Phloem-Mobile RNAs as Systemic Signaling Agents

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Abstract

The plant vascular system plays a central role in coordinating physiological and developmental events through delivery of both essential nutrients and long-distance signaling agents. The enucleate phloem sieve tube system of the angiosperms contains a broad spectrum of RNA species. Grafting and transcriptomics studies have indicated that several thousand mRNAs move long distances from source organs to meristematic sink tissues. Ribonucleoprotein complexes play a pivotal role as stable RNA-delivery systems for systemic translocation of cargo RNA. In this review, we assess recent progress in the characterization of phloem and plasmodesmal transport as an integrated local and systemic communication network. We discuss the roles of phloem-mobile small RNAs in epigenetic events, including meristem development and genome stability, and the delivery of mRNAs to specific tissues in response to environmental inputs. A large body of evidence now supports a model in which phloem-mobile RNAs act as critical components of gene regulatory networks involved in plant growth, defense, and crop yield at the whole-plant level.

Contents

INTRODUCTION

The plant vascular system, comprising xylem and phloem, functions as a transport conduit for the delivery of materials essential for the integrated development and physiological functioning of distant tissues and organs (69, 70). In the angiosperm xylem, tracheids and vessel elements form the conduits for transport of water and mineral nutrients from the soil to the aerial regions of the plant (**Figure 1*a***); this xylem transpiration stream travels through dead cells, and the thermodynamic driving force is a tensional gradient. By contrast, in the phloem, files of physiologically competent companion cell (CC)–sieve element (SE) complexes form the sieve tube system (STS) through which nutrients (sugars and amino acids) and signaling molecules (hormones, RNA, etc.) are transported to developing regions of the plant (**Figure 1*a***); this phloem translocation stream moves through mature enucleate SEs, with the flow being driven by a pressure gradient (49).

From an evolutionary perspective, the angiosperm phloem STS reflects the acquisition of several important developmental and physiological attributes. Certainly, for the topic under discussion, it is essential to understand the development and maintenance of the phloem STS. In brief, the CCs and SEs are derived from the vascular cambium and undergo a unique developmental progression, during which nuclei and vacuoles in the SEs are degraded, the content of the cytoplasm becomes reduced in complexity, and plasmodesmata (PDs) in the cell walls connecting adjoining SEs become modified to form sieve plate pores. The physiological competence of these mature enucleate SEs is maintained by their neighboring, nucleate CCs to allow the plasma membrane of the SEs to remain functional; this gives rise to operational CC-SE complexes into which molecules are loaded and subsequently translocated, by bulk flow, to sink tissues (38, 70, 71).

CC: companion cell

SE: sieve element

STS: sieve tube system

PD: plasmodesma

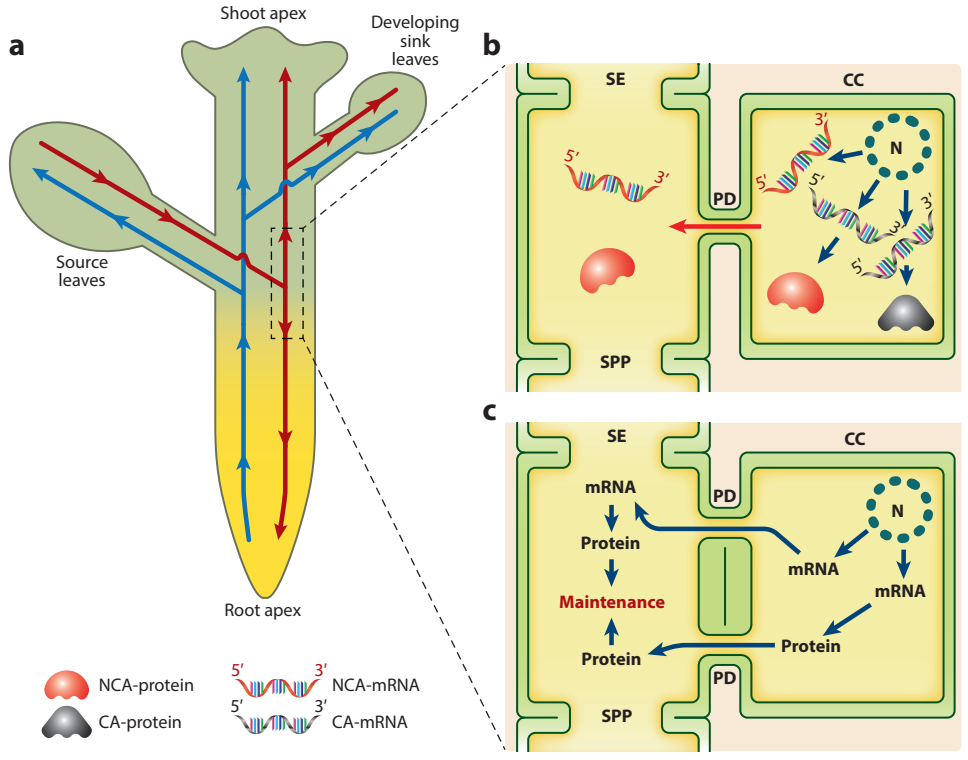


Figure 1

Entry of endogenous information molecules into the phloem. (*a*) Schematic illustration of the phloem sieve tube system (STS) (*red lines*) and xylem (*blue lines*). Mature source leaves deliver various plant resources (fixed carbon, amino acids, RNAs, proteins, etc.) to developing tissues, such as sink leaves and shoot and root apical meristems. Arrows indicate flow directions within the phloem and xylem. (*b*) Transport of non-cell-autonomous proteins (NCA-proteins) and mRNAs (NCA-mRNAs) from companion cells (CCs) into sieve elements (SEs) (*red arrow*). In CCs, transcription and translation of cell-autonomous mRNAs (CA-mRNAs) give rise to proteins that either are confined to CCs (CA-proteins) or move through the CC-SE plasmodesmata (PDs) into the SEs (NCA-proteins). Because mature SEs lack nuclei and hence are not capable of mRNA transcription, mRNAs present in SEs (NCA-mRNAs) come from adjacent CCs. (*c*) Maintenance of mature enucleate SEs, which occurs through provision of essential components for neighboring CCs (*blue arrows*). Proteins associated with SE maintenance are synthesized in CCs and then enter the SEs through PDs, whereas some mRNAs may well be transported into SEs, where they are then translated. Additional abbreviations: N, nucleus; SPP, sieve plate pore.

COMPOSITION OF ENDOGENOUS RNA IN THE PHLOEM SIEVE TUBE SYSTEM

The presence of endogenous mRNA in the angiosperm phloem STS has long been known (50); however, this RNA was generally considered a contaminant, derived from neighboring CCs and phloem parenchyma cells. Analysis of various phloem cell types using such techniques as laser capture microdissection (6, 27, 44, 63, 78) and aphid stylectomy (33) provided solid evidence in support of the notion that the enucleate STS contains a bona fide unique population of mRNAs. Parallel studies of phloem sap collected from naturally "hemophiliac" plants (plants whose phloem sap exudes upon wounding, such as cucurbits, castor bean, and lupin) (29, 35, 80, 87, 90, 117) or

from "nonhemophiliac" plants using an ethylenediaminetetraacetic acid (EDTA) treatment (27, 102) also provided evidence that the phloem STS contains several thousand mRNA molecules that could potentially serve as phloem-mobile systemic signaling agents.

Various plant species appear to contain similar numbers of phloem-mobile mRNAs, yet it would seem that each possesses its own unique mRNA population. Comparative analyses with graft-transmissible mRNA populations identified for *Arabidopsis*, grape, and cucurbits revealed that some 2,006, 3,333, and 3,546 mRNAs were identified as being mobile, respectively (102, 109, 117). However, only 38% of *Arabidopsis* and 33% of grape mobile mRNAs overlap with those identified in cucumber (117). Although, at first sight, this degree of overlap might seem low, it is necessary to take into consideration that these data were derived from plants that were grown under different conditions and were of dissimilar developmental ages.

A comparison of the vascular transcriptomes of cucumber and watermelon indicated that they were almost identical, whereas their phloem transcriptomes had an overlap of approximately 50% (35). These data indicate that, during the evolution of the plant vascular system, plants likely retained a common core of STS mRNAs whose functions are necessary for the effective operation of the phloem. The observed differences in species-specific STS-mRNA and phloem-mobile-mRNA pools may reflect adaptations to growth conditions and/or certain morphological traits, such as those in rosette plants (e.g., *Arabidopsis*) as opposed to annual and woody perennial vines (cucumber and grape, respectively). With the increasing availability of genomes from a broad range of plants, this notion could be tested using a combination of heterografted plants and transcriptomics analyses.

Quantitative analysis of the mRNA population in the phloem STS indicated that many transcripts are highly enriched relative to the surrounding vascular tissues (35, 117). Because mature, functional SEs lack nuclei and therefore are not capable of mRNA transcription, a model has been proposed in which mRNAs present in these cells are derived from their neighboring CCs; to this end, a selective mechanism would need to operate in order to traffic these mRNAs into the SEs (**Figure 1*b***).

Biological Processes Associated with mRNA Present in the Sieve Tube System

Gene Ontology analyses of mRNAs present in the phloem STSs of several plant species (35, 87, 117) have indicated that these transcripts are enriched in processes associated with cellular function, metabolism, development, signaling, and biotic and abiotic stress. Several studies have provided evidence that phloem transcripts function in systemic information networks for plant development. Graft transmission of tomato *Mouse ear* (*Me*) and *gibberellic acid insensitive* (*gai*) transcripts cause leaf morphological changes in wild-type scions (22, 39, 41, 48). Grafting assays have also established that transcripts for *Solanum tuberosum BEL5* (*StBEL5*), encoding a BEL1-like transcription factor, and *S. tuberosum POTATO HOMEOBOX 1* (*StPOTH1*), encoding a KNOX protein, move through the phloem into stolons, where their proteins then interact to mediate tuber formation (11, 64, 74). Here, both the 5′ and 3′ untranslated regions of *StBEL5* proved essential for efficient mRNA entry into and long-distance transport through the phloem (12). Specific *AUXIN/INDOLE-3-ACETIC ACID* (*AUX/IAA*) transcripts have also been proposed to be phloem mobile, and the graft transmissibility of *Arabidopsis* AUX/IAA18 mutant (*miaa18*) transcripts into wild-type roots correlated with defective lateral root formation (79, 80).

These findings offer strong support for a model in which the phloem STS plays a role in transporting RNAs, as information molecules, to sink tissues in order to modulate signaling cascades when plants are exposed to various physiological challenges. In this way, the phloem STS serves

as a unique space to integrate information from various developmental and abiotic- and biotic-stress-related signaling systems. It is also important to consider that the mRNA population within the phloem STS includes mRNAs that function either in local maintenance, physiological, and defense-related processes or in long-distance signaling.

RNP: ribonucleoprotein

RBP: RNA-binding protein

Enucleate Sieve Element Maintenance

Maintenance of the enucleate SEs involves provision of essential materials from their CCs. With respect to proteins, these could be synthesized within the CCs and trafficked into the SEs through the interconnecting PDs, or mRNA could be delivered through PDs for translation in the mature SEs (**Figure 1*c***). In this regard, it is important to note that protein-synthesis-related transcripts are present, at significant levels, in all data sets of phloem sap mRNAs (29, 35, 80, 102, 117). Furthermore, the phloem STS contains the machinery for protein synthesis at the protein level (41, 60, 87), and tRNAs have been detected in pumpkin phloem sap (113). However, efforts to reconstitute protein synthesis within the phloem sap have yet to prove successful (113). In any event, collectively, these findings support the hypothesis that a subpopulation of the mRNAs within the STS undergo translation, with the resultant proteins functioning either in local SE maintenance or, for example, as components of various long-distance signaling systems, such as proteins that assemble into ribonucleoprotein (RNP) complexes.

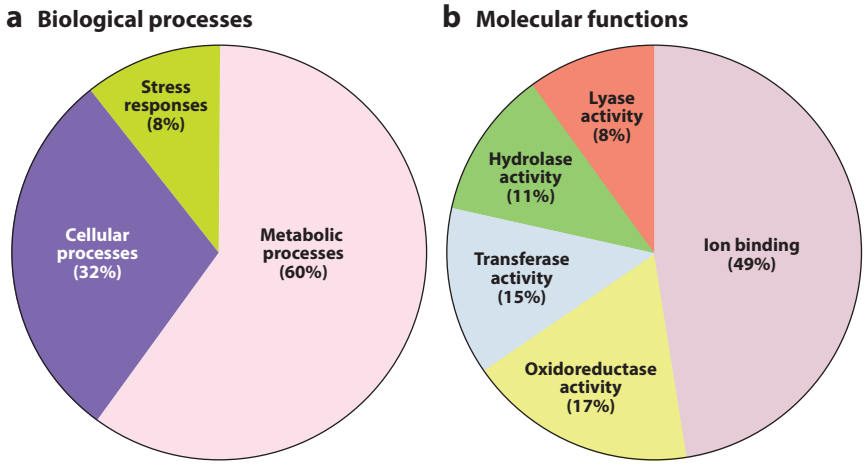
Consistent with this notion, analyses comparing phloem transcriptome and proteome data sets established that a significant number of both transcripts and their protein products are present in the STS (35, 41, 60, 87, 117). These proteins appear to be involved in metabolic, cellular, and stress-related processes (**Figure 2*a***); major molecular functions include ion binding and oxidoreductase, transferase, hydrolase, and lyase activities (**Figure 2*b***). With respect to metabolism, in several cases, transcripts, their enzymes, and the associated metabolites are present in the phloem STS (41, 117) (**Figure 2*c***). This suggests the operation of a signaling system between CCs and their SEs that controls metabolic pathways within the unique symplasmic domain of the phloem STS (41).

MODE OF RNA ENTRY AND TRANSPORT WITHIN THE SIEVE TUBE SYSTEM

In nature, all RNA molecules interact with proteins to form RNP complexes, and this protein-RNA association plays a pivotal role in determining the process and fate of RNAs in living cells. Thus, RNA-binding proteins (RBPs) serve as central components that affect various functional events through recognition, binding, and subsequent recruitment of additional components to assemble into RNP complexes (95). Because thousands of mRNAs have been identified in the phloem translocation stream, a major goal will be to identify the RBPs that function in mediating the trafficking of these RNAs into and out of the SEs along the STS.

Trafficking mRNA from Companion Cells into Sieve Elements

Trafficking of RNA between CCs and SEs occurs through cytoplasmic nano-channels within the PDs that interconnect these two cell types. *Cucurbita maxima* PHLOEM PROTEIN 16 (CmPP16) was the first identified non-cell-autonomous phloem RBP that, in a manner similar to viral movement proteins, binds mRNA in a non-sequence-specific manner, increases the PD size exclusion limit, and traffics cell to cell as an RNP complex (108). Molecular studies have



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Enzyme	Metabolic products
α-Galactosidase	Raffinose → Sucrose; Raffinose → Stachyose → Galactose
Serine acetyltransferase	Serine → *N*-Acetylserine
Tryptophan synthase	Serine → Tryptophan
S-Adenosylmethionine synthase	*S*-Acetylmethionine → Methionine

Figure 2

Examples of the range of both mRNAs and their protein products that are present in the phloem sieve tube system (STS). (*a*,*b*) Analysis of the 719 graft-transmissible mRNAs contained within the cucumber group IV data set (117) and a cucumber phloem proteome database (41), which established the presence of 133 encoded proteins that cover a range of biological processes (panel *a*) and molecular functions (panel *b*). (*c*) An example of mRNAs, their enzymes, and associated metabolic compounds that have been detected in the cucumber phloem STS.

identified a 36-amino-acid motif as being necessary and sufficient for CmPP16 cell-to-cell movement through PDs (100). In addition, this protein interacts with a PD regulatory component, NON-CELL-AUTONOMOUS PATHWAY PROTEIN 1 (NCAPP1) (54), and phosphorylation and glycosylation of recognition motifs on both proteins are necessary to permit CmPP16 entry into the PD cell-to-cell transport pathway (100).

Protein interaction assays showed that NCAPP1 binds to approximately 30% of the proteins contained in pumpkin phloem sap and that this interaction requires the same posttranslational modifications; thus, this pathway likely plays an important role in regulating protein entry into the STS. Consistent with this notion, microinjection assays conducted with size-fractionated pumpkin phloem sap proteins established that many such proteins are capable of interacting with PDs to increase the size exclusion limit and mediate their cell-to-cell movement (10). Furthermore, proteomic analyses of phloem sap have shown that more than 10% of total phloem sap proteins are characterized as RBPs (41, 60, 87). These phloem RBPs presumably function in loading, unloading, or long-distance transport of RNAs within the phloem STS. Future studies that focus on characterizing these phloem RBPs will offer further insight into the molecular mechanisms involved in such RNA-trafficking events.

RNA-Binding-Protein-Mediated mRNA Transport Through the Phloem Sieve Tube System

PTB protein: polypyrimidine-tract-binding protein

Pumpkin has two isoforms of CmPP16 (CmPP16-1 and CmPP16-2), both of which can move long distances through the phloem translocation stream (108). However, this movement is complex in nature. An elegant study, based on introducing tracer-labeled forms of these two proteins into the phloem STS, revealed that efficient rootward movement of CmPP16-1, but not of CmPP16-2, occurs when it is complexed with two other phloem proteins, namely eukaryotic translation initiation factor 5A (eIF5A) and translationally controlled tumor-associated protein (TCTP); shootward movement of both CmPP16-1 and CmPP16-2, by contrast, is not dependent on the presence of these proteins (4). This pivotal study provided evidence for destination-selective long-distance delivery of phloem proteins and clearly revealed that the nature of the proteins that make up a specific RNP complex can contribute to the targeting of bound mRNA to a specific tissue.

The most well characterized phloem RBP to date is *C. maxima* RNA-BINDING PROTEIN 50 (CmRBP50), a polypyrimidine-tract-binding (PTB) protein that is present at high levels in the STS (36). Coimmunoprecipitation assays identified phloem proteins and mRNAs associated with this RNP complex. One of the five bound mRNAs was *C. maxima GIBBERELLIC ACID–INSENSITIVE PHLOEM* (*CmGAIP*), a well-known phloem-mobile mRNA (39, 88), and binding specificity between CmRBP50 and the *CmGAIP* mRNA is conferred by PTB motifs. Here, it is interesting to note that the sequence motifs required for *CmGAIP* mRNA entry into phloem differ from those for its recognition by CmRBP50 (42). This suggests that assembly of each CmRBP50-based RNP complex most likely occurs in SEs.

Proteins comprising this CmRBP50 RNP complex were identified by coimmunoprecipitation assays using phloem sap, and assembly was shown to be dependent on phosphorylation of four CmRBP50 C-terminally located serine residues (36) (**Figure 3*a***). In vitro reconstitution experiments demonstrated that sequential protein binding to CmRBP50 confers both enhanced RNP complex stability and affinity for the cargo mRNA (57). Heterografting assays also confirmed the presence of a fully intact CmRBP50 RNP complex in scion phloem sap, consistent with the notion that this RNP complex functions in the long-distance delivery of its bound mRNAs (36) (**Figure 3*b***).

A recent study of two putative orthologs of CmRBP50, *S. tuberosum* PTB1 (StPTB1) and StPTB6, established their capacity to bind the phloem-mobile *StBEL5* mRNA through 3′-untranslated-region pyrimidine-rich sequences (24). As with *CmRBP50*, the *StPTB1* promoter was active in petiole and stem CCs. Furthermore, this study established a strong correlation between StPTB1 and StPTB6 abundance, *StBEL5* transcript stability and long-distance transport, and tuber yield. This work suggests that a conserved mRNA transport mechanism may well operate in the phloem STSs of cucurbits and potato.

The phloem lectin PHLOEM PROTEIN 2 (PP2) is an abundant protein in the cucurbit phloem sap (41, 60) and has been reported to function as a viroid RBP (34). Similarly to CmPP16, PP2 binds mRNA in a non-sequence-specific manner; increases the PD size exclusion limit; moves long distances through the phloem STS; and, based on heterografting studies, is involved in delivery of viroid RNA from the cucumber stock to a nonhost pumpkin scion (34). These findings raise the possibility that PP2 might well function in shuttling mRNAs from the CC to the SE.

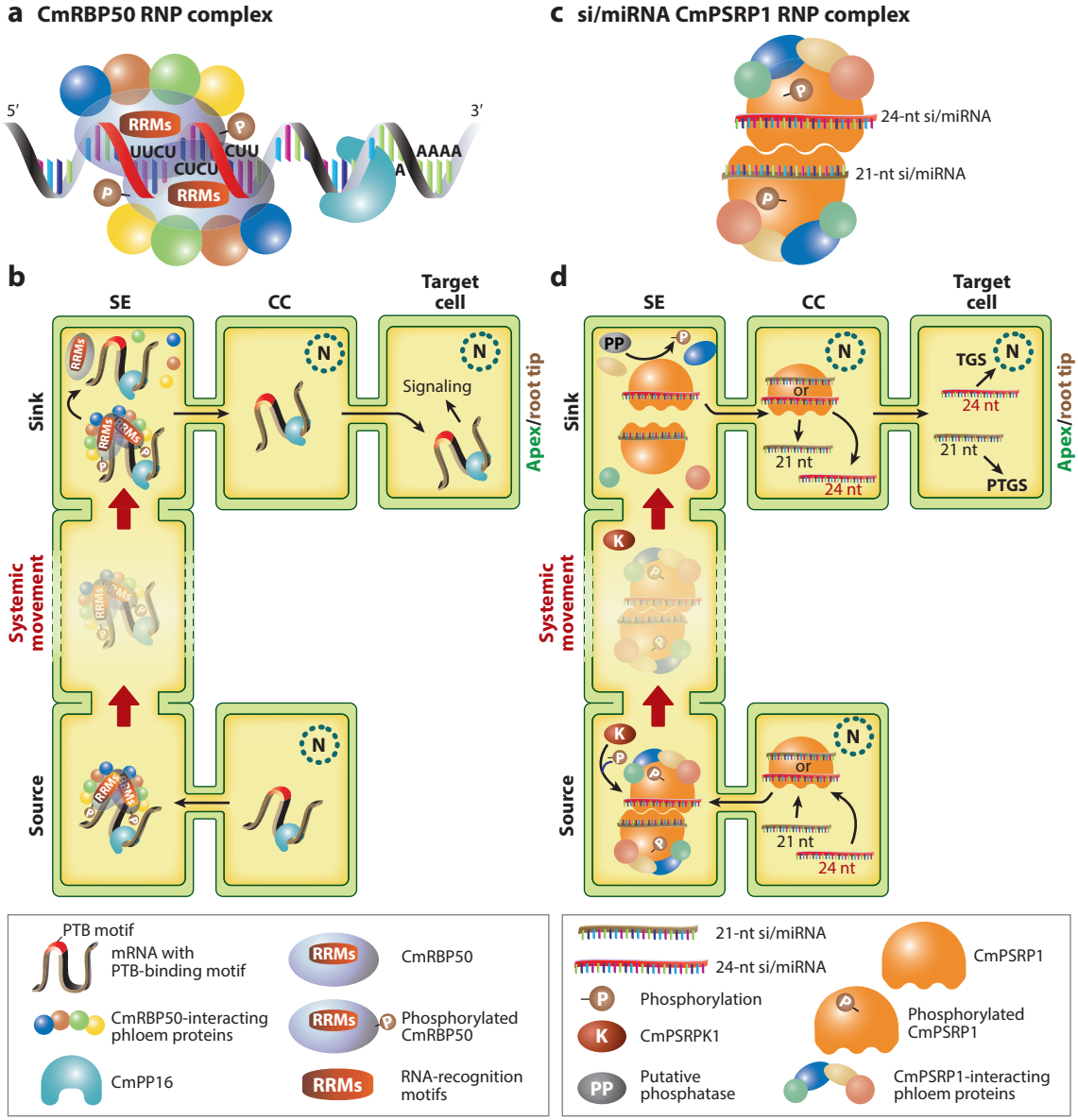
An intriguing aspect of the proteins identified within the CmPP16- and CmRBP50-based protein complexes was the common presence of CmeIF5A. This protein is highly conserved between eukaryotic and prokaryotic organisms and interacts with ribosomal proteins and eukaryotic elongation factor 2 (eEF2) to function in the elongation step of protein translation (91). Interestingly, eIF5A interacts with eEF2 in pumpkin phloem sap (73), raising the possibility that some of

the mRNAs, transported over long distances through the phloem by these RNP complexes, may undergo translation during their journey.

Mechanism for Phloem Loading and Unloading of si/miRNAs

sRNA: small RNA

Small RNA (sRNA)–mediated gene silencing is a conserved mechanism of gene regulation in eukaryotes at the transcriptional and posttranscriptional levels and is involved in a broad range of biological processes. With respect to a systemic function of these sRNAs, a wide range of experiments have established that a gene-silencing-related signal moves through the vascular



system (56, 81, 103, 104). Analyses of RNA extracted from phloem sap provided direct evidence that sRNA species belonging to both small interfering RNA (siRNA) and microRNA (miRNA) gene-silencing pathways are present in the phloem STS (18, 37, 111, 117).

siRNA: small interfering RNA

miRNA: microRNA

Biochemical studies identified a protein in the phloem sap of pumpkin, cucumber, and lupin that is able to bind sRNAs (111). This protein from the pumpkin STS, designated *C. maxima* PHLOEM SMALL-RNA BINDING PROTEIN 1 (CmPSRP1), binds preferentially to single-stranded sRNA but not to double-stranded sRNA (111). CmPSRP1 has the properties of an endogenous movement protein, in that it can increase the PD size exclusion limit and can traffic from cell to cell while carrying single-stranded sRNA (but not double-stranded sRNA or mRNA). Similarly to CmPP16 and CmRBP50, C-terminal serine residues are in vivo phosphorylated and play a critical role in forming RNP complexes with other phloem proteins (37). As CmPSRP1 phosphorylation is not essential for its trafficking into SEs (37, 111), it likely traffics on a different pathway than NCAPP1 does, but this remains to be established.

A combination of gel filtration, coimmunoprecipitation, and sRNA studies indicated that, in the pumpkin STS, the CmPSRP1 RNP complex is present as a homodimer, with a molecular weight of ~350 kDa and 24-nt sRNA as the predominant bound form of sRNA (37) (**Figure 3*c***). Given that the size exclusion limit of CC-SE PDs is on the order of 40–60 kDa (96), this CmPSRP1 RNP complex likely assembles in SEs (37). Consistent with this notion, a protein in the phloem sap, *C. maxima* PHLOEM SMALL-RNA BINDING PROTEIN KINASE 1 (CmPSRPK1), was identified that uniquely and specifically phosphorylates CmPSRP1. Importantly, cucumber orthologs of CmPSRPK1 are present in phloem sap collected from along the translocation pathway (41), providing support for the hypothesis that CmPSRPK1 maintains CmPSRP1 phosphorylation during long-distance transport of the CmPSRP1 RNP complex (**Figure 3*d***).

In sink organs, delivery of bound sRNA to the target tissue(s) would be unlikely to involve PD trafficking of the assembled CmPSRP1 RNP complex. Insight into how this postphloem movement of sRNA might occur was provided by stability studies in which CmPSRP1 RNP complexes were loaded with radiolabeled sRNA. These RNP complexes were then incubated with phloem sap collected from source and sink regions of the plant. Only exposure to sink phloem sap destabilized the CmPSRP1 RNP complex, resulting in its dissociation and release

Figure 3

Schematic models illustrating the proteins contained within the *Cucurbita maxima* RNA-BINDING PROTEIN 50 (CmRBP50) (36) and small interfering RNA/microRNA (si/miRNA) *C. maxima* PHLOEM SMALL-RNA BINDING PROTEIN 1 (CmPSRP1) (37) ribonucleoprotein (RNP) complexes, along with the steps of the associated phloem-based RNA signaling pathways. (*a*) The structure of the CmRBP50 RNP complex. CmRBP50 RNA-recognition motifs (RRMs) bind polypyrimidine-tract-binding (PTB) sequences in mRNAs (*red ribbons with sequences*). Phosphorylation of CmRBP50 is essential for assembly of the RNP complex. A second RBP, *C. maxima* PHLOEM PROTEIN 16 (CmPP16), binds outside of the PTB domain. (*b*) Schematic illustrating the steps involved in phloem transport of mRNAs mediated by homodimeric CmRBP50 RNP complexes. CmPP16 binds to non-cell-autonomous mRNAs in the companion cells (CCs) to mediate their plasmodesmal trafficking into the sieve element (SE). Phosphorylated CmRBP50 then binds to PTB-containing mRNAs, which is followed by assembly of stabilized CmRBP50-based RNP complexes for translocation into sink tissues (apex/root tip). Disassembly of the RNP complex is followed by CmPP16-mediated export of mRNA into target cells. (*c*) The structure of the si/miRNA CmPSRP1 RNP complex. CmPSRP1 binds 21/24-nucleotide (nt) single-stranded si/miRNAs. As with CmRBP50, phosphorylated CmPSRP1 recruits phloem proteins to form homodimeric RNP complexes. (*d*) Schematic illustrating the steps involved in phloem transport of si/miRNAs mediated by CmPSRP1 complexes. CmPSRP1 binds 21/24-nt si/miRNA in CCs and mediates their entry into the sieve tube system (STS). In the STS, a specific kinase, *C. maxima* PHLOEM SMALL-RNA BINDING PROTEIN KINASE 1 (CmPSRPK1), phosphorylates CmPSRP1, which is followed by assembly of stabilized RNP complexes; CmPSRPK1 maintains the phosphorylation status of CmPSRP1 during translocation to sink tissues. In the target sink tissue, CmPSRP1 is dephosphorylated by a phloem-located phosphatase (37), with subsequent dissociation of the CmPSRP1 RNP complex, followed by delivery of cargo si/miRNA into target cells for posttranscriptional gene silencing (PTGS) or transcriptional gene silencing (TGS). Additional abbreviation: N, nucleus.

P_i: inorganic phosphate

of the labeled sRNA (37). Proteomics studies have identified a wide range of phosphatases in phloem sap (41, 60), and it will be interesting in future studies to characterize the phosphatase or phosphatases that dephosphorylate CmPSRP1 for RNP complex disassembly. Here, we assume that, once released from the RNP complex, CmPSRP1 would then traffic through the CC-SE PDs to deliver its cargo sRNA for posttranscriptional or transcriptional gene silencing of the target loci or transcript (**Figure 3*d***).

Because PSRP1 homologs have yet to be identified in other plant genomes (37), it is possible that CmPSRP1-mediated systemic sRNA delivery may be specific to pumpkin and cucumber. A recent study using sRNA-affinity chromatography identified additional sRNA-binding proteins in the phloem sap from pumpkin, cucumber, and watermelon, and homologs were identified in other plant genomes, including those of *Arabidopsis*, tomato, and rice (B.-K. Ham & W.J. Lucas, unpublished results). The characterization of these proteins may well provide insight into why mutants defective in systemic gene silencing have yet to be identified.

FUNCTIONS OF PHLOEM-MOBILE RNA IN NUTRIENT SIGNALING

Plants have evolved complex signaling networks to regulate nutrient homeostasis at the cellular, tissue, and whole-plant levels, and the vascular system plays a central role in this process. Split-root experiments, in which the root system of an individual plant is separated into two media chambers containing different nutrient levels, have provided irrefutable evidence that signaling agents are transported from root to shoot and from shoot to root to achieve systemic regulation of nutrient homeostasis (19, 20, 30, 31, 62, 66, 67, 89, 101). By this system, the root responds to local differences in soil mineral nutrient status to generate and transmit signals through the xylem transpiration stream to the shoot. The shoot system perceives these root-derived signals and then generates response signals that are delivered through the phloem STS to the root and developing vegetative apices in order to coordinate the processes for nutrient uptake and plant development (17, 68–70, 116).

Regulation of the Phosphate Signaling Cascade by Phloem-Mobile miRNAs

Phosphate is an essential macronutrient, and as such, it has been the subject of intense investigation from the perspective of both uptake from the soil and homeostasis within the body of the plant (23, 116). A pivotal role for the phloem in regulating both processes has been established through studies of the regulatory role of the phloem-mobile miRNA miR399 in the activity of PHOSPHATE 2 (PHO2) in roots.

Expression of miR399 occurs predominantly in the vascular tissue (7, 9, 117) and has been detected in the phloem STS (16, 82, 83, 117), with its level increasing under conditions of inorganic phosphate (P_i) deficiency (18, 82, 83, 117). Proof of systemic action was provided by grafting studies in which scions overexpressing miR399 were grafted onto wild-type *Arabidopsis* rootstocks. High levels of miR399 accumulated in the wild-type roots, where it targeted the 5′ untranslated region of *PHO2* to suppress the level of this ubiquitin-conjugating E2 enzyme that acts as a negative regulator of P_i transport (2, 32, 61, 82, 97). Experiments in which wild-type *Arabidopsis* scions were grafted onto *pho2* rootstocks resulted in overaccumulation of P_i in scion leaves (13), consistent with the notion that PHO2 downregulates P_i acquisition by the root.

These pioneering studies established that miR399 serves as an important shoot-to-root signal for controlling root expression of *PHO2* and that this control may contribute to P_i homeostasis. Furthermore, because antagonistic expression patterns between miR399 and *PHO2* have been observed in various plant species (51), miR399-*PHO2*-mediated P_i homeostasis appears to be

an evolutionarily conserved pathway in plants. However, promoter–β-glucuronidase (GUS) and time-course analyses have shown that miR399 is expressed in shoots under P_i-sufficient conditions (9), but under P_i stress, upregulation of miR399 expression can occur in both shoots and roots (61). This raises the question of the role of phloem-mediated miR399 translocation in P_i-stress signaling. Studies performed with cucumber showed that the level of miR399 in the phloem STS increased significantly during the early (12–24-h) stage of the P_i-stress response (117). Thus, phloem delivery of miR399 may be especially important during the early stage of information exchange between the root and shoot systems of the P_i-stressed plant.

Deep sequencing and microarray analyses have identified additional P_i-stress-responsive miRNAs (40, 62, 72, 83). In *Arabidopsis*, miR827 targets *NITROGEN LIMITATION ADAPTATION* (*NLA*) transcripts, whereas in rice, the target is *Oryza sativa SYG1/Pho81/XPR1–MAJOR FACILITATOR SUPERFAMILY 1* (*OsSPX-MFS1*) and *OsSPX-MFS2* (also called *OsNLA1* and *-2*, respectively) (40, 62, 83, 85, 94). The *Arabidopsis NLA* encodes a ubiquitin E3 ligase with SPX and RING domains and mediates degradation of PHOSPHATE TRASPORTER 1 (PHT1) proteins (62, 65). The predicted target genes of miR2111 encode an E3 ligase and a calcineurin-like phosphoesterase (40, 83). Evidence is lacking regarding the involvement of miR827 and miR2111 in systemic regulation under P_i-stress conditions; future studies need to critically address this aspect.

Tissue-Specific Delivery of Phloem-Mobile mRNAs Under Phosphate Stress

A recent comprehensive study of the early P_i-stress response in cucumber offered important insights into the dynamic nature of the mRNA population moving within the phloem STS (117). Heterografted cucumber and watermelon plants were first grown under a P_i-sufficient condition (control) and then given a P_i-stress treatment. Transcriptomic analyses of watermelon sink tissues (apex, developing leaves, and developing roots) revealed that several thousand cucumber mRNAs were graft transmissible. Importantly, these cucumber transcripts were detected in the mRNA extracted from grafted watermelon sink tissues, and their presence therefore cannot be attributed to wound-induced contamination of the phloem at the site of tissue sampling. Furthermore, because the distance of the sampling sites from the graft union was generally 30 cm and the tissues were excised 24 h after the P_i stress was applied, it would be expected that these cucumber transcripts were delivered through the phloem STS. When small transport distances are combined with long treatment times, one cannot discount a contribution from PD-mediated cell-to-cell trafficking of mRNA along the plant axis (58).

A remarkable finding from this study was that, under P_i-sufficient (control) conditions, discrete populations of cucumber phloem-mobile mRNAs were being delivered to the watermelon apex, developing leaves, or developing roots (117). Upon exposure to P_i-stress conditions, these mRNA populations underwent dramatic changes; only a few graft-transmissible mRNAs were held in common for each tissue sampled from control and P_i-stressed plants. This feature of selective transport of mobile mRNAs into specific recipient sink tissues is consistent with destination-selective delivery of CmPP16-1 to roots (4) and phloem-mediated trafficking of *GAIP* mRNA, which plays an important role in tomato leaf development but not fruit development (39).

The findings of Zhang et al. (117) raise several important questions. First, how might the phloem STS distinguish between general (control) and P_i-stress-related signaling mRNAs? Second, what mechanism imparts specificity in terms of mobile-RNA delivery to its target sink? As discussed above, various mechanisms appear to operate in order to allow selective trafficking of mRNA into the SE for long-distance transport through the STS. Once in the SE, sequence elements or structural motifs in each mRNA are thought to allow recognition and binding by one or more specific RBPs that, in a manner similar to the CmRBP50 RNP complex, then proceed

to assemble into a multiprotein RNP complex (**Figure 3*a***). The presence in the SE of proteins that form these RNP complexes could be governed by both endogenous and environmental inputs at the level of transcription, translation, and recognition or trafficking through CC-SE PDs. Through such a mechanism, the phloem STS could well contain subgroups of RNP complexes whose bound transcripts are associated with general physiological or developmental processes (control conditions), abiotic stress, pathogen defense, and so forth.

Given that the phloem translocation stream moves through the STS by bulk flow, delivery rates for RNP complexes could be adjusted through the influence of sink metabolism on the overall pressure gradient between the source region and a particular sink. However, sink strength would not be able to exert control over which RNP complexes enter the STS of, for example, a developing sink leaf. A more plausible mechanism for the observed selective delivery of transcripts to specific sinks might involve a surveillance system that operates within the sink region of the STS to recognize appropriate as opposed to mislocated RNP complexes. This may be achieved by recognition of bound peripheral proteins on mislocated RNP complexes (4), which could target them for disassembly and protein turnover using the ubiquitin–26S proteasome system located in the STS (60).

Systemic Communication in Nitrogen Signaling for Homeostasis

Nitrogen (N) is another macronutrient that is essential for plant growth and metabolism. Similar to P_i-stress signaling, vasculature-mediated long-distance signaling systems are important for establishing N homeostasis in plants (67). In this regard, it has been suggested that peptides could well function in both xylem- and phloem-mediated N-stress signaling systems. The C-TERMINALLY ENCODED PEPTIDEs (CEPs), identified by in silico screening, are produced in the stele of the root under N-stress conditions. These CEPs then move through the apoplasm to enter the xylem transpiration stream for delivery to source leaves in the shoot, where they are recognized by CEP receptors that are members of the leucine-rich-repeat receptor kinase family (98). Similarly to CEPs, rhizobium-induced CLAVATA 3/ENDOSPERM SURROUNDING REGION (ESR)–RELATED (CLE) peptides appear to function as root-derived signals and are recognized in the shoot by a *Glycine max* NODULE AUTOREGULATION RECEPTOR KINASE (GmNARK), a soybean CLAVATA 1–like protein (5, 93). These receptor kinases would then activate signal cascades, resulting in shoot-to-root signaling through the phloem to coordinate N availability in the soil or through N fixation in root nodules, with N requirements in the shoot.

Information is lacking on RNA-mediated shoot-to-root signaling under N-stress conditions. However, recent studies have established that a range of mRNAs are mobile under N-limiting conditions (102) and that phloem sap contains N-stress-responsive miRNAs (18, 59, 84, 117). These findings are consistent with the notion that phloem-mediated RNA signaling plays a role in N homeostasis. In addition, a deep sequencing analysis of *Arabidopsis* seedlings exposed to N-starvation conditions identified a set of N-stress-responsive miRNAs, including miR399, miR827, and miR2111, that are also involved in the P_i-stress response (59). Thus, the possibility exists that miRNA-mediated P_i- and N-stress signals act in concert to form an integrated local and systemic signaling network.

Role of miR395 in Sulfate Systemic Signaling

Sulfur (S) is an essential nutrient in plants (55). Under S-deficiency conditions, sulfate uptake and the activity of ATP sulfurylase (APS) (the first enzyme in the sulfate assimilation pathway) are enhanced, and split-root experiments have indicated that one or more phloem-translocated signals

modulate *APS* expression in the root system (52). In this regard, computational and experimental studies have identified miR395 as a potential regulator for *APS* expression (43, 45). Grafting studies, using a combination of wild-type *Arabidopsis* (scions) and *hen1-1* (rootstocks) in which the miR395 level was dramatically decreased, provided evidence that scion-derived miR395 can move through the graft union into the root, resulting in downregulation of a member of the *APS* gene family (17). Because miR395 is present in *Brassica napus* and cucumber phloem sap (18, 117) and is graft transmissible in *Arabidopsis*, it could well act as a phloem-mobile signaling agent in S homeostasis (17). The caveat here is that, although miR395 expression is confined primarily to phloem CCs, S-deficiency conditions result in its upregulation in both leaves and roots (45). Thus, additional studies are needed to further elucidate the role of miR395 in the systemic regulation of S metabolism.

SYSTEMIC EPIGENETIC CONTROL OF GENE EXPRESSION AND GENOME STABILITY

sRNA molecules are essential controllers of gene silencing and genome defense in both animals and plants. Excellent reviews are available on sRNA biogenesis (8, 15, 16) and on the mechanisms underlying posttranscriptional and transcriptional gene silencing by means of 21- and 24-nt sRNAs, respectively (15, 75). Here, we discuss the role of the phloem STS in mediating systemic epigenetic control over gene expression and genome stability.

Early plant studies on gene silencing established that a sequence-specific signal is able to move across a graft union, without the need for signal amplification, to induce silencing in recipient scion (sink) tissues (81, 104). The sequence-specific nature of this silencing implicated the movement of nucleic acids. Movement through the phloem was supported by the observation that the spread of gene silencing follows the source-to-sink pattern of the translocation stream and that of virus infection (69).

Insight into the actual nature of this silencing signal was afforded by studies performed using transgenic squash lines expressing a viral capsid protein. Analysis of phloem sap collected from a spontaneously silencing line identified capsid-protein-specific 21-nt siRNAs (111). These siRNAs were graft transmissible and induced systemic transgene silencing in grafted scions. Parallel studies performed on wild-type pumpkin showed that the phloem sap of this species contained sRNAs in the 21- and 24-nt size classes. The 21-nt sRNA population was enhanced in virus-infected plants, consistent with their role in the systemic response of these plants to viral challenge (104, 111).

The 24-nt sRNAs represent the dominant size class in the phloem STS of healthy plants (18, 37, 111). The role of endogenous phloem-mobile 24-nt sRNAs has been probed through grafting experiments using a combination of wild-type *Arabidopsis* (Col and C24 genotypes) and mutant lines defective in 24-nt biogenesis (77). Here, a homograft between a Col-*dcl2*,*3*,*4* scion and Col-*dcl2*,*3*,*4* rootstock [in which 24-nt sRNA biogenesis is defective owing to the loss of DICER-LIKE 3 (DCL3)] established the absence of 24-nt sRNAs in sampled roots. By contrast, analysis of roots from a C24 (scion):Col-*dcl2*,*3*,*4* (rootstock) heterograft detected 24-nt sRNAs, and this size class represented the major population of sRNAs.

Importantly, grafting C24-*sde-1* scions (lacking the functional RNA polymerase IV required for 24-nt sRNA biogenesis) onto either C24 or Col wild-type rootstocks resulted in a very low level of 24-nt sRNAs in these roots. These findings provided strong support for the hypothesis that the source region represents the major site in the plant for endogenous 24-nt sRNA production and, further, that these 24-nt sRNAs move as the dominant species through the phloem into the root (77). Sequence analysis of graft-transmissible 24-nt sRNAs established that the great majority were associated with transposable elements (TEs) and methylated DNA; these studies established

that phloem-mobile 24-nt sRNAs target sequence-specific loci within sink meristematic tissues for transcriptional gene silencing through the RNA-directed DNA methylation pathway (76, 77).

In many plants, a large proportion of the genome is made up of TEs, which, when active, can relocate and thereby cause changes in gene expression (25). Because TE activity could have a serious impact on genome stability, the presence in the phloem STS of sRNA directed against specific TEs (111), in conjunction with their capacity for graft transmissibility and transcriptional gene silencing of target TE loci, suggests an important role in maintaining genome stability by restricting TE activity in meristematic tissues. Transcriptomics and genome-wide methylation analyses conducted on Col, C24, and Col-*dcl2,3,4 Arabidopsis* root tissues obtained from various grafting combinations offered strong support for this model (56). This study revealed that the systemic delivery of 24-nt sRNAs led to the targeting of thousands of loci, many of which were retroelement-superfamily TEs located in euchromatic regions of the *Arabidopsis* genome.

An intriguing aspect of these studies of *Arabidopsis* was that, although thousands of loci were targeted by these phloem-mobile sRNAs, the expression of only a very small number of genes changed (77). This result may reflect that *Arabidopsis* has a small genome and would thus have fewer TEs, whose activity may also be low, which would be consistent with the finding that, in *Arabidopsis*, loss of function in RNA-directed DNA methylation pathway genes has a limited effect on transposon activity and gene expression (75). Thus, for plants with larger genomes and, hence, expanded TEs (28, 106), phloem delivery of sRNAs may well have more profound roles in modifying gene expression as a result of TE silencing.

Grafting has long been utilized as an important tool in agriculture, yet much remains to be established in terms of the molecular basis for positive (or negative) traits afforded through specific heterograft combinations (92, 105). In this regard, Lewsey et al. (56) showed that unique patterns of methylation in the C24 genome, termed epialleles, could be detected in the grafted Col genome and, furthermore, that these naturally unmethylated target genes in Col encode various enzymes. This important finding suggests that heterografting could alter expression patterns in crop plants, thereby affecting traits important for food quality or yield potential. Heterografting assays performed using tomato, eggplant, and pepper supported this notion by demonstrating extensive changes in DNA methylation patterns (107).

There seems to be little doubt that the source region of plants serves as an important site for the generation of TE-specific 24-nt sRNAs. This location seems plausible, considering that TE transposition can be influenced by environmental inputs (14). Thus, this location would allow 24-nt TE-derived sRNAs to enter the phloem for delivery to shoot and root meristems. Such a system would provide a systemic genome-wide defense against those TEs that are activated by specific environmental or growth conditions. Finally, because sRNA is delivered to developing floral organs (114), this system would have the potential to provide an epigenetic transgenerational memory in seeds.

RNA EXCHANGE BETWEEN PARASITIC PLANTS AND THEIR HOSTS

Several plant families have evolved the ability to parasitize various host plants. Through the establishment of vascular connections, these parasites can acquire water and nutrients from their hosts. Multiple studies have reported that, in addition to nutrients, parasitic plants can receive a wide range of RNA species from their hosts, delivered through connections between the phloem systems of the parasites and their hosts (26, 46, 47, 53, 88, 102).

In agriculture, parasitic plants can cause significant reductions in crop yield and seed quality. The finding that host RNA can be delivered into the parasitic plant presented a novel strategy for controlling parasitism. For example, the growth and development of parasitic broomrape

(*Orobanche aegyptiaca*) on its host plant requires broomrape mannose 6-phosphate reductase, a key enzyme involved in mannitol accumulation. When transgenic tomato plants expressing an engineered sRNA to target the broomrape mannose 6-phosphate reductase were used as hosts for broomrape, this key enzyme level was reduced compared with wild-type tomato plants, as was the development of this parasitic plant (3, 110).

A similar strategy was tested for controlling dodder (*Cuscuta pentagona*) parasitizing tobacco. In this plant parasite, dodder *KNOTTED 1*–like genes are involved in haustorium formation with its host plant. Transgenic tobacco expressing siRNA directed against these key genes blocked the ability of dodder to form haustorial connections with the host plants (1).

At a functional level, little is known concerning the roles played by the host RNAs that are delivered through the phloem to the plant parasite. Similarly, the exchange of RNAs between a parasite and its host—in terms of both the pathway involved and the influence of these transcripts on host defense, control over resource allocation, and so forth—remains to be explored. These studies could provide important insights into the evolutionary events underlying the emergence of parasitism in various plant families. Lastly, such studies have potential for developing effective strategies to control yield losses associated with parasitism of important agricultural crops.

BIDIRECTIONAL RNA MOVEMENT IN PLANTS

Systemic gene-silencing studies have utilized transgenic plants expressing a silencing construct [e.g., a green fluorescent protein (GFP) hairpin sequence (hpGFP RNA)] to deliver siRNA into recipient (GFP-expressing) tissues. For plants such as tobacco, stem-grafting assays could be performed using an hpGFP RNA stock plant (containing the root system and mature source leaves as the site for generating the GFP siRNA) and a scion (generally vegetative sink tissues expressing GFP). Here, movement of the silencing signal followed a pathway from source to sink tissues, equivalent to that of phloem delivery of photosynthate. However, for equivalent studies based on *Arabidopsis*, a rosette plant of small stature, hypocotyl grafts must necessarily be made at the seedling stage. In these assays, efficient silencing signals moved from the scion (the source region of the plant) to the rootstock (the hypocotyl and root system), presumably through the phloem (77).

In an elegant study that used an inducible promoter to drive hpGFP RNA in the *Arabidopsis* rootstock, Liang et al. (58) showed that slow but effective root-to-shoot spread of silencing can occur by cell-to-cell trafficking of siRNA through vascular and cortical PDs. The efficiency of this movement was due in large part to the close proximity of the apical meristem to silencing signals generated in the hypocotyl. In this way, silencing can spread throughout the plant and, depending on the timing of siRNA induction, can penetrate to inflorescence meristems (58, 114). Taken together, these findings revealed a capacity for long-distance shoot-to-root and root-to-shoot movement of siRNA through the phloem and cell-to-cell through PDs, respectively, indicating that siRNA can move in a bidirectional manner in plants.

Equivalent grafting studies using two evolutionarily diverse *Arabidopsis* ecotypes similarly revealed that a large population of several thousand mRNAs can cross the hypocotyl graft union from shoot to root and vice versa (102). The major difference here is that, in contrast to the silencing studies, in which GFP expression in the scion was necessary for silencing to propagate from the root to the shoot, no such requirement was necessary for long-distance movement of endogenous mRNA. This would not be expected for phloem delivery of mRNA, but it raises an important question as to how mRNAs generated in the root can move across so many cell boundaries.

The steep gradient in transcripts detected along the *Arabidopsis* axis likely reflects the effect of PD trafficking of mRNA through files of cells, with commensurate lowering of transcript

concentration with increasing distance. In this regard, the ability of an mRNA to move from root to shoot may be controlled in part by its abundance within the hypocotyl (21). As in the case of P_i-stress signaling (117), subpopulations of mRNA were detected in specific organs, such as rosette leaves, the lower and upper stem, and flowers (102), consistent with destination-selective trafficking. Taken together, these findings support the hypothesis that, for small rosette-type plants such as *Arabidopsis*, bidirectional movement of mRNAs, involving phloem-mediated shoot-to-root and cell-to-cell-based root-to-shoot transport systems, can play a role in orchestrating developmental and physiological processes at the whole-plant level.

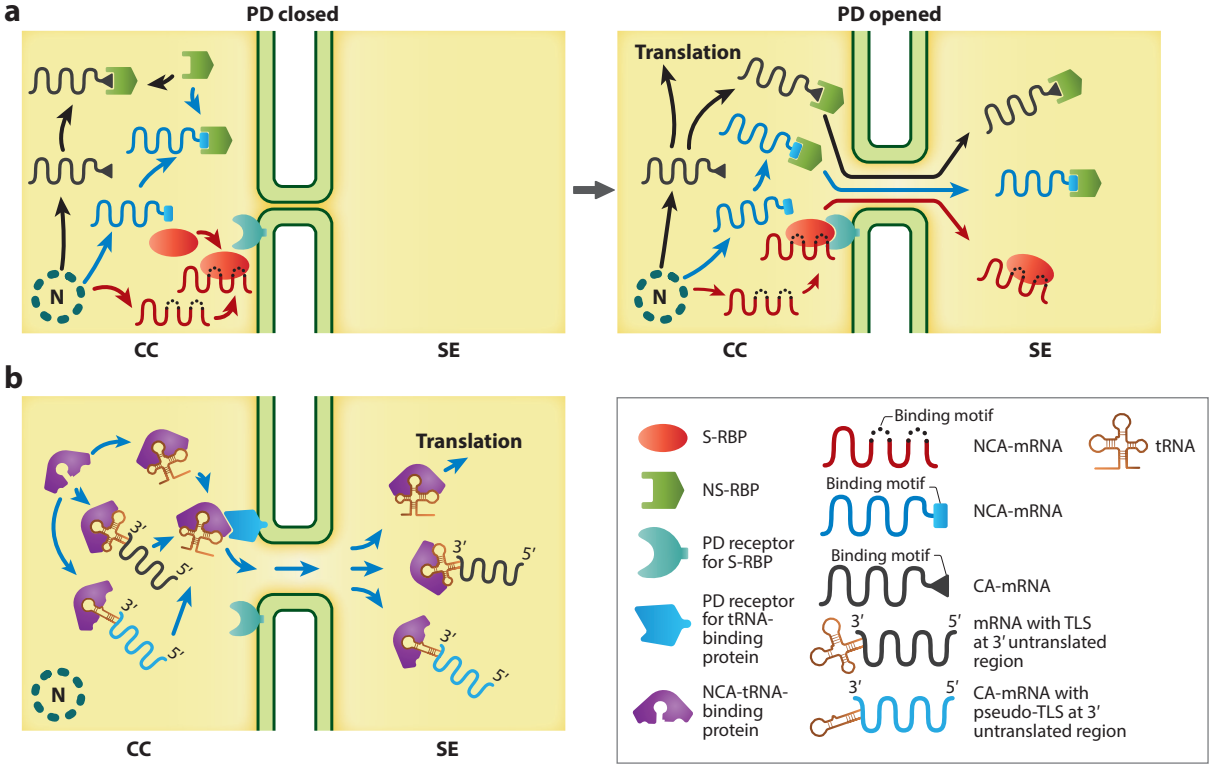


Figure 4

Models illustrating potential mechanisms by which thousands of mRNAs could gain entry into the phloem sieve tube system (STS). (*a*) Model for transcription in companion cells (CCs) generating both cell-autonomous mRNAs (CA-mRNAs) and non-cell-autonomous mRNAs (NCA-mRNAs). The NCA-mRNAs are loaded into the STS through CC–sieve element (SE) plasmodesmata (PDs), which involves either the selective (S) or nonselective (NS) trafficking pathway (71, 96, 112). In the former, S-RBPs recognize and bind to a specific mRNA sequence element or structural motif (binding motif; *black dots*) to form S-RBP–NCA-mRNA complexes. The S-RBP then binds a PD receptor to open the CC-SE PD and allow entry of the RNP complex into the SE. The NS trafficking pathway is mediated by NS-RBPs, which similarly recognize and bind a different mRNA sequence element or structural motif (*blue rectangle*) on the NCA-mRNAs. These NS-RBP–NCA-mRNA complexes enter the SE when PDs are open. Highly abundant CA-mRNAs containing a motif (*black triangle*) that is erroneously recognized by NS-RBPs are mistakenly bound and enter the SE as loading contaminants, as opposed to authentic long-distance signaling mRNAs. (*b*) Model for phloem entry of tRNAs and mRNAs carrying a tRNA-like structure (TLS). An NCA-TLS-RBP forms a complex with tRNA, or with mRNAs carrying such a TLS in the 3′ untranslated region, to mediate entry into the STS (115). CA-mRNAs carrying pseudo-TLSs also enter and move through the STS; this NCA-tRNA-binding protein could also contribute to the entry of CA-mRNAs as loading contaminants. Additional abbreviation: N, nucleus.

PHLOEM ENTRY OF AUTHENTIC AND CONTAMINANT mRNA

Phloem loading of RNA involves RBPs that mediate trafficking of bound RNA through CC-SE PDs (**Figure 3**). As mentioned above, studies of individual mRNAs have identified sequence motifs that are necessary for entry into the STS. Research on viroids has also shown that sequence motifs control the passage of viroid RNA across specific cellular boundaries (86, 99, 118–121). In addition, a recent study demonstrated that many phloem transcripts contain tRNA-like structures and, furthermore, that cell-autonomous mRNAs carrying a tRNA-like structure acquire a capacity for long-distance movement (115). These findings are consistent with a mechanism for selective entry of mRNA into the STS.

Recent RNA-sequencing studies of *Arabidopsis* (102) and cucumber (117) suggest that such a mechanism may not account for the mode of entry for all mRNAs present in the phloem STS. In a P_i-stress-signaling study, a combination of transcriptomics assessment of cucumber source vascular tissue, phloem sap, and watermelon sink tissues and Gene Ontology analysis revealed that many graft-transmissible mRNAs were associated in both stock and scion tissues with general housekeeping activities (117). However, a phloem mRNA subpopulation whose expression level was higher in stock compared with sink tissues was enriched in signaling pathways associated with P_i stress. Collectively, these studies raised an important question as to the nature and function of graft-transmissible mRNAs. **Figure 4** proposes two models to account for the presence of authentic and putative contaminant mRNAs in the phloem STS. These models could be tested through a combination of transcriptomics and molecular analysis of transcripts bound to phloem-mobile RBPs.

SUMMARY POINTS

1. The phloem enucleate sieve tube system (STS) contains a unique population of mRNAs that serve as mobile systemic signaling agents. RNA entry into the STS can occur through a selective or nonselective mechanism.
2. Phosphorylation of phloem RNA-binding proteins allows formation of stable RNA-protein complexes for delivery of cargo RNA to sink tissues.
3. As a specialized symplasmic domain, the phloem STS serves to integrate developmental and abiotic- and biotic-stress-related signaling systems throughout the plant.
4. Systemic movement of small RNAs can exert epigenetic control over gene expression and genome stability.
5. Nutrient homeostasis involves long-distance tissue-specific delivery of small interfering RNA, microRNA, and mRNA.
6. Bidirectional movement of RNA can occur in rosette plants through the phloem (source to sink) and by trafficking cell to cell through plasmodesmata in the reverse direction (sink to source).
7. In agriculture, heterografting of plants can lead to changes in gene expression patterns that may affect traits important for yield and food quality.

FUTURE ISSUES

1. Further studies are needed to evaluate the long-distance signaling roles of a greater number of phloem-mobile mRNAs.

2. Grafting and transcriptomics studies, performed using a diverse set of plants grown under a range of environmental conditions, should offer additional insights into the nature of phloem-mobile mRNAs held in common as well as those unique to each species, along with mRNAs that are responsive to changes in growth conditions.
3. A major effort is needed to characterize the population of RNA-binding proteins detected in the phloem STS to elucidate their individual roles in RNA transport to sink organs.
4. Destination-specific delivery of RNA is an important discovery, and the molecular mechanism or mechanisms underlying this process need to be elucidated.
5. A growing body of evidence supports the notion that protein synthesis occurs in mature sieve elements, but definitive experimental evidence is lacking.

DISCLOSURE STATEMENT

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LITERATURE CITED

1. Alakonya A, Kumar R, Koenig D, Kimura S, Townsley B, et al. 2012. Interspecific RNA interference of SHOOT MERISTEMLESS-like disrupts *Cuscuta pentagona* plant parasitism. *Plant Cell* 24:3153–66
2. Allen E, Xie Z, Gustafson AM, Carrington JC. 2005. MicroRNA-directed phasing during *trans*-acting siRNA biogenesis in plants. *Cell* 121:207–21
3. Aly R, Cholakh H, Joel DM, Leibman D, Steinitz B, et al. 2009. Gene silencing of mannose 6-phosphate reductase in the parasitic weed *Orobanche aegyptiaca* through the production of homologous dsRNA sequences in the host plant. *Plant Biotechnol. J.* 7:487–98
4. Aoki K, Suzui N, Fujimaki S, Dohmae N, Yonekura-Sakakibara K, et al. 2005. Destination-selective long-distance movement of phloem proteins. *Plant Cell* 17:1801–14
5. Araya T, von Wiren N, Takahashi H. 2016. CLE peptide signaling and nitrogen interactions in plant root development. *Plant Mol. Biol.* 91:607–15
6. Asano T, Masumura T, Kusano H, Kikuchi S, Kurita A, et al. 2002. Construction of a specialized cDNA library from plant cells isolated by laser capture microdissection: toward comprehensive analysis of the genes expressed in the rice phloem. *Plant J.* 32:401–8
7. Aung K, Lin SI, Wu CC, Huang YT, Su CL, Chiou TJ. 2006. *pho2*, a phosphate overaccumulator, is caused by a nonsense mutation in a microRNA399 target gene. *Plant Physiol.* 141:1000–11
8. Axtell MJ. 2013. Classification and comparison of small RNAs from plants. *Annu. Rev. Plant Biol.* 64:137–159
9. Baek D, Kim MC, Chun HJ, Kang S, Park HC, et al. 2013. Regulation of *miR399f* transcription by AtMYB2 affects phosphate starvation responses in Arabidopsis. *Plant Physiol.* 161:362–73
10. Balachandran S, Xiang Y, Schobert C, Thompson GA, Lucas WJ. 1997. Phloem sap proteins from *Cucurbita maxima* and *Ricinus communis* have the capacity to traffic cell to cell through plasmodesmata. *PNAS* 94:14150–55

11. Banerjee AK, Chatterjee M, Yu Y, Suh SG, Miller WA, Hannapel DJ. 2006. Dynamics of a mobile RNA of potato involved in a long-distance signaling pathway. *Plant Cell* 18:3443–57
12. Banerjee AK, Lin T, Hannapel DJ. 2009. Untranslated regions of a mobile transcript mediate RNA metabolism. *Plant Physiol.* 151:1831–43
13. Bari R, Datt Pant B, Stitt M, Scheible WR. 2006. PHO2, microRNA399, and PHR1 define a phosphate-signaling pathway in plants. *Plant Physiol.* 141:988–99
14. Bennetzen JL, Wang H. 2014. The contributions of transposable elements to the structure, function, and evolution of plant genomes. *Annu. Rev. Plant Biol.* 65:505–30
15. Bologna NG, Voinnet O. 2014. The diversity, biogenesis, and activities of endogenous silencing small RNAs in *Arabidopsis*. *Annu. Rev. Plant Biol.* 65:473–503
16. Borges F, Martienssen RA. 2015. The expanding world of small RNAs in plants. *Nat. Rev. Mol. Cell Biol.* 16:727–41
17. Buhtz A, Pieritz J, Springer F, Kehr J. 2010. Phloem small RNAs, nutrient stress responses, and systemic mobility. *BMC Plant Biol.* 10:64
18. Buhtz A, Springer F, Chappell L, Baulcombe DC, Kehr J. 2008. Identification and characterization of small RNAs from the phloem of *Brassica napus*. *Plant J.* 53:739–49
19. Burleigh SH, Harrison MJ. 1999. The down-regulation of *Mt4*-like genes by phosphate fertilization occurs systemically and involves phosphate translocation to the shoots. *Plant Physiol.* 119:241–48
20. Caetano-Anolles G, Gresshoff PM. 1991. Plant genetic control of nodulation. *Annu. Rev. Microbiol.* 45:345–82
21. Calderwood A, Kopriva S, Morris RJ. 2016. Transcript abundance explains mRNA mobility data in *Arabidopsis thaliana*. *Plant Cell* 28:610–15
22. Chen JJ, Janssen BJ, Williams A, Sinha N. 1997. A gene fusion at a homeobox locus: alterations in leaf shape and implications for morphological evolution. *Plant Cell* 9:1289–304
23. Chiou TJ, Lin SI. 2011. Signaling network in sensing phosphate availability in plants. *Annu. Rev. Plant Biol.* 62:185–206
24. Cho SK, Sharma P, Butler NM, Kang IH, Shah S, et al. 2015. Polypyrimidine tract-binding proteins of potato mediate tuberization through an interaction with *StBEL5* RNA. *J. Exp. Bot.* 66:6835–47
25. Cui X, Cao X. 2014. Epigenetic regulation and functional exaptation of transposable elements in higher plants. *Curr. Opin. Plant Biol.* 21:83–88
26. David-Schwartz R, Runo S, Townsley B, Machuka J, Sinha N. 2008. Long-distance transport of mRNA via parenchyma cells and phloem across the host-parasite junction in *Cuscuta*. *New Phytol.* 179:1133–41
27. Deeken R, Ache P, Kajahn I, Klinkenberg J, Bringmann G, Hedrich R. 2008. Identification of *Arabidopsis thaliana* phloem RNAs provides a search criterion for phloem-based transcripts hidden in complex datasets of microarray experiments. *Plant J.* 55:746–59
28. Dodsworth S, Leitch AR, Leitch IJ. 2015. Genome size diversity in angiosperms and its influence on gene space. *Curr. Opin. Genet. Dev.* 35:73–78
29. Doering-Saad C, Newbury HJ, Couldridge CE, Bale JS, Pritchard J. 2006. A phloem-enriched cDNA library from *Ricinus*: insights into phloem function. *J. Exp. Bot.* 57:3183–93
30. Forde BG. 2002. Local and long-range signaling pathways regulating plant responses to nitrate. *Annu. Rev. Plant Biol.* 53:203–24
31. Franco-Zorrilla JM, Martin AC, Leyva A, Par-Ares JP. 2005. Interaction between phosphate-starvation, sugar, and cytokinin signaling in Arabidopsis and the roles of cytokinin receptors CRE1/AHK4 and AHK3. *Plant Physiol.* 138:847–57
32. Fujii H, Chiou TJ, Lin SI, Aung K, Zhu JK. 2005. A miRNA involved in phosphate-starvation response in *Arabidopsis*. *Curr. Biol.* 15:2038–43
33. Gaupels F, Buhtz A, Knauer T, Deshmukh S, Waller F, et al. 2008. Adaptation of aphid stylectomy for analyses of proteins and mRNAs in barley phloem sap. *J. Exp. Bot.* 59:3297–306
34. Gómez G, Pállas V. 2004. A long-distance translocatable phloem protein from cucumber forms a ribonucleoprotein complex in vivo with *Hop stunt viroid* RNA. *J. Virol.* 78:10104–10
35. Guo S, Zhang J, Sun H, Salse J, Lucas WJ, et al. 2013. The draft genome of watermelon (*Citrullus lanatus*) and resequencing of 20 diverse accessions. *Nat. Genet.* 45:51–58

36. Ham BK, Brandom JL, Xoconostle-Cázares B, Ringgold V, Lough TJ, Lucas WJ. 2009. A polypyrimidine tract binding protein, pumpkin RBP50, forms the basis of a phloem-mobile ribonucleoprotein complex. *Plant Cell* 21:197–215
37. Ham BK, Li G, Jia W, Leary JA, Lucas WJ. 2014. Systemic delivery of siRNA in pumpkin by a plant PHLOEM SMALL RNA-BINDING PROTEIN 1-ribonucleoprotein complex. *Plant J.* 80:683–94
38. Ham BK, Lucas WJ. 2014. The angiosperm phloem sieve tube system: a role in mediating traits important to modern agriculture. *J. Exp. Bot.* 65:1799–816
39. Haywood V, Yu TS, Huang NC, Lucas WJ. 2005. Phloem long-distance trafficking of *GIBBERELLIC ACID-INSENSITIVE* RNA regulates leaf development. *Plant J.* 42:49–68
40. Hsieh LC, Lin SI, Shih AC, Chen JW, Lin WY, et al. 2009. Uncovering small RNA-mediated responses to phosphate deficiency in Arabidopsis by deep sequencing. *Plant Physiol.* 151:2120–32
41. Hu C, Ham BK, El-Shabrawi HM, Alexander D, Zhang D, et al. 2016. Proteomics and metabolomics analyses reveal the cucurbit sieve tube system as a complex metabolic space. *Plant J.* 87:442–54
42. Huang NC, Yu TS. 2009. The sequences of Arabidopsis *GA-INSENSITIVE* RNA constitute the motifs that are necessary and sufficient for RNA long-distance trafficking. *Plant J.* 59:921–29
43. Jones-Rhoades MW, Bartel DP. 2004. Computational identification of plant microRNAs and their targets, including a stress-induced miRNA. *Mol. Cell* 14:787–99
44. Kanehira A, Yamada K, Iwaya T, Tsuwamoto R, Kasai A, et al. 2010. Apple phloem cells contain some mRNAs transported over long distances. *Tree Genet. Genomes* 6:635–42
45. Kawashima CG, Yoshimoto N, Maruyama-Nakashita A, Tsuchiya YN, Saito K, et al. 2009. Sulphur starvation induces the expression of microRNA-395 and one of its target genes but in different cell types. *Plant J.* 57:313–21
46. Kim G, LeBlanc ML, Wafula EK, dePamphilis CW, Westwood JH. 2014. Genomic-scale exchange of mRNA between a parasitic plant and its hosts. *Science* 345:808–11
47. Kim G, Westwood JH. 2015. Macromolecule exchange in *Cuscuta*-host plant interactions. *Curr. Opin. Plant Biol.* 26:20–25
48. Kim M, Canio W, Kessler S, Sinha N. 2001. Developmental changes due to long-distance movement of a homeobox fusion transcript in tomato. *Science* 293:287–89
49. Knoblauch M, Knoblauch J, Mullendore DL, Savage JA, Babst BA, et al. 2016. Testing the Münch hypothesis of long distance phloem transport in plants. *eLife* 5:e15341
50. Kollmann R, Dörr I, Kleinig H. 1970. Protein filaments—structural components of the phloem exudate: I. Observations with *Cucurbita* and *Nicotiana*. *Planta* 95:86–94
51. Kuo HF, Chiou TJ. 2011. The role of microRNAs in phosphorus deficiency signaling. *Plant Physiol.* 156:1016–24
52. Lappartient AG, Vidmar JJ, Leustek T, Glass AD, Touraine B. 1999. Inter-organ signaling in plants: regulation of ATP sulfurylase and sulfate transporter genes expression in roots mediated by phloem-translocated compound. *Plant J.* 18:89–95
53. LeBlanc M, Kim G, Patel B, Stromberg V, Westwood J. 2013. Quantification of tomato and *Arabidopsis* mobile RNAs trafficking into the parasitic plant *Cuscuta pentagona*. *New Phytol.* 200:1225–33
54. Lee JY, Yoo BC, Rojas MR, Gomez-Ospina N, Staehelin LA, Lucas WJ. 2003. Selective trafficking of non-cell-autonomous proteins mediated by NtNCAPP1. *Science* 299:392–96
55. Leustek T, Martin MN, Bick JA, Davies JP. 2000. Pathways and regulation of sulfur metabolism revealed through molecular and genetic studies. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 51:141–65
56. Lewsey MG, Hardcastle TJ, Melnyk CW, Molnar A, Valli A, et al. 2016. Mobile small RNAs regulate genome-wide DNA methylation. *PNAS* 113:E801–10
57. Li P, Ham BK, Lucas WJ. 2011. CmRBP50 protein phosphorylation is essential for assembly of a stable phloem-mobile high-affinity ribonucleoprotein complex. *J. Biol. Chem.* 286:23142–49
58. Liang D, White RG, Waterhouse PM. 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
59. Liang G, He H, Yu D. 2012. Identification of nitrogen starvation-responsive microRNAs in *Arabidopsis thaliana*. *PLOS ONE* 7:e48951

60. Lin MK, Lee YJ, Lough TJ, Phinney BS, Lucas WJ. 2009. Analysis of the pumpkin phloem proteome provides insights into angiosperm sieve tube function. *Mol. Cell. Proteom.* 8:343–56
61. Lin SI, Chiang SF, Lin WY, Chen JW, Tseng CY, et al. 2008. Regulatory network of microRNA399 and *PHO2* by systemic signaling. *Plant Physiol.* 147:732–46
62. Lin SI, Santi C, Jobet E, Lacut E, El Kholti N, et al. 2010. Complex regulation of two target genes encoding SPX-MFS proteins by rice miR827 in response to phosphate starvation. *Plant Cell Physiol.* 51:2119–31
63. Lin T, Lashbrook CC, Cho SK, Butler NM, Sharma P, et al. 2015. Transcriptional analysis of phloem-associated cells of potato. *BMC Genom.* 16:665
64. Lin T, Sharma P, Gonzalez DH, Viola IL, Hannapel DJ. 2013. The impact of the long-distance transport of a *BEL1*-like messenger RNA on development. *Plant Physiol.* 161:760–72
65. Lin WY, Huang TK, Chiou TJ. 2013. Nitrogen limitation adaptation, a target of microRNA827, mediates degradation of plasma membrane-localized phosphate transporters to maintain phosphate homeostasis in *Arabidopsis*. *Plant Cell* 25:4061–74
66. Liu CM, Muchhal US, Uthappa M, Kononowicz AK, Raghothama KG. 1998. Tomato phosphate transporter genes are differentially regulated in plant tissues by phosphorus. *Plant Physiol.* 116:91–99
67. Liu Q, Chen XB, Wu K, Fu XD. 2015. Nitrogen signaling and use efficiency in plants: What's new? *Curr. Opin. Plant Biol.* 27:192–98
68. Liu TY, Chang CY, Chiou TJ. 2009. The long-distance signaling of mineral macronutrients. *Curr. Opin. Plant Biol.* 12:312–19
69. Lough TJ, Lucas WJ. 2006. Integrative plant biology: role of phloem long-distance macromolecular trafficking. *Annu. Rev. Plant Biol.* 57:203–32
70. Lucas WJ, Groover A, Lichtenberger R, Furuta K, Yadav SR, et al. 2013. The plant vascular system: evolution, development and functions. *J. Integr. Plant Biol.* 55:294–388
71. Lucas WJ, Ham BK, Kim JY. 2009. Plasmodesmata—bridging the gap between neighboring plant cells. *Trends Cell Biol.* 19:495–503
72. Lundmark M, Korner CJ, Nielsen TH. 2010. Global analysis of microRNA in *Arabidopsis* in response to phosphate starvation as studied by locked nucleic acid-based microarrays. *Physiol. Plant.* 140:57–68
73. Ma Y, Miura E, Ham BK, Cheng HW, Lee YJ, Lucas WJ. 2010. Pumpkin eIF5A isoforms interact with components of the translational machinery in the cucurbit sieve tube system. *Plant J.* 64:536–50
74. Mahajan A, Bhogale S, Kang IH, Hannapel DJ, Banerjee AK. 2012. The mRNA of a Knotted1-like transcription factor of potato is phloem mobile. *Plant Mol. Biol.* 79:595–608
75. Matzke MA, Kanno T, Matzke AJM. 2015. RNA-directed DNA methylation: the evolution of a complex epigenetic pathway in flowering plants. *Annu. Rev. Plant Biol.* 66:243–67
76. Melnyk CW, Molnar A, Bassett A, Baulcombe DC. 2011. Mobile 24 nt small RNAs direct transcriptional gene silencing in the root meristems of *Arabidopsis thaliana*. *Curr. Biol.* 21:1678–83
77. Molnar A, Melnyk CW, Bassett A, Hardcastle TJ, Dunn R, Baulcombe DC. 2010. Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. *Science* 328:872–75
78. Nakazono M, Qiu F, Borsuk LA, Schnable PS. 2003. Laser-capture microdissection, a tool for the global analysis of gene expression in specific plant cell types: identification of genes expressed differentially in epidermal cells or vascular tissues of maize. *Plant Cell* 15:583–96
79. Notaguchi M, Wolf S, Lucas WJ. 2012. Phloem-mobile *Aux/IAA* transcripts target to the root tip and modify root architecture. *J. Integr. Plant Biol.* 54:760–72
80. Omid A, Keilin T, Glass A, Leshkowitz D, Wolf S. 2007. Characterization of phloem-sap transcription profile in melon plants. *J. Exp. Bot.* 58:3645–56
81. Palauqui JC, Elmayan T, Pollien JM, Vaucheret H. 1997. Systemic acquired silencing: transgene-specific post-transcriptional silencing is transmitted by grafting from silenced stocks to non-silenced scions. *EMBO J.* 16:4738–45
82. Pant BD, Buhtz A, Kehr J, Scheible WR. 2008. MicroRNA399 is a long-distance signal for the regulation of plant phosphate homeostasis. *Plant J.* 53:731–38
83. Pant BD, Musialak-Lange M, Nuc P, May P, Buhtz A, et al. 2009. Identification of nutrient-responsive Arabidopsis and rapeseed microRNAs by comprehensive real-time polymerase chain reaction profiling and small RNA sequencing. *Plant Physiol.* 150:1541–55

84. Paul S, Datta SK, Datta K. 2015. miRNA regulation of nutrient homeostasis in plants. *Front. Plant Sci.* 6:232
85. Peng M, Hannam C, Gu H, Bi YM, Rothstein SJ. 2007. A mutation in *NLA*, which encodes a RING-type ubiquitin ligase, disrupts the adaptability of *Arabidopsis* to nitrogen limitation. *Plant J.* 50:320–37
86. Qi Y, Pelissier T, Itaya A, Hunt E, Wassenegger M, Ding B. 2004. Direct role of a viroid RNA motif in mediating directional RNA trafficking across a specific cellular boundary. *Plant Cell* 16:1741–52
87. Rodriguez-Medina C, Atkins CA, Mann AJ, Jordan ME, Smith PM. 2011. Macromolecular composition of phloem exudate from white lupin (*Lupinus albus* L.). *BMC Plant Biol.* 11:36
88. Roney JK, Khatibi PA, Westwood JH. 2007. Cross-species translocation of mRNA from host plants into the parasitic plant dodder. *Plant Physiol.* 143:1037–43
89. Ruffel S, Poitout A, Krouk G, Coruzzi GM, Lacombe B. 2016. Long-distance nitrate signaling displays cytokinin dependent and independent branches. *J. Integr. Plant Biol.* 58:226–29
90. Ruiz-Medrano R, Xoconostle-Cázares B, Lucas WJ. 1999. Phloem long-distance transport of *CmNACP* mRNA: implications for supracellular regulation in plants. *Development* 126:4405–19
91. Saini P, Eyler DE, Green R, Dever TE. 2009. Hypusine-containing protein eIF5A promotes translation elongation. *Nature* 459:118–21
92. Schwarz D, Rouphael Y, Colla G, Venema JH. 2010. Grafting as a tool to improve tolerance of vegetables to abiotic stresses: thermal stress, water stress and organic pollutants. *Sci. Horticult.* 127:162–71
93. Searle IR, Men AE, Laniya TS, Buzas DM, Iturbe-Ormaetxe I, et al. 2003. Long-distance signaling in nodulation directed by a CLAVATA1-like receptor kinase. *Science* 299:109–12
94. Secco D, Wang C, Arpat BA, Wang Z, Poirier Y, et al. 2012. The emerging importance of the SPX domain-containing proteins in phosphate homeostasis. *New Phytol.* 193:842–51
95. Singh G, Pratt G, Yeo GW, Moore MJ. 2015. The clothes make the mRNA: past and present trends in mRNP fashion. *Annu. Rev. Biochem.* 84:325–54
96. Stadler R, Wright KM, Lauterbach C, Amon G, Gahrtz M, et al. 2005. Expression of GFP-fusions in *Arabidopsis* companion cells reveals non-specific protein trafficking into sieve elements and identifies a novel post-phloem domain in roots. *Plant J.* 41:319–31
97. Sunkar R, Zhu JK. 2004. Novel and stress-regulated microRNAs and other small RNAs from Arabidopsis. *Plant Cell* 16:2001–19
98. Tabata R, Sumida K, Yoshii T, Ohyama K, Shinohara H, Matsubayashi Y. 2014. Perception of root-derived peptides by shoot LRR-RKs mediates systemic N-demand signaling. *Science* 346:343–46
99. Takeda R, Petrov AI, Leontis NB, Ding B. 2011. A three-dimensional RNA motif in *Potato spindle tuber viroid* mediates trafficking from palisade mesophyll to spongy mesophyll in *Nicotiana benthamiana*. *Plant Cell* 23:258–72
100. Taoka K, Ham BK, Xoconostle-Cázares B, Rojas MR, Lucas WJ. 2007. Reciprocal phosphorylation and glycosylation recognition motifs control NCAPP1 interaction with pumpkin phloem proteins and their cell-to-cell movement. *Plant Cell* 19:1866–84
101. Thibaud MC, Arrighi JF, Bayle V, Chiarenza S, Creff A, et al. 2010. Dissection of local and systemic transcriptional responses to phosphate starvation in Arabidopsis. *Plant J.* 4:775–89
102. Thieme CJ, Rojas-Triana M, Stecyk E, Schudoma C, Zhang W, et al. 2015. Endogenous *Arabidopsis* messenger RNAs transported to distant tissues. *Nat. Plants* 1:15025
103. Voinnet O, Baulcombe DC. 1997. Systemic signalling in gene silencing. *Nature* 389:553
104. Voinnet O, Vain P, Angell S, Baulcombe DC. 1998. Systemic spread of sequence-specific transgene RNA degradation in plants is initiated by localized introduction of ectopic promoterless DNA. *Cell* 95:177–87
105. Warschefsky EJ, Klein LL, Frank MH, Chitwood DH, Londo JP, et al. 2016. Rootstocks: diversity, domestication, and impacts on shoot phenotypes. *Trends Plant Sci.* 21:418–37
106. Wendel JF, Jackson SA, Meyers BC, Wing RA. 2016. Evolution of plant genome architecture. *Genome Biol.* 17:37
107. Wu R, Wang X, Lin Y, Ma Y, Liu G, et al. 2013. Inter-species grafting caused extensive and heritable alterations of DNA methylation in *Solanaceae* plants. *PLOS ONE* 8:e61995
108. Xoconostle-Cázares B, Xiang Y, Ruiz-Medrano R, Wang HL, Monzer J, et al. 1999. Plant paralog to viral movement protein that potentiates transport of mRNA into the phloem. *Science* 283:94–98

109. Yang YZ, Mao LY, Jittayasothorn Y, Kang YM, Jiao C, et al. 2015. Messenger RNA exchange between scions and rootstocks in grafted grapevines. *BMC Plant Biol.* 15:251
110. Yoder JI, Gunathilake P, Wu B, Tomilova N, Tomilov AA. 2009. Engineering host resistance against parasitic weeds with RNA interference. *Pest Manag. Sci.* 65:460–66
111. Yoo BC, Kragler F, Varkonyi-Gasic E, Haywood V, Archer-Evans S, et al. 2004. A systemic small RNA signaling system in plants. *Plant Cell* 16:1979–2000
112. Zambryski P. 2004. Cell-to-cell transport of proteins and fluorescent tracers via plasmodesmata during plant development. *J. Cell Biol.* 164:165–68
113. Zhang S, Sun L, Kragler F. 2009. The phloem-delivered RNA pool contains small noncoding RNAs and interferes with translation. *Plant Physiol.* 150:378–87
114. Zhang W, Kollwig G, Stecyk E, Apelt F, Dirks R, Kragler F. 2014. Graft-transmissible movement of inverted-repeat-induced siRNA signals into flowers. *Plant J.* 80:106–21
115. Zhang W, Thieme CJ, Kollwig G, Apelt F, Yang L, et al. 2016. tRNA-related sequences trigger systemic mRNA transport in plants. *Plant Cell* 28:1237–49
116. Zhang Z, Liao H, Lucas WJ. 2014. Molecular mechanisms underlying phosphate sensing, signaling, and adaptation in plants. *J. Integr. Plant Biol.* 56:192–220
117. Zhang Z, Zheng Y, Ham BK, Chen J, Yoshida A, et al. 2016. Vascular-mediated signalling involved in early phosphate stress response in plants. *Nat. Plants* 2:16033
118. Zhong X, Archual AJ, Amin AA, Ding B. 2008. A genomic map of viroid RNA motifs critical for replication and systemic trafficking. *Plant Cell* 20:35–47
119. Zhong X, Tao X, Stombaugh J, Leontis N, Ding B. 2007. Tertiary structure and function of an RNA motif required for plant vascular entry to initiate systemic trafficking. *EMBO J.* 26:3836–46
120. Zhu Y, Green L, Woo YM, Owens R, Ding B. 2001. Cellular basis of potato spindle tuber viroid systemic movement. *Virology* 279:69–77
121. Zhu Y, Qi Y, Xun Y, Owens R, Ding B. 2002. Movement of potato spindle tuber viroid reveals regulatory points of phloem-mediated RNA traffic. *Plant Physiol.* 130:138–46