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Pathways for mRNA localization in the cytoplasm

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Studies of the intracellular localization of mRNA have clearly demonstrated that certain subsets of mRNA are concentrated in discrete locations within the cytoplasm. Localization is one aspect of the post-transcriptional control of gene expression, and is intertwined with the translation and turnover of mRNA to achieve the goal of local protein production. Different mechanisms have been identified that enable localized mRNAs to target different subcellular compartments, and recent advances in understanding these pathways is reviewed here.

Introduction

Owing to the time needed to transduce a signal from an extracellular stimulus through to the resulting changes in the transcriptome and/or proteome, it is evident that transcriptional control alone cannot produce extremely rapid responses for synthesis of new proteins. Instead, cells rely heavily on post-transcriptional control for regulating gene expression. mRNA localization is one such post-transcriptional control mechanism and its investigation indicates that the role of the machinery of mRNA localization in regulating gene expression is intertwined with control of translation and mRNA turnover. mRNA localization is involved in many cellular processes, although most involve cellular asymmetry (the requirement for creating and maintaining cell polarity, which has a role during development and differentiation) [1]. The demand for a particular protein factor to be Asymmetrical within the cytoplasm of the cell can be met by several means; however, a high overall production of protein throughout the cytoplasm might not suffice and might even be detrimental. Therefore, mRNA localization provides a powerful way to produce proteins at specific local concentrations.

Building localized mRNA complexes

Regardless of the mechanism of targeting, sequences within the mRNA account for mRNA localization. These sequences are referred to as localization elements (LEs), Zipcodes and targeting elements (TEs). Here, we refer to these mRNA-targeting sequences as LEs. They function through *trans*-acting factors that specifically bind to them. Here, we review the recent developments in understanding these LEs: their recognition by *trans*-acting factors; how localization meshes with translational control; how mRNAs concentrate locally in the cytoplasm (examining the role of the cytoskeleton, including that of molecular motors kinesins, dyneins and myosins); and the potential interactions of mRNA with organelles undergoing cytoplasmic trafficking.

LE-containing mRNAs, together with the bound *trans*-acting factors required for localization (primarily RNA-binding proteins that specifically recognize the LE), create complexes we refer to here as localizing ribonucleoprotein particles (L-RNPs). A review of all localizing mRNAs and *trans*-acting factors involved in LE binding is beyond the scope of this article (reviewed in Refs [2–4]), but formation of L-RNPs can involve multiple RNA-binding proteins in addition to LEs of several hundred nucleotides. In such cases, it is likely that LE function is provided by a series of many interactions within a specific complex, as in the localization of Vg1 mRNA (a transforming growth factor-β superfamily signaling molecule required for mesoderm induction during embryogenesis) to the vegetal pole in *Xenopus laevis* oocytes [5,6]. Multiple studies have identified several specific Vg1 LE RNA-binding proteins [6,7], and, together, the data suggest ordered addition of multiple RNA-binding proteins is required to promote localization of Vg1 to the vegetal pole. Specific association of at least one factor, 40LoVe, depends on prior binding of two other RNA-binding proteins – heterogeneous nuclear ribonucleoprotein I (hnRNP I) and Vg1 RNA-binding protein (Vg1RBP; also known as Vera) – to the Vg1 LE [7,8] (Figure 1). However, less complicated LE-binding interactions have been reported, such as those of the myelin basic protein (MBP) mRNA, which can be found localized to the myelin compartment of oligodendrocytes. A short 21-nucleotide sequence within MBP mRNA (A2RE) that binds to hnRNP A2 [9] has been identified as necessary and sufficient for targeting of A2RE-containing mRNA to both the processes of oligodendrocytes [10] and the dendrites of cultured neurons [9].

L-RNP formation and the nucleus

Injection of a localizing mRNA into the cytoplasm of a cell has revealed that the mRNA need not originate in the nucleus to localize properly in the cytoplasm [5,10,11]. However, in conflict with this evidence, many results indicate that L-RNP formation initiates in the nucleus, and that nuclear factors are involved in the process of mRNA localization [12,13]. Many factors that bind specifically to various LEs are present within the nucleus, and 40LoVe can be localized to actively transcribing

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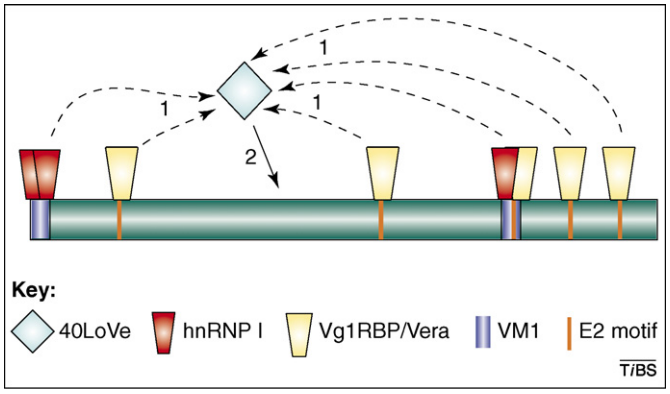

Figure 1. The LE of Vg1 mRNA. Vg1 is a TGF-β superfamily protein involved in mesoderm induction during *Xenopus* embryogenesis. The Vg1 mRNA localizes to the vegetal pole of oocytes through a 360-nucleotide LE in the 3′ untranslated region. The LE is depicted in green; the motifs that have been identified as required for localization are highlighted, as are the *trans*-acting factors that are known to bind these sequences. The binding of 40LoVe (pale blue diamond) to an unidentified site occurs after previous recognition of the Vg1 LE by the RNA-binding proteins hnRNP I and Vg1RBP/Vera (as indicated by the numbered arrows).

chromosomes in oocytes [7], suggesting that L-RNP formation might even occur co-transcriptionally. Consistent with this possibility, Zipcode-binding protein 1 (ZBP1), a protein that binds specifically to the LE of β-actin mRNA, can be seen accumulating at the β-actin transcription site during serum stimulation [14].

A recent illustration of this conflict was provided by localization of *oskar* mRNA to the *Drosophila* oocyte posterior pole (*oskar* encodes a determinant of germ cell fate and posterior polarity). This event involves splicing of the first intron of the mRNA, suggesting that nuclear formation of this L-RNP might be a requirement in this case [15]. Because mutations in the exon-junction complex (EJC) also show defects in *oskar*-mRNA localization, it is likely that splicing of the first intron is required to recruit the EJC to the mRNA for its role in *oskar*-mRNA localization [16]. However, previous results showed that *oskar*-mRNA reporters can localize without an intron. Interestingly, multimerization of *oskar* transcripts might account for this observation because intronless *oskar*-reporter mRNA can localize by binding to endogenous *oskar* mRNA that has undergone nuclear splicing [17] (Figure 2). Thus, when injection makes it seem that only cytoplasmic components are required for localization, the potential for multimerization with endogenous localizing mRNAs confounds such results.

How prevalent transcript multimerization is in mRNA localization is unknown but, within this topic, it is appropriate to note that mRNAs localizing to the processes of neurons and oligodendrocytes can be observed to form large microscopically observable structures called 'granules' or 'particles' that probably contain many mRNAs, *trans*-acting factors and ribosomes [18,19]. For MBP

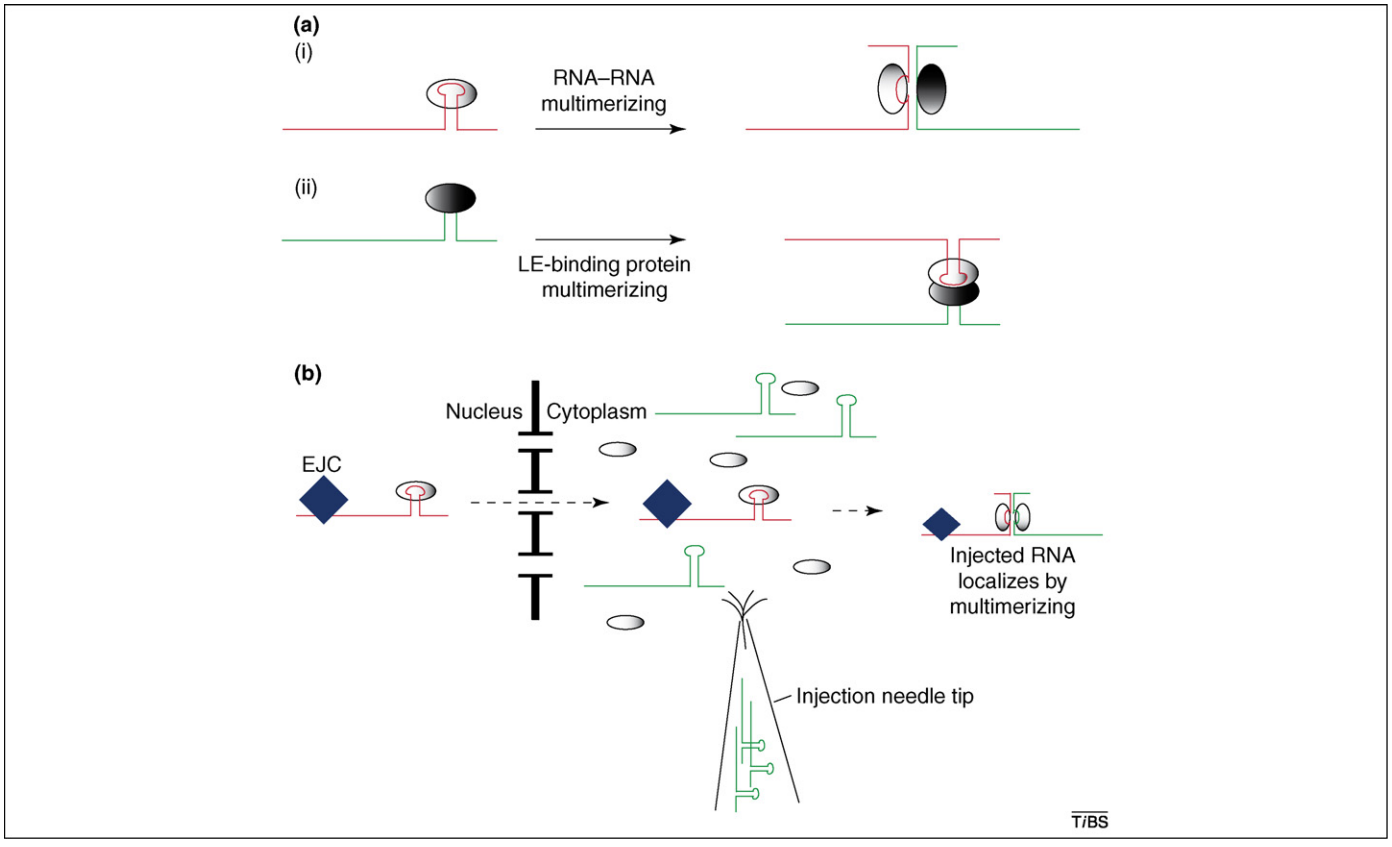

Figure 2. mRNA can localize as multimeric RNPs. **(a)** Two possible explanations for multiple mRNAs combining into one RNP particle, leading to localization of multiple mRNAs are shown. (i) Two localizing mRNAs might have the potential to anneal through complementarity in mRNA sequence, or (ii) LE-binding proteins (depicted, for simplicity, as the gray and black ovals bound to the stem–loop structure of mRNA) might multimerize to join multiple mRNAs indirectly into a localizing multimer. These pathways are not mutually exclusive and might operate together in the same RNP. **(b)** Cytosolic injection might not discriminate a requirement for nuclear factors in the localization process. In this scenario, cytoplasmically injected mRNA (green) can still associate with factors from the nucleus (e.g. the EJC) that have emerged with the endogenous mRNAs (red) owing to multimerization by one of the mechanisms described in (a).

mRNA, multimerization of hnRNP A2 has been proposed to account for granule formation of MBP mRNA [9] (Figure 2), although how the formation of L-RNPs relates to transcript multimerization and the presence of granule and particle structures awaits clarification.

Repressing translation from the start

L-RNPs formation in the nucleus possibly arises from the need to repress the translation of the mRNAs that comprise these complexes. A constant theme throughout studies of localized mRNAs is that they must localize before translation, otherwise protein sorting by localized synthesis is obviated [20–23]. Once in the cytoplasm, mRNAs have immediate access to the translation apparatus, which might interfere with the localization machinery (Figure 3). Export from the nucleus of L-RNPs that are unable to translate is an efficient way to eliminate premature translation initiation. In this model, L-RNPs require local translational activation, which has been observed for several localized mRNAs. Signal transduction has been demonstrated to have an important role in local activation [21,24,25]. For instance, β-actin mRNA localizes to the leading edge of a lamellipod (a cellular structure that facilitates cell motility) in fibroblasts and other motile cells owing to the LE sequence [26]. This particular LE can also target the mRNA to the distal processes of neurons in culture in which β-actin mRNA is found in growth cones and dendritic spines [27]. ZBP1, in addition to localizing β-actin mRNA, also represses translation of β-actin [24]. Phosphorylation of a conserved tyrosine residue in ZBP1 by membrane-associated Src family kinases releases it from the mRNA, thereby relieving translational inhibition and locally activating synthesis of β-actin.

Another example of a mechanism whereby translation follows localization is for *ASH1* mRNA, which localizes to the tip of the growing bud in *Saccharomyces cerevisiae* to restrict expression of Ash1 protein to the daughter cell nucleus [28]. This results in asymmetric expression of mating-type components so that the daughter cells can be of opposite mating type from the mothers and enabling sexual reproduction. *ASH1* mRNA uses multiple methods to repress translation during transport to the bud tip. The first method is impeding ribosomal elongation by the presence of RNA secondary structure within the LEs (all four LEs are located within the open reading frame of the *ASH1* mRNA), thus delaying translation until the mRNA reaches its destination [29]. A second mechanism involves translational repression by binding of Puf6p to the 3′ untranslated region of the *ASH1* mRNA [30]. Puf6p is a member of the PUF family of proteins, all of which share a common RNA-binding domain and are involved in translational repression [31]. In the absence of Puf6p, Ash1 is translated before arrival at the bud tip and, as a result, the asymmetry of the protein is affected. Puf6p probably does not

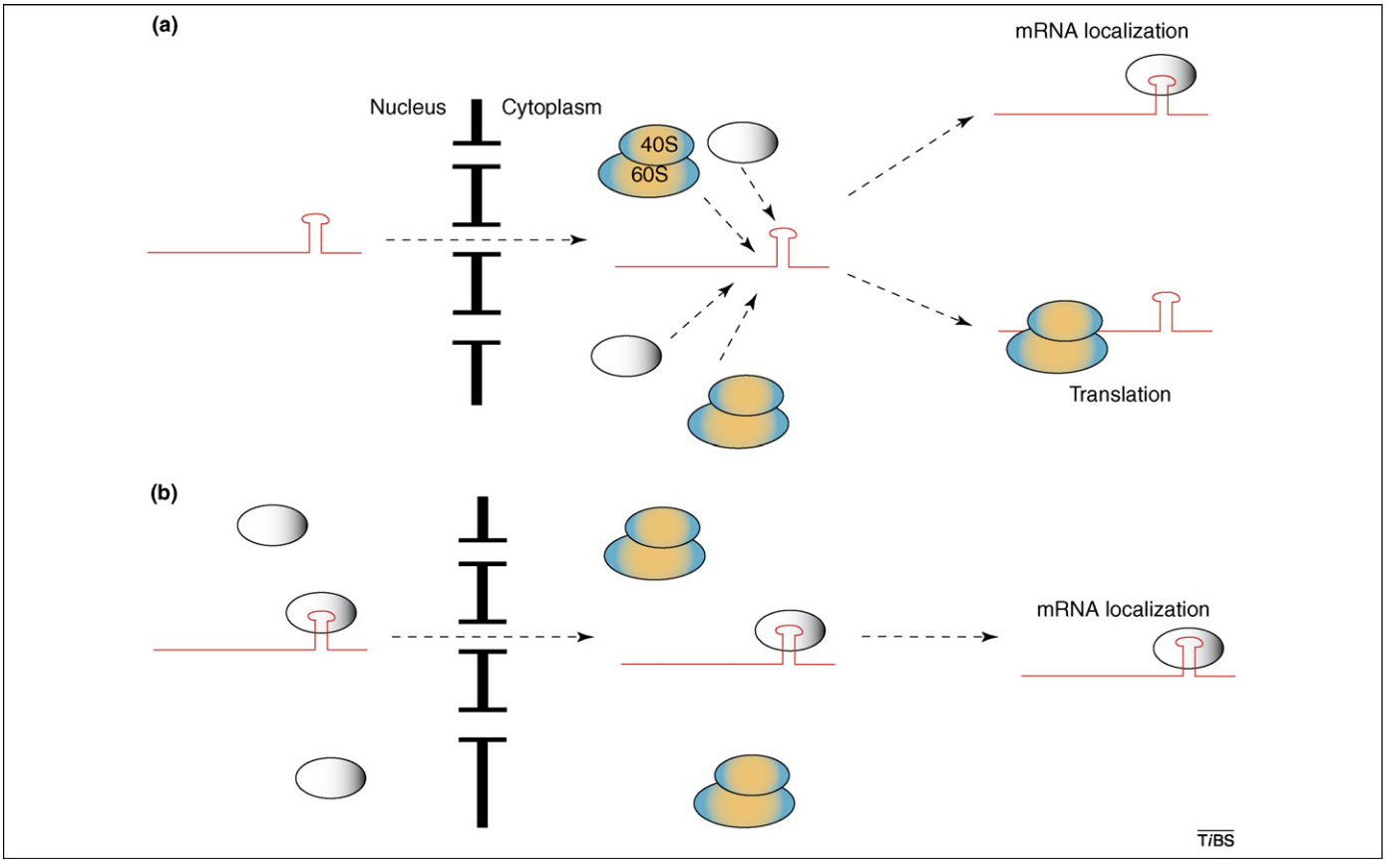

Figure 3. Model for the formation of translationally repressed L-RNPs. **(a)** Upon export from the nucleus into the cytoplasm, mRNAs can interact with the translation apparatus (depicted, for simplicity, as just the 40S and 60S ribosomal subunits). Competition with the translation machinery for exported mRNA at this point could interfere with LE-binding factors (represented, for simplicity, as a single gray oval bound to the mRNA LE). **(b)** An L-RNP formed within the nucleus to be translationally repressed eliminates competition with the translation apparatus during formation, leading to more efficient localization once exported to the cytoplasm.

function alone in this pathway because at least one other RNA-binding protein, Khd1, has been demonstrated to similarly effect *ASH1* mRNA distribution [32].

Localizing mRNA in the cytoplasm

There is no single mechanism by which mRNA becomes asymmetrically distributed within the cell: multiple mechanisms are involved. For instance, protection from mRNA degradation in the posterior pole of early embryos enables the *Drosophila* Heat Shock protein 83 kD (Hsp83) mRNA to concentrate at this site, whereas its degradation occurs everywhere else throughout the cytoplasm [33]. In this system, when Smaug (an RNA-binding protein in *Drosophila* embryos) binds to *Hsp83* mRNA and recruits the cytoplasmic de-adenylase, this ultimately leads to destabilization of unlocalized *Hsp83* mRNA. Hence, Hsp83 will be produced locally at the posterior pole [34]. However, most characterized examples of mRNA localization are thought to involve directed movement of L-RNPs, and much effort in the field of mRNA localization in recent years has attempted to define this process. The rationale for directed movements stems from the dependence of mRNA localization on the cytoskeleton (reviewed in Ref. [35]). Models for mRNA localization invoke one or two primary cytoskeleton dependent steps: active transport of the L-RNP to the site of localization and/or a local anchoring.

Interactions with myosin motors and microfilaments

ASH1 mRNA localization requires directed transport. Genetic analysis has revealed that the process involves the motor protein myosin (Myo4p/She1p) plus two additional proteins. The myosin motor interacts with actin microfilaments and actively transports the L-RNP cargo via an adaptor protein called She3, which associates, in turn, with an RNA-binding protein, She2, that binds directly to the LEs within the *ASH1* mRNA [28]. Interestingly, in this case, RNA cargo seems to be required to localize the motor to the bud tip [36]. The dependence on the myosin motor in yeast illustrates the importance of both microfilaments and molecular motors in the localization process, and, to date, represents the best-characterized example of motor–L-RNP interaction (Figure 4).

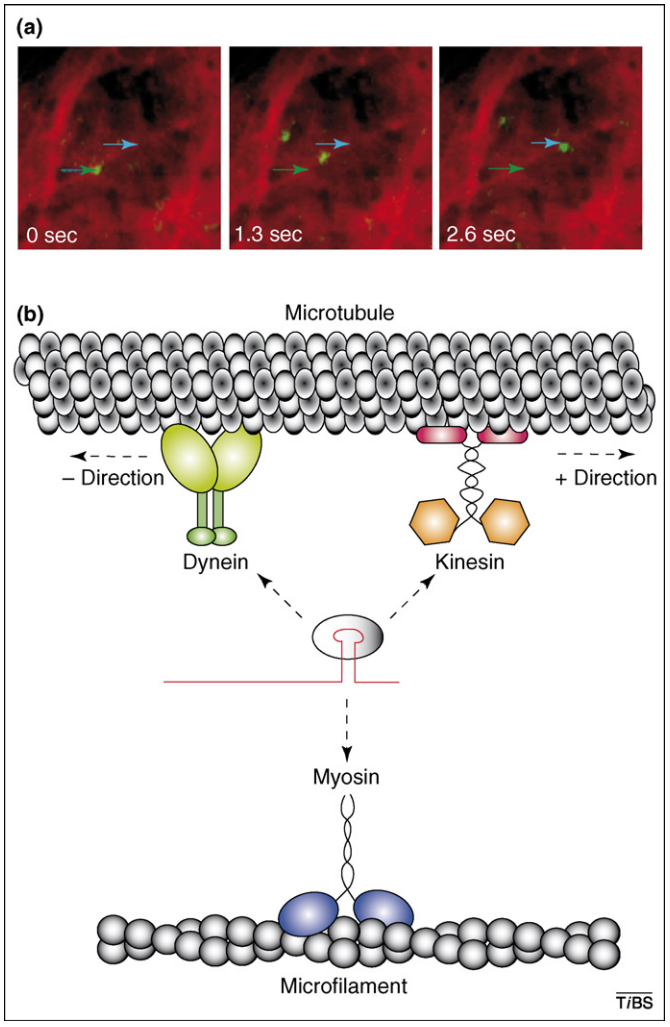

Figure 4. Interaction with cytoskeletal motors during mRNA localization. **(a)** A LE-containing mRNA labeled *in vivo* with a GFP tag shows tracking along a microtubule in time-lapsed images. Red, tubulin; green arrow, starting position; blue arrow, ending position [70]. **(b)** The interactions of L-RNPs with microtubule motor complexes or microfilament motor complexes have been implicated in localization. All three types of cytoskeleton-dependent motors (kinesin, and dynein for microtubules and myosin for microfilaments) have been suggested to have roles in either directed movement or local anchoring. Based on the identified examples of L-RNP–motor protein complexes, localizing mRNA is depicted here as interacting with motors indirectly through LE-binding proteins as a RNP complex (represented for simplicity as a gray oval bound to a localizing mRNA, but can in fact be extremely large with multiple RNA-binding proteins and mRNAs). In metazoans, the molecular details of these interactions are not known.

Interactions with microtubule motors

Dynein. Although mRNA localization seems to be independent of microtubules in yeast, in many higher eukaryotes localization involves microtubules for the transport of several L-RNPs (Figure 4). The involvement of microtubules in mRNA transport has fueled many experiments examining the roles of their dependent motors (kinesins and dyneins). In *Drosophila* oocytes, mRNA encoding Gurken (a transforming growth factor-α family protein) moves to the antero-dorsal position in oocytes after microinjection, recapitulating the localization of the endogenous *gurken* mRNA [37]. Inhibiting dynein (a microtubule minus-end directed motor) activity results in loss of localization of *gurken* mRNAs. Also, late during oogenensis, dynein activity is involved in localizing *bicoid* mRNA to the anterior end by continuous transport to this region of the oocyte [38]. Similarly, interiorly injected *wingless* and *pair-rule* mRNAs localize to the apical surface in developing fly embryos in a dynein-dependent process [11]. In adult tissues of *Drosophila*, dynein is also implicated in localizing *inscuteable* mRNA to the apical surface of developing neuroblasts [39]. Therefore, dynein seems to function as an essential motor for localization of these mRNAs, and several dynein–dynactin complex interacting factors have roles in localizing mRNAs [40–42].

Kinesin. Similar to dynein, mutations in *Drosophila* kinesin have been examined for effects on mRNA localization. Kinesins are a large family of microtubule-dependent molecular motors containing at least one polypeptide that harbors the motor domain. This subunit, kinesin heavy chain (KHC), of conventional kinesin (KIF5) can interact with cargo through an adaptor protein called the kinesin light chain (KLC) [43]. The loss of KHC

function in *Drosophila* oocytes disrupts the localization of *oskar* mRNA [44] but KLC is dispensable for its localization [45], which is inconsistent with previously demonstrated models for kinesin-cargo transport. This indicates that novel unidentified kinesin-adaptor interactions might function in the localization of this L-RNP. Interestingly, kinesin might not actually direct movement of the *oskar* L-RNPs towards the posterior pole but, rather, in any direction away from the cortex of the oocyte except for the posterior pole where it is localized. This suggests that *oskar* mRNA localizes due to being actively excluded from locations along the oocyte cortex other than the posterior pole [46]. Furthermore, in *Drosophila* S2 cells, microscopically visible 'granules' of green fluorescent protein (GFP)-dFMR, an RNA-binding protein that is proposed to be a marker for localizing mRNA, move bi-directionally and demonstrate both kinesin- and dynein-dependent movement, as determined by RNA interference of these motors [47]. Notably, the kinesin-dependent movement is also independent of KLC in these experiments.

There remains uncertainty regarding how closely the observed effects on mRNA localization lie to targeted loss of motor function because motors might also have roles in cytoskeletal architecture in addition to other active-transport pathways. In several experimental systems for which genetic analysis is not practical, microscopic and biochemical analyses have made inroads towards defining the mRNA–motor complex interactions that account for cytoplasmic mRNA localization. For example, by localizing MBP mRNA in the presence of kinesin antisense oligonucleotides to depress kinesin activity, it was demonstrated that MBP mRNA transport into the processes of oligodendrocytes was impaired [48]. Moreover, using time-lapse imaging of labeled mRNA, the observed movement was calculated to occur at speeds matching those of kinesin-mediated transport [48]. In another experiment, two alternative strategies to disrupt kinesin-II activity were used to assess its role in Vg1-mRNA localization to the vegetal pole of *Xenopus* oocytes. A blocking antibody and expression of a dominant negative kinesin-II fragment both inhibited localization of fluorescently labeled Vg1 mRNA, thus emphasizing the generic significance of kinesin-mediated transport in mRNA localization [49].

Association of LE-binding factors with kinesin

Physical association of localizing mRNA and/or binding proteins with kinesin has also been reported, primarily by co-fractionation experiments. The RNA-binding protein Staufen is of particular interest among these LE-binding factors. Staufen is a *Drosophila* protein that is involved in several mRNA-localization events in both oogenesis and adult tissues. Staufen consists of five double-stranded RNA-binding domains (dsRBDs,). Most vertebrate organisms seem to express two different Staufen homologs, stau1 and stau2, with several identified mammalian isoforms each arising from alternative splicing [50]. Although specificity of binding to a LE has not been demonstrated for any Staufen homolog, data far too extensive to review here indicate that the genes encoding Staufen have some role in many localization pathways from several different organisms as an integral part of L-RNPs. Mammalian Staufen has been demonstrated to interact with tubulin [51], and this interaction has been proposed to be involved in mRNA localization. However, if L-RNPs are actively driven by motor complexes, motors should contact the microtubules directly; therefore, it is not clear how a Staufen–microtubule interaction could effect movement of L-RNP complexes.

Staufen in L-RNP function has been used as a marker for complexes in microscopic and biochemical analyses. *Staufen* (and *oskar* mRNA) in *Drosophila* fails to localize to the posterior pole of *Drosophila* oocytes in the absence of KHC activity [44]. Moreover, in rat whole-brain extracts, distinct stau1 and stau2 fractions, which correlate to microscopically observable 'particles' and 'granules' that move in a microtubule-dependent manner in cultured neurons, co-fractionate with both KHC and localizing mRNA [50]. Similarly, *Xenopus* Staufen proteins co-fractionate with *Xenopus* kinesin I and with vegetal localizing mRNA [52,53]. Because blocking kinesin II has been demonstrated to impair vegetal localization [49], this observation suggests that multiple kinesin proteins can have a role in localizing mRNAs to the oocyte vegetal pole, either directly or indirectly.

All of these experiments are consistent with kinesin being the motor that drives mRNA localization when the primary direction is to the plus-end of microtubules, but the nature of the RNA–motor connection remains elusive. Models for L-RNP movement suggest that the interaction between localizing RNA and motor is indirect because it is mediated through multiple proteins. Possibly, the complex associates with organelles that are trafficked by motors through the cytoplasm (see later).

KIF5 associates with cargo through a C-terminal tail domain, therefore, to obtain evidence of direct RNP–cargo binding by kinesin, an affinity matrix of the KIF5 cargo-binding tail domain was used to isolate interacting proteins from mouse-brain extracts. One region of the tail identified a >1000S complex containing at least 42 proteins, many of which are believed to be participants in mRNA localization [54]. As productive as these experiments are, the molecular connection between kinesin and L-RNP remains undefined owing to the large number of proteins identified.

Anchoring the RNA

The second part of a two-step cytoskeleton-dependent localization model involves cytoskeleton anchoring, and all three types of cytoskeletal elements can be involved in anchoring. For example, β-actin mRNA anchors in primary fibroblasts dependent on microfilaments [55], and actin depolymerization releases ZBP1 from cytoskeletal association [56]. Genetic mutations in regulators of the actin cytoskeleton also effect anchoring. For example, loss of *oskar*-mRNA localization is seen in both tropomyosin II and moesin mutant oocytes. Likewise, *ASH1*-mRNA localization is affected by *bni1* mutants, a formin involved in actin-filament stabilization [57–59]. A potential role for EF1α (a translation elongation factor that helps deliver tRNA to the ribosomal A-site) in anchoring through its interaction with actin has also been proposed to provide another potential site for mRNA anchoring [60,61].

One recent report demonstrated that dynein, in addition to being the motor that drives directed transport, functions as a microtubule-dependent anchor in *Drosophila* embryos. Thus, motor activity can be used by L-RNPs for multiple roles in the pathway of mRNA localization [42].

Directed transport need not be a requirement for localization because local mRNA anchoring that is independent of such movement has been observed. For instance, in *Drosophila* embryogenesis, *nanos* mRNA can be found throughout the oocyte cytoplasm in a translationally repressed state, but concentrates at the posterior pole by diffusion coupled with a local anchoring mechanism [62].

Intermediate filament (IF)-dependent anchoring of mRNAs to the vegetal pole of *Xenopus* oocytes has also been observed. Interestingly, it has been reported that some vegetal-localized mRNAs might also function as structural components of the cytokeratin meshwork at the vegetal cortex because destruction of these mRNAs with antisense oligodeoxynucleotides perturbs the structure of the meshwork [63]. Moreover, ribosomal protein mRNAs might also associate with IFs [64]. This is interesting in light of nucleic acid binding by IF proteins [65], and the potential for these to be involved in mRNA localization remains largely unexplored.

Organelle association for localizing mRNA

The association of localizing mRNA with cellular organelles that localize in a motor-dependent manner has support in several systems. We direct the reader to a recent review that provides a more-detailed discussion of the linkage between organelle trafficking and mRNA localization [66]. Studies of ER inheritance in yeast revealed that the She3 and Myo4/She1 proteins are each required for the segregation of a subdomain of endoplasmic reticulum (ER) residing next to the plasma membrane, called cortical ER, to the bud tip of the daughter cell [67]. However, She2, which is essential for *ASH1*-mRNA localization, is dispensable for cortical ER inheritance to the daughter cell. Therefore, She3 and Myo4/She1 function in this pathway independently of She2. Several studies have found a conserved RNP complex that resembles the Sm proteins associates with translational control complexes and is required for the function of subdomains of ER. Mutations in factors of the Scd6 family of RNA-binding proteins from both *C. elegans* and *Drosophila* have phenotypes indicative of functional defects within the ER, suggesting some connection between RNP complexes and the function of ER (reviewed in Ref. [68]). Interestingly, analysis of ER-associated mRNAs revealed the association of many mRNAs encoding soluble proteins, indicating that ER association can be independent of secretory translation [69]. The association between these two fields of study suggests that there is still quite a bit to learn about both.

Concluding remarks

The past several years have seen advances in our understanding of how mRNAs localize in the cytoplasm, aided by developments in technology that enable researchers to visualize mRNA movements in real time, even with single-molecule resolution. These advances add the dimension of time to classical molecular studies, and the results indicate that this is crucial to understanding the mechanism of localization. Studies clarify the nature of RNA movement and provide insights not only for the numerous areas of cell biology that mRNA localization impacts but also for RNA biology in general.

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