CHAPTER

SAFE KEEPING THE MESSAGE
mRNP Complexes Tweaking after Transcription

Said Hafidh,*[,1] Věra Čapková[1] and David Honys[1,2]

[1]*Laboratory of Pollen Biology, Institute of Experimental Botany AS CR, Rozvojová, Czech Republic.* [2]*Department of Plant Experimental Biology, Faculty of Science, Charles University in Prague, Viničná, Czech Republic.*
**Corresponding Author: Said Hafidh—Email: hafidh@ueb.cas.cz*

Abstract: The mRNA-protein complexes (mRNPs, Messenger ribonucleoprotein particles) are the "couriers" of the modern eukaryotes that process, store and deliver messages (transcripts) from the nucleus to the appropriate subcellular compartments and beyond. Presence of mRNPs arbitrates the posttranscriptional control of gene expression by editing the precursor RNA to maturity, postulate its subcellular localization and/or storage and dictate its fate once in the cytoplasm; either to be translated or dispensed through mRNA degradation. Initiation of transcription is coupled with processing of the transcribed message and the immediate association of the transcript with a set of structural and regulatory proteins. *Per se*, mRNP complexes sub-optimize transcription by recruiting RNA-binding proteins which are the core component of the RNP activities that culminate overall distribution and abundance of individual proteins. This asymmetric distribution of the mRNA is the determinant of protein gradient and is known to influence cell polarity, cell fate and overall patterning during development. Embryo patterning in Drosophila, polarization of maternal mRNA to daughter cell in budding yeast and directional growth of mammalian neural cell and pollen tubes of flowering plants, are the most prominent examples of mRNP facilitated posttranscriptional control, influencing cell fates and patterns of development.

This chapter addresses the current knowledge on the mechanisms of posttranscriptional control reinforced by the formation of RNP particles and reviews differences in the underlying mechanisms. The outline of the chapter encompasses step-wise cellular processes leading to the formation of mRNPs and its implication to cellular activities. A dedicated section is also integrated discussing the recent findings on the unique mechanism of RNP formation in the male gametophyte of *Nicotiana tabaccum*. A proposed model outlines the network of posttranscriptional control with a focus on the role of RNPs is also presented aiming to stimulate future research with a perspective of advancing our knowledge on the subject and its plausible application in improving food quality.

RNA Infrastructure and Networks, edited by Lesley J. Collins.

INTRODUCTION

The complexity of the eukaryotic genome presents a challenging task for a cell to selectively "display" precise genetic information at a given time and space during development. Deployment of specific transcription factors and marking of the genome with epigenetic patterns has allowed eukaryotic cells to select and modulate the amount of genetic information in different cell types. Similarly, several classes of noncoding RNAs (ncRNAs) have been well characterized as posttranscriptional modulators of gene expression regulating protein-coding RNAs through transcription, splicing, mRNA turnover, nucleotide modification and translational repression (reviewed in ref 48). Commitment of the coding RNAs through these multiple fates is dynamic, as a result, a complex network of posttranscriptional processes that interconnect regulatory ncRNAs and the target RNAs exist and is referred as RNA-infrastructure.[48] The building blocks of the RNA-infrastructure are first initiated when noncoding and coding RNAs are assembled into RNA-protein complexes, ribonucleoprotein particles (RNPs), immediately following transcription in the nucleus. Depending on the nature of the bound proteins, RNPs can direct multiple fates of the RNA cargo and thus forms an exclusive pathway of RNA regulation. The networks of RNP mediated-RNA regulation alone represent a major component of the RNA-infrastructure and we referred it as RNP-infrastructure. In this chapter we will focus on the different features of RNP mediated posttranscriptional control of gene expression and how these processes intertwine with different aspects of development.

RNP-INFRASTRUCTURE; POSTTRANSCRIPTIONAL MODULATOR OF GENE EXPRESSION

Developmental architecture in many eukaryotes from embryonic patterning to maturity is preceded by a precise accumulation of specific macromolecules leading to polarity, cell fate establishment, patterning and organ specification. Across all diverse species of living organisms, temporal and spatial control of gene expression is imposed at the transcriptional and posttranscriptional level through chromatin modification, modulated transcriptional initiation and RNA processing, RNA localization and storage and controlled translation and protein turnover. At the centre of these transcriptional events is the assembly of the mRNA-ribonucleoprotein (mRNP) complexes that facilitate all downstream activities to determine the destination of the encoded transcript (Fig. 1). By regulating the quality and destiny of premRNAs, regulation mediated by mRNPs provides qualitative and quantitative assessment of gene expression posttranscriptionally, thereby regulating overall cellular activities. Through this coordinated network of regulation, a foundation for developing fully functional complex structures with distinct biological function is laid and propagated throughout the development of an organism. Thus, it's crucial for mRNAs with specific message to be delivered at the right location, at the right time and at the right dosage.

The exhilarating journey of a transcript following transcription starts in the nucleus where the message is wrapped with a cohort of *trans*-acting factors binding to specific *cis*-elements as it is copied from its DNA template. The message is scanned for its quality and undergoes maturation to its functional form. This complex of mutually interacting mRNA and proteins (termed mRNP for ribonucleoprotein) is fine-tuned by aggregating additional

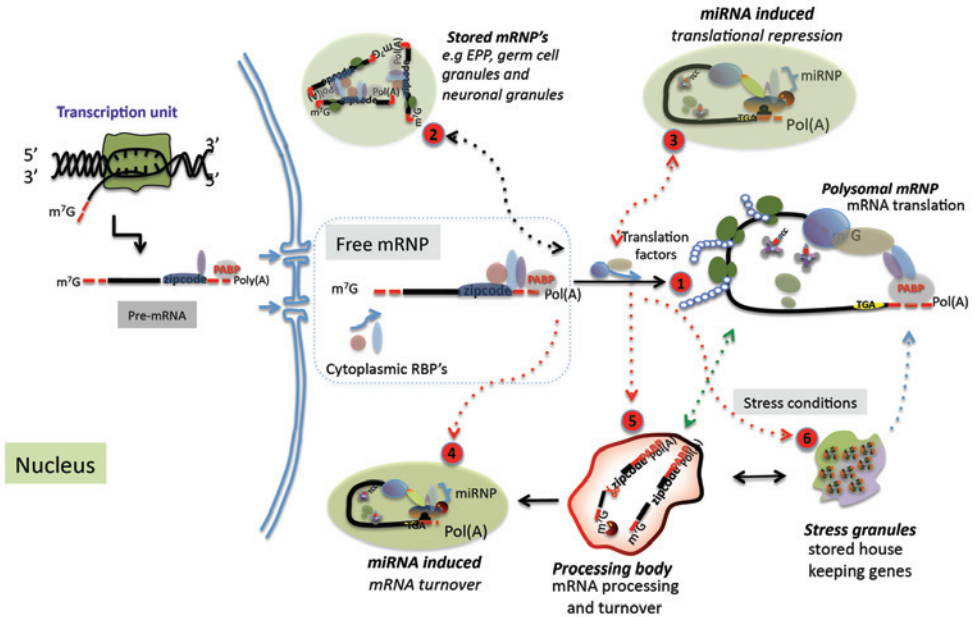

Figure 1. mRNP complexes mediate mRNA posttranscriptional fate. Transcription in the nucleus is immediately coupled with the binding of RBPs (steel blue and purple) to the appropriate *cis*-elements of the premRNA (coding region in black; 5′ and 3′-*UT*R in red) to facilitate the maturation processes and imminent transport through the nuclear pore. Once cytoplasmic, additional proteins (sky blue and brown) are recruited to the mRNP that determines the fate and proper subcellular localization of the mRNA. Several fates are imminent for the aggregated mRNP; (1) translation factors together with ribosomal subunits (olive green) might be recruited to the complex and remodel the mRNP into a translationally active polysomal mRNP. (2) Emerging mRNP complexes can be aggregated into dormant translationally sequestered granules (stored RNPs) either to be transported or stored to be utilized at later times. (3) Similarly, mRNPs can be aggregated and stored in a miRNA-induced translationally-silent state. (4) For aberrant transcripts or for modulation of gene expression, some mRNAs undergo degradation and are discarded from translation. (5) Sequestered mRNPs can be gathered in processing bodies to be processed for degradation and recycled or to be shuttled back to the cytoplasm for translation. (6) Occasionally under stress conditions, mRNPs are assembled into stress granules and sequestered from translation. Active shuttling of mRNPs between PBs, SGs and polysomal mRNP promote regulation of gene expression linked with cellular development. All features shown are not drawn to scale. Abbreviations: mRNP, mRNA ribonucleoprotein; RBPs, RNA binding proteins; UTR, un-translated region; miRNA, micro-RNA; miRNP, microRNA ribonucleoprotein complex; PBs, processing bodies; SGs, stress granules.

specialized proteins prior to its migration to the cytoplasm. Such a routine is renowned to be one of the established mechanisms of posttranscriptional regulation, necessary for the temporal and spatial localization and distribution of the target transcript within the cell, to ensure localized protein synthesis and delivery to the site of action. Messages that are transcribed but only to be translated at later times, or at later stages of developmental, or in a case of urgency such as stress or tissue damage, are stored in a form of stored RNPs and kept away from translational initiation machinery (Fig. 1).[1,2] Furthermore, their storage has to be "cost-effective" and easily accessible upon the initiation of translation, particularly in response to external stimuli. Aberrant transcripts or those accumulated in excess are directed to processing bodies (PBs) and undergo RNA degradation.[3] As such the mechanism of RNA storage and mobilization in conjunction with RNA turnover provides exquisite control of gene products, advancing the specificity installed in the promoter. The integration of newly encoded transcripts into the mRNP complexes is also critical for the subcellular localization of the mRNA and allows on-site protein synthesis. A considerable number of encoded proteins do not possess a localization signal and thus depend entirely on the localization of their transcript. The importance of correct localization and storage of mRNA during development has been studied intensively in *Drosophila melanogaster* during oogenesis. mRNA encoded by *bicoid* [*bcd*] and *nanos* [*nos*] genes are localized on the interior and posterior cortex of the Drosophila oocyte until the completion of oogenesis.[4,5] The distribution of these two sets of mRNAs is an outcome of opposing protein gradients essential for the initiation of asymmetry along the interior-posterior axis in the early developmental stages of the Drosophila embryo.[4] Localization studies have shown that polarization of the *bcd* mRNA is solely dependent on the specific sequences located within the 3′-UTR of the transcript, together with the intact polarized network of microtubule cytoskeleton.[6] Subcellular localization of mRNA is also a prevailing concept in plants and there is cumulative evidence demonstrating its significance in many aspects of cellular activities. Besides starch, rice accumulates two major classes of proteins, prolamines and globulin-like glutelins.[7] These two proteins are both synthesised on the endoplasmic reticulum (ER) and then translocated to the ER lumen. Whereas prolamines are retained and arranged into protein bodies, glutelins are transported and stored in protein storage vacuoles.[7] This fundamental differential localization has been shown to be the result of RNA-embedded subcellular RNA localization signal.[7,8]

An exquisite model of mRNP mediated posttranscriptional regulation has also emerged in the development of the male gametophyte of Tobacco (*Nicotiana tabacum*).[1,9] In all flowering plants, the development of the male gametophyte is governed by precisely orchestrated cellular activities leading to developmental maturity accompanied by cellular and morphological changes. The progression through the stages of development is propelled by underlying molecular changes including chromatin remodelling, gene expression profile and posttranscriptional regulation.[10] Once matured, the male gametophyte (pollen) undergoes dramatic progamic changes during the formation of a pollen tube. Initiation of these changes is accomplished through storage and precise localization of specific macromolecules and a `burst` of translation at a local site to promote directional growth of the pollen tube. As such, the architecture of the male gametophyte is ideal to pinpoint and understand mechanisms imposed in the progamic phase switch leading to differential gene expression, protein accumulation and cell signalling with respect to mRNA storage and simulated localization. Transcriptomic studies of the male gametophyte developmental stages have identified a subset of genes which are expressed early during development and those which are detected only at the later stages of pollen development.[11,12] Studies

in the developing tobacco pollen have established that some of the early expressed genes accumulate the message at the early stages of pollen development,[13] but, their translation was postponed to the time of pollen tube growth.[10,14] Furthermore, analyses of protein extracts in a time course of pollen development has indicated that the male gametophyte of flowering plants has evolved a more stringent system in which the late-translated messages are stored in aggregated multiple-mRNP particles and sequestered from translation.[1]

PRE-mRNA PROCESSING AND THE INITIATION OF mRNP FORMATION

Activation of gene expression is the first implicated step that determines the accumulation of a protein in a cell. Gene expression starts in the nucleus and the transcripts are ultimately mobilized to the cytoplasm for translation. Local or targeted chromatin modification involves methylation and acetylation exposing *cis*-elements of the gene to be transcribed, resulting in the binding of the transcription-activating complex and transcription of the DNA template. The transcribed RNA is immediately bound with a cohort of noncoding small nuclear RNAs with the appropriate binding proteins (snRNPs) associated with other RNA binding proteins to form a spliceosome complex responsible for splicing of the introns and maturation of the transcript. In addition to intron removal, other nuclear RNA-binding proteins (RBPs) are involved in premRNA 5′- capping and polyadenylation, mRNA export, degradation of some of the transcripts and synthesis of small interfering RNAs (siRNAs) and microRNAs (miRNAs). Proteomic studies in several plant species have identified numerous RBPs possessing RNA binding domains such as the RNP motif, RNA recognition motif (RRM), Pumilio (PUM), pentatricopeptide (PPR) and other putative motifs that have also been predicted to bind RNA.[3]

The majority of RBPs in eukaryotes have now been characterised and are known to participate in diverse roles of mRNA management including nuclear processes such as the activity of the exon junction complex (EJC) in premRNA splicing and nuclear export, initiation of translation, formation of PBs and SGs, translational repression by miRNAs and siRNAs and mRNA subcellular translocation. The precise role of EJC and the impact the nuclear processes has on correct localization of the subsequent mRNA, was apparent from studies of Drosophila *OSKAR* mRNA localization and investigation of the role of the EJC in mammalian neurons. EJC protein composition between the two organisms remained conserved and the complex was shown to posses four core proteins; RNA helicase eIF4AIII, Barentsz, Mago Nashi and Tsunagi (Y14) (as well as the human orthologs MLN51, Magoh and Y14).[15] In the course of the nuclear processes, the core proteins are anchored at the exon-exon junctions of the *OSKAR* premRNA, actively involved in its splicing and remain bound until the first round of translation is initiated.[16] The correct localization of *OSKAR* mRNA requires the inclusion of the first intron together with the localization motif in the 3′-UTR.[15] The necessity of intron 1 retention in *OSKAR* mRNA localization was demonstrated by sequential deletions of the three introns as well as deletion of more than one intron.[15] Results of these studies further demonstrated that localization of *OSKAR* mRNA was prompted by correct splicing at first exon-exon junction and was independent of the intron sequence identity.[15] As such, precise spatial distribution and binding of EJC between exon-exon junctions is not only critical for correct splicing but also essential for posterior localization of the *OSKAR* mRNA, linking EJC role in splicing and its influence on correct mRNA localization. Identification of many other RBPs in plants associated with mRNP particles and their functional significance within the mRNP complex still remains to be scrutinised.

The characteristics of the RNPs, as determined by the nature and composition of the associated proteins, dictate the fate of the loaded message as the assembled complex exits the nuclear pore. Some of the messages undergo nonsense-mediated decay (NMD),[63] whereas the majority are transported to the cytoplasm for localization, storage or immediate translation (Fig. 1). Thus, the existence of the mRNP complexes is not only essential for intracellular protein distribution and storage, but also provides an opportunity to study a snapshot of the life history of the transcript from the nucleus to its fate in the cytoplasm and subsequently highlight the link with posttranscriptional mode of regulation. Since the formation of the mRNPs is immediately initiated in the nucleus, the fate of the mRNA is predetermined well before its entry into the cytoplasm, directed by the composition and the architecture of the mRNP.

The binding of specific RBPs to the mRNA is solely dependent on *cis*-acting elements, also known as "zipcodes", which are present on the mRNA and are known to depict the fate and subcellular targeting of the message. In most specifically localized mRNAs, the zipcodes are located in the 3′-untranslated region, frequently forming secondary structures and are sometimes present as multiple repeats. *Vg1* mRNA from Xenopus oocyte is one good example.[17] Occasionally, the zipcodes can also be located in the 5′-UTR or even in the coding sequence of the mRNA as in the case of *ASH1* mRNA from yeast, in which three localization repeats have been identified in the coding sequence and an additional repeat embossed in the 3′-UTR.[18] Each *ASH1* sequence motif was confirmed to have a capacity to localize a bound transcript alone, although the presence of multiple repeats enhances the correct localization of the message. Arn et al[19] proposed a functional model that multiple clusters of localization motifs are likely to promote regional concentrations of RBPs, which in turn attract other proteins through protein-protein interactions necessary for RNA localization.[19] One of the best characterized RNA-binding proteins utilizing localization elements is the chicken zipcode binding protein 1 (ZBP1) which binds to a conserved zipcode of the β-actin mRNA in its 3′-UTR and enables the translocation of the message to actin-rich protrusions in primary fibroblasts and neurons.[22] Characterization of the ZBP1 have led to the identification of two RNA recognition motifs (RRM) and four hnRNP K homology (KH) RNA binding domains.[23] The authors[23] identified distinct roles for each of the found domains in which the KH domains were identified to mediate binding to the zipcode and formation of an RNP, whereas, the RRM motifs were responsible for the subcellular localization of the β-actin RNPs. An equivalent of ZBP1 in plants, OsTudor-SN, is known to be involved in the subcellular trafficking of prolamine and glutelin mRNAs along the actin filaments.[24]

Another important set of proteins associated with mRNP particles are those dedicated for modulating gene expression and mRNA translation, as well as dictating the rate of protein accumulation. Several have been identified including Argonaute proteins (AGO) and Argonaute-like Piwi proteins which together with Piwi interacting RNAs (piRNAs) direct cleavage of the target mRNA, sequestered transcripts from translation and represses transposon translocation in somatic cells and germ cells respectively (Fig. 1).[25,57,58] These proteins are known to interact with miRNAs, siRNAs, or piRNAs preloaded in the mRNP to induce transcript cleavage via the RISC complex. Alternatively, some (e.g., human let-7 miRNA)[59] can also impose translational repression via the interaction of AGO protein with the 7-methyl-guanine (m7G) cap of the mRNA outcompeting translation initiation factor elF4E.[26] This alternate role of the RISC complex is a result of partial complementarities between miRNA and the miRNA binding sites, which leads to translational repression or accelerating the degradation of the target mRNA within Processing bodies, PB (see below and refer to Table 1). In numerous studies, miRNA or siRNA-targeted transcripts

Table 1. Glossary of the cellular components used in the chapter

Name	Localization	General Description	RNA Processing Role	Refs
Polysomes (Polyribosomes)	Cytoplasm	Cluster of ribosomes bound to mRNA. Exist in free, cytoskeletal or membrane-bound form.	Involved in protein synthesis on the associated mRNA. Can initiate translation at the 5′-UTR or through specific internal motifs.	25,37
mRNA ribonucleoprotein particles (mRNPs)	Cytoplasm	A complex of proteins bound to mRNA associated with ribosomes (polysomal mRNPs) or without (free mRNPs).	Splicing, cytoplasmic translocation, subcellular localization, transcript storage, dictate fate on translation and protein abundance.	9,37
EDTA/puromycin-resistant particles (EPPs)	Cytoplasm	Aggregated mRNPs with preloaded translation machinery first identified in the tobacco male gametophyte as large RNPs co-sedimenting with polysomes and resistant to polysome-destabilising substances.	Provide robust storage of translationally silenced mRNAs in the early stages of pollen development and promotes immediate translation upon pollen tube growth.	1,9
Stress granules (SGs)	Cytoplasm	Generated in response to stress-induced polysomes disassembly and translational silencing.	Stores silenced aggregated mRNPs and resupply ‘preloaded’ mRNPs for immediate translation.	3,37,48
Processing bodies (PBs)	Cytoplasm	Sites of mRNA quality control and decay driven by 5′→3′ exoribonuclease	Decapping, degradation and recycling of mRNAs and aberrant RNA’s. Interplay with translation machinery.	3,25,37, 48
Exon junction complex (EJC)?	Nucleus	Associate with precursor RNA and possess helicase activities	Intron splicing, mRNA fate and quality control.	16
Cytoskeleton	Cytoplasm	Scaffolding of the cell made of actin fillaments and microtubules.	Intracellular transport of RNA and proteins.	6,20,28, 29,30,49
Endoplasmic reticulum (ER)*	Cytoplasm	Interconnected network of tubular membranes and vesicles with an extension to Golgi apparatus (cisternae).	Rough-ER: site of protein synthesis, packaging and transport.	7,8,20, 27,49

*Three forms of ER; Rough endoplasmic reticulum (protein synthesis and transport), Smooth endoplasmic reticulum (lipids and steroids synthesis, control of cellular metabolism), Sarcoplasmic reticulum (regulate calcium concentration).

were demonstrated to be sequestered to PBs and many translationally repressed mRNAs have been localized within these granules.[59-61] Under certain conditions such as stress, some of the repressed transcripts are relieved from inhibition and re-enter the translation machinery. A well-demonstrated example is the human *CAT-1* gene that is expressed in the hepatoma Huh7 cells. The *CAT-1* mRNA is naturally sequestered from translation by miR122 to the PBs, however, this inhibition is lifted during stress and prompt recruitment of *CAT-1* mRNA to polysomes and subsequent translation.[62] Presence of other repressing RBPs also results in the lack of translation through yet unknown mechanisms and their repression is relieved upon posttranslational modification.

MOBILIZATION TO THE SITE OF STORAGE AND mRNA LOCALIZATION

mRNA localization provides an efficient means of establishing a targeted protein synthesis at specific subcellular location and thus generating a gradient of protein accumulation and a subsequent local function. Although several mechanisms of mRNA localization have been proposed that concurrently accomplish the subcellular localization of several transcripts, the most common pathway involves the translocation of mRNPs and anchoring at the site of translation.[6] It is now known that during the mRNP formation in the nucleus, a class of accessory proteins are also bound to the transcript that mark its intracellular localization. Several of these proteins have been identified in mammals and a well described example is the family of heterogeneous nuclear ribonucleoprotein proteins (hnRNP) of which some putative homologs have already been identified in *Arabidopsis thaliana* and rice.[27] It is likely that hnRNPs are recruited to the mRNP particles via zipcodes recognition. Since there is no defined general localization consensus sequence, hnRNPs are unlikely to provide specificity during mRNA translocation, instead other cohorts of transacting factors might be responsible in playing this role in concert with bound hnRNPs. The significance of hnRNPs in mRNA localization was demonstrated in neuronal cells whereby hnRNAP A2 was identified to be involved in the localization of the myelin basic protein in oligodendrocytes of the mammalian neurone and in Drosophila in which Hrp48 is required for subcellular localization and a subsequent translational regulation of many RNA molecules.[27]

Once the nuclear mRNPs are translocated into the cytoplasm (cytoplasmic mRNPs), they are packaged into a transported form through the recruitment of cytoplasmic RBPs to the complex, leading to the transformation of the nuclear mRNP into transported granules or particles. At this stage, the mRNP bears several sets of transacting factors involved in mRNA translocation, anchoring and those involved in translational regulation and subsequent protein synthesis. The packed mRNP is sequestered from translation by specific set of bound proteins during translocation until it reaches its destination.

CYTOSKELETON: ACTIN AND MICROTUBULE DYNAMICS AS A FLOATING RAFT FOR mRNPs SUBCELLULAR LOCALIZATION

The cytoplasmic mRNPs are conveyed to their destination via the cytoskeleton, most commonly through microtubules and occasionally through actin microfillaments which provide a basic "road map" interconnecting different parts of the cell, as well as act as a

scaffold for translational purposes. The initial understanding that the cytoskeleton is the framework that provides cell shape has expanded to a much wider role particularly in subcellular mRNA localization and control of localized protein synthesis. A combination of biochemical studies together with advances in cellular imaging, has allocated a central role of cytoskeleton in mediating movements of macromolecules. *Per se*, this promotes on-site translational regulation and protein function, which consecutively establishes a local gradient of cellular proteins. This differential protein accumulation influence cellular patterning as well as facilitates mRNA translocation to cellular organelles and the extracellular matrix.[28] Deployment of mRNAs over a short distance along the cytoskeleton network operates through actin filaments, whereas, long distance trafficking such as in oocytes, neural cells and likely in other directionally growing structures utilizes microtubules to move along the mRNP granules.[29] It is not yet known how the mRNPs are translocated along the cytoskeleton in plants, but the involvement of motor proteins has been well demonstrated in other eukaryotes. For instance, the subcellular localization of *ash1* mRNA to the bud tip of the daughter cell in yeast is known to be mediated by myosin, whereas, similar mechanisms involving kinesin and dynein-mediated movement have been proposed in the Drosophila oocyte and human oligodendrocytes.[30] However, a more pinpointed mechanism behind mRNPs trafficking along the cytoskeleton in plants remains to be unravelled. It is tempting to envision and there is already a strong indication pointing towards the involvement of RBPs together with cytoskeleton interacting proteins in tethering and facilitating the movement of the mRNPs along actin and microtubule railings to promote their localization.

INTRA- AND INTERCELLULAR TRANSLOCATION OF mRNPs

The fact that plants are sessile and instead respond to environmental stimuli to induce necessary physiological changes, means that cell-to-cell signalling becomes one of the critical steps to communicate and promote overall changes. Unlike animals, plant cells are gated (made of discontinuous cell wall that forms plasmodesmata openings) suggesting possible exchanges of macromolecules and other cellular components between adjacent cells. Indeed, several examples demonstrate the movement of RNA and other particles through plasmodesmata as a long distance migration of transcripts (signaling molecules) influencing cellular activities of the neighboring cells. One classical example is the cell-to-cell movement of viruses following an infection, which results in the systematic spreading of the infection. The mechanism behind this viral cell-to-cell RNA translocation is the work of the movement proteins, which interact with the plasmodesmata and alters their size-exclusion limit promoting RNA intercellular trafficking.[31] In another example, *KNOTTED1* (*KN1*) and *SUCROSE TRANSPORTER1* (*SUT1*) are two plant genes whereby their encoded transcripts are also translocated between neighbouring cells. The intercellular movement of *KN1* has been shown to mimic the mechanism imposed by viral movement proteins and it is now known to be involved in the initiation and maintenance of meristem in shoot apex as well as between cell layers within the leaf.[32] *In situ* hybridization studies have localized *SUT1* mRNA in companion cells and in nuclear-less sieve elements (SE) of the phloem system. Since SE lack nuclei, *SUT1* mRNA is transcribed in companion cells and then translocated to the SE with an as yet unknown mechanism. The detection of SUT1 protein in SE also signifies a likelihood of intercellular trafficking of core translational components.[32] Similarly, the plant vascular system has also been proposed as a passage for a long distance transport of molecules.

The phloem system is responsible for delivering a variety of signalling molecules such as hormones, whereas the xylem system is involved in the transport of water and nutrients. The ability of the small interfering RNAs (siRNAs) to exert a systematic wide spread distribution throughout the plant suggest an existence of an effective system in which these small molecules can be transported. Indeed, Bartel et al[33] and Kidner et al[34] in their analyses of phloem sap identified diverse species of miRNAs, firming up the model of wide spread siRNA induced effect in regulating plant development through the vascular system. A well-demonstrated example of long distance mRNA translocation in plants is that of the *Fowering locus T* (*FT*), which responds to day length and induces flowering. Perceived light signal on the leaf leads to the transcription of the *FT* gene in leaves. However, its effect is imposed in a distantly located floral meristem in the shoot apex.[35] Trafficking of the *FT* transcript along the phloem was later demonstrated by transiently expressing *FT* on a single Arabidopsis leaf using a heat shock promoter. Following induction, expressed transcripts were detected at the shoot apex verifying signal perception in a form of mRNA via phloem system. A similar mode of action has been recently described for the BEL1-like family of transcription factors in potatoes in which *StBEL5* (a gene that regulates tuber formation) is induced in leaf veins and petioles but exerts its effect in stolon tips.[36] Thus, plants have developed sophisticated mechanisms of dispatching the information between cells and between organelles and are able to induce a long distance effect by translocating necessary mobile signals. It is yet to be demonstrated how transcripts are packaged for long distance migration, although future studies are anticipated to uncover stored mRNAs in a form of RNPs.

The co-existence and the symbiotic relationship between plant cells and cellular organelles is also governed by the exchange of macromolecules. The partial transcriptome encoded by the chloroplast and mitochondrial genomes is insufficient for a complete biogenesis and function of these organelles. Nuclear encoded proteins are known to be imported into the chloroplasts and mitochondria (represent >90% of the required proteins), through interaction of peptide motifs (*trans*-peptides) of the imported proteins and the envelope membrane channel import complex (protein translocons). Nonetheless, nuclear encoded RNAs such as *ATM1* from yeast and ChL H and CHL 42 in plants are also targeted to these organelles. The *ATM1* possesses two repeats of mitochondrial zipcodes located in the 3′-UTR and 48-nucleotide sequence in the coding sequence necessary for its import to the mitochondria. Although the precise mechanism of intercellular RNA trafficking still remains elusive, more and more evidence are now emerging demonstrating the movement of RNAs within the cell and beyond, deployed as part of signal transduction or for other cellular functions.

STORAGE OF mRNPs AND mRNA TURNOVER

Although a significant proportion of the mRNAs are translated immediately once they have localized, a subset of the transcripts are redirected to be stored and inactivated from translation in various types of stored RNP particles, stress granules or discarded into processing bodies where they undergo a nonsense-mediated decay and eventually are degraded and recycled (Fig. 1). Likewise, other types of stored RNP granules have also emerged including neuronal granules (found in neurons) and polar or germinal granules that have been identified in germ cells compartments in flies.[37] These types of RNA granules have a similar role to that of SGs, however, they are not produced in response to stress.

Stress granules and processing bodies are active sites of mRNP processing in eukaryotes. SGs are a class of RNA granules that are highly up-regulated during translational repression predominantly in response to stress (Table 1). Stress response induces shuttling of transcripts from translationally active polysomes to mRNPs that aggregates into large cytoplasmic foci and sequesters transcripts from translation. Stored mRNAs are shuttled back to polysomes for rapid translation following recovery from stress. Thus, SGs are the centre of mRNA processing providing emergency storage and protection of functional transcripts and their immediate resupply following recovery from stress. Several proteins have been identified that constitute SGs some of which are in common with PBs and also those that are exclusive to SG's. Some of the identified proteins includes T-cell intracellular antigen 1 (TIA1), TOA1-related (TIA1R), eIF4E, eIF4G, eIF4A, eIF3, PABP, G3BP1 and 40S ribosomal subunits.[3] TIA1 has been characterised as a multitask component serving as a translational silencer, a regulator of alternative splicing and mRNA decay. Plants orthologs of TIA1 have also been identified and a mutation in *Nicotiana plumbaginafolia* TIA1-like oligouridylate-binding protein 1 (UBP1) prevents SGs formation.[3]

Processing bodies on the other hand (Table 1), are the sites for mRNA degradation prompted by the polyA-tail deadenylation, RNA-mediated gene silencing and translational repression. PBs are generally characterised for possessing 5′-m^{7}G decapping enzymes (DCPs), 5′-3′ exonuclease (XRN1), a class of Ago proteins and Ago-like (GW182) in animals cells.[37] Arabidopsis null mutant *xrn4-5* which has reduced cytosolic 5′-3′ exonuclease activities shows increase accumulation of PBs foci demonstrating linkage of PBs with their role in mRNA degradation.[3]

Characterisation of another form of stored RNP particles, germ cell granules, with respect to their protein and RNA constituents in many organisms showed that they resemble both mRNA storage properties of SGs and RNA decay characteristics of PBs. Mammalian germ cell granules (chromatoids) possess several components involved in small RNAs processes and mRNA decay. The Dicer enzyme, Argonaute proteins, GW182, MIWI (a homologue of Drosophila PIWI), several species of miRNAs, DCP1A and XRN1 represent several identified components with known function.[25] In part, it is hypothesised that the mammalian germ cell granules are equipped to modulate gene expression and to dictate the initiation and efficiency of protein translation, although a much wider role has been also implemented. Similar germ cell granules have been also found in the Drosophila germ line linking them with the control of protein translation and miRNA mediated transcriptional control of maternally expressed genes. Distinctively, RNA granules recently identified in the gonadal syncytium (a large cell-like structure consisting of multiple haploid sperm cell nuclei following germ cell division without cytokinesis) of *Caenorhabditis elegans* have been annotated to be compositionally similar to both PBs and SGs.[25] They contain PB markers CGH-1 (conserved germline helicase 1) and CAR-1 (cytokinesis, apoptosis, RNA-associated 1) as well as SG-associated proteins like PAB-1 (poly(A) binding protein 1) and ATX-2 (ataxin-related 2). This suggests their possible dual role in stabilizing maternal mRNAs and simultaneously repressing their translation.[38] Occurrence of similar factors in the germ cell granules of flowering plants remains to be demonstrated, however, their presence is imminent.

The ontogenic development of the male gametophyte in which a small daughter cell (germ cell) is enclosed within a larger daughter cell (vegetative cell), provides a unique model in understanding the mechanism of transcriptional and translational control, in which the two cell types acquires two distinct fates. This distinct specification of the

two cell types suggests differences in the underlying molecular pathways leading to cell differentiation. Consistent with this objective, the earlier expression of male gametophytic genes and a need for high rate of translation during pollen tube growth, suggests a demand for a robust storage system that could withstand a long-term storage and yet to deliver the message efficiently. A number of pollen mRNAs were shown to be bound to pollen stored mRNP particles.[9] However, the nature of the isolated mRNPs was "indestructible" in a buffer constituting strong detergents in comparison to other universal forms of RNPs. Furthermore, these two types of mRNPs showed different densities in a sucrose gradient. This novel class of RNP particles were annotated as EDTA/puromycin-resistant particles or EDTA/puromycin-resistant particles (EPPs).[1,9] With NTP303 as a model example,[1,9,13,39] EPP complexes seem to offer that secure storage. The authors hypothesised that the EPP complex is assembled gradually during development by agglomeration of the mRNP monomers following initial assembly of the *ntp303* mRNP particles.

Recent identification of the EPP particles in the tobacco male gametophyte highlights the presence of germ cell-like granules in flowering plants.[1] Just like the role played by Drosophila germinal granules in delivering maternal mRNAs and its role during initial stages of embrogenesis, plants EPP particles demonstrate nicely the significant role of RNA granules (in the form of mRNP) as a developmental "clock" that induces programic and morphological changes in response to activating stimulus (Fig. 2). EPP particles represent preloaded complex machinery devoted to mRNA processing, transport, subcellular localization and protein synthesis. Evidence from the analysis of isolated EPP fractions identified several proteins associated with protein metabolism including; protein synthesis (eIF4A-8, eukaryotic initiation factor 4A-8), protein fate (BiP4, laminal-binding protein 4 precursor) and protein transport (Rab11a, Ras related protein) and those associated with RNA localization and translation including cytoskeletal proteins, protein kinases and phosphotases. The composition of EPP particles also consists of set of mRNAs that are stored and translationally silenced at earlier stages of development and travels with the maturation processes of the pollen grain. Some of the stored messages are massively translated either at the late stages of pollen development and/or transported to the growing tip of pollen tube to be translated and deposited at the tip region (Fig. 2). This potential role of EPPs particularly resembles that of the growing dendrites of the neuronal cells in human. The directional growth in neurons is facilitated by the transport of sequestered mRNAs by neuronal granules to the synaptic surfaces for translation. Similar to EPPs, neuronal granules are also preloaded with translational machinery and several regulatory RBPs. Among the identified components of neuronal granules includes silenced mRNAs, RBPs such as; HuD, G3BP, Sam68, SYNCRPI, hnRNP A2, RNG105, FMRP and Staufen, as well as translation initiation factors and small and large ribosomal subunits.[25] As such, the neuronal granules are the mediators of nerve cell networking depositing transcripts to the growing tip and catalyze their efficient translation thereby promoting directional growth. Such a role is already emerging in plant EPPs and pollen tube growth,[1] though further experiments are necessary to validate this role and address other potential functions of EPP's that arbitrates the dynamics of gametophyte development.

Moreover, a recent study by Bayer et al[40] have identified a paternally expressed *SHORT SUSPENSOR* (*SSP*) which promotes elongation of the embryonic suspensor cells by activating *YODA-MAPK* pathway (*YDA*). *SSP* is expressed at the mature pollen stage however the protein can only be detected in the zygote and the endosperm post fertilization suggesting delivery of the transcript by the twin sperm cells. Since the EPP mode of organisation seems to be a male phenomenon so far, the *SSP* transcript is likely

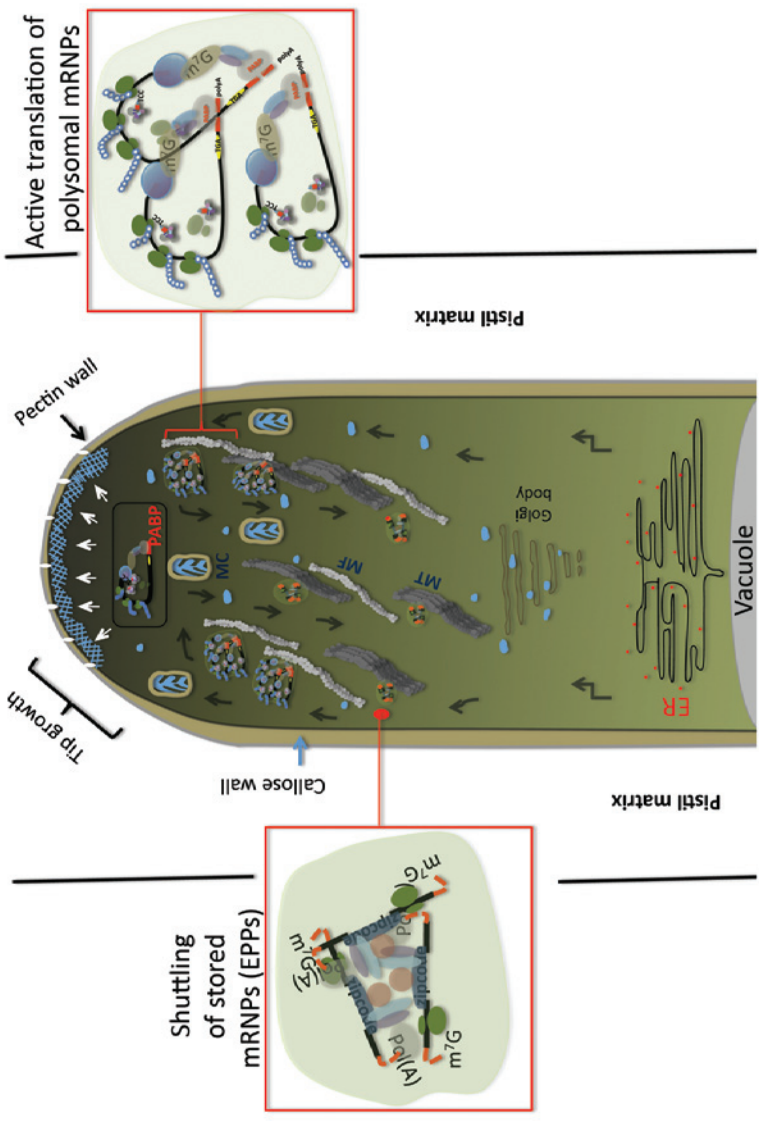

Figure 2. Stored mRNPs facilitate pollen tube tip growth. Directional growth as seen during pollen tube growth as well as in dendritic extension in neuronal cells are preeminent examples of stored mRNA utilization in a form of mRNPs (or EPPs as in tobacco male gametophyte) that provides dynamic and spatial control of gene expression during development. Sequestered stored particles are targeted to the tip region (site of growth) along an array of microtubules (MT) and microfilaments (MF) and delivered to the site of active translation and protein concentration. Neuronal studies have also demonstrated the existence of large RNA granules resembling the characteristics of EPP particles of the tobacco male gametophyte. Features in the diagram are not drawn to scale. ER; endoplasmic reticulum.

to be governed by a similar mechanism as the *NTP303* prior to its delivery in the female gametophyte. In view of the fact that both examples are drawn from genes critical for fertilization and zygotic development, the "indestructible" nature of EPPs points to the notion that EPPs are likely to be a step higher to the role played by regular mRNPs and thus offering maximum storage security and assuring the delivery of the important transcripts.

It remains uncertain how cells control the fate of each transcript, nevertheless, there is an accumulative wealth of evidence that the presence of polyA tails influences mRNA fate either to be stored, decapped and degraded, or returned into the translational machinery for synthesis.[41] An insight into the mechanisms that regulates mRNA shuttling between polysomes and RNA granules has started to emerge and further studies on mRNP characteristics would enlighten the complete network of regulation imposed by mRNPs posttranscriptionally.

SYSTEMATIC TRANSLATION REPRESSION BY SMALL RNAs

The continuous effort that has been focused in the understanding of the mechanisms imposed in posttranscriptional control of gene expression, has lately deciphered a complex network of choreographed interaction between mRNAs, microRNAs, proteins and the surrounding cytosolic structures. Since the discovery of the RNA interference phenomenon in plants, research studies over the last decade have evidently proven that double stranded small RNA molecules are universal and effective regulators of gene expression during development in all eukaryotes. RNA interference (RNAi) particularly the subtype microRNA and small interfering RNA, have long been implicated to modulate expression of their target genes posttranscriptionally by inducing endonucleolytic cleavage of the target transcripts.[42] Similar classes of the small RNAs in animals are now known to be involved in the degradation of the target transcripts as well as in the inhibition of translation initiation.[43] FOG-2 is a transcription factor required for cardiac development in animals and it is regulated by miR-130a.[43] Northern analysis showed steady accumulation of the *FOG-2* RNA transcripts predominantly in the heart and brain, however, immunodetection studies showed the dynamic peak of the protein accumulation at embryonic day 16.5 and its diminution at the neonate stage. This dynamic accumulation profile of the FOG-2 protein corresponds to the alternate accumulation of the miR-130a, pointing to the translational regulation of FOG-2 by miR-130a.[43] Another good example of miRNA translational regulation in animals, is that of the dendritic miRNA-134 required for the outgrowth of hippocampal neurons.[44] The miRNA-134 promotes this outgrowth by inhibiting translation of the translational repressor *PUMILIO-2*. Until recently, the notion of translational repression induced by miRNAs was unique to the animal miRNA pathway. The simplest explanation of this particular feature of miRNA action in animals was the result of imperfect hybridization with their target transcripts, whereas plant miRNAs show perfect complementarity with their target sites. Various studies in plants have now emerged demonstrating the "full-throttle action" of the small RNAs pathways from mere RNA cleavage to the block of translation initiation, hinting that the phenomena of translational repression imposed by miRNAs also exist in plants (Fig. 1). Two independent surveys of flowering abnormalities in Arabidopsis have uncovered a posttranscriptional control of floral organ identity genes named *APETALA2* (*AP2*) and *AP2*-like genes, to be under the regulation of miRNA172.[45,46] It was identified that miRNA172 controls *AP2* activity by modulating *AP2* mRNA levels through cleavage

of the mRNA as well as by translational repression of the same transcripts. In respect to these findings, Brodersen et al[47] presented a functional verification of the miRNA role in translation repression in plants, by measuring the abundance of a green fluorescent protein (GFP) integrated with the miRNA171 binding site. Thus, the emerging picture from these findings together with the currently ongoing experiments collectively suggest that miRNAs in plants just like in animals also execute their posttranscriptional regulatory actions at the translational level, independent of directing mRNA cleavage, or even incorporate both levels of posttrancriptional regulation for a more advance control of the target gene expression. Such advance control of gene expression has been hypothesised to be more apparent for genes having a developmental role for the reason that their misregulation can be detrimental during development. Therefore, the dual posttranscriptional control imposed by the formation of mRNP particles together with the action of small RNAs molecules, provide a mechanistic control of gene expression that influence cell fate decisions marking and sustaining the blueprint of development.

REINITIATING TRANSLATION OF THE LOCALIZED, TRANSLATIONALLY SEQUESTERED mRNAs

The innovation of mRNP formation as a posttranscriptional mediator of gene expression and mRNA translation might have occurred primarily to sequester mRNAs from immediate translation. Imminent repression of the mRNP prior to its entry into the cytoplasm is a must, otherwise preloaded translational machinery would initiate premature translation and thus, interfere with the localization "machinery" precluding subcellular localization and preventing delivery to other intended destinations. This particular characteristic of mRNPs has been a key determinant of morphogen gradient and cell fate specification, polarised subcellular activities, in addition to promoting intra- and intercellular mRNA trafficking. Although there is not enough evidence specifically from plants, a firm model depicting numerous mechanisms of translational initiation and reinitiation has been developed generally for prokaryotes and eukaryotes.[48] Once the transcript is anchored to its subcellular location, interaction of cytoskeleton binding proteins with the bound RBPs has been hypothesised to fuel the reinitiation of translation of the sequestered transcript. Recent reports have also provided evidence for the role of posttranslational modification of RBPs in the repression and reinitiation of translation. For instance, a Drosophila ZBP1 protein is required for the localization of β-actin mRNA to the leading edge of a lamellipod (a cytoskeletal actin projections of the moving cell) in fibroblasts and to distal ends in growth cones and dendritic spines in neurons.[49] Binding of ZBP1 to the β-actin zipcodes represses untimed translation during the localization process. This translational inhibition is relieved upon localization of β-actin. On-site phosphorylation of ZBP1 by a member of the family of membrane-associated kinases (*Src*), thereby spatially and temporally separates localization and translational initiation.[50] A similar mechanism has been also implicated for *Ash1* mRNA in growing buds of the yeast *Saccharomyces cerevisiae* to promote mating type by restricting translation of *Ash1* mRNA in the daughter cell nuclei. This repression is attained through the association of the Puf6p protein with the 3′-UTR of *Ash1* mRNA. The deficiency of Puf6p activities results in en-route *Ash1* mRNA translation and subsequent lack of protein symmetry.[51] In instances where the translational repression was an outcome of miRNA binding, no clear studies have emerged to address how the actual repression is lifted and how the mRNP

is routed back to the active translational machinery. However, what is known is that in an unrepressed form, the absence of miRNA allows the binding of translation initiation factor eIF4E to the 7-methyl-guanine (m^7G) cap and, through eIF4G, its interaction with poly(A) binding protein (PABP) to form a closed loop necessary for efficient translation. Equally, binding of miRNA to the target mRNA could result in RNA cleavage or create a competition for cap binding between the associated Ago protein and eIF4E, thereby releasing eIF4E/G and halting the initiation of translation.[52] Due to the nature of inhibition, it is tempting to speculate that posttranslational modification most likely of the Ago protein, would be one of the mechanisms that de-represses the inhibition of translation prompted by miRNA binding.

CONCLUSION AND FUTURE PERSPECTIVES

Advances in the survey of RNP infrastructure have highlighted the extent through which the role of mRNP complexes becomes indispensable, seemingly orchestrating cellular processes in coordination to developmental cues. Diverse complexes of mRNP particles govern the second level of posttranscriptional control, modulating the abundance of gene expression in response to morphological changes during development. The stepping stones of this type of regulation involve binding of a cohort of RBPs, leading to the titration of the message away from the translation machinery, first localized and then stored at the allocated position for later use or dispensed through several mRNA decay mechanisms. The mRNP-mode of regulation and the repertoire of events thereafter are the major drivers promoting asymmetric distribution of cell fate determinants, mobile signalling simulating developmental and environmental response, as well as concentrating and supplies specific proteins in the localized regions of development. Continued effort in this field is still uncovering a cascade of regulated mRNAs and previously missing components of the network. The sheer scale through which mRNPs operate is well demonstrated in the developing male gametophyte of *Arabidopsis thaliana* in which several independent studies have identified a combined maximum of 48.4% total proteome at the mature pollen stage of the actively expressed late genes.[53-56] These findings suggest a large scale translational repression of the encoded transcripts. From this perspective, comparative studies of the EPP transcriptome would explain "where all the transcripts go" and simultaneously validating translational activation in the growing pollen tube. The rapid growth of the pollen tube, which also resembles that of the human neuronal dentrites, demands a fast delivery of functional proteins to the tip region. Although pollen tubes deliver sperm cells with a negligible amount of cytoplasm, cytoplasmic inheritance through the ovules plays a significant role in transmitting genetic information and reinforcing parental epigenetic patterns. As such, assured storage of the messages becomes vital for inducing programic developmental changes and for stable inheritance of the genetic information. Modern eukaryotes seem to have achieved this by utilizing stored messages in a form of mRNP localized in the proximity of site of action where proteins are synthesised in an instant, guaranteeing fast delivery. Thus, the invention of mRNP particles and its high order arrangement (i.e., EPPs) in the male gametophyte of flowering plants seems to deliver that message without any major pitfalls.

Recent efforts combining genetic, transcriptomic and proteomic studies have led to the identification of more RNA-binding proteins and gave more insight to the mechanism of posttranscriptional control. Similarities and differences to the nature of

mRNP-mediated posttranscriptional regulation deserve additional attention to appreciate the significance of the dynamics of the RNPs infrastructure. The application of advanced genomic and proteomic technologies, together with modern histological techniques, is anticipated to shed more insight to this molecular connection of RNPs with patterns of cellular development. Targeted studies of the mechanism of EPP formation and translational repression during male gametophyte development and its significance to the pollen transcriptome and consequently proteome, will be of considerable interest adding the dimension to the mechanisms of posttranscriptional regulation in connection with gametophytic development.

ACKNOWLEDGMENT

The authors gratefully appreciate the financial support from the Grant Agency of the Czech Republic (grant no. 522/09/0858) and from the Ministry of Education, Youth and Sports of the Czech Republic (projects no. LC06004, OC08011 and OC10054).

REFERENCES

1. Honys D, Reňák D, Feciková J et al. Cytoskeleton-associated large RNP complexes in tobacco male gametophyte (EPPs) are associated with ribosomes and are involved in protein synthesis, processing, and localization. J Proteome Res 2009; 8(4):2015-2031.
2. Wood AJ, Oliver MJ. Translational control in plant stress: the formation of messenger ribonucleoprotein particles (mRNPs) in response to desiccation of Tortula ruralis gametophytes. The Plant Journal 1999; 18(4):359-370.
3. Bailey-Serres J, Sorenson R, Juntawong P. Getting the message across: cytoplasmic ribonucleoprotein complexes. Trends Plant Sci 2009; 14(8):443-453.
4. Ephrussi A, Dickinson LK, Lehmann R. Oskar organizes the germ plasm and directs localization of the posterior determinant nanos. Cell 1991; 66(1):37-50.
5. Gavis ER, Lehmann R. Localization of nanos RNA controls embryonic polarity. CELL-CAMBRIDGE MA- 1992; 71:301-301.
6. Kloc M, Zearfoss NR, Etkin LD. Mechanisms of subcellular mRNA localization. Cell 2002; 108(4):533-544.
7. Yamagata H, Sugimoto T, Tanaka K et al. Biosynthesis of storage proteins in developing rice seeds. Plant Physiol. 1982; 70(4):1094.
8. Choi SB, Wang C, Muench DG et al. Messenger RNA targeting of rice seed storage proteins to specific ER subdomains. Nature 2000; 407(6805):765-767.
9. Honys D, Combe JP, Twell D et al. The translationally repressed pollen-specific ntp303 mRNA is stored in nonpolysomal mRNPs during pollen maturation. Sex Plant Reprod 2000; 13(3):135-144.
10. Twell D. Pollen developmental biology. In: O'Neil SD, ed. Plant Reproduction. Annual Plant Reviews Sheffield, UK: Sheffield Academic Press; 2002. p. 86-153.
11. Honys D, Twell D. Comparative analysis of the Arabidopsis pollen transcriptome. Plant Physiol 2003; 132(2):640.
12. Honys D, Twell D. Transcriptome analysis of haploid male gametophyte development in Arabidopsis. Genome Biol 2004; 5(11):R85.
13. Čapková V, Štorchová H, Tupý J. Protein synthesis in pollen tubes: preferential formation of new species independent of transcription. Sex Plant Reprod 1988; (1):150-155.
14. Štorchová H, Čapková V, Tupý J. A Nicotiana tabacum mRNA encoding a 69-kDa glycoprotein occurring abundantly in pollen tubes is transcribed but not translated during pollen development in the anthers. Planta 1994; 192(3):441-445.
15. Hachet O, Ephrussi A. Splicing of oskar RNA in the nucleus is coupled to its cytoplasmic localization. Nature 2004; 428(6986):959-963.
16. Tange TØ, Nott A, Moore MJ. The ever-increasing complexities of the exon junction complex. Curr Opin Cell Biol 2004; 16(3):279-284.

17. Lewis RA, Kress TL, Cote CA et al. Conserved and clustered RNA recognition sequences are a critical feature of signals directing RNA localization in Xenopus oocytes. Mech Dev 2004; 121(1):101-109.
18. Gonzalez I, Buonomo SBC, Nasmyth K et al. ASH1 mRNA localization in yeast involves multiple secondary structural elementsand Ash1 protein translation. Curr Biol 1999; 9(6):337-340.
19. Arn EA, Cha BJ, Theurkauf WE et al. Recognition of a bicoid mRNA localization signal by a protein complex containing Swallow, Nod and RNA binding proteins. Dev Cell 2003; 4(1):41-51.
20. Lipshitz HD, Smibert CA. Mechanisms of RNA localization and translational regulation. Curr Opin Genet Dev 2000; 10(5):476-488.
21. Martin KC, Ephrussi A. mRNA localization: gene expression in the spatial dimension. Cell 2009; 136(4):719-730.
22. Ross A, Oleynikov Y, Kislauskis E et al. Characterization of a beta-actin mRNA zipcode-binding protein. Mol Cell Biol 1997; 17(4):2158.
23. Farina KL, Huttelmaier S, Musunuru K et al. Two ZBP1 KH domains facilitate {beta}-actin mRNA localization, granule formation and cytoskeletal attachment. J Cell Biol 2003; 160(1):77.
24. Wang C, Washida H, Crofts AJ et al. The cytoplasmic-localized, cytoskeletal-associated RNA binding protein Os Tudor-SN: evidence for an essential role in storage protein RNA transport and localization. The Plant Journal 2008; 55(3):443-454.
25. Anderson P, Kedersha N. RNA granules: posttranscriptional and epigenetic modulators of gene expression. Nat Rev Mol Cell Biol2009; 10(6):430-436.
26. Kiriakidou M, Tan GS, Lamprinaki S et al. An mRNA m^7G cap binding-like motif within human Ago2 represses translation. Cell 2007; 129(6):1141-1151.
27. Crofts AJ, Washida H, Okita TW et al. Targeting of proteins to endoplasmic reticulum-derived compartments in plants. The importance of RNA localization. Plant Physiol 2004; 136(3):3414.
28. Muench DG, Park NI. Messages on the move: the role of the cytoskeleton in mRNA localization and translation in plant cells. Botany 2006; 84(4):572-580.
29. de Heredia ML, Jansen RP. mRNA localization and the cytoskeleton. Curr Opin Cell Biol 2004; 16(1):80-85.
30. St Johnston D. Moving messages: the intracellular localization of mRNAs. Nat Rev Mol Cell Biol 2005; 6:363-375.
31. Lazarowitz SG, Beachy RN. Viral movement proteins as probes for intracellular and intercellular trafficking in plants. The Plant Cell Online 1999;11(4):535.
32. Okita TW, Choi SB. mRNA localization in plants: targeting to the cell's cortical region and beyond. Curr Opin Plant Biol 2002; 5(6):553-559.
33. Bartel DP. MicroRNAs genomics, biogenesis, mechanism and function. Cell 2004; 116(2):281-297.
34. Kidner CA, Martienssen RA. The developmental role of microRNA in plants. Curr Opin Plant Biol 2005; 8(1):38-44.
35. Why cells move messages: the biological functions of mRNA localization. Seminars in Cell and Developmental Biology: Elsevier; 2007.
36. Hannapel DJ. A model system of development regulated by the long-distance transport of mRNA. Journal of Integrative Plant Biology 2010; 52(1):40-52.
37. Anderson P, Kedersha N. RNA granules. J Cell Biol 2006; 172(6):803.
38. Boag PR, Atalay A, Robida S et al. Protection of specific maternal messenger RNAs by the P body protein CGH-1 (Dhh1/RCK) during Caenorhabditis elegans oogenesis. J Cell Biol 2008; 182(3):543.
39. Weterings K, Reijnen W, Aarssen R et al. Characterization of a pollen-specific cDNA clone from Nicotiana tabacum expressed during microgametogenesis and germination. Plant Mol Biol 1992; 18(6):1101-1111.
40. Bayer M, Nawy T, Giglione C et al. Paternal control of embryonic patterning in Arabidopsis thaliana. Science's STKE 2009; 323(5920):1485.
41. Brengues M, Parker R. Accumulation of Polyadenylated mRNA, Pab1p, eIF4E and eIF4G with P-Bodies in Saccharomyces cerevisiae. Mol Biol Cell 2007; E06.
42. Baulcombe D. RNA silencing in plants. Nature 2004; 431(7006):356-363.
43. Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. Nat Rev Mol Cell Biol 2009; 10(2):126-139.
44. Fiore R, Siegel G, Schratt G. MicroRNA function in neuronal development, plasticity and disease. Biochim Biophys Acta 2008; 1779(8):471-478.
45. Aukerman MJ, Sakai H. Regulation of flowering time and floral organ identity by a microRNA and its APETALA2-like target genes. The Plant Cell Online 2003; 15(11):2730.
46. Chen X. A microRNA as a translational repressor of APETALA2 in Arabidopsis flower development. Science's STKE 2004; 303(5666):2022.
47. Brodersen P, Sakvarelidze-Achard L, Bruun-Rasmussen M et al. Widespread translational inhibition by plant miRNAs and siRNAs. Science 2008; 320(5880):1185.

48. Collins LJ, Penny D. The RNA infrastructure: dark matter of the eukaryotic cell? Trends Genet 2009; 25(3):120-128.
49. Czaplinski K, Singer RH. Pathways for mRNA localization in the cytoplasm. Trends Biochem Sci 2006; 31(12):687-693.
50. Huttelmaier S, Zenklusen D, Lederer M et al. Spatial regulation of b-actin translation by Src-dependent phosphorylation of ZBP1. Nature 2005; 438:512-515.
51. Gu W, Deng Y, Zenklusen D et al. A new yeast PUF family protein, Puf6p, represses ASH1 mRNA translation and is required for its localization. Genes Dev 2004; 18(12):1452.
52. Meister G. miRNAs get an early start on translational silencing. Cell 2007; 131(1):25-28.
53. Holmes-Davis R, Tanaka CK, Vensel WH et al. Proteome mapping of mature pollen of Arabidopsis thaliana. Proteomics 2005; 5(18):4864-4884.
54. Noir S, Bräutigam A, Colby T et al. A reference map of the Arabidopsis thaliana mature pollen proteome. Biochem Biophys Res Commun 2005; 337(4):1257-1266.
55. Sheoran IS, Sproule KA, Olson DJH et al. Proteome profile and functional classification of proteins in Arabidopsis thaliana (Landsberg erecta) mature pollen. Sex Plant Reprod 2006; 19(4):185-196.
56. Grobei MA, Qeli E, Brunner E et al. Deterministic protein inference for shotgun proteomics data provides new insights into Arabidopsis pollen development and function. Genome Res 2009; 19(10):1786.
57. Forstemann K. Transposon defense in Drosophila somatic cells: A model for distinction of self and nonself in the genome. RNA Biol 2010; 7(2).
58. Malone CD, Brennecke J, Dus M et al. Specialized piRNA pathways act in germline and somatic tissues of the Drosophila ovary. Cell 2009; 137(3):522-535.
59. Pillai RS, Bhattacharyya SN, Artus CG et al. Inhibition of translational initiation by Let-7 MicroRNA in human cells. Science 2005; 309(5740):1573.
60. Sheth U, Parker R. Decapping and decay of messenger RNA occur in cytoplasmic processing bodies. Science 2003; 300(5620):805.
61. Sen GL, Blau HM. Argonaute 2/RISC resides in sites of mammalian mRNA decay known as cytoplasmic bodies. Nat Cell Biol 2005; 7(6):633-636.
62. Bhattacharyya SN, Habermacher R, Martine U et al. Relief of microRNA-mediated translational repression in human cells subjected to stress. Cell 2006; 125(6):1111-1124.
63. Chang Y, Imam JS, Wilkinson MF. The Nonsense-Mediated Decay RNA Surveillance Pathway. Annu Rev Biochem. 2007; 76(1):51-74.