

Review

Traveling with purpose: cell-to-cell transport of plant mRNAs

Munenori Kitagawa,¹ Thu M. Tran,² and David Jackson^{1,2,*}

Messenger RNAs (mRNAs) in multicellular organisms can act as signals transported cell-to-cell and over long distances. In plants, mRNAs traffic cell-to-cell via plasmodesmata (PDs) and over long distances via the phloem vascular system to control diverse biological processes – such as cell fate and tissue patterning – in destination organs. Research on long-distance transport of mRNAs in plants has made remarkable progress, including the cataloguing of many mobile mRNAs, characterization of mRNA features important for transport, identification of mRNA-binding proteins involved in their transport, and understanding of the physiological roles of mRNA transport. However, information on short-range mRNA cell-to-cell transport is still limited. This review discusses the regulatory mechanisms and physiological functions of mRNA transport at the cellular and whole plant levels.

New advances in research on noncell-autonomous mRNAs in plants

In the central dogma of molecular biology, DNA makes (messenger) RNA makes protein. More recently, the term messenger RNA (mRNA) has taken on a more profound meaning, since RNAs in plants – including mRNAs – can transmit short- and long-range signals ('messages') between different cells, tissues and organs [1,2]. **Plasmodesmata (PDs)** (see [Glossary](#)) [3] and the **phloem** mediate short- and long-distance RNA transport, respectively. Long-distance mRNA transport can act like an animal nervous system, transmitting exogenous stimuli to distant organs and inducing physiological, developmental, and defense responses [4]. Over the past decade our understanding of this process has significantly improved, while our knowledge of short-distance or cell-to-cell mRNA transport via PDs is still limited. For mRNAs to be transported over long distances, they need to be imported into the phloem at their source, and exported from the phloem at their destination. The **companion cells** serve as stations for this purpose [5]; they are connected by PDs and transport signals to the phloem **sieve elements**. PDs are highly regulated channels [2,6,7], and may confer selectivity between companion cells and sieve elements for long-distance mRNA transport [4,8]. PDs also selectively transport specific transcription factors from cell-to-cell to control the fate of recipient cells in diverse tissues [7]. In many cases, proteins rather than mRNAs were thought to be the mediators for signaling, and mRNAs were once believed to be nonmobile [9–12]. Despite the discovery of the first cell-to-cell mobile mRNA in plants 30 years ago [13], updates to our knowledge of this process have been limited. Recently, however, the association of mobile mRNAs with PDs and their regulatory factors and physiological functions have been revealed, confirming the importance of mRNAs as noncell-autonomous signals [14–16]. This review first summarizes findings on long-distance mRNA transport that provide important insights into the PD transport mechanism. We then discuss our current understanding of the mechanism and physiological roles of short-distance mRNA transport.

Long-distance transport of mRNAs through the phloem

Long-distance mRNA transport via the phloem has been widely studied [4,5,17]. **Grafting** experiments combined with transcriptome analysis identified hundreds to thousands of mobile mRNAs

Highlights

mRNAs traffic between plant cells/tissues/organs via plasmodesmata (PDs) and the phloem to act as noncell-autonomous signals controlling diverse biological processes.

Some RNA features and RNA-binding proteins important for long-distance mRNA phloem transport have been identified.

Transcription factor mRNAs and proteins selectively traffic from cell-to-cell via PDs to regulate cell fates and tissue patterning. Although their transport mechanisms are less well understood, integration of the available information allows the prediction of possible mechanisms.

The endomembrane system, cytoskeleton, motor proteins, RNA helicases, and RNA-processing proteins all participate in the cell-to-cell transport of mRNAs.

Cell-to-cell transport of mRNAs contributes to the propagation of mobile transcription factor signals.

¹National Key Laboratory for Germplasm Innovation & Utilization of Horticultural Crops, Huazhong Agricultural University, Wuhan 430070, PR China

²Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA

*Correspondence: jacksond@cshl.edu (D. Jackson).

[4]. These mRNAs could be transported in a selective or a nonselective manner [8,17]. Indeed, computational modeling found that the mobility of many mRNAs depends on their abundance, half-life, and transcript length, suggesting that their movement is nonselective [8]. However, some mRNAs appear to be transported selectively to regulate organ development and growth [17], and the underlying mechanism of this transport is an area of active research. For example, RNA motifs such as polypyrimidine (poly-CU) sequences, secondary structures such as stem-loops in untranslated regions, transfer RNA (tRNA)-like tertiary structures (TLSs), and RNA modifications such as methylated cytosines (m5C) are enriched in mobile mRNAs, and in some cases are found to be sufficient for transport [2,18]. Specific RNA-binding proteins (RBPs) appear to interact with these different features to promote the transport of certain mRNAs [4,18]. For example, polypyrimidine tract binding (PTB) proteins identified in pumpkin, potato, and pear bind to poly-CU sequences in target mRNAs, and promote their phloem transport to regulate organ development [18,19]. PTBs have diverse functions, including in stabilization and selective intracellular transport of mRNAs in animal systems [20]. In plants, overexpression of PTBs promotes the stability and transport of their target transcripts, and PTB downregulation has an opposite effect [21]. Thus, PTBs may localize target mRNAs to PDs for their phloem import and/or stabilize mRNAs for long-distance transport. Phloem, however, lacks RNase activity [4,5], and many mRNAs that do not have CU-rich sequences can traffic nonspecifically [8,22]. Therefore, PTBs may facilitate transport of CU-rich mRNAs in addition to stabilizing them.

Possible functions of long-distance transported mRNAs

Plants lack a central nervous system, but can transmit localized internal and external stimuli to distant organs [23]. Signaling via the phloem is one way to systemically coordinate growth and stress responses. Some mRNAs act as mediators in this signaling [4]. For example, potato **BEL1-like transcription factor** *StBEL5* mRNA is transported from leaves to roots and **stolons** through the phloem to regulate tuberization [24]. PTBs bind to the poly-CU motif of *StBEL5* mRNA to stabilize it and promote its phloem transport. Short days upregulate the expression of both *StBEL5* and PTBs, and activate *StBEL5* mRNA transport, promoting root and tuber growth. Thus, *StBEL5* mRNA communicates changes in day-length perceived in the leaves to the underground organs to regulate their development. In addition, grafting experiments have identified mRNAs whose transport between shoot and root depends on nutrient conditions [22,25,26]. Such mRNAs include ones that function in hormone metabolism and root architecture. Therefore, mRNAs may communicate stresses caused by nutrient limitation to distant organs via the phloem. However, how the transported mRNAs function in destination organs remains to be confirmed.

Stabilizing mRNAs in the phloem assists in effectively transmitting external stimuli, as PTB does for *StBEL5* mRNA. For example, phloem mobile chaperone proteins bind to their mRNAs and impact developmental events such as flowering and root growth in *Arabidopsis thaliana* [27]. The binding of these chaperone proteins to their mRNAs facilitates phloem transport and represses their translation. Therefore, chaperone mRNA mobility and negative feedback control of translation may maintain systemic chaperone homeostasis to coordinate the growth of distant organs when plants are exposed to localized external stimuli.

Knowledge of mobile RNA signals has been exploited to improve clustered regularly interspaced short palindromic repeats (CRISPR) gene editing. Specifically, single guide RNAs (sgRNAs) were fused with an RNA mobility element, such as a TLS, and transferred into leaves of CRISPR-associated protein 9 (Cas9)-expressing plants using an RNA virus. These engineered mobile sgRNAs were transported systemically via the phloem and could access the germline to produce heritable genome edits [28–30]. In a more recent study, both Cas9 mRNA and sgRNAs were fused with TLS signals, leading to their transport from transgenic rootstocks to grafted wild-type scions to

Glossary

Apoplast: the extracellular space, including the cell wall, outside the plasma membrane of a plant cell.

BEL1-like transcription factor: a plant homeodomain transcription factor that interacts with KNOTTED1-like homeodomain transcription factors to control diverse developmental processes, such as meristem and floral development.

Circular RNA (circRNA): a class of noncoding RNAs that are covalently closed to form circular molecules.

Companion cell: a cell that forms part of the phloem system and mediates the loading and unloading of molecules into the phloem sieve element cells.

Grafting: an approach used in a wide range of agricultural species to improve crop traits. Two or more segments of tissue derived from different plants are joined and connected by tissue regeneration to grow as a single plant.

Meristem: an indeterminate structure containing stem cells that divide and generate diverse tissues and organs.

Moonlighting function: a noncanonical role of a protein beyond that predicted by its sequence homology.

MS2 system: a technique for visualizing RNA *in vivo* that relies on the interaction between MS2 bacteriophage coat protein (MCP) and a specific RNA motif: the MS2-binding sequence (MBS). The RNA of interest is tagged with MBS and coexpressed with MCP fused with a fluorescent protein, resulting in the fluorescent labeling of the RNA.

Phloem: a vascular tissue that systemically transports sugars, metabolites, proteins, and nucleic acids in plants.

Plasmodesmata (PDs): membrane-lined nano-channels that traverse the cell wall and connect the cytoplasm of most plant cells. PDs transport various signals necessary for plant physiology, development, and defense. They also provide access to the phloem for systemic transport.

RNA exosome complex: a multiprotein complex that processes and degrades diverse classes of RNA.

ROTAMASE CYCLOPHILINS: organelle-localized RNA-binding proteins that belong to the cyclophilin family. They are ubiquitously expressed and possess peptidyl-prolyl *cis-trans* isomerase activity.

Sieve element: a living phloem cell that lacks many organelles, including a

produce genome-edited transgene-free seeds [31]. Thus, RNA transport studies can be applied in crop engineering and improvement, with the potential to engineer varieties that are recalcitrant to transformation. Furthermore, Cas9–TLS mRNA transport provides a deeper insight into the mechanism of selective phloem transport. The molecular size of Cas9 mRNA (~1400 kDa) is enormous compared to the size exclusion limit of PD connecting to the phloem (~70 kDa) [32]. It is likely that specific RBPs that bind to TLS convert the entire mRNA structure, including Cas9, into a mobile form, allowing PD transport [1]. TLS sequences also confer directionality, for example in allowing mRNAs to traffic from root to shoot against bulk phloem flow [31]. Mobile mRNAs form ribonucleoprotein (RNP) complexes in the phloem during long-distance transport [5], and these complexes may contain proteins that confer destination information [22]. Proteome analysis using TLS-containing RNAs as baits is one approach to identify RBPs that mediate PD transport and selective organ targeting [33].

Cell-to-cell transport of mRNAs through PDs

As we have discussed, recent insights have emerged into mechanisms of long-distance mRNA transport, while knowledge of short-distance transport via PDs is limited due to a lack of examples and technical constraints. Many proteins are transported cell-to-cell and act as mobile signals [7,34]. In contrast, mRNAs were once assumed to be nonmobile, because protein mobility was inferred by comparing mRNA and protein localization [9–12]. However, it has long been known that viral RNAs can move between cells. A viral movement protein (MP) was the first macromolecule found to move cell-to-cell in plants. MPs interact with PDs to enhance PD transport and facilitate the transport of the viral RNA or DNA genome [35]. This discovery provided insight into identifying the first plant endogenous mRNA that traffics cell to cell, encoded by the maize *KNOTTED1* (*KN1*) gene [13]. *KN1* was the first homeobox transcription factor to be identified in plants, and is expressed in shoot **meristems** where it functions to maintain stem-cell identity [36]. *KN1* mRNA mobility between cells was demonstrated by microinjection experiments in tobacco leaves [13]. Fluorescently labeled *KN1* mRNA did not traffic to neighboring cells when injected alone into single mesophyll cells, but it did traffic when injected with *KN1* protein. This result suggests that *KN1* mRNA is noncell-autonomous, and its transport is facilitated through interaction with *KN1* protein. This finding was confirmed after developing a ‘**trichome** rescue system’ [37]. This system converts the *KN1* mobility between cells into an easy visual phenotype of trichome formation [38]. In a modified system, where *KN1* mRNA is expressed but not translated, trichomes did not form. However, adding back *KN1* protein expression resulted in trichome formation [37]. These results support the idea that *KN1* protein interacts with its mRNA to promote its trafficking between cells. In support of this idea, homeobox transcription factors have the capacity to bind mRNAs in animal systems and form RNP complexes, in addition to their well-known sequence-specific DNA-binding activity [39]. Therefore, it is likely that *KN1* protein and its mRNA traffic via PDs as an RNP complex. SHOOT MERISTEMLESS (*STM*) is the functional *KN1* homolog in arabidopsis, since *KN1* can complement arabidopsis *stm* mutants [40,41]. *STM* protein and mRNA are also mobile [14,42]; thus, the noncell-autonomous action of homeobox transcription factors may require cell-to-cell transport of both their mRNAs and proteins [14,43].

mRNA mobility can be inferred by comparisons between promoter activity and mRNA localization. For example, the promoter of the sucrose transporter *SUT1* drives expression only in companion cells, but *SUT1* protein and mRNA are also found in sieve elements [44]. Sieve elements lose most of their organelles (including nuclei) during maturation, and no transcriptional activity has been reported. Hence, *SUT1* mRNA is thought to traffic from companion cells to sieve elements [44,45]. PD localization of *SUT1* mRNA further supports this idea [44]; however, this interpretation remains controversial [46]. In the case of *STM*, two groups independently generated promoter constructs driving cell-autonomous fluorescent protein reporters, and found expression to be restricted to the peripheral region of the meristem [47,48]. However, *STM* mRNA and mobile YELLOW

nucleus, and mediates long-distance transport.

Stele: the central part of the root or stem in vascular plants. The stele contains vascular tissues, pith, and pericycle tissues, and is surrounded by an endodermis.

Stolons: underground shoots of plants, such as potatoes, that store carbohydrates and undergo tuberization.

Trichome: a small hair-like structure, generated from the epidermis, which protects against biotic and abiotic stresses.

FLUORESCENT PROTEIN (YFP)-fused STM protein (YFP~STM) expressed with its native promoter are detected throughout the meristem [41,47]. Therefore, they could move from the meristem periphery to the meristem center. However, YFP~STM expressed in the outer (L1) layer of the meristem moves only one cell layer, to the L2 [42]. It is possible that the increased molecular weight of the YFP~STM fusion reduces its mobility, although YFP~STM expressed with its native promoter fully complements an *stm* null allele [42]. It is difficult to prove whether promoter reporters reflect true endogenous expression patterns. The reported constructs used a ~5 kb STM promoter region, but did not include introns or downstream genomic sequences, so may not accurately reflect expression [47,48]. In the case of the rice KN1 ortholog, ORYZA SATIVA HOMEBOX1 (OSH1), conserved *cis*-elements in an intron are required for proper OSH1 expression by autoregulation [49]. Hence, at least for STM, incorporating additional genomic regions in addition to the promoter may be necessary to identify the correct expression. A promoter reporter for KN1 in maize has not also been established.

Possible non-PD-dependent pathways of mRNA cell-to-cell transport

In plants, PDs are membrane-lined channels that traverse the cell wall to connect the cytoplasm between neighboring cells. A tightly compressed cylinder of endoplasmic reticulum (ER), the so-called desmotubule, passes through the PD and creates a region called the cytoplasmic sleeve between the plasma membrane (PM) and ER membrane [3]. This sleeve provides cytoplasmic continuity and mediates molecular transport between cells. Some mobile mRNAs, including *SUT1* and *KN1*, associate with PDs, supporting the idea that PDs mediate their cell-to-cell transport [14,16,44].

In animal cells, RNAs move between cells via gap junction channels, tunneling nanotubes (TNTs)/membrane nanotubes (mNTs) and extracellular vesicles (EVs) [50]. Gap junctions are intercellular channels formed by connexin or innexin protein subunits [51], and can transport small RNAs (sRNAs) [52–55]. TNTs are membrane nanochannels, similar to PDs, connecting the cytoplasm of neighboring cells. Various cargos containing mRNAs can move between cells through these channels along actin filaments, presumably powered by myosin motors [50,56–58]. TNTs have been reported to form between human breast cancer cells cocultured with immortalized mouse embryo fibroblasts, and most human transcripts moved nonselectively through TNTs to the mouse cells [57]. Although the physiological consequence of this movement is unknown, the expression of cancer- and immune-related genes changed in the recipient cells, suggesting that mRNA transport via TNTs is biologically relevant.

Another possible route for mRNA transport between animal cells is via EVs, also known as exosomes. These structures are secreted by cells and transport cargo – including sRNAs, long non-coding RNAs (lncRNAs), and full-length or fragmented mRNAs – to other cells [59,60]. Short motifs have been found in EV-enriched mRNAs and facilitate mRNA loading into EVs [61,62]. mRNAs transported by EVs can be translated and function in destination cells [60]. Plant cells also secrete EVs containing RNAs into the **apoplast** [63]. However, intact mRNAs have not been detected in the apoplast, despite efforts to identify them using poly-A enrichment followed by RNA sequencing [64]. This result supports the idea that plant mRNAs move between cells primarily via PDs, rather than via the apoplast or EVs. However, it remains possible that mRNAs are transported via the apoplast in some cases. Despite the lack of evidence for mRNA association, plant EVs do contain sRNAs, including microRNAs (miRNAs), small interfering RNAs (siRNAs), and 10–17-nucleotide tiny RNAs of unknown function [64–67]. However, most miRNAs and siRNAs appear to exist outside of EVs as RNPs in the apoplast [63,64]. These apoplastic sRNAs may act as signals between plant cells, or may even translocate to other organisms (such as fungal pathogens) to mediate interkingdom RNA silencing [68–71]. lncRNAs, including **circular RNAs (circRNAs)**, are also present in the apoplast as RNPs, and may function in sequestering sRNAs

secreted by pathogens to prevent them from entering the plant cell [63,64]. Some RNA-binding proteins have been identified from EV-enriched apoplast fractions, and may function in the packaging of sRNAs and lncRNAs into EVs or their secretion [64,66]. For example, RNA-binding proteins ARGONAUTE (AGO) 2 and GLYCINE-RICH PROTEIN (GRP) 7 are secreted into the apoplast and can bind to lncRNAs [64]. Eliminating these proteins reduces the circRNA content in the apoplast, suggesting that AGO2 and GRP7 function in RNA secretion and/or stabilization.

Mechanism of mRNA targeting to PDs

Mobile mRNAs associate with PDs, and this targeting could be essential for their movement [14,16]. For example, FLOWERING LOCUS T (FT) encodes florigen, a signal that moves long-distance from the leaves through the phloem to the shoot apex to induce flowering [72]. While several reports have found that FT protein is mobile [73–75], FT mRNA is also mobile and controls flowering time in arabidopsis [76]. Fluorescently tagged FT mRNA forms cytoplasmic puncta that are targeted to PDs when expressed in tobacco leaf cells [16]. A preprint suggests that FT mRNA uses the endomembrane trafficking system to pass through PDs [15]. FT mRNA is anchored by **ROTAMASE CYCLOPHILINS** on the surface of organelles such as the trans-Golgi network and multivesicular bodies. It then moves through the cytoplasm by associating with actin, and is targeted to PDs by microtubules [15]. Indeed, depolymerization of actin suppressed the intracellular transport of FT mRNA, and mRNA targeting to PDs was impaired in *rotamase cyclophilin* mutants and when microtubules were depolymerized. To prove the physiological roles of FT mRNA targeting to PD, however, it will be necessary to ask whether this impaired PD targeting affects long-distance transport of FT mRNA and flowering.

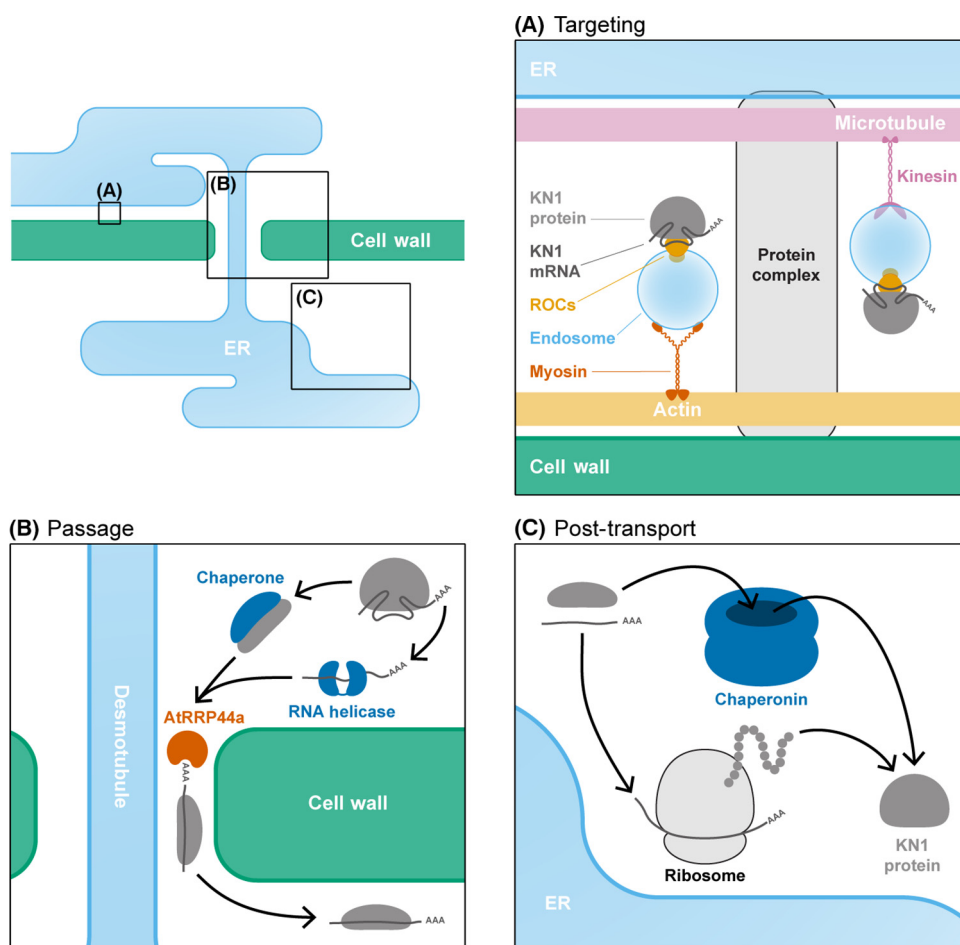
The endomembrane and cytoskeleton also participate in PD targeting of a mobile transcription factor, SHORT-ROOT (SHR). SHR traffics from the root **stele** to the endodermis and functions in root patterning [77,78]. SHR associates with endosomes through interaction with its transport regulator SHR-INTERACTING EMBRYONIC LETHAL (SIEL) [79], which moves along actin filaments, perhaps via myosin, and pauses on microtubules at points where a KINESIN, KinG, localizes. This pause is facilitated by the interaction between SIEL and KinG, which seems critical for PD targeting of SHR [80]. The ER might scaffold this step [81]. It is unknown whether SHR mRNA traffics cell to cell, but SIEL homologs act as nuclear RNA-processing proteins [82], so SIEL might also control an mRNA component of the noncell-autonomous SHR signal.

The intracellular trafficking of STM protein between cell compartments, and to PDs, is similarly regulated by microtubule- and endosome-associated proteins [47,83]. Since STM protein likely interacts with its mRNA, similarly to KN1, STM mRNA may also traffic using microtubule- and endosome-associated pathways. KN1 mRNA has been visualized *in vivo* using a fluorescent tag associated with the viral **MS2 system** [14]. Tagged KN1 mRNAs form puncta that move dynamically through the cytoplasm and pause at or near PDs. This temporal pausing of KN1 mRNA at specific compartments is similar to that seen when SHR interacts with KinG [80], implying that they may share a common PD-targeting mechanism. However, the fluorescently labeled mRNAs do not appear to pass through PDs, presumably due to their large size; it therefore remains to be seen whether this pausing is a critical step in PD transport.

Microtubules also participate in the cell-to-cell transport of miRNAs. Specific miRNAs move from cell to cell, forming activity gradients that result in gradients of target gene expression that determine specific cell fates across a tissue [84]. miRNA mobility can be restricted by their loading onto cell-autonomous AGO proteins [85]. miRNA loading onto AGOs is enhanced, and their mobility is suppressed, in mutants of genes that function in microtubule organization, including KATANIN1 (KTN1) and MICROTUBULE ORGANIZATION 1 (MOR1) [86]. Thus, microtubule

organization impacts the noncell-autonomous action of miRNAs. Microtubules may directly regulate miRNA–AGO interaction, such as in the formation of RNA-induced silencing complexes [86]. Alternatively, when microtubules are disrupted, miRNAs may fail to reach the PDs, and subsequently accumulate in the origin cells, increasing their chances of interacting with AGO proteins.

In summary, for RNAs to move between cells, they must first reach the PDs. Mobile mRNAs are anchored on organelles such as the trans-Golgi network and multivesicular bodies by RBPs such as ROTAMASE CYCLOPHILINS, and traffic toward PDs via the endomembrane transport pathway associated with the cytoskeletal system. Motor proteins such as myosin and KinG may also be involved in this intracellular transport (Figure 1A). PDs also organize ER–PM contact sites



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Figure 1. A putative model for the plasmodesmal (PD) transport mechanism of mobile transcription factor messenger RNA (mRNA). (A) mRNAs of mobile transcription factors, such as those encoded by the KN1 homeobox gene, interact with their proteins (e.g., by forming a ribonucleoprotein complex) to traffic from cell-to-cell. mRNA may be anchored to the endosomal surface by proteins such as ROTAMASE CYCLOPHILINS (ROC), and targeted to the PD via actin and microtubules. The interaction of actin filaments, microtubules, and endoplasmic reticulum (ER) via a specific protein complex in ER–plasma membrane contact sites may be involved in this process, but the mechanism is still unknown. (B) Before PD passage, mRNAs or proteins of mobile transcription factors may be converted to mobile forms by RNA helicases and chaperones, respectively, and trafficked to the neighboring cell via the PD. Arabidopsis RIBOSOMAL RNA-PROCESSING PROTEIN 44A (AtRRP44A) may facilitate mRNA transport as a carrier. (C) After PD passage, mobile transcription factors can be refolded by a chaperonin complex. mRNAs may undergo translation in the recipient cell, amplifying the transported signal.

(EPCSs) where membranes are tethered with specific proteins and the cytoskeleton [35]. EPCSs may regulate PD transport, as a complex of specific actin- and microtubule-binding proteins bridges the actin–microtubule network to maintain ER/cytoskeletal structure and cell morphology [87]. PD-associated EPCS may transport mRNAs between actin filaments and microtubules, switching intracellular transport to PD targeting. Visualization of *in vivo* cellular dynamics and PD targeting of mobile mRNAs in transport pathway mutants will help dissect the transport process.

Regulatory factors for cell-to-cell transport of mRNAs

Similar to their role in long-distance phloem transport, specific RBPs may also facilitate the cell-to-cell transport of mRNAs. Indeed, the trichome rescue screen isolated mutants in *arabidopsis* RIBOSOMAL RNA-PROCESSING PROTEIN 44A (AtRRP44A) that were defective in KN1 cell-to-cell transport [14]. RRP44A is a catalytic subunit of the **RNA exosome complex** and participates in RNA degradation and processing [88]. However, a point mutation that blocks RRP44A catalytic activity did not affect *KN1* mRNA transport, suggesting that it has a noncanonical function in trafficking. While RRP44A protein localizes predominantly in the nucleus, as expected, a modified version with mutated nuclear localization signals and a fused nuclear export signal associates with PDs, suggesting that it may function directly in transport. RRP44A can also bind *STM* mRNA and mediates its cell-to-cell transport [14]. These results suggest that RRP44A facilitates KN1/*STM* function by facilitating the cell-to-cell transport of their mRNAs, and this hypothesis is supported by the observation that weak *rrp44a* alleles enhance *stm* phenotypes. Therefore, RRP44A may act as a carrier to target mRNAs to and/or through PDs. As mentioned earlier, it is fascinating that SIEL, which facilitates SHR transport, also has homology with RNA-processing proteins. Therefore, some RNA-processing proteins may have a **moonlighting function** to regulate the noncell-autonomy of transcription factors and their mRNAs. As mentioned previously for the transport of TLS-fused Cas9 mRNA, the unwinding of higher-order mRNA structure by RBPs may be necessary for mRNAs to pass through the microscopic PD channels [1]. The conformation of mobile proteins may also be regulated to allow passage through PDs. For example, chaperones and chaperonins participate in the PD transport of the KN1 protein [43,89]. Chaperones could unfold the protein to a mobile form, followed by its refolding by chaperonins after passing through PDs (Figure 1B,C) [43]. Specific RBPs might also interact with mobile mRNAs to facilitate their transport by inhibiting mRNA translation and degradation [90].

Mobile mRNAs likely require specific sequences, structures, and/or modifications for PD transport between cells. For example, the potato spindle tuber viroid (PSTVd) is a circular noncoding RNA with 27 RNA loop structures, and at least six of them are required for cell-to-cell transport via PDs in leaves, supporting the idea that transport depends on structure [91]. For mRNAs, however, such features have not yet been identified. For example, *FT* mRNA has a 102-bp sequence at its 5' end that is essential for long-distance phloem transport [92], but it is unclear whether it functions in PD targeting. While *KN1* and *STM* mRNAs can traffic cell-to-cell via PDs, RNA features required for their transport have not yet been found [93,94]. Thus, identifying such features is a pressing challenge. Since only ~10% of phloem-mobile mRNAs harbor TLS or m5C motifs [93,94], there are likely to be additional mRNA features that facilitate PD and phloem transport.

Possible functions of cell-to-cell transported mRNAs

In a small number of cases, the mobility of transcription factor mRNAs appears to be essential for their biological function [14]. How does mRNA transport contribute to transcription factor signaling between cells? Visualizing *KN1* mRNA *in vivo* using the MS2 system coincidentally found that once *KN1* mRNA is tagged by multiple MCP-tagged fluorescent proteins, its transport via PDs is suppressed [14]. Similar mRNA transport inhibition has been reported in the animal TNT pathway [56]. When tagged *KN1* mRNA transport in the trichome rescue system is suppressed, KN1

protein transport is also reduced, suggesting that KN1 mRNA transport facilitates KN1 protein transport [14]. *KN1* mRNA also requires KN1 protein to traffic between cells [13,37], so the protein and mRNA likely interact to make a transport-competent complex. Transport of mRNA, in addition to protein, may be beneficial because it can amplify the signal by being translated in destination cells (Figure 1C) [36]. Animal cells localize some mRNAs to subcellular compartments to form translation hotspots, allowing for spatiotemporal fine tuning of gene expression [95]. In plants, PD transport may localize specific mRNAs to certain tissues or organs and establish their similar translation hotspots, thereby enabling spatiotemporal control of development and environmental responses.

It may be evolutionarily advantageous to have mRNA mobility rather than protein mobility. RNA features facilitating mRNA transport are often found in 3' untranslated regions (UTRs) [18]. Since mutations in UTRs are less likely to affect the function of translation products, their molecular evolution can be faster. Many tRNA genes are dispersed throughout the genome, and could be rearranged, resulting in transcripts with TLSs in their 3' UTRs relatively frequently [93]. These mobile transcripts would establish different protein concentrations in different cells, tissues, and organs, increasing the chance of plants to evolve advantageous traits through phenotypic diversification.

Concluding remarks

Cell-to-cell transport of mRNAs is an emerging field that raises many questions (see Outstanding questions). The critical questions include what mRNAs are transported, what RNA features promote their transport, what proteins regulate their transport, and what are the biological functions of mRNA transport. RNA immunoprecipitation and single-cell mRNA sequencing can be powerful tools to answer these questions [96,97]. In addition, MS2 tagging can label mRNAs *in vivo* with a fluorescent protein [16]. Combining MS2 tagging with immunoprecipitation–mass spectrometry analysis could be used to identify mobile mRNA-specific binding proteins as candidate transport regulators [96]. The MS2 system uses a large tag, which appears to inhibit mRNA transport via PDs, so could be used to investigate its biological relevance [14]. However, to observe the cell-to-cell trafficking of mRNAs, a new reporter system that does not affect movement is needed. For example, the spinach RNA–fluorophore complex is a smaller tag that resembles green fluorescent protein (GFP) and has been applied to plants [98], and an RNA labeling system using a bacterial RBP has been used to visualize the cell-to-cell movement of MP RNA [99].

Acknowledgments

This research was supported by the National Science Foundation (IOS 2224874 and IOS 2131631).

Declaration of interests

The authors declare no competing interests.

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Outstanding questions

What mRNAs are transported cell-to-cell via PDs in plants? How is their transport important for development and environmental responses?

Where are *KN1* and *STM* mRNAs expressed and transported in meristems? How does their mobility contribute to meristem development?

What factors regulate mRNA cell-to-cell transport via PDs?

What RNA properties (sequence, structure, or modification) are essential for mRNA transport via PDs? How do transport regulators such as RBPs recognize these RNA properties and facilitate mRNA transport?

How are mobile mRNAs recruited to PDs before transport? How do actin filaments, microtubules, and ERs interact to deliver mobile mRNA to PDs?

How are some mobile mRNAs transported long distances against the phloem bulk flow to specific tissues or organs?

In long-distance transport, what RBPs recognize TLS and m5C in mobile mRNAs and regulate their transport?

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