsis*, caused by deletion in the conserved DELLA domain of GRAS family transcription factors, exhibits a dwarf phenotype [36]. By employing this mutant and the pumpkin *Cmgaip* which is engineered to mimic *Arabidopsis gai-1* in heterografting studies, Haywood et al. [20] demonstrated that these mutant transcripts can move through heterograft junctions and accumulate in the shoots apex of the scion (Fig. 1b). Importantly, these mutant transcripts could alter the morphology of the scion leaves [20]. Moreover, they were shown to accumulate in shoot apex and flower buds but not in developing fruit, a strong sink organ, supporting the idea that phloem transport of specific NCARs is a selective process.

Phloem-Mobile mRNA Can Regulate Tuberization

BEL5 mRNA, which encodes a transcription factor, was shown to regulate tuber formation by accumulating within stolon tips in response to the photoperiod that induces tuber formation [3]. *BEL5* transcript is ubiquitously expressed in potato, but the level of its expression under short-day conditions has been shown to increase, accumulating more highly in the stem and stolon tips compared to the shoot tips. This, together with *in situ* hybridization and promoter analysis, which demonstrated that *BEL5* mRNA accumulates in phloem cells, led the authors to speculate that *BEL5* may act as a mobile signal which systemically traffics from the aerial tissue to the stolon tips to induce tuber formation. Subsequently, heterografting and overexpression studies demonstrated that *BEL5* transcript not only moves through phloem but can also impact the induction of tuber formation and tuber yields (Fig. 1c), supporting the idea that *BEL5* mRNA may play a role as a long-distance signal for tuberization.

Phloem Sap Transcriptomes: Gold Mines for NCARs

Examples described above highlight that specific mRNAs are loaded into the phloem for long-distance transport and accumulate within specific target tissues. These observations imply that the entry into and exit from the phloem system as well as local cell-to-cell movements following unloading from the phloem are highly regulated processes. Numerous studies performed by employing various techniques have so far identified several hundred mRNAs that are present in phloem saps of *Ricinus* [12], *Arabidopsis* [10], barley [18], and melon [31]. The phloem transcripts identified encode proteins that are involved in cellular processes widely ranging from basic metabolism, signal transduction, stress, and pathogen responses to maintenance of cellular structures. Consistent with the earlier observations suggesting that the accumulation of phloem-mobile transcripts in specific target tissues is a controlled process, not all the phloem transcripts that were tested had capacity to cross heterograft junctions [31]. Perhaps, some of the transcripts that are found in phloem saps may prove to reflect a passive diffusion through the PD between the CC–SE complex, which has a larger size exclusion limit [33]. In this regard, plants must have developed an active mechanism or surveillance system that prevents incidental exit of transcripts that are potentially subversive to the plants from the phloem main stream [17]. Understanding the biological role of these mobile transcripts and the mechanism by which these information molecules enter and exit the phloem will be an important future task.

Systemic Signaling by Non-cell Autonomous Small RNAs

Analyses of phloem saps from various plant species indicated that mobile RNAs in phloem are not limited to protein-coding RNAs [28]. Yoo et al. [45] have identified from pumpkin phloem sap numerous non-coding RNAs, including microRNAs (miRNAs) and small interfering RNAs (siRNAs), which range from 18 to 25 nucleotides in length. Systemic gene silencing mediated by siRNAs transported through the phloem is well documented [43, 44]. By contrast, systemic role of miRNAs in plants has received less attention due to very few examples presented

so far and the suggestion that they are relatively immobile and cell autonomous [1]. However, a new paradigm seems to be emerging that highlights essential roles for miRNAs in long-distance signaling [23]. These new findings denote the possibility that they may act as small NCARs involved in physiological, developmental, or defense responses in plants.

Role of Small RNAs in Post-transcriptional Gene Silencing

In addition to the canonical transcriptional regulation, plants have developed an elegant mechanism to modulate endogenous gene expression by utilizing post-transcriptional gene silencing (PTGS) or RNA interference. PTGS plays an important role in modulating gene expression by inducing degradation or translational inhibition of the target mRNAs that are complementary to the small silencing RNAs [6, 7, 16]. PTGS is mediated by two major types of ~21–24 nucleotide (nt) small RNAs: siRNAs and miRNAs. These small double-stranded (ds) RNAs are processed from different precursor dsRNAs by specialized RNAses called Dicers. Mature siRNAs or miRNAs guide the RNA-induced silencing complex (RISC) to cleave complementary target mRNAs, leading to down regulation of the target genes. siRNAs produced from aberrant dsRNAs and processed by RNA-dependent RNA polymerase mediate PTGS as a defense mechanism against infection by RNA viruses and the activity of transposable elements. A mature miRNA duplex composed of antisense miRNA and sense miRNA* strands is processed within the nucleus from the stem region of its non-coding RNA precursor containing ds-hairpin structures [5]. Only the antisense miRNA strand forms a complex with RISC to guide cleavage of complementary target mRNA. Many miRNAs are shown to be involved in controlling various developmental and physiological processes.

miRNA as a New Player in Shoot–Root Interorgan Signaling: miR399 in Phosphate Homeostasis

Balanced aerial and root growth in plants is supported by coordinated nutrient distribution via shoot–root or root–shoot signaling through phloem or xylem transport systems. Mineral and nitrogen nutrients taken up by roots from soil are reallocated to shoots, whereas the carbon fixed by green tissue is redistributed to roots. Recent findings demonstrate that maintenance of inorganic phosphate (Pi) homeostasis, an essential mechanism supporting growth of sink tissues such as developing leaves and floral organs, involves miR399 as a long-distance signaling molecule [2, 4, 27, 35]. Mutation in *Arabidopsis PHO2*, which encodes the ubiquitin E2 conjugase 24, results in a higher uptake and translocation of Pi, causing the overaccumulation of Pi in leaves [4]. *PHO2* is a target of miR399, the expression of which is upregulated by Pi deficiency. Overexpression of miR399 phenocopies the *pho2* mutant [4, 27]. A systemic role for *PHO2* is suggested by the finding that the *pho2* mutant phenotype in the scion can be restored by wild type rootstock.

Subsequently, it was determined that miR399 is the long-distance signal traveling from shoot to root to deliver the message that the shoot is under low Pi stress by downregulating the expression of *PHO2* in the root [27, 35]. Mature miR399 was shown to accumulate in phloem saps of rapeseed and pumpkin plants at a higher level relative to that in roots, leaves, and stem in response to a limited phosphate level and to accumulate in the rootstock grafted with miR399-overexpressing scion [35]. By contrast, the precursor of miR399 was found phloem-immobile based on its lack of accumulation in the rootstock across the graft union. These results provided strong evidence that the mature miR399 is a systemic signal.

Intriguingly, the sense or star strand of miR399 (miR399*) was not detected in roots where the miR399, transported from shoots via phloem, was shown to accumulate. This raised the possibility that mature miR399 might travel along the phloem as a single-stranded (ss) small RNA. Supporting experiments will be necessary to confirm that miR399* is not present within the phloem sap. However, this finding is consistent with the earlier report by Yoo et al. [45], which showed that small RNAs present in pumpkin phloem sap were found to be highly susceptible to ssRNA-specific RNase treatment, indicating that they may predominantly exist as ssRNAs. In addition, it was shown that small RNAs are not capable of moving cell to cell on their own but rather require non-cell autonomous small RNA-binding proteins, such as ss-specific phloem small RNA-binding protein1 (CmPSRP1) isolated from pumpkin phloem sap [45]. A functional homologue of CmPSRP1 remains to be isolated in other plant species including *Arabidopsis*; however, such a chaperone is likely required for miR399 and other phloem-specific small RNAs to enter into and exit from phloem as well as to translocate within the SEs.

Elusive Role of siRNA in Non-cell Autonomous Gene Silencing

Systemic PTGS was initially observed by grafting experiments in which gene silencing signals were found graft transmissible, in a sequence-specific manner, from tobacco rootstock displaying spontaneous gene silencing to the non-silenced scion [34]. It is now established that the PTGS induced by transgene expression as well as by virus infection is mediated by siRNAs [43, 44]. Their roles as systemic silencing signals are suggested by the findings that siRNAs can move cell to cell and perhaps systemically [21] and that they are present within phloem sap [45]. The observation that transgene silencing spreads throughout the

leaf tissue except into symplastically isolated mature guard cells [21] strongly suggests that the silencing signal is likely moving through PD as plant viruses do. Intriguingly, the genetic components that are involved in local intercellular silencing are different from those recruited for systemic gene silencing [14], and currently, molecular identity of the systemic silencing signal remains elusive.

In a comprehensive study performed on transgenic tobacco and *Arabidopsis* that ectopically express green fluorescent protein (GFP), Himber et al. [21] showed that the gene silencing triggered in a small group of cells can spread or diffuse cell to cell over ten to fifteen cell layers. By employing viral gene silencing suppressors that have different effects on the production of 21 nt, 24 nt, or both classes of siRNAs, they found that the accumulation of 21 nt siRNAs, but not the 24 nt, within the region where gene silencing is initiated correlates with the observed local spreading of the silencing signal. They also showed that an extensive gene silencing beyond the initial spreading can occur in *Arabidopsis* through secondary 21 nt siRNAs produced by a putative RNA-dependent RNA polymerase (RdRp), SDE1/SGS2. This transitivity-mediated extensive gene silencing, however, was not observed in gene silencing that is induced against endogenous targets. Subsequently, screening for the suppressor mutations that reduce or block spreading of gene silencing led to isolation of several components involved in this process: Dicer-like 4 (DCL4); an RdRp, RDR2; and NRPD1a, the subunit of DNA-dependent RNA polymerase IV [14, 13]. Based on these studies, it was proposed that the DCL4 function is necessary to initiate the production of 21 nt siRNAs in the primary cells and that the RDR2 and NRDP1 are involved in cell-to-cell movement or the recognition of 21 nt siRNAs within the recipient cells (Fig. 2) [13].

Another elegant study performed by applying micrografting techniques to various *Arabidopsis* mutants has identified the components of heterochromatin silencing in the nucleus as cellular factors that are essential to trigger systemic gene silencing within the scion [8]. In this study, only the 5′ part of the *GFP* gene was used to produce dsRNA in the rootstock to silence the *GFP* carried by the scion (see Fig. 2). Intriguingly, it was found that the siRNAs produced in the silencing scion was exclusively derived from the 3′ part of the *GFP*, suggesting that long-distance silencing signals activated a seemingly unidirectional transitivity different from that in the case of local spreading [15]. By employing reciprocal grafting, it was found that components of transcriptional silencing, including DNA-dependent RNA polymerase Iva RDR2 and DCL3, are required for the production of 24 nt siRNAs which possibly feed into AGO4 to guide cleavage of the *GFP* target transcript, generating decapped and polyadenylated RNA. Moreover, RDR6, which produces dsRNA from decapped RNA, was shown to be necessary to activate systemic gene silencing in the scion (see Fig. 2). Surprisingly, these components, especially DCL1-4, were found dispensable in the rootstock for the transmission of systemic gene silencing to the scion. Based on these results, Brosnan et al. [8] proposed that the phloem-mobile signal may not be the products of the Dicer members but rather dsRNA precursors.

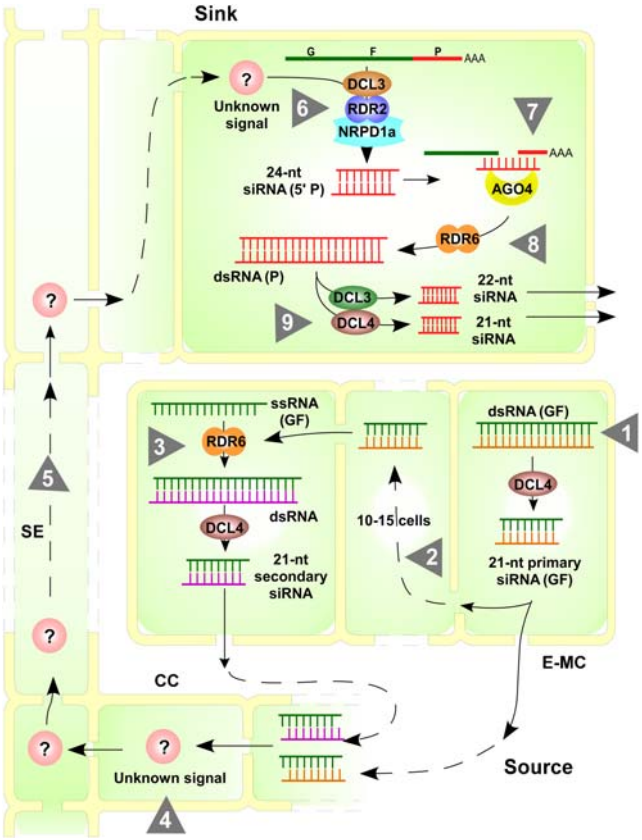

Fig. 2 Local and systemic silencing by siRNAs against the trangene GFP. Local silencing is induced by ectopic expression of dsRNA (specific to GF domain), which produces 21-nt primary siRNAs by DCL4 in epidermal/mesophyll cells (E-MC) (*1*). The primary siRNAs can move cell-to-cell over ten to fifteen cell layers (*2*). The siRNAs can guide RDR6 to synthesize new dsRNAs, which are then processed to 21-nt secondary siRNAs; this process results in extensive amplification of gene silencing signals (*3*). An as yet unknown signal enters into the phloem (CC–SE; *4*) and travels through SEs (*5*) for systemic gene silencing to occur in scions (sink tissues). The systemic signal stimulates the production of 5′-P domain-specific 24-nt siRNAs from *GFP* mRNA, which requires DCL3, RDR2, and NRPD1a (*6*). The loading of the 24-nt siRNAs to AGO4 leads to the cleavage of *GFP* mRNA, producing P-specific, decapped, and polyadenylated RNAs (*7*). These RNAs can be used by RDR6 to synthesize dsRNAs (*8*), which are then further processed into new 21- or 22-nt siRNAs by DCL4 or DCL3, respectively (*9*). This model is drawn based on the studies by Dunoyer [14, 15] and Brosnan [8]

Systemic Movement of Exogenous Non-coding RNAs

Viroids represent unique, small (~250–400 nt), single-stranded circular RNA molecules that can command not

only their own replication but also their spreading throughout the host cells even though they are non-coding RNAs [11]. As viroids do not encode their own proteins, they must have evolved to contain all the structural information essential for recruiting and hitch-hiking host molecules to guide their replication, cell-to-cell trafficking, and systemic phloem transport. Recent studies on the structure and function of *Potato spindle tuber viroid* (PSTVd) highlight that the viroid genome contains secondary structural elements essential for its infectivity [19, 47, 48, 50].

PSTVd belongs to the *Pospiviroidae* family, which replicates in the nucleus by subverting endogenous transcriptional machinery [9]. Viroid replication occurs via a rolling-circle mechanism by which a viroid genome, the (+) stand, is transcribed into the replication intermediate and the (−) strand to synthesize a new viroid genome. The resulting concatameric (+) strand undergoes cleavage and ligation followed by nuclear export and cell-to-cell trafficking to initiate systemic infection. By using *in situ* hybridization on infected solanaceous plants, Zhu et al. [50] have detected the replication intermediate within the nuclei of companion and parenchyma cells, providing strong evidence that PSTVd replicates in the phloem during systemic infection. It was also demonstrated that viroid trafficking does not simply follow the mass flow of the phloem stream, but rather that viroid genomes contain specific structural motifs to unlock the entry into and exit from SEs [51]. Mutations in the lower part of the conserved central region of PSTVd were shown to be replication competent in the mesophyll and phloem cells but were unable to exit from the phloem. Further dissection of viroid genomes has revealed other sequential and structural motifs necessary for short-distance transport, including a bipartite motif necessary for bundle sheath-to-mesophyll trafficking in young leaves [37] and another motif within the left terminal domain of PSTVd required for transport from the bundle sheath into the phloem [49]. A comprehensive genome map that illustrates numerous motifs important for replication or systemic trafficking is now available for the PSTVd (Fig. 3) [47]. Collectively, these studies well demonstrate that viroids, like their virus cousins, have evolved an elegant mechanism through which they can exploit endogenous nuclear and cell-to-cell trafficking machineries to sustain their replication and systemic infection throughout the whole plant.

A Model for the Potential Mechanism Underlying RNA Trafficking

Currently, most of the molecular players that chaperone various RNA molecules, regardless of their size or origin, remain to be discovered. However, accumulating evidence seems to support the hypothesis that cell-to-cell trafficking of NCAR molecules has to be tightly coupled with their nuclear trafficking for the biosynthesis and systemic movement. In this view, either a relay or concerted action between nuclear and PD trafficking components is likely to operate, and a wide range of ss-, ds-, and/or sequence-specific RNA-binding proteins with or without specific enzymatic activities may prove to play big roles in this process.

The model presented in Fig. 4 illustrates a potential mechanism by which local cell-to-cell and phloem-mediated long-distance trafficking of NCARs may occur. In this simplified model, NCARs interact with RNA-binding proteins that transport the RNAs through PD. A specific, relatively high-affinity interaction with the chaperone would be necessary for a given NCAR in order to prevent unwanted recognition by the cellular system responsible for destroying aberrant RNA molecules. To enter the phloem long-distance transport system, NCARs must first reach the CCs and pass through the PD between CC and SE. It is possible that NCARs may undergo amplification or biosynthesis within CCs along the vasculature to counteract potential dilution during their phloem transport. Once loaded into the SEs, NCARs would need to find a correct exit point, possibly

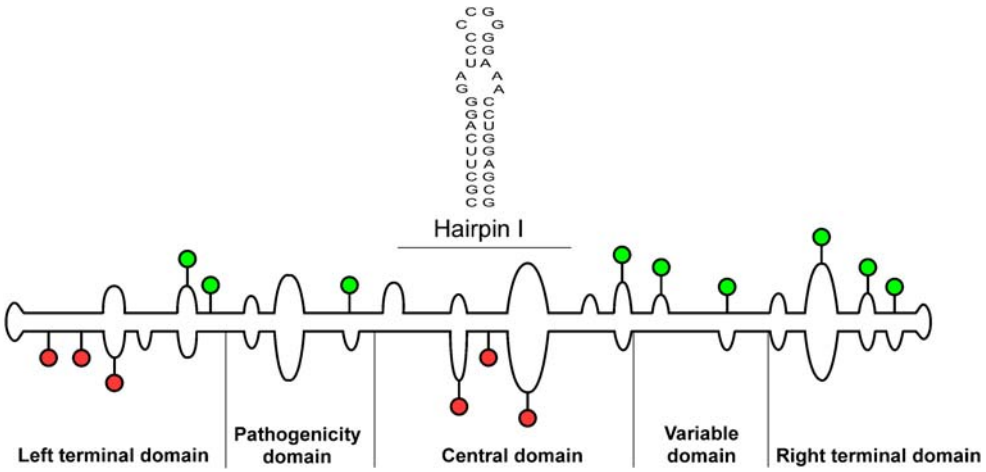

Fig. 3 A genome map illustrating motifs important for replication or systemic trafficking of PSTVd. The viroid can be divided into five structural domains. *Red circles* indicate the motifs critical for replication of the viroid, and *green circles* indicate those essential for systemic trafficking

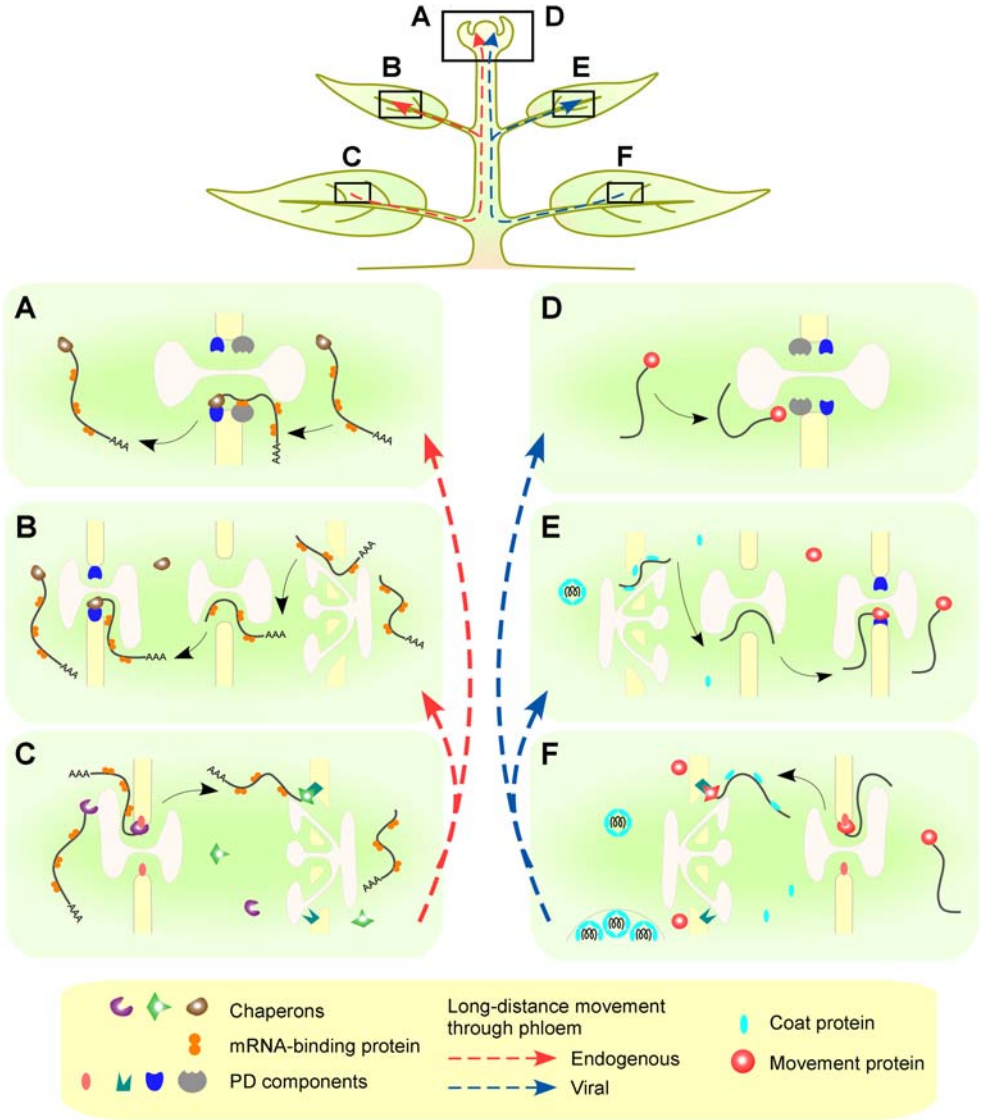

Fig. 4 A model for the molecular mechanism underlying local and long-distance NCAR trafficking. Cell-to-cell movement of endogenous mRNAs (*A–C*) or viral RNAs (*D–F*). At the source tissue (*C* and *F*), mRNAs and viral RNAs enter the phloem through companion cells. This process requires appropriate endogenous chaperons or movement proteins and/or coat proteins for viruses. Once in the phloem, the RNAs may follow mass flow of the phloem stream before exit into the sink. At the sink tissue (*B* and *E*), chaperons or movement proteins help RNAs to move cell to cell. An RNA "surveillance system" at the shoot meristem (*A* and *D*) excludes the entry of viral RNAs while allowing certain endogenous mobile mRNAs

through interactions with a new set of chaperones. Again, within the CCs, reamplification of NCARs may occur before they find their way out to shoot or root apexes and/or other developing tissues if they have a proper signal to pass through the RNA surveillance system.

Future Perspectives

It seems now evident that NCARs play important roles in long-distance signaling in plants, and as more genome information becomes available, novel NCARs are likely to be uncovered from various plant species. One of the remaining questions is how this signaling works at the molecular level. Answers to this question will be important not only for advancing our knowledge of plant biology but also for agricultural applications. For example, the knowledge gained from the studies on NCARs may allow for plant biologists to better control the changes in developmental phases of plants and resistance to pathogen infections which would help produce new crops with desirable qualities and higher yields. Future challenges in this exciting field of research will be identifying the molecular players and mechanisms by which RNA-based long-distance signaling occurs as well as elucidating temporal and spatial regulations of NCAR trafficking at the whole-plant level. Technical development will be also necessary to aid the detection of NCARs at a higher resolution and to map the structural specificity that potentiates their cell-to-cell and long-distance trafficking.

Acknowledgments We apologize to our colleagues whose important works could not be directly cited in this review due to the page limitations. We appreciate Lee lab members R. Sager and F. Pan for proofreading this manuscript and for helpful suggestions, respectively. This work was supported by the National Science Foundation (grant no. MCB 0445626 to J.-Y. L.).

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