Localization of mRNAs at the subcellular level is an essential mechanism for specific protein targeting and local control of protein synthesis in both eukaryotes and bacteria. While mRNA localization is well documented in metazoans, somatic cells, and microorganisms, only a handful of well-defined mRNA localization examples have been reported in vascular plants and algae.

This review summarizes the function and mechanism of mRNA localization and highlights recent studies of mRNA localization in vascular plants. While the emphasis focuses on storage protein mRNA localization in rice endosperm cells, information on targeting of RNAs to organelles (chloroplasts and mitochondria) and plasmodesmata is also discussed.

WHAT IS MRNA LOCALIZATION?

Localization of mRNAs was initially discovered in the 1980s, when β-actin mRNA was found to be asymmetrically distributed in ascidian eggs and embryos (Jeffery et al., 1983). This nonuniform spatial distribution pattern was later observed for maternal mRNAs in *Xenopus* (Rebagliati et al., 1985) and *Drosophila* oocytes (Frigerio et al., 1986; Berleth et al., 1988) and supported the proposal of prelocalized RNA during early development (Davidson, 1971; Kandler-Singer and Kalthoff, 1976). Subsequently, mRNA localization was observed in a variety of somatic cells such as fibroblasts (Lawrence and Singer, 1986), oligodendrocytes (Trapp et al., 1997), and neurons (Garner et al., 1988). Today, mRNA localization is prevalent in bacteria, yeast, algae, vascular plants, and metazoans and, therefore, is an ancient, universal, evolutionarily conserved mechanism.

Our understanding of mRNA localization stems mainly from research in *Xenopus*, *Drosophila*, budding yeast, fungi, and structurally polarized animal cells (Holt and Bullock, 2009; Martin and Ephrussi, 2009; Medioni et al., 2012; Shahbabian and Chartrand, 2012; Weatheritt et al., 2014; Weil, 2014; Chin and Lécuyer, 2017; Lazzaretti and Bono, 2017; Teimouri et al., 2017). By contrast, only a handful of examples for mRNA localization have been observed in plants. Nevertheless, substantial progress has been made over the years. Here, we discuss the importance and mechanism of mRNA localization, summarize mRNA localization studies in land plants, and provide suggestions for future research in plants.
WHY DOES mRNA LOCALIZATION MATTER?

The importance of mRNA localization is best exemplified in early metazoan development, where more than 70% of the expressed mRNAs are asymmetrically distributed in the *Drosophila* embryo (Lécuyer et al., 2007). As one of the key steps in posttranscriptional gene regulation and protein synthesis, localization of mRNA serves several functions during cell growth and development. First, the direct consequence of mRNA localization is to save cellular energy. As local translation from a single mRNA molecule can yield many copies of protein molecules at strategic locations in the cells, mRNA localization undoubtedly provides a more energy-efficient way to achieve specific targeting and asymmetrical distribution of proteins. Second, mRNA localization is an effective mechanism of concentrating proteins at specific intracellular locations. This is especially true for the rice (*Oryza sativa*) prolamine mRNAs, which are concentrated on the endoplasmic reticulum (ER) membrane that delimits the prolamine intracisternal granules, to form a spherical protein body (Li et al., 1993; Choi et al., 2000; Hamada et al., 2003a, 2003b). Third, local translation of specific proteins at the site of action can prevent proteins from forming protein-protein interactions that impede function or are harmful to the cell. One of the best examples is the localization of myelin basic protein (MBP) mRNA to the myelinating compartments of oligodendrocytes (Shan et al., 2003; Boggs, 2006). Due to its nonspecific membrane-binding properties, randomly distributed MBP in the cytoplasm would disrupt the integrity of the endomembrane system (Du et al., 2007). By transporting its mRNA to the cell’s myelin compartment, local translation at this site avoids this potential problem. Lastly, mRNA localization aids in the assembly and formation of multiprotein complexes (Kramer and McLennan, 2019). Stochastic modeling of protein-protein interactions indicates that the rate of assembly of interacting proteins randomly distributed in the cell would be very low unless the proteins were colocalized during translation. Ribosome-profiling studies readily demonstrate that the initial steps leading to the formation of many multiprotein complexes occur by the coassembly of nascent polypeptides during translation (Shiber et al., 2018).

Coupling with the precise spatiotemporal control of gene expression and protein synthesis at the site of action, mRNA localization plays a critical role in regulating many diverse processes during normal cell physiology and development, in disease, host-pathogen responses, and cellular adaptation to stress. More information about the effects of dysregulation of mRNA localization in animal and fungi systems is available in other reviews (Cody et al., 2013; Fallini et al., 2016; Wang et al., 2016; Chin and Lécuyer, 2017).

HOW DOES mRNA LOCALIZATION WORK?

The localization of mRNAs is a multistep process involving events in both the nucleus and cytoplasm. The initial step is the recognition and binding of cis-acting zipcode elements by trans-acting factors, usually RNA-binding proteins (RBPs), during transcription in the nucleus, forming heterogenous nuclear ribonucleoprotein complexes. These RBPs may also serve in mRNA processing (i.e. splicing and polyadenylation) during posttranscription in the nucleus. The association of a set of RBPs with mature mRNAs forms the primary messenger ribonucleoprotein (mRNP) complex, mediating nuclear export of mRNAs. After export into the cytoplasm, the primary mRNP complexes undergo extensive remodeling where one or more proteins are removed and others are added (Lewis and Movry, 2007; Niedner et al., 2013). For example, once in the cytoplasm, the mRNP is modified to include an adaptor protein, which links the mRNP complex to a cytoskeletal motor protein enabling transport on the cytoskeleton. At its destination, the mRNP undergoes further remodeling where it is anchored and subsequently activated for translation (Jansen et al., 2014). Alternatively, the mRNP can be processed in P-bodies or stored in stress granules.

The zipcode sequences contain the necessary information for the successful transport of the corresponding mRNAs. They are usually located in the untranslated regions (UTRs), mainly in the 3’ UTR, but may also be present in the coding region (Chabanon et al., 2004; Tian and Okita, 2014). Although zipcodes may be sequence dependent, the secondary/tertiary structure of zipcode RNA sequences may be the signal recognized by trans-acting factors. For example, the yeast *ASH1* RNA contains four zipcodes that share weak sequence homology (Niedner et al., 2014). Three of the zipcodes are located in the coding region, a location that may constrain their evolution. Although one zipcode is sufficient for *ASH1* localization, all four work synergistically to ensure efficient transport on microfilaments (Niedner et al., 2014). These zipcodes also serve to arrest translation, as the hairpin loop structure impedes ribosome movement.

The zipcodes are recognized by specific RBPs. Typical RBPs contain one or more conserved RNA-binding motifs. These include the RNA recognition motif (RRM), K homology motif, zinc finger, pentatricopeptide repeat, cold shock domain, RGG (Arg-Gly-Gly) box, Puf RNA-binding repeats, and RNA helicase DEAD/DEAH box (Git and Standart, 2002; Lunde et al., 2007; Glisovic et al., 2008; Bailey-Serres et al., 2009; Lorković, 2009; Lee and Kang, 2016). Many proteins that have RNA-binding activity, however, lack a conserved RNA-binding motif. A notable example is the yeast *ASH1* RNA and its two RBPs, She2P and She3P. Although She2P and She3P lack any recognizable canonical RNA-binding domain, they specifically recognize and directly bind to the zipcode elements of *ASH1* RNA (Böhl et al., 2000; Niedner et al., 2014). Much more atypical is the binding of VPS36, an Endosomal Sorting Complexes Required for Transport protein II (ESCRT-II) subunit, via its N-terminal GRAM-like ubiquitin-binding in EAP45 (GLUE).
domain, to *Drosophila bicoide* mRNA (Irion and St Johnston, 2007).

In addition to RNA-binding motifs, RBPs usually contain a variety of auxiliary sequences or domains. Examples include peptide domains enriched in Gly, Arg, Pro, and Arg-Ser repeats (Biamonti and Riva, 1994; Fedoroff, 2002; Maris et al., 2005; Lunde et al., 2007; Ambrosone et al., 2012; Lee and Kang, 2016). These additional modules afford RBPs diverse binding abilities to other proteins, which can recruit other relevant proteins or factors to further specify RNA recognition and mediate mRNA transport.

While there are examples where mRNAs are transported passively by diffusion to an anchoring site (Forrest and Gavis, 2003; Chang et al., 2004; Palacios, 2007), most are actively transported by molecular motors along the cytoskeleton. Molecular motors can move bidirectionally along the cytoskeleton and are widely used to transport organelles and other cargos, including mRNP complexes (Bullock, 2007; Palacios, 2007). The active transport of mRNAs requires the core mRNP machinery, specifically RBPs and other adaptor proteins, that recruit the corresponding motor proteins to transport mRNAs toward their final destination. All three families of molecular motors (myosins, dyneins, and kinesin) have been shown to transport various mRNAs (Bullock, 2007; Palacios, 2007; Ryder and Lerit, 2018). In many cases, however, how mRNP particles are attached to molecular motors has yet to be resolved and requires further investigation.

**MRNA LOCALIZATION IN PLANT CELLS**

Localization of mRNAs has been poorly investigated in vascular plants simply because most plant cells are not amenable for study. While most plant cells are large enough to be studied by light microscopy, they invariably contain a large vacuole compartment that squeezes the cytoplasm to the periphery of the cell (Chou et al., 2019). Meristematic cells are cytoplasmically dense, but their small sizes preclude them from being a system to study by light microscopy. An ideal plant cell type is the cereal endosperm cell, which is large and cytoplasmically dense. In this section, we mainly focus on storage protein mRNA localization in rice developing endosperm cells, a well-defined model for mRNA localization study in vascular plants. We briefly summarize studies of mRNA targeting to chloroplast and mitochondria and targeting to the plasmodesmata, the entry point for cell-to-cell and long-distance movement.

**mRNA Localization to the Cortical ER in Cereal Endosperm Cells**

An advantage of studying endosperm cells from rice is that, unlike other cereals that predominantly accumulate one type of storage protein in developing grains, this cereal synthesizes two major types of storage proteins, glutelin and prolamine, and moreover deposits them in separate intracellular compartments. Although both storage protein types are synthesized on the rough ER, prolamines are retained as intracisternal inclusions in the ER lumen, forming an ER-derived protein body I (PB-I), whereas glutelins are exported to the Golgi and are subsequently transported to the protein storage vacuoles (PSVs) to form PB-II. The biogenesis of a prolamine intracisternal granule gives rise to two distinct morphological domains of the cortical ER membrane complex: protein body ER (PB-ER) and the interconnecting cisternal ER (cis-ER) network consisting of cisternal and tubular membranes (Li et al., 1993).

These unique traits make rice endosperm cells an ideal system to study mRNA targeting to the ER. Biochemical evidence obtained in the 1980s and 1990s revealed the asymmetric distribution of storage protein mRNAs on the ER (Kim, 1988; Li et al., 1993); prolamine mRNAs are concentrated on the PB-ER, whereas glutelin mRNAs are localized on the adjoining cis-ER. As mRNA localization was not dependent on the synthesis of storage protein, a series of transgenic rice lines expressing hybrid storage protein mRNA sequences were constructed. Confocal microscopic analysis of seed sections subjected to in situ reverse transcription-PCR hybridization showed that the distinct distribution of prolamine and glutelin mRNAs to the PB-ER and cis-ER, respectively, is dependent on specific prolamine and glutelin cis-RNA sequences or zipcodes (Hamada et al., 2003b; Washida et al., 2009, 2012). Based on these findings, a model of mRNA localization featuring three mRNA transport and localization pathways to subdomains of the cortical ER in rice endosperm cell was proposed (Fig. 1; Crofts et al., 2005; Doroshenko et al., 2012; Tian and Okita, 2014; Chou et al., 2019). Prolamine (as well as α-globulin) and glutelin mRNAs are transported to the PB-ER and cis-ER, respectively, using regulated pathways requiring zipcodes. A third zipcode-independent default pathway was also evident. Genetic-biochemical studies showed that the three mRNA transport pathways are not independent but are instead arranged in a hierarchical interrelationship (Fig. 2). The glutelin mRNA localization pathway is dominant over the prolamine mRNA transport pathway that, in turn, is dominant over the default pathway (Crofts et al., 2005; Doroshenko et al., 2012; Tian and Okita, 2014; Chou et al., 2019). The mutant rice lines *glup4* and *glup6* exhibit mislocalization of glutelin mRNAs from the cis-ER to PB-ER, while *glup2* mislocalizes prolamine mRNAs from the PB-ER to cis-ER. The interrelatedness of the three RNA transport pathways suggests that they share common trans-factors for targeting to the ER membrane, while glutelin and prolamine pathways require additional factors for selective transport (Chou et al., 2019). Indeed, our studies showed that prolamine and glutelin zipcode sequences bind identical trans-factors, Tudor-SN (Chou et al., 2017; Tian et al., 2018, 2019), RBP-2 (Chou et al., 2017; Tian et al., 2018, 2019).
Both prolamine and glutelin RNAs contain multiple cis-acting zipcode elements (Fig. 3). The two cis-acting signals of prolamine are found in the coding region and 3' UTR. Both contain a conserved 27-nucleotide motif in each zipcode element (Hamada et al., 2003b; Washida et al., 2009, 2012). By contrast, glutelin RNA has three zipcode regions containing two sequence motifs, 23 and 27 nucleotides in length. Zipcode 1, located at the end of

![Figure 1](https://plantphysiol.org) Working model of mRNA transport pathways to the cortical ER in developing rice endosperm cells (adapted from Tian and Okita, 2014). In the nucleus, storage protein mRNAs are recognized and bound by various sets of RBPs, forming heterogenous nuclear ribonucleoprotein (hnRNP) complexes where they are exported from the nucleus. In the cytoplasm, the mRNP complexes undergo dynamic remodeling and are actively targeted to the distinct ER subdomain via the cytoskeleton by three pathways. Prolamine (blue) and globulin (orange) mRNAs are targeted to the PB-ER, where the mRNAs are translated and their coded proteins are translocated into the ER lumen. Prolamine polypeptides assemble as an intracisternal granule to form PB-I, while globulins are rapidly exported to the Golgi for subsequent transport to the irregularly shaped PSVs by dense vesicles. Glutelin mRNAs (red) are localized to the cis-ER, and the coded glutelin proteins are transported to the PSV via the Golgi complex. An additional default pathway mediates general mRNA (green) transport to the cis-ER.

![Figure 2](https://plantphysiol.org) The interrelationship among the three mRNA transport pathways in developing rice endosperm (adapted from Chou et al., 2019). The glutelin mRNA localization pathway (red) is dominant over the prolamine mRNA transport pathway (blue), which, in turn, is dominant over the default pathway (gray). Analyses of mutant rice lines expressing defective Rab5 (glup4), Rab5-GEF (glup6), and Got1 (glup2) suggest the involvement of membrane trafficking in the transport of mRNAs to the cis-ER or PB-ER. The dominant mutation, glup5, misdirects only globulin mRNAs to the cis-ER without affecting the targeting of prolamine mRNAs to the PB-ER. The hierarchical interrelationship of the three RNA-transport pathways suggests that they may share common trans-factors for targeting to the ER membrane, while additional factors are required for selectively transporting glutelin and prolamine mRNAs.
exon 4, is composed of three copies of motif 1. Zipcode 2 contains a single motif 2 located within the 3' UTR, while zipcode 3 at exon 1 contains both motifs 1 and 2 (Washida et al., 2009). Deletion analysis showed that removal of these zipcode elements resulted in the mislocalization of the corresponding mRNAs (Hamada et al., 2003b; Washida et al., 2009, 2012). Individually, these zipcode elements functioned weakly on their own, as two zipcodes are required for restricted localization of prolamine and glutelin mRNAs to the PB-ER and cis-ER, respectively (Hamada et al., 2003b; Washida et al., 2009, 2012).

Asymmetric distribution of storage mRNAs is also observed in other cereal endosperm cells. Similar to the pattern seen in rice endosperm, zein (a prolamine family protein) mRNAs are targeted to PB-ER while legumin-1 (an 11S globulin family protein) mRNAs are localized to the cis-ER in maize (Zea mays; Washida et al., 2004). The spatial distribution of storage protein mRNAs in the distinct ER domain may directly contribute to the deposition of zein in the ER-derived protein body and legumin-1 in PSV (Washida et al., 2006). The findings in maize and rice endosperms suggest that mRNA localization may be a prevalent and conserved mechanism to sort storage proteins in cereal endosperm cells.

**Identification of Trans-factors that Recognize Storage Protein Zipcodes**

Using the prolamine zipcode sequences as bait, 15 RBPs were selectively captured under stringent binding-elution conditions (Crofts et al., 2010). Follow-up functional studies of five of these captured proteins, RBPs A, I, J, K, and Q, exhibited direct binding to prolamine zipcode RNA and assembled into at least three zipcode RNA-protein complexes (Yang et al., 2014). RBPs A-J-K and I-J-K formed two complexes, while Q formed a third complex with other unidentified proteins (Yang et al., 2014). The finding of multiple complexes is consistent with the dynamic remodeling of mRNP complexes in the multistep process of mRNA localization.

Although similar affinity chromatography studies failed to capture any specific RBPs using the glutelin zipcode RNA as bait, a northwestern-blotting approach identified a key glutelin RNA-binding protein, RBP-P, which was also captured by prolamine zipcode sequences (Crofts et al., 2010). RBP-P contains two RRMs flanked by Ala/Glu-rich N-terminal and Gly-rich C-terminal domains. The two RRMs in RBP-P directly contribute to its strong binding to glutelin and prolamine mRNAs (Tian et al., 2018). The auxiliary domains flanking the RRMs, especially the Gly-rich region, mediated both RNA binding and protein-protein interaction (Tian et al., 2018). Point mutations in the interdomain (A252T) that separated the two RRM motifs and the C-terminal Gly-rich region (G373E and G401S) reduced the association of RBP-P with prolamine and glutelin mRNAs in vivo and caused mislocalization of these mRNAs (Tian et al., 2018), suggesting an essential role of RBP-P in mRNA localization.

The role of RBP-P in glutelin and prolamine mRNA localization also requires its association with other RBPs to form one or more multiprotein complexes. A yeast two-hybrid screening analysis of a rice seed cDNA library identified two major interacting partners, RBP-L and RBP208 (Tian et al., 2018). These RBPs, which contain three RRM motifs, coassembled with RBP-P into multiprotein complexes that specifically bind to prolamine and glutelin zipcode RNAs, as revealed by in vivo RNA-immunoprecipitation studies (Tian et al., 2018). The following lines of evidence further substantiated the involvement of RBP-L in glutelin and prolamine mRNA localization. First, RBP-L exhibited strong binding to storage protein zipcode RNAs. Second, a microscopic analysis combining in situ reverse transcription-PCR and immunofluorescence labeling showed that RBP-L colocalized with both glutelin and prolamine mRNAs in developing endosperm cells.
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Tian et al., 2019). Third, knockdown of RBP-L expression induced by a T-DNA insertion in its 3′ UTR disrupted the efficient assembly of the required mRNP complexes, which in turn caused partial mislocalization of glutelin and prolamine mRNAs in rice endosperms (Tian et al., 2019). The RBP-P/RBP-L protein complex may serve as a scaffold that recognizes and binds to prolamine and glutelin zipcodes for stringent selection of prolamine and glutelin mRNAs from other unlocalized mRNAs.

Although the relationship of RBP-P and RBP-L to RBPs A, I, J, and K (A-J-K and I-J-K) for selective transport of prolamine mRNA localization remains unresolved, RBP208 may serve as a link between these two RBP groups. RBP208 interacts with both RBP-P and RBP-L, forming distinct complexes: the RBP-P/RBP208 complex mainly locates in the nucleus, while RBP208 only interacts with RBP-L in the cytoplasm (Tian et al., 2018). Additionally, RBP208 interacts with RBP-Q in both the nucleus and cytoplasm (L. Tian and T.W. Okita, unpublished data). As mentioned earlier, RBP-Q forms a third multicomplex on the prolamine zipcode sequences. Hence, its interaction with RBP208 may serve as a link between the prolamine and glutelin transport pathways. These findings reflect the multiple functions of these RBPs in RNA metabolism and resonate with the aforementioned interrelationship of prolamine and glutelin mRNA transport pathways.

NONZIPCODE TRANS-FACTORS

In addition to the zipcode-binding RBPs, other factors are also involved in RNA localization. One such protein is OsTudor-SN. This RBP was initially identified as a cytoplasm-localized, cytoskeleton-associated protein in developing rice grains (Sami-Subbu et al., 2001; Wang et al., 2008). It contains four tandem Staphylococcus nuclease domains (4SN module) and a Tudor domain followed by an additional truncated SN domain in the C terminus (Tsn module; Chou et al., 2017). The N-terminal 4SN module directly binds to glutelin and prolamine mRNAs, while the Tsn module possesses the capacity to interact with eight other RBPs (Chou et al., 2017). These binding activities collectively contribute to the involvement of Tudor-SN in glutelin and prolamine mRNA localization (Wang et al., 2008; Chou et al., 2017), as reflected by the partial mislocalization of storage protein mRNAs in rice lines expressing Tudor-SN possessing mutations in the fourth SN and Tudor domains (Chou et al., 2017).

DOES RNA LOCALIZATION COOPT MEMBRANE TRAFFICKING?

The involvement of cytoskeleton-associated OsTudor-SN protein in mRNA localization raised the possibility of active transport of prolamine and glutelin mRNAs. Indeed, employment of the GFP-based RNA movement system (Okita and Choi, 2002; Hamada et al., 2003a) showed that glutelin and prolamine RNAs were detected as moving particles. Movement of the particles was overall unidirectional in a stop-and-go manner, although bidirectional, random, and oscillatory movement patterns were also observed. These movement patterns and the velocity of the RNA transport particle suggest the role of a cytoskeletal motor protein (Okita and Choi, 2002; Hamada et al., 2003a). Indeed, drugs that disrupt the integrity of actin filaments suppressed particle movement, suggesting the involvement of myosin motor protein (Hamada et al., 2003a).

The shape of the RNA-transport particles during movement was not uniform but pleomorphic, suggesting that the particle’s structure was not a rigid ribonucleoprotein but rather a membrane vesicle. Such a view is consistent with the observations that storage protein RNA localization requires several membrane-trafficking factors. In glup4 and glup6 mutant lines, glutelin mRNAs are mislocalized from the cis-ER to PB-ER (Doroshenk et al., 2010; Tian and Okita, 2014; Chou et al., 2019). Moreover, in both mutant rice lines, an extramural paramural body formed by aborted endocytosis is conspicuous. In addition to containing secreted glutelin and α-globulin storage proteins, the paramural body contains marker proteins for various endomembranes and mRNAs, the latter suggesting mRNA transport via membrane trafficking. Glup4 codes for the small GTPase Rab5 while Glup6 codes for the guanine nucleotide exchange factor of Rab5 (Rab5-GEF). Given the functions of Rab5 in endosomal trafficking and the finding that large amounts of mRNAs were trapped into extracellular paramural bodies (Yang et al., 2018), glutelin mRNA transport may involve endosomal trafficking. A third mutant line, glup2, which contains a defective Golgi Transport1 (Got1), mislocalizes prolamine and α-globulin mRNAs from the PB-ER to cis-ER (Fig. 2). Got1 is a membrane-related protein found on COPII vesicles that mediates cargo transport from the ER to the Golgi (Lorente-Rodriguez et al., 2009; Fukuda et al., 2016). Apparently, prolamine and glutelin mRNA transport are assigned to different membrane-trafficking pathways.

The detailed molecular mechanism of how storage protein mRNA localization coopts membrane trafficking requires further study. In budding yeast and filamentous fungi, mRNA transport has been directly linked to membrane trafficking (Kraut-Cohen and Gerst, 2010; Jansen et al., 2014; Haag et al., 2015; Niessing et al., 2018). A subset of yeast mRNAs are cotransported with the ER (Aronov et al., 2007; Fundakowski et al., 2012), while in the smut fungus Ustilago maydis, mRNAs are cotransported on microtubule-powered endosomes (Niessing et al., 2018). There is also evidence that mRNA localization is associated with the ER in metazoans. Xenopus Vg1 RBP (Vera), the trans-factor of the Vg1 zipcode, colocalizes with the ER (Deshler et al., 1997; King et al., 2005). In mammalian cells, a substantial number of mRNAs that code for cytoplasmic proteins are closely associated with the ER,
suggested their direct transport from the nucleus (Chen et al., 2011). Indeed, the RBP p180 may serve as an ER receptor that anchors mRNAs independent of ribosomes and translation (Cui et al., 2012).

**mRNA Targeting to Chloroplasts and Mitochondria**

In addition to mRNA localization on the ER membrane, nucleus-encoded mRNAs are also targeted to or near the surface of chloroplasts and mitochondria (Weis et al., 2013; Tian and Okita, 2014). Although chloroplasts and mitochondria contain their own genomes, nearly all of the proteins that constitute these functional organelles are nucleus encoded. These proteins are synthesized in the cytosol and then imported into the organelles posttranslationally (Martin et al., 2002; Timmis et al., 2004). Emerging evidence over the years, however, indicates that directed mRNA localization coupled with cotranslation is also involved in protein targeting to these endosymbiotic organelles.

Viruses use various intracellular membranes for replication. The single-stranded (+) plant RNA viruses replicate on the chloroplast envelope (Budziszewska and Obrepska-Steplowska, 2018). Although the mechanism accounting for the localization of the replication complex on the chloroplast membranes has yet to be elucidated, the targeting signal to chloroplast has been suggested to be a 41-residue sequence located in the two amphipathic helices located in the 140K/98K protein domains of *Turnip yellow mosaic virus* (Moriceau et al., 2017).

Several plant viruses and viroids exploit the chloroplast as the site of replication. Replication of RNA viroids of the Asunviroidea family occurs in the chloroplasts, although the import mechanism has yet to be determined (Gómez and Pallás, 2010a, 2010b; Budziszewska and Obrepska-Steplowska, 2018). A 110-bp chloroplast-specific RNA-targeting signal was identified at the central region of the viroid RNA sequence, while the remaining 5′-end and 3′-end sequence contributed to RNA folding into a specific secondary structure for enhancing mRNA targeting and the subsequent protein translation in the chloroplast (Gómez and Pallás, 2010b). The trans-factors that recognize this targeting signal have yet to be identified.

The minus-strand RNA of the *Bamboo mosaic virus* (BaMV) is also imported into chloroplasts. The nucleus-encoded, plastid phosphoglycerate kinase interacts with the 3′ UTR of BaMV RNA and together with heat shock proteins translocate the viral nucleoprotein complex across the chloroplast membranes (Cheng et al., 2013). In this instance, transport of the viral RNA may occur by hitchhiking along with the import of the plastid phosphoglycerate kinase into the stroma.

While the overall contribution of mRNA targeting to the chloroplast surface for its biogenesis and function remains to be determined, this process is an essential mechanism for mitochondria. The mRNAs of many nucleus-encoded mitochondrial proteins are enriched on the mitochondrial surface (Ahmed and Fisher, 2009). In general, mRNA targeting to the mitochondria is regarded as an essential process of gene expression and regulation closely linked to cellular functions and the formation of protein complexes that are involved in metabolic pathways (Margot et al., 2005). In vascular plants, this view is exemplified by the voltage-dependent anion channel (VDAC), an abundant mitochondrial outer membrane protein. In Arabidopsis (*Arabidopsis thaliana*), VDACs are synthesized from two mRNA types that differ in the length of their 3′ UTR. While the expression of the shorter mRNA species in a VDAC mutant line restores a normal mitochondria phenotype, expression of the longer variant alters mitochondria size and number (Michaud et al., 2014). Recently, a genome-wide study of potato (*Solana*um *tuberosum*) mitochondria-targeted mRNAs via RNA sequencing demonstrated that targeted mRNAs specifically encoded mitochondrial proteins involved in metabolic pathways, suggesting a coordinated control of mRNA localization within particular cellular functions (Vincent et al., 2017).

Based on extensive studies in yeast, several parameters of mitochondria targeting are well established. mRNA targeting to the mitochondrial surface is almost invariably dependent on translation, as an important mRNA localization signal is the mitochondrial targeting sequence (MTS). The synthesis of this peptide signal suggests its interaction with the translocase of the outer membrane (TOM) complex (e.g. Tom20 and Tom70), which is also required for mRNA localization to mitochondria (Eliyahu et al., 2010; Gadir et al., 2011). A second independent targeting signal is located in the 3′ UTR. About half of mitochondria-enriched mRNAs contain recognition sites for Put3 RBP, which may serve to anchor the localized mRNA to the mitochondrial surface (Gerber et al., 2004; García-Rodríguez et al., 2007; Saint-Georges et al., 2008). A prime example of these two localization signals is observed for the yeast *ATM1* mRNA, whose localization is dependent on either its MTS sequence or its 3′ UTR (Corral-Debrinski et al., 2000).

In addition to MTS and 3′ UTR signals, required for binding TOM components and Put3, respectively, there are likely other factors yet to be identified. The Gerst laboratory (Zabezhinsky et al., 2016) demonstrated that COPI, the complex that transports membrane vesicles from the Golgi to the ER, has an essential role for mRNA localization to the mitochondria. COPI inactivation was found to decrease the amount of *OXA1* mRNA associated with the mitochondria while redirecting these mRNAs to the ER, suggesting a close connection between mRNA localization and membrane trafficking.

Although mRNA targeting to plant mitochondria is not as well studied as that in yeast, studies of the Arabidopsis VDAC3 mRNA and potato malate dehydrogenase mRNA suggest that the cis-elements located in the 3′ UTR are a key signal for mRNA localization to the mitochondria surface (Michaud et al., 2014; Vincent et al., 2017). Except for the 3′ UTR, the upstream
AUGs have also been proposed as potential regulators for targeting the associated mRNAs to the potato mitochondrial surface (Vincent et al., 2017).

Symplastic Transport and Long-Distance mRNA Movement via the Phloem: Targeting to the Plasmodesmata

In addition to the transport and localization of mRNAs at specific intracellular locations, RNAs are also transported between cells or long distances to different tissues via a plasmodesmata-mediated symplastic route in plants. These mobile RNAs include mRNAs, small RNAs, small interfering RNAs, micro-RNAs, and tRNAs. As long-distance movement has been extensively reviewed in several recent articles (Saplaoura and Kragler, 2016; Kehr and Kragler, 2018; Liu and Chen, 2018; Morris, 2018), we instead focus on why and how mRNAs are transported.

In plants, cell-to-cell communication plays an essential role in regulating plant development, growth, disease resistance, and responses to various environmental stresses. Intercellular communication involves the transport and movement of a large number of molecules, such as metabolites, proteins, and RNAs, and is operated by a plant-specific symplasmic pathway mediated by plasmodesmata. Cell-to-cell trafficking of mRNAs was first observed for maize KNOTTED1 (Lucas et al., 1995) and later for the potato Suc transporter SUC1 (Kühn et al., 1997). It is now well established that regulators of gene expression (i.e., transcriptional factors and small RNAs) are transported between cells via the plasmodesmata to control organ development and tissue patterning (Otero et al., 2016).

Considerable effort has been directed at understanding long-distance transport via the phloem. While plasmodesmata mainly mediate the cell-to-cell movement of molecules, the plant vascular phloem system conducts long-distance trafficking of photoassimilates and macromolecules from source to sink tissues (Otero et al., 2016). Since the first discovery of mRNA species in rice phloem sap (Sasaki et al., 1998), increasing evidence reveals that a considerable number of mRNAs move non-cell-autonomously through the phloem (Liu and Chen, 2018; Winter and Kragler, 2018).

The composition of phloem-mobile mRNAs is extensive, covering possibly one-third to one-half of a transcriptome (Morris, 2018). Hence, many phloem-mobile mRNAs may not serve any specific function, as they are constitutively expressed in many tissues or encode organelle-localized proteins. Therefore, the phenomenon of phloem-mediated long-distance movement of some mRNAs could be considered as nonspecific bulk flow. Alternatively, many of the mRNAs may originate from young sieve cells, which empty their contents when they mature within the phloem (Knoblauch et al., 2018). However, recent evidence from high-throughput sequencing analyses indicates that mRNA transport may be mediated by a sequence motif recognition pathway (Thieme et al., 2015; Zhang et al., 2016a; Yang et al., 2019). One such element is the tRNA-like structures (TLSs) embedded in the UTR. Transcripts harboring TLS motifs were found to be enriched in the phloem-mobile mRNA population (Zhang et al., 2016a), suggesting that the TLS with predicted stem-bulge-stem-loop structure is sufficient to mediate mRNA transport through graft junctions.
mobile mRNAs such as Arabidopsis family proteins, is present in several known phloem-mobile mRNAs such as Arabidopsis GIBBERELLIC ACID INSENSITIVE (GAI) and potato StBEL5 (Haywood et al., 2005; Huang and Yu, 2009; Cho et al., 2015). However, due to the high abundance of PTB motifs throughout the genome, PTB was questioned to be a specific motif enriched in graft-mobile transcripts (Zhang et al., 2016b; Yang et al., 2019). A more recent study from Arabidopsis seedlings suggested that although PTB and TLS motifs were enriched in small subsets of mobile transcripts, both motifs were not sufficient to predict the mobility of an mRNA (Yang et al., 2019). The study also discovered that m^3C methylation might guide systemic mRNA mobility over graft junctions, as the modification was found to be common in the mRNAs previously described as mobile (Yang et al., 2019).

Cooccurrence of mRNAs and RBPs (Kehr and Krägler, 2018) revealed that mRNAs are transported as mRNP complexes in phloem flow. RBPs are the main binding proteins to mRNAs through recognition of RNA sequence motifs and thereby mediate their transport and/or protect them from degradation. One example of the link between the transported mRNA and its RBPs is the potato StBEL5 mRNA. Over-expression of its binding proteins, StPTB1 and StPTB6, stabilized and enhanced the long-distance movement of StBEL5 mRNA, while both its stability and phloem transport were decreased in suppression lines (Cho et al., 2015). Proteomics analysis on phloem exudates from Arabidopsis (Tetyuk et al., 2013), rice (Aki et al., 2008), pumpkin (Cucurbita maxima); Haebel and Kehr, 2001; Lin et al., 2009), cucumber (Cucumis sativus; Walz et al., 2004), castor bean (Ricinus communis; Barnes et al., 2004), and Brassica napus (Giavalisco et al., 2006) show that a significant protein class consists of RBPs, including the previously characterized pumpkin phloem Small RNA-binding Protein1 (PSRP1; Yoo et al., 2004), the 16-kD pumpkin phloem protein (CmPP16; Xoconostle-Cázares et al., 1999), the eukaryotic translation initiation factor 5A (eIF-5A; Pallas and Gómez, 2013), and the 50-kD RNA-binding protein RBP50 (Ham et al., 2009). In instances where the binding sequences recognized by these RBPs have been established, such as for eIF-5A, which specifically recognizes CCUACCA and AAUGUCACAC (Xu and Chen, 2001), it provides a facile approach to identify potential mRNA partners.

OUTSTANDING QUESTIONS

- Except for a few examples established in plant cells, how fundamental is mRNA localization in plant growth and development?
- Do mRNAs co-opt specific membrane trafficking processes to transport mRNAs to distinct subdomains of the endoplasmic reticulum, chloroplast, mitochondria, and plasmodesmata?
- What are the cis-determinants and trans-factors responsible for RNA localization to the plasmodesmata?
- Are mRNAs that code for proteins that share function or intracellular location co-transported as regulons?
- How could we further combine biochemical methods and high-throughput approaches to decipher mRNP architecture and dissect the machinery involved in mRNA localization in plant cells?
Ule, 2018). RNA-protein immunoprecipitation in tandem followed by high-throughput sequencing can footprint the occupancy sites of RBPs and mRNP complexes (Singh et al., 2014). Ribosome profiling can globally monitor the translation status of mRNA during the localization process to allow investigation of dynamic mRNP complexes (Brar and Weissman, 2015). Proteomic profiling can reveal the global protein occupancy on mRNAs to characterize the mRNA-protein interactome and, in turn, elucidate the binding sites and targets of RBPs during mRNA localization (Baltz et al., 2012).

Further employment of computational approaches will assist in quantifying the localized mRNAs, deciphering mRNP architecture, and constructing feasible models for the mRNA localization process (see Outstanding Questions). Another point that needs consideration is the modification of mRNAs. Cytosine methylation is involved in the regulation of mRNA mobility across graft junctions via the phloem and is worthy of investigation for its possible role in mRNA localization. The above-mentioned biochemical methods, in combination with high-throughput sequencing, will advance efforts to dissect the machinery involved in mRNA localization in plant cells.

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Targeting of mRNAs


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