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Taking a cellular road-trip: mRNA transport and anchoring

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Abstract

mRNA localization is a crucial mechanism for post-transcriptional control of gene expression used in numerous cellular contexts to generate asymmetric enrichment of an encoded protein. This process has emerged as a fundamental regulatory mechanism that operates in a wide range of organisms to control an array of cellular processes. Recently, significant advancements have been made in our understanding of the mechanisms that regulate several steps in the mRNA localization pathway. Here we discuss progress made in understanding localization element recognition, paying particular attention to the role of RNA structure. We also consider the function of mRNP granules in mRNA transport, as well as new results pointing to roles for the endocytic pathway in mRNA localization.

Introduction

Over 25 years ago, a new cellular regulatory mechanism was revealed with the observation that β-actin mRNA is asymmetrically distributed within the cytoplasm of *Ascidian* eggs [1]. As later demonstrated, the function of mRNA localization is the generation of polarized gene expression through local protein synthesis [2]. Since this discovery, and the realization of the importance of mRNA localization in many aspects of multi-cellular life, including embryonic development [3], cell motility [4], and synaptic plasticity [5], inroads have been made into understanding the mechanisms controlling this process. A combination of powerful genetic and biochemical approaches has led to the discovery of *trans*-acting protein factors that function in mRNA localization [6]. In addition, new cell biological techniques for imaging RNA movement in live cells have enabled unprecedented views of this process in a number of systems [7]. Moreover, a global analysis underscored the importance of mRNA localization during *Drosophila* development, with over 70% of the ~3,000 transcripts analyzed displaying distinct localization patterns [8]. We now know that mRNA localization is a mechanism used to spatially regulate gene expression that is conserved not only throughout the Eukarya, but also in the Bacteria, emphasizing its importance in all domains of life [9].

The ultimate goal of mRNA localization is the subsequent spatial restriction of protein expression (Box 1). Mechanisms to accomplish this include degradation of protein products outside of the specified region, entrapment of a freely diffusing mRNA at the site of local protein synthesis, or active transport of mRNAs to specific cellular destinations (with the

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two latter mechanisms requiring a static anchor for the mRNA [6]). Importantly, to faithfully restrict protein accumulation to one part of a polarized cell, an mRNA must remain translationally silent during active transport or diffusion; this topic has been reviewed recently [10] and will not be covered here. Instead, our focus will be on the cellular mechanisms used to identify, package, and anchor mRNAs that are actively transported to specific domains within the cell cytoplasm.

Box 1

Functions of mRNA localization in eukaryotes

Cytoplasmic localization of mRNAs ultimately results in asymmetric distribution of protein expression, which can generate both cellular and developmental polarity. Some of the earliest examples of mRNA localization were identified in developmental systems. Early *Drosophila* development relies on maternally localized mRNAs, with *bicoid* mRNA at the anterior [55] and *oskar* mRNA at the posterior [56] providing spatial determinants that define the anterior-posterior axis (Fig. 1B). In *Xenopus* oocytes, selected transcripts are localized along the animal-vegetal axis of the oocyte during early oogenesis (Fig. 1D). Some of these mRNAs go on to specify the germ line [57], while others are involved in defining the primary germ layers, and thus are critical for patterning of the early embryo [58,59]. mRNA localization is also critical for generating cellular polarity. For example, in the yeast *Saccharomyces cerevisiae ASH1* mRNA is localized to the tip of a budding daughter cell during mitosis (Fig. 1E; [12,60]). Local translation of Ash1p prevents mating-type switching, thus specifying daughter cell fate [12]. β-actin mRNA is localized to the leading edge of migrating fibroblasts (Fig. 1F) where local translation results in cytoskeletal remodeling necessary for cell movement [4]. mRNA localization is also critical for neuronal function [61]. Studies in *Xenopus* [62], *Drosophila* [63], and mammalian [64] neurons have shown that local protein synthesis is involved in neuronal growth during development, and in faithful synaptic function in the adult (Fig. 1G; [65]). These selected examples of mRNA localization represent some of the most widely studied model systems, with each offering unique advantages and distinct yet overlapping insights.

Localization Elements: Structure, not sequence, gives a message direction

The importance of *cis*-acting signals within localizing transcripts has been widely documented in a number of systems [11–13]. These localization elements (LEs) are generally found within the 3′-untranslated region (UTR) of localized mRNAs, and function predominantly to engage *trans*-acting protein factors [14]. Recruited proteins, including molecular motors [15–17] and translational repressors [18,19], thus function to direct the correct spatial and temporal distribution of the mRNA and its encoded protein product. Indeed, numerous proteins and protein families have been shown to be involved in mRNA localization. Perhaps the most widely characterized of these are a family of RNA binding proteins that includes ZBP-1, which was first described as a critical regulator of *β-actin* mRNA localization in chick embryo fibroblasts [20]. Related proteins have been shown to be required for mRNA localization in *Xenopus* oocytes (Vg1RBP/Vera; [21,22]), local translation in *Drosophila* oocytes and synapses (IMP; [23]), and translational regulation in mammalian fibroblasts (IMP-1,2,3; [24]). However, the molecular mechanisms governing how these proteins act in mRNA localization remain unclear. Given the conservation of mechanism and involved protein factors [25], it is surprising that a concordant conservation of LE sequences has not been observed [26]. Instead, it has been proposed that LEs fold into conserved secondary and tertiary structures that are recognized by RNA-binding proteins [26–28].

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Until recently, this hypothesis was based on secondary-structure predictions, with stem-loop conformations as the likely candidate for defining an active LE [29]. However, in 2010 Bullock and colleagues succeeded in solving an NMR structure for the *Drosophila K10* mRNA LE [30••], which localizes to the anterior of the oocyte and to the apical surface of the syncitial blastoderm in embryos (Fig. 1A, C; [31]). The 44-nucleotide *K10* LE adopts a hairpin structure (Fig. 2A), but the helices do not conform to the expected A-form RNA. Instead, they form an A′-form helix, which, similar to B-form DNA, has a much wider major groove than A-form helices (Fig. 2B). In functional assays the widened major groove, the result of purine-purine base-stacking interactions, proved to be the key feature of the *K10* LE [30••].

For the *Drosophila oskar* transcript, which is localized to the posterior pole of the developing oocyte and egg (Fig. 1A, B), a *cis*-LE has, until very recently, evaded identification [32••]. While the *osk* 3′-UTR was known to play a role [33], splicing of the first intron in the *osk* pre-mRNA and deposition of the exon-junction complex (EJC) are also required for *osk* localization [34]. Ghosh *et al.* [32••] have now reconciled these data into a model whereby splicing results in the formation of a spliced *oskar* localization element (SOLE); the two juxtaposed exons form a hairpin structure that directs *osk* mRNA localization (Fig. 2C, D). Interplay between the SOLE and the EJC also appears to be important, but the underlying mechanism is not yet clear. These findings underscore the importance of structural elements in mRNA localization, and also emphasize the complexity of identifying them through comparative computational analyses.

While short LEs can fold into discrete stem-loop structures, other much longer LEs have the potential to form complex structural conformations. With the discovery [35] that Fragile X Mental Retardation Protein (FMRP) is required for dendritic localization (Fig. 1G) of a number of mRNAs, came a renewed interest in the RNA-recognition potential of this protein. FMRP shows a propensity for binding intramolecular G-quadruplex structures [36], but a biological role for this interaction had yet to be shown. Subramanian *et al.* tested this interaction by examining the putative G-quadruplexes in the 3′-UTRs of two post-synaptically-localizing mRNAs, *PSD-95* and *CamKIIa* (Figs. 1G, 2E-F; [37•]). They found that the structural conformation of the G-quadruplex is essential for mRNA localization, and that this activity was independent of sequence information. In addition, the G-quadruplex motif was found in almost a third of all neurite-targeted mRNAs, suggesting its function as a general LE.

Although the importance of structural motifs in LE function is gaining significant support, it remains a challenge to predict novel mRNA secondary and tertiary structures, especially in the context of longer localization signals (such as the 340-nt LE in *Xenopus Vg1* mRNA [13]). However, this year saw the publication of the first real test of RNA tertiary structure prediction, *RNA-Puzzles* [38•]. Similar to the contribution of the *CASP* competition to *in silico* protein structure prediction [39], the goal of *RNA-Puzzles* is to improve RNA tertiary structure prediction through applying various computer programs to RNAs of known structure. The success of this project in coming years will be of great interest to the mRNA localization field, and has the potential to improve our ability to identify structural LEs.

Localizing mRNAs can go it alone

An increasingly accepted model to explain motor-dependent RNA transport proposes that, after LE recognition, multiple localizing mRNA species associate to form a localization-competent mRNP. The resulting large RNP granule (Box 2), containing multiple mRNAs, could then recruit molecular motors, thus transporting many mRNAs in a single trip [40]. Potentially, different mRNA species with similar LEs could be loaded into the same mRNP and co-transported to the same cellular location; an idea that was validated in yeast using

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fluorescent protein-tethering to track movement of *ASH1* and *IST2* mRNAs *in vivo* [41•]. While co-transport is also evident in other systems, recent studies using quantitative high-resolution microscopy have challenged the view that mRNP granule formation is obligatory for transport.

Box 2

Cytoplasmic mRNP granules

mRNA, from transcription to degradation, is associated with a vast array of RNA-binding proteins in ribonucleoprotein particles or mRNPs. These associations are far from random, with specific mRNP configurations observable in different cellular circumstances. Several such mRNPs—termed granules due to their large size—have been intensely studied, including processing-bodies (P-bodies [66]), stress granules [67], and GW bodies [68]. These cytoplasmic granules are composed of overlapping sets of proteins and mRNAs, with some apparently common functions. For example, in both stress granules and P-bodies mRNAs can be stored in a translationally inactive state to be subsequently returned to translation [69], or degraded [66]. RNA-protein interactions and the formation of mRNP granules are crucial events in the life of an mRNA, and localizing mRNAs are no exception. Several localized mRNAs have been shown through both biochemical and cell biological techniques to form granule-like structures, including *Drosophila oskar* [46•] and *Xenopus Vg1* [70]. How granule formation may aid in localization is unclear, although translational repression, protection of the mRNA from the degradation machinery, and transport of multiple mRNA species in a single localizing particle, are all attractive, and not mutually exclusive, hypotheses.

In neurons, synaptic plasticity and neuronal growth are dependent on local synthesis of proteins from a variety of transported mRNAs [26]. That these mRNAs would localize in distinct granules containing only one type of mRNA makes sense, as the spatio-temporal distribution throughout the neuron is different for each protein. This idea was tested by Kiebler and co-workers, who showed that *MAP2* and *CamKIIα* mRNAs were transported in distinct particles [42•], and that these particles contained only one or a few mRNA molecules [43•]. Similar results were reported by Batish *et al.*, who used high-resolution *in situ* hybridization to assess neuronal transport particles [44•]. Interestingly, this study also showed that mRNAs containing the same recognition motifs within their LEs were no more likely than those with apparently dissimilar LEs to co-localize. In the *Drosophila* blastoderm embryo as well, formation of large granules containing multiple RNAs does not appear to be required for localization. Amrute-Nayak and Bullock [45•] differentially labeled *K10* LE RNA (Fig. 1C) to determine whether more than one mRNA assembled into a single localizing particle. No co-localization of red- and green-labeled RNAs was observed in embryos, indicating that the injected RNAs do not co-assemble into the same transport particle [45•].

Although it is apparent that some mRNAs localize singly, others are transported in large granules containing several transcripts; a prominent example of this is seen in *osk* mRNA transport in the *Drosophila* oocyte (Fig. 1A). In this instance, formation of granules of 50–80S in size is proposed to function in translational repression of *osk* during transport to the posterior pole of the oocyte [46•]. Similarly, in the *Xenopus* oocyte *Vg1* mRNA has been observed to localize to the vegetal pole in granules, and recent data suggest that *VegT* is co-transported in these particles (T. Wood, personal communication). These examples, together with the data for *ASH1* and *IST2* RNAs in yeast [41•], indicate that co-transport of localized mRNAs in RNP granules has potential biological importance, thus raising the question: What controls whether an mRNA is transported in a large granule or alone? Cellular context

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likely plays an important role, where RNA-binding proteins could alternatively promote or prevent the formation of higher order structures. Indeed, in neurons, the RNA-binding protein Staufen appears to control the number of mRNAs in a localizing mRNP [43•] suggesting that the composition of granules, whether small or large, is tightly regulated. However, information within the localizing mRNA itself must be considered. For example, mRNAs that are localized in a group might contain multiple copies of a motif that would allow for mRNP formation by multi-domain RNA-binding proteins, protein-protein, or even inter-molecular RNA-RNA interactions (as has been suggested for *osk* [47]). Further complicating matters, these mechanisms need not function in a mutually exclusive manner, with a network of *cis*- and *trans*-factors potentially working together.

The functional consequences of mRNA localization in granules are also important. First, the transport of multiple mRNAs in a single particle results in a greater net polarization of the transcript using fewer molecular motors. Secondly, granule formation has been linked to translational control [46•], thus coordinately controlling protein expression of multiple mRNAs. The converse situation could also be beneficial, however, since individual transport allows for transcript-specific regulation. These alternatives are perhaps best exemplified by comparing neurons, where synaptic translation of single mRNAs is tightly controlled, with oocytes, where large numbers of mRNAs are localized and translated later at specific developmental time points. Further analysis in such different cell types may shed some light on the mechanisms that orchestrate assembly of transport mRNPs, be they large or small.

mRNA anchoring and the endocytic pathway

Regardless of how an actively transported mRNA reaches its cellular destination, mechanisms must be in place to anchor the mRNA in order to prevent it from diffusing back into the bulk cytoplasm. This critical feature of the mRNA localization pathway remains one of its least understood parts. However, a recent report has implicated the endocytic pathway in anchoring of the *Drosophila* pole plasm through an interaction with posteriorly-localized *osk* mRNA [48•]. Oskar protein had previously been shown to stimulate endocytosis upon localization, an activity that when disrupted resulted in diffusion of pole plasm [49]. The molecular culprit behind this activity, it appears, is Mon2, a conserved protein that is associated with the Golgi apparatus and the endosomal compartment. While Mon2 is dispensable for *osk*-induced endosomal cycling and maintenance of the polarized microtubule cytoskeleton, it is critical for the formation of the long actin projections that are necessary for anchoring pole plasm components [48•]. This induction of actin remodeling requires the activity of three proteins previously shown to regulate actin dynamics, Cappuccino, Spire, and the small GTPase Rho [50], as well as additional as yet uncharacterized regulators [48•]. Thus, Mon2 links posterior *osk* mRNA localization and anchoring of pole plasm with the endocytic pathway, the activity of which is in itself posteriorly polarized [48•].

The involvement of the endocytic pathway in the anchoring of a localized RNA is perhaps not unexpected. ESCRT-II (endosomal sorting complex required for transport-II) was previously shown to play a role in *bicoid* mRNA localization at the anterior pole of the *Drosophila* embryo [51]. However it is not yet clear how ESCRT-II promotes *bicoid* accumulation at the anterior of the embryo, although it has been shown to be distinct from its role in endosomal cycling [51]. In the pathogenic yeast *Ustiglio maydis*, various mRNAs are localized in a microtubule-dependent manner as the cell creates invasive filaments [52]. This process was recently linked to the endocytic pathway, with mRNPs observed to “hitch-hike” onto endosomes. Both constituents are then transported through an association with molecular motors towards the tip of the growing filament [53•]. These data hint at the idea that mRNA localization pathways can intersect with other cellular processes, such as endosomal sorting, and may utilize shared machinery (albeit in distinct ways). Indeed, a

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diverse array of functions, including cytokinesis, cell migration, and the generation of polarity, rely on the endocytic pathway, leading to the emerging hypothesis that endosomal sorting acts as a multi-purpose platform for a variety of cellular activities [54].

Conclusions and Future Directions

From recognition to anchoring the life of a localizing mRNA is a complex and still rather mysterious one. The recent elucidation of *bone fide* structural elements in the 3′-UTR of several mRNAs has opened a new chapter in the delineation of LEs, one that the field has long been working towards. It will be exciting to see whether advances in computational approaches will contribute to the discovery of additional structured LEs. It will also be important to tease apart the significance of RNP granule formation in mRNA localization. With the recent data in some systems highlighting the fact that mRNAs can travel alone, comparison of mRNP assembly in multiple cell types will be critical. And, as has been the case for some time, understanding the mRNA anchoring process remains a particular challenge. Recent studies aimed at understanding the involvement of endocytic pathway components offer a tantalizing glimpse into the overlap of cellular trafficking mechanisms. Understanding precisely how these pathways interact represents a particularly exciting area of future investigation in the field of mRNA localization.

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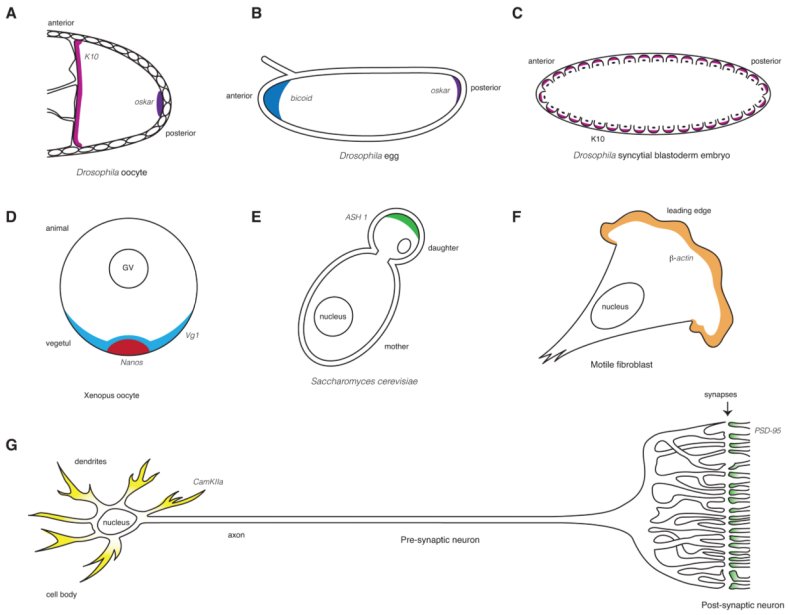

Figure 1. mRNA localization in *Drosophila* oocytes and embryos, and animal neurons

A. *K10* mRNA (magenta) localizes to the anterior and *oskar* mRNA to the posterior (purple) of a developing *Drosophila* oocyte. The oocyte is depicted on the right, surrounded by somatic follicle cells. **B.** *bicoid* mRNA (blue) localizes to the anterior and *oskar* (purple) mRNA to the posterior of the *Drosophila* egg. **C.** *K10* mRNA (magenta) is found at the apical side of the blastoderm nuclei (black), which are partially separated from one another by invaginated cell membranes. **D.** *Nanos* (red) and *Vg1* (pale blue) localize to the vegtal pole of the *Xenopus* oocyte utilizing two alternative localization pathways referred to as the early (active in stages I and II of oogenesis) and late (active in stages II–IV) pathways, respectively. The oocyte depicted is in stage IV of oogenesis. **E.** *ASH1* mRNA (green) localizes to the distal tip of a budding daughter cell during mitosis in the yeast *Saccharomyces cerevisiae.* **F.** *β-actin* mRNA (orange) localizes to the leading edge of motile fibroblast. **G.** *CamKIIα* mRNA (yellow) localizes to the dendrites of a neuron, whereas *PSD-95* mRNA (pale green) is found at the post-synaptic side of a synapse.

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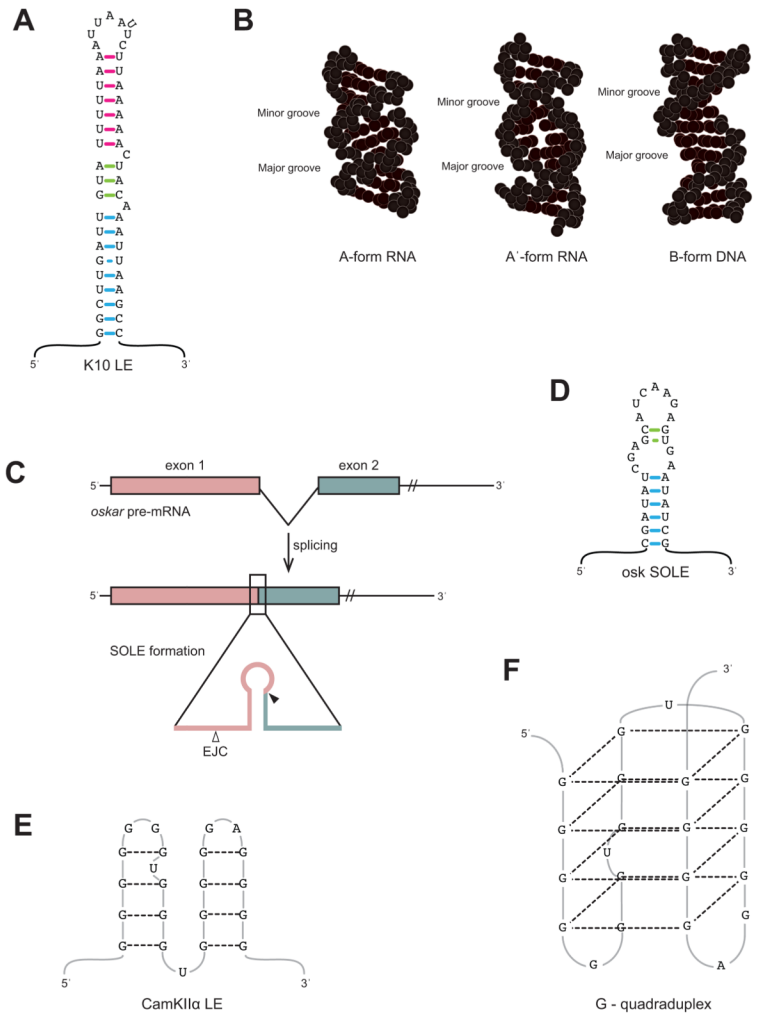

Figure 2. Structural motifs involved in mRNA localization

A. Secondary structure of the *K-10* LE. The three helical sections of the hairpin are shown with colored base-pairs (proximal blue, medial green, and distal pink). A flipped out U is present in the loop. This structural representation is based upon Figure 1 of [30••]. **B.** Depiction of A-form RNA, A′-form RNA, and B-form DNA helices. The *K-10* LE stem folds into A′-form helices, in which the size of the major groove is similar to that of B-form DNA. This is in contrast to the small major groove found in the more common A-form RNA helix. This image is again based upon Figure 1 of [30••]. **C.** The SOLE is formed after splicing of the first intron in *osk* pre-mRNA [32••]. Exon 1 is shown as pink and exon 2 is green in both the pre-mRNA (top) and the spliced product (below). The inset shows the exon 1 (pink) and exon 2 (green) sequences within the SOLE and the position of the EJC (open arrowhead), 20–24nts upstream of the splice junction (filled arrowhead). **D.** The *oskar* SOLE is predicted to form a hairpin, with the proximal and distal stems shown with blue and green basepairs, respectively. **E.** Secondary structure of the *CamKIIα* LE in mammals, as depicted in [37•]. **F.** *CamKIIα* LE folds into a G-quadruplex through hydrogen-bond interactions indicated by dashed lines.