ANNUAL REVIEWS

Annual Review of Plant Biology

Long-Distance Transported RNAs: From Identity to Function

Julia Kehr,[1] Richard J. Morris,[2] and Friedrich Kragler[3]

[1]Department of Biology, Institute for Plant Sciences and Microbiology, Universität Hamburg, Hamburg, Germany; email: julia.kehr@uni-hamburg.de

[2]Computational and Systems Biology, John Innes Centre, Norwich, United Kingdom; email: richard.morris@jic.ac.uk

[3]Department II, Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany; email: kragler@mpimp-golm.mpg.de

Annu. Rev. Plant Biol. 2022. 73:457–74

First published as a Review in Advance on December 15, 2021

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

https://doi.org/10.1146/annurev-arplant-070121-033601

Keywords

phloem, long-distance transport, microRNA, mRNA, grafting, RNA signaling

Abstract

There is now a wealth of data, from different plants and labs and spanning more than two decades, which unequivocally demonstrates that RNAs can be transported over long distances, from the cell where they are transcribed to distal cells in other tissues. Different types of RNA molecules are transported, including micro- and messenger RNAs. Whether these RNAs are selected for transport and, if so, how they are selected and transported remain, in general, open questions. This aspect is likely not independent of the biological function and relevance of the transported RNAs, which are in most cases still unclear. In this review, we summarize the experimental data supporting selectivity or nonselectivity of RNA translocation and review the evidence for biological functions. After discussing potential issues regarding the comparability between experiments, we propose criteria that need to be critically evaluated to identify important signaling RNAs.

Contents

1. DISCOVERY OF RNA TRANSPORT

The first evidence for intertissue transfer of macromolecules was found in plant viruses. These pathogens, which have minimal genomes, produce specialized proteins required to transfer their genomes to neighboring cells and, via the phloem, to distant tissues. In the beginning of the nineteenth century, it became clear that plant viruses differed in their transmissibility. Some viruses could be transmitted by inoculation using phloem or leaf sap from infected plants, while transmission of others was only detected after grafting with infected plants. Pioneering studies by Bennett (6, 7) led to the discovery that *Cucumber mosaic virus* (CMV), which has an RNA genome, can be transmitted from an infected to a healthy plant through a bridging *Cuscuta* plant parasitizing via its haustoria on the phloem of two distinct host plants. More than 75 years later, researchers showed that *Cuscuta* spp. feeding on *Arabidopsis thaliana* also take up functional host proteins (47), protein-encoding messenger RNA (mRNA) (17, 40, 74), and small interfering RNA (siRNA) (1).

Today, we recognize that all RNA viruses produce specialized movement proteins, forming larger viral RNA–protein complexes that interact with host factors to facilitate intercellular transfer of viral DNA or RNA genomes via pores, called plasmodesmata (PD), that cross the cell walls (51). Most viruses can move from infected sugar-producing (photosynthetically active) source tissues via the sugar-conducting phloem vessels to sugar-requiring (photosynthetically inactive) sink tissues; i.e., they can follow the flow of sugar through the plant. Researchers proposed that most, if not all, macromolecules detected in the phloem and in phloem-feeding *Cuscuta* are imported and exported from phloem vessels in a similar fashion via PD channels. PD are intercellular conduits that contain and connect the cytoplasm, the endoplasmic reticulum, and the plasma membrane of most adjacent cells. Depending on the tissue type, PD can be structurally highly complex, forming one or several branches and a central cavity. PD are formed either at the cell plate during cell division (primary PD) or newly at existing cell walls (secondary PD). The symplasmic connection

mRNA: messenger RNA

PD: plasmodesmata

established by PD allows plant cells enclosed by cellulosic walls to exchange large and small molecules that otherwise cannot be transferred between and within tissues. In the plant body, dynamically closing and opening PD channels form symplasmic (cytosolic-connected) domains that change during the diurnal cycle and developmental transitions, permitting a dynamic and controlled exchange of PD-transported signaling molecules. As most tissues are symplasmically connected by PD pores, an individual plant should be conceived—at least in part—as a symplastic entity (for reviews, see 50, 68, 89). An important feature of PD is their spatiotemporal dynamics that allow for transport to be regulated (for reviews, see 2, 13), enabling complex intercellular communication, as postulated more than 100 years ago by Eduard Tangl (72), who first described PD while he was working at Czernowitz University during the Austro-Hungarian Monarchy.

Convincing evidence for a selective endogenous PD-based transport system for RNAs was first found in viroids. These tiny, infectious, plant-specific pathogens have a small (~200-nt-long), circular, nonencapsidated RNA genome that is stably folded. Groundbreaking work by Biao Ding (21) at Ohio State University revealed that viroid RNAs form distinct pseudoknot/hairpin motifs that are necessary for replication and moving to specific cell types via PD (67, 93). Viroid RNAs do not encode for proteins and thus must hitchhike via the host endogenous intercellular transport system.

Initial evidence for intercellular transport of both endogenous mRNA and protein was found with the homeodomain transcription factor KNOTTED1 (KN1) expressed in the meristem inner cell layers but detected in the outer layers of *Zea mays* (71). Based on this observation, researchers proposed that KN1 protein, and potentially its mRNA, moves across cell layers via PD. Indeed, fluorescently tagged recombinant KN1 RNA—binding its own protein—moved to neighboring cells after being microinjected into a single mesophyll cell (52). In a similar line, a tomato mutant, *mouse ears*, was described producing an anomalous homeodomain fusion protein whose mRNA moved from rootstock to scions, where it accumulated in leaf primordia. The presence of the anomalous mRNA induced leaf shape changes (41). Again, these insights support the notion of a selective PD-based transport system for endogenous RNAs and translation of the transported mRNA.

2. METHODS TO IDENTIFY MOBILE RNA POPULATIONS

In general, it seems that the identified population of potentially mobile RNAs strongly depends on the experimental approach. The most comprehensive information about (potentially) phloem-mobile RNAs was obtained from phloem exudate (sap) samples and from grafting experiments (**Figure 1**).

2.1. Phloem Sap Sampling

In most species, phloem sampling is difficult, and the amounts that can be obtained are small. Sampling methods mainly differ in the length of the harvest period and the volume and purity of phloem content that can be collected but also in the degree of injury imposed, defining the level of potential contaminations and artefacts (20).

Aphid stylectomy is the most elegant approach, yielding very small volumes of phloem that allow only limited analyses. It is minimally invasive, although insect-induced effects cannot be fully excluded (19, 20). Using this technique, only a limited number of RNAs have been identified (22, 70, 77). Even fewer reports have successfully identified RNAs from samples obtained through ethylenediaminetetraacetic acid (EDTA)-facilitated exudation (18), an easy sampling technique prone to contamination and degradation (20).

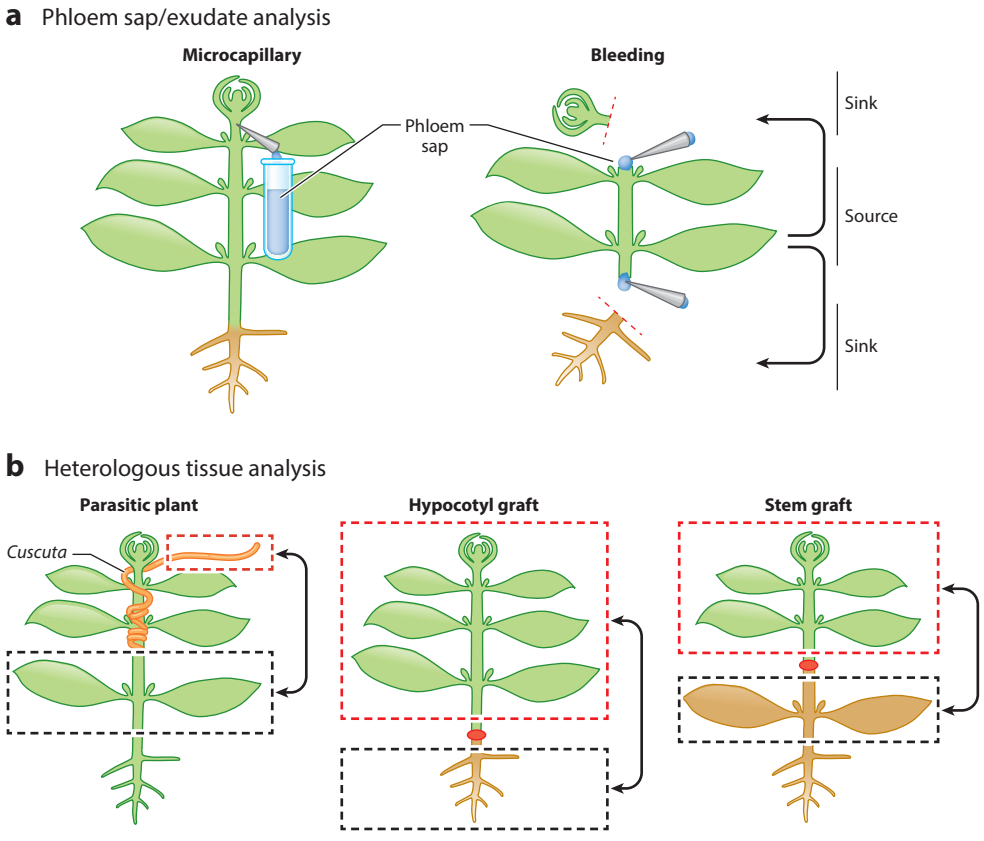

Figure 1

Approaches to identify mobile RNAs moving over long distances. (*a*) Phloem sap is collected from either small punctures or cut whole plant parts. Emerging phloem droplets are harvested and analyzed using RNA sequencing (RNA-seq) or specifically confirmed by reverse transcription–polymerase chain reaction (RT-PCR) and Northern blot assays. (*b*) Alternatively, mobile RNAs can be identified by using parasitic plants (e.g., *Cuscuta*) feeding on the vasculature of host plants or by using heterografted plants. Here, mobile RNAs are identified based on their naturally occurring sequence differences such as single-nucleotide polymorphisms (SNPs) and insertions/deletions (INDELs). Note that this approach allows for the identification of macromolecules moving unidirectionally or bidirectionally [from source to sink and/or vice versa (*a*)] and moving to specific tissues.

Most phloem sap RNA-profiling studies have been performed in plants showing spontaneous exudation. In these studies, to avoid contamination by cell debris produced by the injury, the first exudate is removed, and samples are tested for the presence of marker transcripts. The approaches used to collect phloem exudate differ significantly in invasiveness, ranging from cutting whole organs (e.g., leaves) or complete stems (25, 26, 60, 88, 92) to making tiny incisions (11, 12, 64, 65) (**Figure 1**).

Also, phloem anatomy can influence sample content. Cucurbits, for example, have long been used for studying phloem exudate composition because of the large amount of sap that can be easily obtained from cut petioles or stems. However, members of the Cucurbitaceae family show an unusual phloem anatomy with fascicular and extrafascicular phloem, and sap composition is

dependent on its origin from one or the other phloem type (49). Because of this unusual anatomy, RNAs found in phloem exudates from cucurbits might not necessarily be representative of those of other vascular plants (49).

Differences in plant species, developmental stages, sampling techniques, and sampling sites make a direct comparison challenging. Nevertheless, available studies of phloem content reveal a picture of a large and dynamic set of RNA molecules from all major classes, including mRNAs, ribosomal RNAs (rRNAs), transfer RNAs (tRNAs) and fragments thereof, small nucleolar RNAs (snoRNAs), siRNAs, and microRNAs (miRNAs) (reviewed in 39). Moreover, although several attempts were made, no translation or RNase activity could be detected within several different phloem exudates (22, 27, 70, 90), suggesting that the phloem stream is a safe environment for RNA transport.

miRNA: microRNA

RBP: RNA-binding protein

SE: sieve element

2.2. Grafting

Grafting is a classical agricultural technique used for more than 2,000 years to improve agricultural performance of dicotyledonous crops. The simplest approach to create a grafted plant is to combine a rootstock with a heterologous scion. In a successful graft, the cells will connect via secondary PD and vascular continuity will be reestablished (reviewed in 79), allowing an efficient systemic transfer of nutrients, water, and signaling molecules.

Grafting also has a long tradition in plant research. Here, different grafting methods, e.g., cleft grafting or flat grafting, are used, depending on plant species and research. In plants with rosette leaves, like *Arabidopsis*, the most suitable grafting positions are the inflorescence stem and the hypocotyl (79, 80) (**Figure 1**).

To study long-distance RNA transport, different combinations of mutants, transgenics with wild type, cultivars, and even plant species have been used over the last decades. By exchanging scion and rootstock, directional long-distance transport events can also be addressed. If grafting and sampling sites are sufficiently distant, artefacts caused by lost cell integrity, such as callus formation at the graft union, can be excluded. In small plants or seedlings that are micrografted, this can be a challenging task (5). Here, great care must be taken to avoid and control for cross-contamination of the grafted tissues as highly sensitive deep sequencing approaches are used to identify the transported RNA population.

Different grafting approaches in different plants have identified thousands of RNAs that are able to cross graft junctions, including mRNAs, miRNAs, and siRNAs (reviewed in 39). Researchers must consider that approaches differ according to grafting techniques, tissue and time of sampling, growth conditions (soil versus in vitro), plant age (juvenile versus adult), and the parts from which plants were combined. Therefore, a comparison of the results is not a simple task, and may be impossible.

The following sections provide a short overview about the populations of miRNAs and mRNAs identified as (potentially) phloem-mobile, using the different approaches described above, and also highlight the RNA-binding proteins (RBPs) that have been found in phloem samples.

3. LONG-DISTANCE TRANSPORTED RNAS AND PHLOEM RNA-BINDING PROTEINS

3.1. Messenger RNAs

After the first discovery of evidence for mRNA presence in phloem sieve elements (SEs) (43, 70), research during recent decades has identified a remarkably large number of potentially translocated transcripts, driven by advances in sequencing technology.

In phloem samples, a limited number of mRNAs has been identified in different plant species (18, 22, 23, 37, 60), but only a few studies using more global approaches have recently identified 955 differential transcripts in mulberry phloem sap during phytoplasma infection by full-length RNA sequencing (25).

Most insights that we have about mobile transcripts to date come from grafting experiments combined with deep sequencing and relatively complex bioinformatic analysis to identify heterologous transcripts present in grafted plant parts. Here, the identification of mobile mRNAs based on sequence differences [single nucleotide polymorphisms (SNPs) and insertions/deletions (INDELs)] between rootstocks and scions has revealed a genomic dimension to mRNA transport. In a study between two hypocotyl-grafted ecotypes of *Arabidopsis*, 2,006 mobile transcripts were identified, and an estimated 20% of the complete transcriptome might have the potential to move (74). The hypocotyls of small seedlings, six days after germination, were micrografted and harvested two to three weeks later when they were bolting. Surprisingly, ~25% of the identified mobile transcripts moved from roots to shoots (74), opposite to the expected direction of phloem flow. Researchers proposed that these mRNAs might move either in the phloem against the source-to-sink bulk flow or cell to cell and not through the translocation stream (39, 74). Interestingly, a significant fraction of mobile *Arabidopsis* RNAs overlapped with graft-mobile mRNAs found in grapevine and cucumber/watermelon, suggesting a certain degree of conservation (78). Another study between soybean and common bean found 1,322 soybean and 874 common bean mobile mRNAs and, in contrast to small RNAs, no strong accumulation in recipient tissues. In this study, 7-day-old seedlings were grafted and sampled 10 days after grafting (44). In watermelon/bottle gourd grafts, 130 and 1,144 mRNAs moved upwardly, and 167 and 1,051 mRNAs were downwardly transmitted under normal and chilling-stress conditions, respectively. Seedlings were grafted and collected 17 days after grafting (81).

In grapevine, more than 2,600 transcripts were reported to be mobile in in vitro cultured young grafts. Interestingly, far fewer graft-mobile transcripts (987) were identified in field-grown mature grafts (87). By wedge grafting *Nicotiana benthamiana* stem scions into a slit of *Arabidopsis* bolting stems, only 138 mobile *Arabidopsis* transcripts were identified moving from stock to scion (57). In this interspecies graft setting, larger, four-week-old plants were used, stems were grafted on stems of flowering *Arabidopsis* well above the rosette, and samples were collected three weeks after grafting. Also using a *N. benthamiana*/tomato heterograft system, only 183 *Nicotiana* mRNAs were moving from shoot to root. Here, three-week-old plants were used for hypocotyl grafting and harvested several weeks after (83). These numbers and results suggest that the translocated mRNA population might depend on the plant species, their age and developmental stage, growth conditions, and the time point of harvest after grafting. Also, the number of biological replicates and used bioinformatic pipelines probably play an important role in how many mobile transcripts were identified and considered significant. While the knowledge about mobile transcripts is steadily increasing, there are still only a few cases where a function could be demonstrated.

3.2. MicroRNAs

Since the first detection of selected endogenous miRNAs and siRNAs in phloem exudates of pumpkin, cucumber, castor bean, and yucca (88), researchers suggested that these small RNAs could be systemically mobile. Meanwhile, a much larger set of miRNAs has been identified from the phloem sap of more plant species, including different cucurbits, rapeseed, apple, mulberry, and lupin (11, 12, 26, 48, 65, 69, 77). For example, in rapeseed phloem sap, up to 161 miRNAs from 37 different families have been found using microarrays and RNA-seq (11, 65), and in mulberry, 86 conserved and 19 novel miRNAs were identified by deep sequencing (26). The phloem miRNA

population observed contains miRNAs involved in development (e.g., miR156, miR159, miR173) or in responses to nutrient deprivation (e.g., miR395, miR398, miR399, miR2111). miRNA mobility seems to be rather conserved, as several miRNAs, for example, miR156, miR167, miR169, miR390, and miR398, are phloem enriched in different species, such as rapeseed, cucurbits, apple, and mulberry, while others are absent (26, 77).

RNP: ribonucleoprotein

In addition to miRNAs, other small RNAs, such as siRNAs, tRNAs and tRNA halves, and rRNAs, have been shown to be enriched in phloem sap, but these are not the focus of this review (39). Moreover, genomic analyses of grafted tissues identified numerous transposon-derived and phased secondary small interfering RNAs (phasiRNAs) that were capable of trafficking from shoots to roots and inducing genomic methylation changes or precursor cleavage in target tissues, respectively (44, 56).

3.3. Phloem RNA-Binding Proteins

It is well established that viral RNAs in the phloem are always found in ribonucleoprotein (RNP) complexes with viral proteins and that host factors can assist virus phloem transport (34). Also, the first plant endogenous phloem RBPs interacting with mobile RNAs were found by studying host factors interacting with viroids and viruses. The abundant phloem lectin PHLOEM PROTEIN 2 (PP2) seemed to bind RNA in a non-sequence-specific manner and facilitated the translocation of viroid RNA in Cucurbitaceae heterografts through the phloem (29).

Researchers estimate that 10% of the phloem proteome are RBPs, and the binding of RBPs to phloem-mobile RNAs may be important for loading, transport, and unloading of RNAs (45, 69, 88). A significant part of the phloem proteome encompasses RBPs with known RNA-binding domains, including ribosomal proteins and proteins involved in translation initiation and elongation. Furthermore, glycine-rich RBPs and several uncharacterized proteins with unknown functions have been identified (28). It seems highly likely that these phloem RBPs are involved in RNA transport. In *Cucurbita maxima*, CmPP16 has been shown to bind to its own RNA to form an RNP complex. This complex interacts with a PD-associated protein, NCAPP1, and can thereby facilitate RNA entry into the phloem (73, 84).

CmRBP50 from pumpkin has been shown to preferentially bind to mRNAs harboring the polymer-pyrimidine CUCU domain. This leads to the formation of a large RNA–protein complex with additional proteins that was proposed to facilitate long-distance RNA transport via the phloem (30). The RBP50 homologs in potato, StPTB1 and StPTB6, can bind to the 3′ UTR region of StBEL5 mRNA to facilitate phloem transport and improve tuber yield (15). However, again highlighting the complexity of the RNA transport system, no significant enrichment of CUCU sequences was found in the graft-mobile cucurbit and *Arabidopsis* mRNA population when compared to the overall mRNA population (86, 87). Additionally, studies in other plant species suggest that long-distance translocation via the phloem occurs in the form of RNPs (29, 30, 62, 63).

4. SELECTIVITY OF RNA TRANSPORT

There are different interpretations of the term selective. We use one of the common definitions (55) of selective: the discriminatory power of a process to act on a defined subset. For an RNA transport process to be selective, we would expect that from a pool of different RNA molecules only a subset would be transported. If a process is 100% selective (in this case meaning that only those RNA molecules with the same properties are transported), it is said to be specific (55).

Since the discovery of the high numbers of RNAs detected in phloem sap and identified as graft-mobile, researchers have been debating the origin of mobile RNAs and whether transport is selective, and the following sections will discuss these open questions. Selectivity could occur

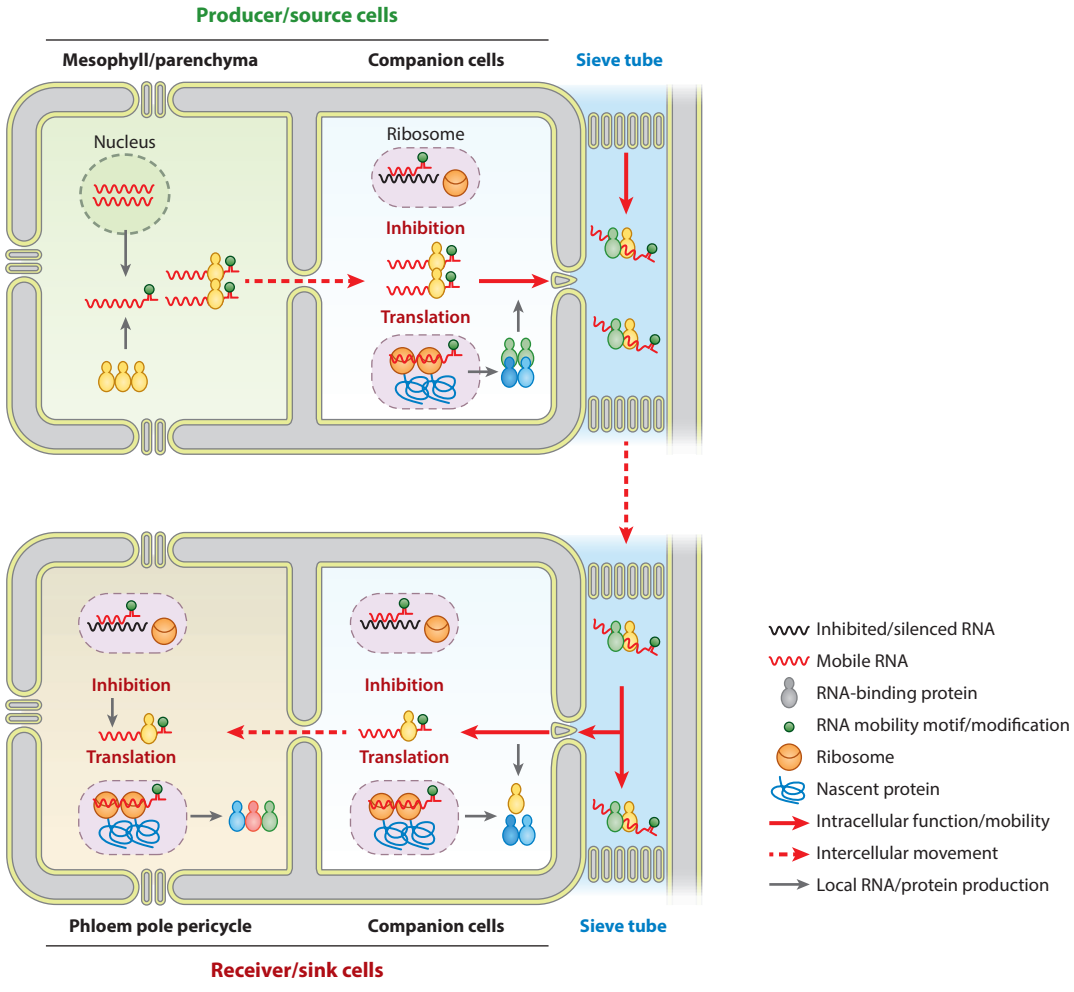

Figure 2

Schematic drawing showing the transport of RNAs and RNA-binding proteins via the phloem. Small RNAs and messenger RNAs (mRNAs) move as RNA–protein complexes from source cells, such as mesophyll and/or companion cells, into the sieve tubes. The origin is likely dependent on the phloem loading type. The exported RNA–protein complexes move via the phloem bulk flow from producer/source cells to receiver/sink cells. In the receiver/sink cells, the phloem-mobile RNA–protein complexes are unloaded. Here, the delivered microRNAs negatively regulate protein translation, whereas mobile mRNAs can be translated to proteins.

at any stage of transport: translocation to and movement through PD, entry into the phloem, transport in the phloem, or export from the phloem into target tissue (**Figure 2**).

4.1. What Is the Origin of Mobile RNAs?

The phloem transport complex consists of companion cells (CCs) and SEs that are the result of the division of a common mother cell. They stay intimately connected by specialized PD called pore–plasmodesma units (PPUs) that have, compared to other PD, a high size exclusion limit (SEL), allowing the passage of macromolecules of up to about 70 kDa or larger depending on their Stokes radius (66). To fulfil their function as transport conduits, SEs differentiate and lose their nuclei and remodel their organelles and cytoplasmic structures when they maturate.

CC: companion cell

Therefore, it cannot be ruled out that the contents of the formerly intact SEs may still be present in SEs, and this might explain the high number and diversity of macromolecules observed in these conduits (42, 61). In addition, the SEL of PPUs should prevent the passage of particles as large as the ribosome or proteasome components identified in exudates (63). This should also hold true for large RNAs bound to RBPs. If loading through PD from neighboring cells into SEs is impossible, large complexes can only originate from within the sieve tube system itself. Knoblauch et al. (42) estimated the expected concentration of macromolecules in phloem exudate based on the assumption that they are remnants from immature SEs. Their estimates suggest that components of SEs prior to differentiation might contribute significantly to the composition of phloem exudates and that the mere presence of a molecule in the translocation stream does not imply a specific function in the phloem or the target tissues (42). However, several observations cast doubt on all mobile RNAs being cellular leftovers.

4.2. Is Loading of Graft-Mobile Messenger RNAs Selective?

For diffusion, molecular size is a key determinant of movement, yet such correlations are at best only very weak for long-distance mRNA transport (14). Given current estimates of size exclusion limits and molecular stoke radiuses of transported macromolecules, mRNA molecules would likely be too large to diffuse from cell to cell and into SEs and would require an active transport mechanism. Accordingly, the transport mechanisms for small RNAs and mRNAs might be different (44). Furthermore, different phloem anatomy and loading modes might also be important. In *N. benthamiana*/*Arabidopsis* (57) and *N. benthamiana*/tomato (83) heterografts, belonging to the mainly apoplastic loading species like most herbaceous plants, no correlation between the mobile RNA and the level of the transcripts in source leaves was found. However, in grapevine, a woody species where symplastic loading is driven by diffusion through PD, 17 of the 33 highly abundant leaf transcripts were graft mobile (87). Researchers proposed that highly expressed mRNAs are more likely to reach the phloem only in symplastic loaders with numerous PD connections between mesophyll and phloem cells. In apoplastic loading species with a CC–SE complex highly isolated from the surrounding leaf cells, such as *A. thaliana*, the expression levels in CCs might be the suitable reference (74, 83). In watermelon/bottle gourd grafts, researchers also observed that most mobile mRNAs are lowly or very lowly abundant in tissues (81). Cucurbitaceae belong to the third category of phloem loading called polymer trapping, and the RNA loading mechanism could again differ from the two other loading types.

For *Arabidopsis*, researchers have postulated that mRNAs in CCs with higher abundances are more likely to be mobile (14). However, studies that overexpressed a few selected mRNAs did not observe increased mobility in mRNAs or mobility of reporter transcripts (66, 83, 86, 91). Despite this, detection of mRNA mobility could be influenced by RNA stability, secondary modifications, RNA motif(s), interactions with RBPs, or retention mechanisms. Comparing the relatively small number of mobile transcripts in *Arabidopsis*/*N. benthamiana* grafts, no transcript length dependence and no previously known sequence motifs in promoter or transcript sequences were found (57). However, others reported that tRNA-like sequence (TLS) motifs are significantly enriched in and make up 11% of the graft-mobile mRNA population (92). Deletion of such motifs could abolish the mobility of the *CK1* transcript, while the addition of the same or related TLS motifs to the normally immobile *GUS* or *DMC1* transcripts triggered mobility (92). However, many transcripts with a predicted TLS motif in leaves of *N. benthamiana*/tomato heterografts did not seem to move to roots. Although only a very small number of mobile transcripts were identified in these grafts and dicistronic occurring TLS motifs were not considered, the authors concluded that TLS motifs are only effective in mRNAs transcribed in CCs (83).

Also, posttranslational modifications are suspected to influence RNA transport selectivity. Five-methylcytosine (m^5C) methylation was found to be highly enriched in graft-mobile mRNAs, and two transcripts, *TCTP1* and *HSC70.1*, were not graft mobile in RNA methylation-deficient mutants in the juvenile growth phase (86).

Recent research using fluorescence-based RNA labeling also suggests that some mobile mRNAs are selectively and actively targeted to PD, whereas nonmobile mRNAs are not. It was suggested that plant RBPs could be involved in PD targeting (53). These RBPs could recognize specific sequence or structural motifs and facilitate transport (53), which is in line with the observation that transcript mobility of, e.g., TCTP1 depends on a relatively small sequence stretch and not on its abundance (86). Also, modifications like methylation could influence RNA structure and RBP interactions. As described above, many RBPs could be identified in phloem exudates, and their involvement in targeting mRNAs to PD and facilitating phloem transport remains to be investigated.

From the existing data, we conclude that the phloem import of at least some mRNAs appears to be selective but not all mobile mRNAs are the result of selectivity. Accordingly, researchers have proposed that RNA mobility has both regulated and nonregulated components (83) similar to those suggested for protein mobility (13, 32, 89). While there is evidence for motifs as well as for methylation playing a role in import (47, 86), what this selection is based on remains an open question.

If a subset of mRNAs were being selected, researchers might expect that this would be based on their functionality, yet Gene Ontology (GO) analyses of mobile mRNA species report over-representations of very different and diverse GO terms. This broad distribution of functions does not suggest that specific molecular processes are being selected for.

4.3. Is Loading of Mobile MicroRNAs Selective?

Researchers have suggested that macromolecules up to a given size can enter phloem sieve tubes by unselective leakage from the phloem-associated CCs (14, 66). Given their small size, miRNAs would be good candidates for diffusion through the PPUs that have an exceptionally high SEL (66). Such a passive mechanism would, however, not rule out selectivity. For instance, if transport through phloem PD was diffusive (passive), the level of miRNAs translocated from CCs to SEs still might not directly reflect their level of expression in CCs, but selectivity might still depend on their retention, their degradation rate in CCs, or their posttranscriptional modifications that might affect the interaction with RBPs facilitating transport.

Phloem sap has been shown to contain a larger population of miRNAs that differs from that of leaves and roots. Also, miRNA responses to nutrient stress differed from those of the other tissues (11). However, since the translocated miRNAs, at least in apoplastic loaders (see Section 4.2), can be expected to be synthesized mainly in CCs, a comparison between crude extract and phloem sap might be misleading, and cellular resolution would be required. Some miRNAs have not been found in phloem samples at all, e.g., miR171 (11, 12, 65, 88). This could be most easily explained if miR171 is not expressed in CCs. Selectivity could, of course, also be achieved by specific modifications that alter intercellular localization, PD permeability, and/or interactions with RBPs. In addition, selective retention mechanisms could be active in CCs. In plant crude extracts, for example, miRNAs with highly different AGO loading capacities were found by size-separating small RNA pools. Some mature miRNAs appeared in the small fraction that was not bound in protein complexes (16). It seems likely that the efficiently AGO-bound miRNAs could be excluded from movement unless they are highly expressed. This loading efficiency

differed between leaves and flowers (16), and one could speculate that it is also cell-type specific in CCs.

4.4. What Happens After Loading?

After entering SEs, imported molecules are expected to be passively swept away with the translocation stream driven by bulk flow, although some unexpected root-to-shoot movement was observed in a few grafting studies (see Section 3.1). In addition, in long-stem grafts between *N. benthamiana*/tomato, a significant number of transcripts passed the graft junction but disappeared during their movement from shoot to root, including some of those with TLS motifs (83). Since phloem sap lacks RNase activity (22, 27, 70, 90), this suggests potentially selective mRNA unloading along the transport route. Here, RBPs could provide selectivity and specificity through binding and exporting individual RNA molecules. A similar turnover of phloem proteins was first proposed based on radioactive labeling experiments combined with aphid stylet sampling at different locations along the transport pathway in wheat (24). Fisher et al. (24) proposed a highly selective protein turnover in the transport phloem and a nonselective unloading in sink tissues, which contrasted with findings in similar experiments performed on rice (3). Here, the abundant CmPP16 phloem protein was injected and shown to be specifically allocated to distant tissues. These insights suggest that some small macromolecules can be selectively distributed, whereas most move without restrictions and only large molecules (>50 kDa) require facilitating mechanisms (reviewed in 61).

5. MOBILE RNAS AS SIGNALING AGENTS

Mobile miRNAs and mRNAs have the capacity to act as long-distance signals. However, considering the large number of mobile RNAs, it seems unlikely that all of these RNAs function in long-distance signaling. But no matter how active, passive, or (non)selective RNA long-distance transport is, the mobile molecules might still serve a function in the phloem and/or in distant tissues, but for most mobile RNAs the potential functions are yet unclear. In the easiest interpretation, they could deliver carbon and nitrogen skeletons to sinks (42). However, there is strong evidence that at least some mobile RNAs are involved in coordinating stress responses and growth within the plant body.

5.1. Messenger RNAs as Signals

Some specific mobile mRNAs have been shown to play important regulatory roles in plant development in recipient sink organs such as young leaves, roots, and potato tubers (4, 41, 58). In grafted plants in different species, *GAI* mRNA moved from source to sink over graft junctions and could be correlated to changes in growth (33, 35, 85). Similarly, mobile mutant homeodomain transcription factor fusions were shown to induce changes in leaf shape in tomato (41). In contrast to *GAI* or homeodomain transcription factors, mRNAs encoding *GFP*, *YFP*, *GUS*, or other markers such as herbicide (BASTA) resistance did not move from transgenic *Arabidopsis* rootstocks to wild-type scions (33, 35, 86, 91). Also, the potato *StBEL5* mRNA is known to move from the leaf via the phloem to roots and stolons to induce tuberization (4, 31). In *Arabidopsis*, *AtIAA18* and *AtIAA28* transcripts are synthesized in mature leaves and can move to roots to regulate lateral root development (58). Also, shoot-derived *AtTCTP1* mRNA stimulates the emergence of lateral roots along the primary root (10), and mobile *CK1* enhances shoot growth (91). Although it is compelling to assume that the above examples correlate with the expression of a functional protein from the mobile transcript, currently only two conclusive

reports demonstrate, based on *GUS-TLS* and *YFP-TCTP1* fusion constructs, that translation of phloem mobile transcripts in target tissues can occur (86, 91).

In addition to development, some mRNAs could potentially be involved in nutrient stress responses. In *Arabidopsis*, a subset of translocated mRNAs was reported to be induced under phosphorus or nitrogen starvation (74). Even more than 3,000 mRNAs were phloem-mobile in cucumber/watermelon heterografts in response to phosphorus deficiency (92). A more recent study identified mobile mRNAs specifically induced by low nitrogen, phosphorus, or iron in *N. benthamiana*/tomato heterografts (82). Of the 294 mobile mRNAs identified in total, 112 appeared under all growth conditions, indicating that they are not involved in information transfer. Some of the stress-induced mRNAs could potentially be related to altered root development. A comparison to the results in the other graft systems yielded no overlapping mRNAs to the *Arabidopsis* system and only four to the cucumber/watermelon system. This suggests that, if the transported mRNAs are involved in specific stress responses, these responses are species-specific and not conserved. However, the lack of overlap of stressed-induced mobile mRNAs could also be explained by the different grafting systems, growth conditions, and potential detection issues such as limited number of replicates, depth, and distinguishable SNPs and INDELs or by sequencing quality, bioinformatic pipelines used to identify heterologous RNAs, and the sequencing method used. It should also be noted that most studies did not, or were not able to, assess whether the identified mobile mRNAs are full length and did not include this criterion in the discussion of potential physiological functions.

It is puzzling that many mobile transcripts are transported to tissues where they are already expressed and noteworthy that the fraction of imported mRNA originating from source tissue is extremely small compared to its expression in the destination tissue. Transfer ratios between source and destination tissue have been estimated to be between 0.00001% and 0.76% for mRNA in grapevine grafts, with most transcripts having transfer values of less than 0.01% (87). This suggests that only a small fraction of any one type of mRNA is transported. These transport ratios could be modulated in response to environmental cues, but how can these mobile transcripts arriving in target tissues be distinguished from the locally transcribed mRNAs and thus impose their function? Could differential responses in distal tissues be evoked by secondary RNA modifications that enhance the translation or stability of transported mRNAs, as suggested for m^5C transcripts (9)?

Taken together, the available data show that hundreds to thousands of different mobile mRNAs are transported but in relatively low concentrations compared to their local expression levels. A few specific mobile mRNAs have been shown to be important for the regulation of developmental processes, but the biological functions for most mobile transcripts are yet to be determined.

5.2. MicroRNAs as Signals

The first evidence for a mobile miRNA signal came from two independent reports (46, 64) demonstrating that miR399, a phosphate starvation–inducible miRNA, strongly accumulated in rapeseed phloem sap under stress and could move across graft junctions from miR399-overexpressing scions to wild-type rootstocks to degrade its target transcript *PHOSPHATE 2* (*PHO2*) and thus regulate phosphate homeostasis. Similar observations in wild-type *Arabidopsis* and *hen1-1* mutant heterografts confirmed the shoot-to-root mobility of miR399 and the sulfate deficiency–responsive miR395 under the respective nutrient starvation (11). As observed for miR399, the translocation of miR395 led to the downregulation of one of its target mRNAs, *APS*, in roots. Another study using wild-type/hen1 micrografts showed that in addition to miR399, miR827 and miR2111 were also phosphate responsive and graft transmissible (36). In addition,

miR398 was shown to accumulate in phloem sap during copper starvation, but the study did not attempt to confirm its mobility in grafts (12). In all of these grafting studies in *Arabidopsis*, transport was almost exclusively observed from scions to rootstocks and from high-expressing to low- or no-expressing tissues. Also, in soybean/common bean heterografts, all miRNAs and other small RNAs were synthesized in shoots and transported almost exclusively to roots where they were not expressed, suggesting the functional importance of the transfer (44).

In addition to nutrient stress responses, phloem miRNAs were analyzed in the context of infection and development. miR2111 has been shown to stimulate root nodule formation in leguminous plants during infection by nitrogen-fixing bacteria (59, 76). Mulberry miRNA (mul-miR482a-5p) was upregulated in phloem sap upon infection by phytoplasmas, and researchers suggested that it might negatively regulate resistance to infection (26). Other examples of the involvement of miRNAs in the systemic regulation of development stem from potato, where miR172 could induce tuberization, and this effect is graft transmissible from leaf to stolon (54). In tobacco heterografts, a role of miR172 in the establishment of source–sink relationships was suggested (38). miR156 was shown to move across graft unions in potato from overexpressing scion to wild-type stock and affect the leaf trichome shape, while its precursor was nonmobile (8). Accordingly, no miRNA precursors could be detected in *Brassica napus* phloem samples by Northern blotting (12, 64). These observations support the general notion that mature miRNAs, rather than their precursors, are the transported form. However, a more recent study claimed to find particular primary miRNAs (pri-miRNAs) in RNA-seq data sets from *C. maxima* phloem samples, which they confirmed by RT-PCR (75).

In addition to sense miRNA strands, some of their near-complementary miRNA* strands are also thought to be mobile. One study demonstrated that, in addition to miR399, its miR399* strand was mobile in *Arabidopsis* grafting experiments. In the case of mobile miR827 and miR2111, the miRNA* strands were not graft translocated (36). However, miR399*, miR827*, and miR2111* were detected in phloem samples in *Brassica* (65). Also, other miRNA*s were detected in *Brassica* phloem (12). In all studies, miRNA*s were not present at concentrations corresponding to their sense miRNA strands. Nutrient starvation responses were observed for the phosphate-responsive 399 and 2111 miRNA*s in phloem sap (65) but not for miR395* and miR398* under sulfur or copper starvation, respectively (12). This might suggest that miRNAs are mobile, as single strands and miRNA*s might exert specific functions (36). However, issues with selective instability of one or the other strand during transport or sample preparation cannot be excluded. Taken together, the available data suggest that specific mobile miRNAs (and other small RNAs) seem to qualify well as potential long-distance signals.

6. CONCLUSIONS AND FUTURE DIRECTIONS

An increasing wealth of data demonstrates that different types of RNAs are present in phloem sap and can be transported between tissues of grafted plants. Only a subset of the transcribed RNAs is mobile, suggesting selectivity, but the physical or chemical nature of this selection remains to be determined, as does the biological function of most mobile RNAs. Existing data point to physiological and signaling roles, but it seems unlikely that all mobile RNAs are signals. Identifying potential RNA-signaling molecules from this plethora seems like looking for a needle in a haystack.

But which criteria must be met by a given mobile RNA to qualify as a signaling molecule? (*a*) The mobile RNA population must change over time and in response to a stimulus; i.e., it must encode something. (*b*) The mobile RNA molecule must be produced in source tissues, be present in the phloem, and leave SEs in target tissues; i.e., it must be transmitted. (*c*) The mobile RNA

must be functional after transport (this likely means that the full-length RNA must be mobile); i.e., there must be a decoding process.

For many miRNAs, the signaling role is well established. In the example of miR399, (*a*) its expression changes over time and in response to phosphate starvation; (*b*) it is expressed in the shoot and then transported to the root; and (*c*) the mode of action of miR399 is well known, and its binding to target mRNAs provides signal amplification and readout mechanisms. So the encoding, transmission, and decoding pipeline is instantiated in a way that is consistent with a communication and signaling system.

For transported mRNAs, strong data to support a physiological function are available for only very few cases, and questions remain about the potential function of the hundreds to thousands of mRNAs that are transported over long distances. A signaling role has been hypothesized, but evidence is sparse. Many mRNAs are transported to tissues where they are already transcribed. How might these transported mRNAs be distinguished from the locally produced mRNAs; how might this signal be amplified, and what functionality would be gained? Or are most of the mobile transcripts just playing a role by providing redundancy and thus robustness to the biological system?

Mobile miRNAs play crucial roles in plant development and stress responses, and evidence also supports important functions for mobile mRNAs. The main challenge now is to identify and understand the signaling pathways based on mobile RNA molecules within and across species, to reveal their biological functions, and to understand how miRNA and mRNA molecules are licensed with a travel permit. This will be a tedious task, given that so many transcripts are exchanged between tissues and that RNA modifications and interactions with specific RBPs could modulate not only their transport but also their activity in receiving cells. The development of a predictive framework for identifying mobile RNAs and assessing their impact on signaling or physiological processes will be key for unraveling the mysteries of mobile RNA and for putting our understanding to the test.

SUMMARY POINTS

1. Long-distance transport of RNA in plants is supported by an increasing wealth of experimental data from several species using a variety of techniques.
2. The exchange of RNA between tissues within a plant as well as between species (grafting, parasitic plants) is on a genomic scale.
3. There is evidence to support the theory that the long-distance transport of some RNAs is selective, but the features and mechanisms for this selectivity remain to be determined.
4. Specific mobile messenger RNAs (mRNAs) have been shown to be important for a number of developmental processes.
5. Mobile microRNAs show clear characteristics of signals, while similar roles for mRNAs are possible but are yet to be confirmed.
6. Little is known about the transport mechanisms of mobile RNAs and the involvement of RNA-binding proteins.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We apologize to all colleagues contributing to RNA transport in plants whose valuable work and insights are not discussed in this review due to space limitations. This article is part of a Synergy project that is receiving funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement no. 810131).

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. Amsbury S, Kirk P, Benitez-Alfonso Y. 2017. Emerging models on the regulation of intercellular transport by plasmodesmata-associated callose. *J. Exp. Bot.* 69:105–15
3. Aoki K, Suzui N, Fujimaki S, Dohmae N, Yonekura-Sakakibara K, et al. 2005. Destination-selective long-distance movement of phloem proteins. *Plant Cell* 17:944–56
4. Banerjee AK, Chatterjee M, Yu Y, Suh S-G, Miller WA, Hannapel DJ. 2006. Dynamics of a mobile RNA of potato involved in a long-distance signaling pathway. *Plant Cell* 18:3443–57
5. Bartusch K, Melnyk CW. 2020. Insights into plant surgery: an overview of the multiple grafting techniques for *Arabidopsis thaliana*. *Front. Plant Sci.* 11:613442
6. Bennett CW. 1940. Acquisition and transmission of viruses by dodder (*Cuscuta subinclusa*). *Phytopathology* 30:2
7. Bennett CW. 1944. Studies of dodder transmission of plant viruses. *Phytopathology* 34:905–32
8. Bhogale S, Mahajan AS, Natarajan B, Rajabhoj M, Thulasiram HV, Banerjee AK. 2014. *MicroRNA156*: A potential graft-transmissible microRNA that modulates plant architecture and tuberization in *Solanum tuberosum* ssp. *andigena*. *Plant Physiol.* 164:1011–27
9. Bohnsack KE, Höbartner C, Bohnsack MT. 2019. Eukaryotic 5-methylcytosine (m^5C) RNA methyltransferases: mechanisms, cellular functions, and links to disease. *Genes* 10:102
10. Branco R, Masle J. 2019. Systemic signalling through translationally controlled tumour protein controls lateral root formation in Arabidopsis. *J. Exp. Bot.* 70:3927–40
11. Buhtz A, Pieritz J, Springer F, Kehr J. 2010. Phloem small RNAs, nutrient stress responses, and systemic mobility. *BMC Plant Biol.* 10:64
12. **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**
13. Burch-Smith TM, Zambryski PC. 2012. Plasmodesmata paradigm shift: regulation from without versus within. *Annu. Rev. Plant Biol.* 63:239–60
14. Calderwood A, Kopriva S, Morris RJ. 2016. Transcript abundance explains mRNA mobility data in *Arabidopsis thaliana*. *Plant Cell* 28:610–15
15. Cho SK, Sharma P, Butler NM, Kang I-H, 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
16. Dalmadi Á, Gyula P, Bálint J, Szittya G, Havelda Z. 2019. AGO-unbound cytosolic pool of mature miRNAs in plant cells reveals a novel regulatory step at AGO1 loading. *Nucleic Acids Res.* 47:9803–17
17. 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
18. 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
19. Dinant S, Bonnemain J-L, Girousse C, Kehr J. 2010. Phloem sap intricacy and interplay with aphid feeding. *C. R. Biol.* 333:504–15
20. Dinant S, Kehr J. 2013. Sampling and analysis of phloem sap. *Methods Mol. Biol.* 953:185–94
21. **Ding B. 2009. The biology of viroid-host interactions. *Annu. Rev. Phytopathol.* 47:105–31**
22. Doering-Saad C, Newbury HJ, Bale JS, Pritchard J. 2002. Use of aphid stylectomy and RT-PCR for the detection of transporter mRNAs in sieve elements. *J. Exp. Bot.* 53:631–37

12. Identification of miRNAs in phloem sap as nutrient starvation signals in *Brassica napus*.

21. Discovery that viroid RNAs form distinct motifs that are necessary for movement through plasmodesmata.

23. 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
24. Fisher DB, Wu Y, Ku MSB. 1992. Turnover of soluble proteins in the wheat sieve tube. *Plant Physiol.* 100:1433–41
25. Gai Y-P, Yuan SS, Liu ZY, Zhao HN, Liu Q, et al. 2018. Integrated phloem sap mRNA and protein expression analysis reveals phytoplasma-infection responses in mulberry. *Mol. Cell Proteom.* 17:1702–19
26. Gai Y-P, Zhao H-N, Zhao Y-N, Zhu B-S, Yuan S-S, et al. 2018. MiRNA-seq-based profiles of miRNAs in mulberry phloem sap provide insight into the pathogenic mechanisms of mulberry yellow dwarf disease. *Sci. Rep.* 8:812
27. 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
28. Giavalisco P, Kapitza K, Kolasa A, Buhtz A, Kehr J. 2006. Towards the proteome of *Brassica napus* phloem sap. *Proteomics* 6:896–909
29. Gomez G, Pallas 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
30. Ham BK, Brandom JL, Xoconostle-Cazares 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
31. Hannapel DJ, Banerjee AK. 2017. Multiple mobile mRNA signals regulate tuber development in potato. *Plants* 6:8
32. Haywood V, Kragler F, Lucas WJ. 2002. Plasmodesmata: pathways for protein and ribonucleoprotein signaling. *Plant Cell* (Suppl. 2002):S303–25
33. Haywood V, Yu T-S, Huang N-C, Lucas WJ. 2005. Phloem long-distance trafficking of *GIBBERELLIC ACID-INSENSITIVE* RNA regulates leaf development. *Plant J.* 42:49–68
34. Hipper C, Brault V, Ziegler-Graff V, Revers F. 2013. Viral and cellular factors involved in phloem transport of plant viruses. *Front. Plant Sci.* 4:154
35. Huang N-C, Yu T-S. 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
36. Huen AK, Rodriguez-Medina C, Ho AYY, Atkins CA, Smith PMC. 2017. Long-distance movement of phosphate starvation-responsive microRNAs in *Arabidopsis*. *Plant Biol.* 19:643–49
37. 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
38. Kasai A, Kanehira A, Harada T. 2010. miR172 can move long distances in *Nicotiana benthamiana*. *Open Plant Sci. J.* 4:1–6
39. Kehr J, Kragler F. 2018. Long distance RNA movement. *New Phytol.* 218:29–40
40. 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
41. 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
42. Knoblauch M, Peters WS, Bell K, Ross-Elliott TJ, Oparka KJ. 2018. Sieve-element differentiation and phloem sap contamination. *Curr. Opin. Plant Biol.* 43:43–49
43. Kühn C, Franceschi VR, Schulz A, Lemoine R, Frommer WB. 1997. Macromolecular trafficking indicated by localization and turnover of sucrose transporters in enucleate sieve elements. *Science* 275:1298–300
44. Li S, Wang X, Xu W, Liu T, Cai C, et al. 2021. Unidirectional movement of small RNAs from shoots to roots in interspecific heterografts. *Nat. Plants* 7:50–59
45. 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
46. 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
47. Liu N, Shen G, Xu Y, Liu H, Zhang J, et al. 2020. Extensive inter-plant protein transfer between *Cuscuta* parasites and their host plants. *Mol. Plant* 13:573–85

48. Liu N, Yang J, Guo S, Xu Y, Zhang M. 2013. Genome-wide identification and comparative analysis of conserved and novel microRNAs in grafted watermelon by high-throughput sequencing. *PLOS ONE* 8:e57359
49. Lopez-Cobollo RM, Filippis I, Bennett MH, Turnbull CG. 2016. Comparative proteomics of cucurbit phloem indicates both unique and shared sets of proteins. *Plant J.* 88:633–47
50. Lucas WJ. 1995. Plasmodesmata: intercellular channels for macromolecular transport in plants. *Curr. Opin. Cell Biol.* 7:673–80
51. Lucas WJ. 2006. Plant viral movement proteins: agents for cell-to-cell trafficking of viral genomes. *Virology* 344:169–84
52. Lucas WJ, Bouché-Pillon S, Jackson DP, Nguyen L, Baker L, et al. 1995. Selective trafficking of KNOTTED1 homeodomain protein and its mRNA through plasmodesmata. *Science* 270:1980–83
53. Luo K-R, Huang N-C, Yu T-S. 2018. Selective targeting of mobile mRNAs to plasmodesmata for cell-to-cell movement. *Plant Physiol.* 177:604–14
54. Martin A, Adam H, Diaz-Mendoza M, Zurczak M, Gonzalez-Schain ND, Suarez-Lopez P. 2009. Graft-transmissible induction of potato tuberization by the microRNA *miR172*. *Development* 136:2873–81
55. McNaught AD, Wilkinson A, eds. 1997. *Compendium of Chemical Terminology*: *IUPAC Recommendations*. Oxford: Blackwell Scientific Publications. 2nd ed.
56. Molnar A, Melnyk C, Bassett A, Hardcastle T, Dunn R, Baulcombe D. 2010. Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. *Science* 328:872–75
57. Notaguchi M, Higashiyama T, Suzuki T. 2015. Identification of mRNAs that move over long distances using an RNA-Seq analysis of Arabidopsis/*Nicotiana benthamiana* heterografts. *Plant Cell Physiol.* 56:311–21
58. 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
59. Okuma N, Soyano T, Suzaki T, Kawaguchi M. 2020. *MIR2111–5* locus and shoot-accumulated mature miR2111 systemically enhance nodulation depending on HAR1 in *Lotus japonicus*. *Nat. Commun.* 11:5192
60. 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
61. Oparka KJ, Cruz SS. 2000. The great escape: phloem transport and unloading of macromolecules. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 51:323–47
62. Ostendorp A, Pahlow S, Krüßel L, Hanhart P, Garbe MY, et al. 2017. Functional analysis of *Brassica napus* phloem protein and ribonucleoprotein complexes. *New Phytol.* 214:1188–97
63. Pahlow S, Ostendorp A, Krüßel L, Kehr J. 2018. Phloem sap sampling from *Brassica napus* for 3D-PAGE of protein and ribonucleoprotein complexes. *J. Vis. Exp.* 2018(131):e57097
64. 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
65. 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
66. Paultre DSG, Gustin M-P, Molnar A, Oparka KJ. 2016. Lost in transit: long-distance trafficking and phloem unloading of protein signals in Arabidopsis homografts. *Plant Cell* 28:2016–25
67. 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
68. Robards AW, Lucas WJ. 1990. Plasmodesmata. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 41:369–419
69. Rodriguez-Medina C, Atkins CA, Mann AJ, Jordan ME, Smith PMC. 2011. Macromolecular composition of phloem exudate from white lupin (*Lupinus albus* L.). *BMC Plant Biol.* 11:36
70. Sasaki T, Chino M, Hayashi H, Fujiwara T. 1998. Detection of several mRNA species in rice phloem sap. *Plant Cell Physiol.* 39:895–97
71. Sinha NR, Williams RE, Hake S. 1993. Overexpression of the maize homeo box gene, *KNOTTED-1*, causes a switch from determinate to indeterminate cell fates. *Genes Dev.* 7:787–95

53. Demonstration that targeting of mRNAs to PD is selective.

71. First evidence for intercellular transport of endogenous mRNA and protein.

72. Tangl E. 1880. Ueber offene Communicationen zwischen den Zellen des Endosperms einiger Samen. *Jahrb. Wiss. Bot.* 12:170–90
73. Taoka K-i, Ham B-K, 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
74. 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
75. Tolstyko E, Lezzhov A, Solovyev A. 2019. Identification of miRNA precursors in the phloem of *Cucurbita maxima*. *PeerJ* 7:e8269
76. Tsikou D, Yan Z, Holt DB, Abel NB, Reid DE, et al. 2018. Systemic control of legume susceptibility to rhizobial infection by a mobile microRNA. *Science* 362:233–36
77. Varkonyi-Gasic E, Gould N, Sandanayaka M, Sutherland P, MacDiarmid RM. 2010. Characterisation of microRNAs from apple (*Malus domestica* 'Royal Gala') vascular tissue and phloem sap. *BMC Plant Biol.* 10:159
78. Walther D, Kragler F. 2016. Limited phosphate: Mobile RNAs convey the message. *Nat. Plants* 2:16040
79. Wang J, Jiang L, Wu R. 2017. Plant grafting: how genetic exchange promotes vascular reconnection. *New Phytol.* 214:56–65
80. Wang Y. 2011. Plant grafting and its application in biological research. *Chin. Sci. Bull.* 56:3511–17
81. Wang Y, Wang L, Xing N, Wu X, Wu X, et al. 2020. A universal pipeline for mobile mRNA detection and insights into heterografting advantages under chilling stress. *Hort. Res.* 7:13
82. Xia C, Huang J, Lan H, Zhang C. 2020. Long-distance movement of mineral deficiency-responsive mRNAs in *Nicotiana benthamiana*/tomato heterografts. *Plants* 9:876
83. Xia C, Zheng Y, Huang J, Zhou X, Li R, et al. 2018. Elucidation of the mechanisms of long-distance mRNA movement in a *Nicotiana benthamiana*/tomato heterograft system. *Plant Physiol.* 177:745–58
84. Xoconostle-Cazares 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
85. Xu H, Iwashiro R, Li T, Harada T. 2013. Long-distance transport of *Gibberellic Acid Insensitive* mRNA in *Nicotiana benthamiana*. *BMC Plant Biol.* 13:165
86. Yang L, Perrera V, Saplaoura E, Apelt F, Bahin M, et al. 2019. m^5C methylation guides systemic transport of messenger RNA over graft junctions in plants. *Curr. Biol.* 29:2465–76.e5
87. Yang Y, Mao L, Jittayasothorn Y, Kang Y, Jiao C, et al. 2015. Messenger RNA exchange between scions and rootstocks in grafted grapevines. *BMC Plant Biol.* 15:251
88. Yoo B-C, 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
89. Zambryski P, Crawford K. 2000. Plasmodesmata: gatekeepers for cell-to-cell transport of developmental signals in plants. *Annu. Rev. Cell Dev. Biol.* 16:393–421
90. 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
91. 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
92. Zhang Z, Zheng Y, Ham B-K, Chen J, Yoshida A, et al. 2016. Vascular-mediated signalling involved in early phosphate stress response in plants. *Nat. Plants* 2:16033
93. 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

74. First demonstration of the genomic scale of long-distance mRNA transport across graft junctions in two *Arabidopsis* ecotypes.