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mRNA on the Move: The Road to Its Biological Destiny*[S]

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Cells have evolved to regulate the asymmetric distribution of specific mRNA targets to institute spatial and temporal control over gene expression. Over the last few decades, evidence has mounted as to the importance of localization elements in the mRNA sequence and their respective RNA-binding proteins. Live imaging methodologies have shown mechanistic details of this phenomenon. In this minireview, we focus on the advanced biochemical and cell imaging techniques used to tweeze out the finer aspects of mechanisms of mRNA movement.

mRNA transport and cytoplasmic compartmentalization of protein synthesis allow precise control over spatial and temporal gene expression and are essential for survival and response to extracellular cues. It has been shown that mRNA localization is essential for cell fate determination (1), directed cell movement (2), and tissue functionality (3). About 3 decades ago, it was discovered that mRNA was asymmetrically distributed in eggs and embryos (4) and in oocytes (5–7) and that this localization was necessary to convey proper embryonic patterning during development. Concurrently, it was found that mRNA could also localize in differentiated somatic cells such as migrating fibroblasts (8), oligodendrocytes (9), and neurons (Table 1) (10). It is now understood that the localization of specific mRNA targets, facilitated by RNA-binding proteins (RBPs),[3] is a highly conserved mechanism to spatially restrict protein production, amplify local protein concentration, or even direct integration into macromolecular complexes, sometimes co-translationally (reviewed in Refs. 11–14). RBPs are multifunctional regulators, as they are responsible for processing, localizing, and controlling the translation of a host of mRNA targets. Sometimes, a unique RBP can carry an mRNA from the nucleus to its final destination in a translationally repressed state. Local cues altering its association with the mRNA may ensure compartmentalized translation (15, 16). Many RBPs that govern mRNA localization are preferentially expressed during critical developmental stages, where differentiation, survival, or cell migration is necessary to establish tissue patterning (3). Modifying the expression of *trans*-acting RBPs (*i.e.* knockdown/out or overexpression) or mutating *cis*-acting regulatory elements present in mRNA targets leads to developmental or cognitive deficiencies (17, 18) and a host of disease states (19). Advances in new imaging technologies have allowed the visualization and quantitation of mRNA localization in fixed and living cells, facilitating a more detailed analysis of the molecular mechanisms involved in the process.

In this minireview, we discuss the current knowledge of mRNA movement from its birth to its final destination within the eukaryotic cell and the impact that biochemical approaches complemented by single molecule imaging techniques have made on the field.

How Does mRNA Localize?

mRNA localization is directed by *cis*-acting localization elements (LEs), also known as zipcodes, typically present in the 3′-UTR of the transcript (Fig. 1, *panel IV*). These *cis*-acting regulatory sequences can range from a few nucleotides to >1 kb in length and are recognized by diverse families of RBPs. Some mRNAs have all the information required for successful localization in a simple element. One of the earliest LE studied was the chicken zipcode in β-actin mRNA. In these early experiments, reporter plasmids expressing different elements of the 3′-UTR of β-actin mRNA narrowed the *cis*-acting elements to a 54-nucleotide zipcode region that mediated localization of the transcript to the leading edge of chicken fibroblasts (20). More recently, biochemical and structural characterization of this β-actin zipcode led to the identification of a bipartite LE within 28 nucleotides that is specifically recognized by ZBP1 (zipcode-binding protein 1; see "β-Actin mRNA: The Targeted mRNA") (21, 22). Another example is the myelin basic protein mRNA, which requires an 11-nucleotide element (called A2RE) in its 3′-UTR that is recognized by heterogeneous nuclear ribonucleoprotein (hnRNP) A2 to be properly transported in oligodendrocytes (23).

Zipcodes present in mRNAs may also be recognized by RBPs on the basis of secondary structures or stem-loops. For example, *Drosophila bicoid* mRNA contains a helical region where only secondary structure, not primary sequence, is important for transport (24). The primary sequences of LEs present in *gurken*, *K10*, *hairy*, and the *I* factor retrotransposon mRNAs are all distinct but may have similar three-dimensional structures. These LEs are recognized by Egalitarian, an RNA adaptor protein involved in dynein-directed transport, illustrating that sequence diversity can enable the recruitment of similar localization machinery (25, 26). A comparable phenomenon has been shown in yeast. The *ASH1* (asymmetric synthesis of HO 1) mRNA contains four different zipcodes (E1, E2A, E2B, and E3) spread through the transcript that do not show homology in primary sequence, but each one is able to mediate mRNA local-

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[3] The abbreviations used are: RBP, RNA-binding protein; LE, localization element; hnRNP, heterogeneous nuclear ribonucleoprotein; mRNP, messenger ribonucleoprotein particle; CLIP, cross-linking and immunoprecipitation; FMRP, fragile X mental retardation protein.

TABLE 1

Some mRNAs, RBPs, and types of mRNA movement discussed in text

CamKIIa, Ca^{2+}/calmodulin-dependent protein kinase II; EJC, exon-exon junction complex.

mRNA(s)	*RNA-binding protein(s)*	*Localization Element (LE)*	*Type of Movement*	*Cell type/Organism and Subcellular destination*	*Significance*	*Reference(s)*
ASH 1	She2p	E1, E2A and E2B (ORF) and E3 (end of ORF and 3'UTR)	myosin-directed transport	Yeast: distal bud tip	mating type switching	(27,28,33,34,55-57)
gurken, k10, hairy, I factor	Egalitarian	structural element recognized	dynein-directed transport	*Drosophila* embryo: apical blastoderm	embryonic development	(25,26)
oskar	Y14, Staufen, hnRNP A/B	EJC, 5' and 3'UTR	diffuse and kinesin-directed transport	*Drosophila* oocyte: posterior pole	germ line differentiation	(31,32,69-74)
nanos	multiple	3'UTR	selective degradation, diffuse and trap	*Drosophila* oocyte: posterior pole	embryonic patterning	(75)
bicoid	Staufen	Stem loop IV/V and Domain III (3'UTR)	dynein-directed transport	*Drosophila* oocyte: anterior pole	embryonic patterning	(24,76)
Vg1	40LoVe, hnRNP I, Vg1RBP/Vera, Kinesin-1 and Kinesin-2	VM1 or E2 (3'UTR)	kinesin-directed transport	*Xenopus* oocyte: vegetal pole	embryonic development	(38-41)
β-actin	ZBP1, ZBP2	5'-CGGAC -19nt- C/A-CA-C/U-3' (3'UTR)	active transport; diffuse & capture	Somatic cell periphery; neuron dendrite; axonal growth cones	directed motility; axon guidance; dendrite arborization	(20-22,37,48,53, 85-88,90,95)
CamKIIa	Staufen, hnRNP U, PSF, FMRP	G–quadruplex (3'UTR)	directed/corralled, oscillatory	Mammalian cell: neuron dendrite	memory formation	(80,83)
Arc	multiple	3'UTR	bidirectional transport	Mammalian cell: neuron dendrite	synaptic plasticity	(81,84)
GIRK2	Nova	YCAY element in introns and 3'UTR	active transport	Mammalian cell: neuron dendrite	inhibitory post-synaptic potentials	(43)
MBP	hnRNP A2	A2RE (3'UTR)	active transport	oligodendrocyte processes	myelin membrane morphology	(23)

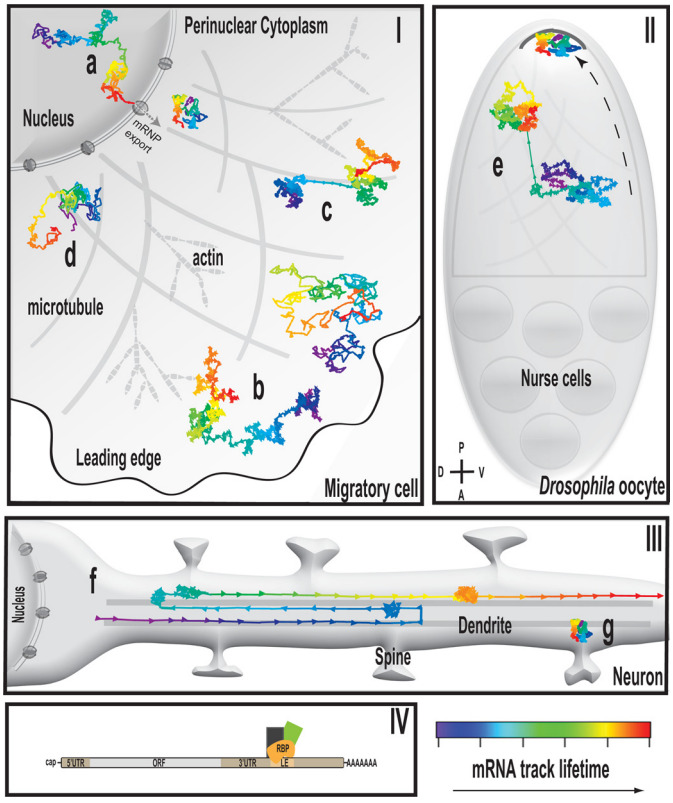

FIGURE 1. **The many roads of mRNA movement.** Shown is a simulation of mRNA motility within cells. mRNA tracks represent mRNA movements as a function of time, coded from *purple*/*blue* to *red*. *Panel I*, fibroblast Cell. *a*, nuclear mRNAs are subjected to corralled diffusion during much of the tortuous path through the nucleus due to chromatin confinement. *b*, in motile cells, mRNA is mainly diffusive, although it occasionally travels along the cytoskeleton. mRNAs localized to the leading edge have larger diffusion coefficients on average (*c*), whereas the movement of perinuclear mRNAs is more confined (*d*). *Panel II*, *Drosophila* oocyte. *e*, although *oskar* mRNA is largely diffusive, localization of the mRNA in oocyte stage 9 is accomplished through a slight bias in active transport of mRNA on microtubules toward the posterior pole (*P*; *arrow*) (74). *A*, anterior; *D*, dorsal; *V*, ventral. *Panel III*, neuron. *f*, neuronal mRNAs depend largely on microtubule-based transport for localization into dendrites. *g*, *Arc* mRNAs are seen to be docked beneath dendritic spines, indicating putative domains that maintain mRNAs in specific locations. *Panel IV*, schematic representation of an mRNA. The cap structure in the 5′-end, the poly(A) tail in the 3′-end, the 5′-UTR, the ORF, the 3′-UTR containing an LE, and the RBPs associated are depicted.

ization to the bud tip using the same RBP, She2p (SWI5-dependent HO expression 2 protein) (27, 28).

A large number of LEs have been identified and characterized in different systems. However, a clear pattern in zipcode primary sequence or structure has not yet emerged, indicating the heterogeneity of these motifs (extensively reviewed in Ref. 29).

Role of Nuclear Encoding in mRNA Localization

Processing of the pre-mRNA in the nucleus influences the cytoplasmic fate of mRNA, illustrating the elegant integration of spatiotemporal events that can occur within an individual cell. Pre-mRNA modifications include the addition of the cap structure to the 5′-end, the addition of poly(A) to the 3′-end, and the deposition of the exon-exon junction complex proteins with the removal of introns during splicing (30). Deposition of RBPs onto the transcript during these events in the nucleus determines its final destination. For instance, splicing at the first intron of *Drosophila oskar* mRNA supports posterior pole cytoplasmic localization of the transcript with Y14, a component of the exon-exon junction complex and essential factor of the transport machinery (31, 32). In yeast, She2p is the RNA adaptor protein involved in myosin-based transport required for *ASH1* mRNA to be exported to the cytoplasm and transported to the daughter cell by binding the mRNA and linking it to the cytoskeleton (33, 34). Once in the cytosol, the messenger ribonucleoprotein particle (mRNP) may gain or lose additional factors that determine whether the mRNA is freely diffusive, actively transported upon the cytoskeleton by molecular motors (kinesins, dyneins, and myosins), protected from degradation, able to translate, or anchored to a compartmentalized domain (Fig. 1 and supplemental figure and movie) (reviewed in Refs. 11 and 13). mRNAs are transported in large and diverse multiprotein complexes. In addition to RBPs, it has been suggested that noncoding RNAs and microRNAs might be components of these large complexes as well (35).

Ripping, Clipping, and Chipping Away at mRNP Composition

Defining the RBPs that associate with specific mRNAs can yield critical information about how mRNA trafficking and localization are regulated in different cellular compartments. Biochemical methodologies to study mRNA interaction with specific RBPs usually rely on standard immunoprecipitation and/or affinity purification techniques, followed by the identification and characterization of the molecules that are part of the complexes. For example, RNA affinity purification methods led to the identification of ZBP1, the founding member of the VICKZ (Vg1 RBP/Vera, IMP-1,2,3, CRD-BP, KOC, ZBP1) family of RBPs (36), in the localization of β-actin mRNA in fibroblasts (37). RNA affinity chromatography also led to the identification of the novel protein 40LoVe, a member of the hnRNP D family of proteins, in the vegetal localization of *Vg1* mRNA in *Xenopus* oocytes. 40LoVe was found to bind specifically to two different late LEs of the *Vg1* mRNA and to be a necessary component of the Vg1 LE RNP (38). hnRNP I and Vg1 RBP/Vera had previously been described as factors associating with *Vg1* mRNA in the nucleus and being exported into the cytoplasm with the transcript (39). In addition, *in vivo* and *in vitro* studies showed that transport of *Vg1* mRNA to the vegetal cortex of the oocyte implicates different microtubule plus-end-directed motors (40, 41). Kinesin-1 associates with *Vg1* LE RNA; blocking its function by using specific antibodies prevents RNA localization, suggesting a direct role for kinesin-1 in vegetal localization of the mRNA. Kinesin-1 also interacts with kinesin-2, and both motor proteins seem to facilitate *Vg1* mRNA movement to its final destination, providing new mechanistic insight into this motor-driven RNA transport process in frog oocytes (41).

Cross-linking and immunoprecipitation (CLIP) (42) is an effective and powerful technique for identifying LEs and has the advantage of isolating RNA-protein complexes under physiological conditions. CLIP coupled with high-throughput sequencing has recently identified an LE in the 3′-UTR of *GIRK2* (G-protein-activated inwardly rectifying potassium (K) channel 2) mRNA that mediates Nova-dependent localization of its transcript in primary neurons (43). This finding suggests an attractive hypothesis in which the loading of the splicing factor Nova onto intronic sequences in the nucleus could be coupled to the mechanism of localization directed by the 3′-UTR of the same mRNA.

Characterization of zipcode sequences that are required for localizing mRNA involves visualizing the subcellular distribution of reporter RNAs carrying putative zipcode fragments. Fusing an essential LE to a reporter RNA should result in a localization pattern within the cell similar to that observed for the endogenous mRNA. Conversely, deletion of regions or point mutations that disrupt localization can be used to see how certain sequences affect mRNA localization. When the RBP is known, putative LEs can be easily characterized on the basis of their binding ability *in vitro* using EMSA (21, 44). Information regarding the binding specificity and affinity is valuable; however, spatial (and temporal) evidence of RNA-protein association within cells is imperative and better determined through imaging methods.

Seeing Is Believing

Originally, intracellular localization of mRNAs was only observed using *in situ* hybridization techniques in fixed samples (8, 45). *In situ* detection remains the standard tool for examining the distribution of mRNAs in fixed cells, tissues, and larger samples like *Drosophila* or zebrafish embryos. Lécuyer *et al.* (46) characterized the distribution patterns of >3000 transcripts during early developmental stages of *Drosophila* embryogenesis by using high-resolution fluorescence *in situ* hybridization procedures. Surprisingly, 70% of the mRNAs analyzed showed different subcellular localization rather than uniform distribution, suggesting that mRNA localization mechanisms are involved in the control of the majority of mRNAs.

Although most of the analysis methods to study mRNA localization in fixed cells have been qualitative (8, 47), recent advances have resulted in more objective and quantitative measurements for mRNA distribution (48, 49). Additionally, due to improvements in sensitivity and resolution of mRNA detection using live microscopy, it is now feasible to visualize single molecules of mRNA to quantify their movements in real time (50–53). Direct observation and quantitation of ensemble mRNA distributions enable further exploration of mRNA dynamics and movement mechanisms.

Prior to the widespread use of high-resolution single mRNA live imaging, mRNA localization was proposed to operate through the following mechanisms: (*a*) directional transport along cytoskeletal elements, (*b*) random diffusion and local trapping of mRNAs, (*c*) vectorial export from the nucleus and trapping, or (*d*) local protection from degradation (13). Thus far, live imaging techniques (reviewed extensively in Ref. 54) in a variety of cell lineages have provided examples of all of these behaviors. Not surprisingly, the method utilized by a cell to produce an asymmetric distribution of mRNA is finely tuned to be appropriate for the particular morphology and time constraints that the cell must overcome. For instance, during yeast cell division, *ASH1* mRNA is preferentially localized into the bud tip of the daughter cell, ensuring asymmetry of *HO* gene expression, which is essential for mating type switching (55, 56). Pioneering the MS2 system to visualize mRNA for the first time in live cells, Bertrand *et al.* (57) demonstrated that *ASH1* mRNA localization was due to movements consistent with myosin-directed motility. Since then, the MS2 technique has been expanded to mammalian cells and even whole organisms (50, 58–60).

RNA Movement within the Nucleus—Shav-Tal *et al.* (61) imaged single reporter mRNAs in the nucleus, and they found that movements of mRNA were governed by the laws of diffusion and not active transport (Fig. 1, *panel I*, *a*, and supplemental figure). Furthermore, they did not observe mRNAs docking in particular nuclear domains, indicating that mRNP assembly likely occurs co-transcriptionally. Additional studies showed that although mRNA length does not affect overall mRNA diffusion kinetics in the nucleus (~0.005–0.02 $\mu m^2/s$), larger transcripts take longer to reach the cytoplasm due to increased frequency of corralled diffusion (62), a consequence of an

increased tortuous path through the chromatin (63). Mor *et al.* (62) also calculated that, on average, mRNA takes ~20 min to travel from the transcription site to the nuclear pore for export (Fig. 1, *panel I*, *a*, and supplemental figure), and interestingly, inclusion of introns expedited export rates but not nucleoplasmic diffusion rates (64). Single molecule tracking analysis of mRNA through labeled nuclear pores utilizing live cell imaging determined that nuclear mRNA export takes less than one-fifth of 1 s (51), an order of magnitude slower than the transport of proteins (65). Remarkably, in 10% of the export events, mRNAs remained docked at the nuclear pores for longer periods of time (approximately seconds), indicating a rate-limiting step, possibly related to quality control (51, 66).

RNA Movement within the Cytoplasm—Similar to the nucleus, movement of mRNAs in the cytoplasm of cells is also largely governed by diffusion, although it can be up to two times faster due to a less restrictive environment (Fig. 1, *panel I*, and supplemental figure and movie) (62). Fusco *et al.* (58) were the first to observe that more than half of *lacZ* reporter mRNAs are freely diffusing with a diffusion coefficient of 0.45×10^{-9} cm^2/s. The remainder of the mRNAs are either static or corralled, corresponding to ~20% associated with the cytoskeleton and the remaining confined by microtubule-based domains. Five percent of the mRNAs move along microtubules in a directional manner at rates of 1–1.5 μm/s for lengths of up to 3 μm (Fig. 1, *panel I*, *b*, and supplemental figure and movie) (58). Interestingly, addition of the β-actin mRNA zipcode element to the reporter mRNA increased the frequency of active transport to 20% as well as the length of transport, implying that *cis*-acting elements may influence localization. A comparable mechanism is exploited during *Drosophila* oogenesis, where localization of *oskar* mRNA to the posterior pole must be accomplished at the appropriate developmental stage to allow germ line differentiation (reviewed in Ref. 67). Fixed cell imaging revealed that intact microtubules (68), kinesin (69), and several *trans*-acting factors such as Staufen and hnRNP A/B (70–73) are necessary for *oskar* mRNA localization, implicating active transport as the localization mechanism. Contrary to expectations of concerted movement to the pole, live cell imaging of *oskar* mRNA revealed that the majority of mRNA diffuses randomly, with only 13% being actively transported (Fig. 1, *panel II*, *e*) (74). Examination of actively transported mRNAs revealed a 7% bias in transport in the direction of the posterior pole due to a subtle bias in microtubule orientation. Likewise, *nanos* mRNA localizes to the posterior pole of *Drosophila* embryos during a critical developmental window, albeit via an alternative mechanism. *nanos* localization relies on diffusion and actin-dependent entrapment of mRNA at the pole, receiving help from the forces of cytoplasmic streaming toward the pole (75). *bicoid* mRNA localization to the anterior *Drosophila* oocyte in the later stages of oogenesis depends on yet another mechanism. Dynein continuously transports *bicoid* mRNA toward the anterior part of the oocyte, as docking there is insufficient to maintain localization (76). It is logical to speculate that the most energetically efficient and thus preferred method of mRNA localization is diffusion and docking. In the absence of these options, or alternatively, if the timing of mRNA localization is a limiting factor, cells such as neurons may rely primarily on active transport to maintain an asymmetric mRNA distribution.

RNA Movement in Neurons—Neurons are highly polarized cells that rely primarily on active transport mechanisms for localizing mRNAs into dendrites (Fig. 1, *panel III*) (77, 78). The field of mRNA kinetics in neuronal dendrites remains largely observational, as investigations under way are still characterizing the movements as a precursor to understanding how they are achieved. One of the earlier visualizations of mRNA in neuronal dendrites revealed that a reporter mRNA (GFP-MS2-Ca^{2+}/calmodulin-dependent protein kinase II 3′-UTR) exhibited kinesin- and microtubule-dependent oscillatory motion (79). Additional imaging studies of fluorescent RBPs have also found that mRNP granules exhibit oscillatory behavior in dendrites at speeds up to 2 μm/s (Fig. 1, *panel III*, *f*) (50, 80). Analysis of single *Arc* (activity-regulated cytoskeleton-associated) mRNAs in dendrites revealed that approximately half of the mRNA population was motile (81). This motile population exhibited both small bidirectional jumps and longer tracks at constant speed. The population of lengthier translocations occurred in both directions, with anterograde movements being longer than the retrograde ones.

Live cell imaging of mRNA movement in neuronal dendrites has been quantitative for studying the contribution of RBPs to localization. Currently, evidence indicates that RBPs in neurons alter the efficacy of active transport and may be an attractive target for regulation of mRNA movement. Mutation of the *Drosophila FMR1* gene (fragile X mental retardation 1) was found to decrease the net distance of labeled mRNAs, implicating fragile X mental retardation protein (FMRP) as a "processivity factor" that increases mRNA interaction with motors (82). However, the total distance traveled was unaltered because the decrease in directional mRNA movement was compensated with an increase in oscillatory behavior in FMRP mutant neurons. Furthermore, the presence of wild-type FMRP in the neuron allowed increased mRNA shuttling from the soma into the dendrites as well as an increased mobile fraction of dendritic mRNA. For *Chic* mRNA, FMRP knockdown resulted in a reversal in the directional bias of movement from 58% anterograde (WT) to 74% retrograde (*Drosophila FMR1* mutants). A subsequent study of FMRP-regulated mRNA localization in neurons confirmed that FMRP increases the fraction of motile particles as well as the displacement of dendritic Ca^{2+}/calmodulin-dependent protein kinase II mRNA (83). An additional dimension of the regulation of mRNA localization in neurons is the synaptic activity-induced alteration of mRNA transport, which is proposed to play a role in synaptic plasticity. Rook *et al.* (79) found that depolarization induces an increase in anterograde mRNA motility into the dendrites. Dictenberg *et al.* (83) studied the increase in dendritic FMRP upon stimulation of metabotropic glutamate receptors and found that following stimulation, FMRP increases its association with its delegated motor, the kinesin KIF5, bringing it into the dendrites. Consistent with this, knock-out of FMRP reduces the steady-state localization of FMRP targets and does not exhibit metabotropic glutamate receptor stimulation-induced mRNA localization, suggesting the role of RBPs in activity-controlled transport of mRNAs essential for synaptogenesis.

The hypothesis that local translation is necessary for synapse-specific modifications suggests that mRNA should be present at the right time in a specific location to immediately contribute to the local protein pool. An example of this behavior is *Arc* mRNA, which is retained in domains directly beneath spines in a UTR-dependent manner (Fig. 1, *panel III*, *g*) (84). This raises intriguing questions as to how specific mRNAs are targeted and retained at the appropriate places within the cell. Dynes and Steward (81) observed that actively transported mRNAs may travel vectorially to a destination at the dendritic spine (Fig. 1, *panel III*). This strongly suggests that transported mRNAs may be responding to local cues to stall or be retained in certain domains.

β-Actin mRNA: The Targeted mRNA

β-Actin mRNA can be transported and targeted to subcellular compartments to undergo local translation (15, 53). Over the last 30 years, β-actin mRNA localization has provided a model system for understanding the mechanisms and purpose of mRNA localization within eukaryotic cells. Biochemical, structural analysis, and imaging approaches have provided an elegant understanding of how the zipcode and zipcode-binding proteins act together to ensure the fate of the transcript once it is synthesized in the nucleus.

β-Actin mRNA requires the presence of a zipcode in its 3′-UTR to be targeted to the leading edge in fibroblasts (20) and in dendritic filopodia and axonal growth cones (85, 86). ZBP1 has been identified by affinity purification methods (37) as the key factor that binds the β-actin mRNA zipcode in the nucleus and is involved in localization as well as translational repression of its mRNA target in the cytoplasm (15, 87–89). ZBP2, the mouse homolog of the human hnRNP protein KSRP (K-homology splicing regulator protein), also binds to the β-actin 3′-UTR in the nucleus and facilitates nuclear ZBP1 association with the transcript and further cytoplasmic localization in fibroblasts (87, 88, 90). Structural studies have recently shown that ZBP1 KH34 (third and fourth hnRNP K-homology domains) specifically binds the bipartite β-actin 3′-UTR element, with KH4 and KH3 recognizing 5′-CGGAC-3′ and 5′-(C/A)CA(C/U)-3′ sequences, respectively (21, 22). Abolition of the function of the zipcode by mutation of the element itself, treatment with specific antisense oligonucleotides, or knockdown/out of ZBP1 protein leads to the mislocalization of β-actin mRNA and subsequent alterations of cell morphology, motility, and adhesion as well as failures in synaptic growth and deficiencies in dendritic spine number, maturation, and arborization (2, 20, 22, 53, 85, 86, 91–94).

Live imaging of β-actin mRNA in different regions of COS cells revealed that restricted mRNA is able to diffuse freely in the leading edge of the cell (Fig. 1, *panel I*, *c*, and supplemental figure), but in the perinuclear region, mRNA diffusion is restricted (Fig. 1, *panel I*, *d*, and supplemental figure). Disruption of the actin cytoskeleton by cytochalasin D delocalizes the mRNA from the leading edge and increases its mobility in the perinuclear region, indicating that the cytoskeletal actin environment strongly contributes to the location of β-actin mRNA within fibroblast cells (48, 95). Once at the leading edge, β-actin mRNA dwells around adhesions in fibroblasts to provide a novel protein source for adhesion maturation, which in turn regulates directed motility (53). In neurons, growth factor stimulation induces β-actin mRNA and ZBP1 protein transport into growth cones (86). Local translation of β-actin mRNA requires phosphorylation on ZBP1 Tyr-396 by Src kinase, a known active component of leading edge adhesions. Similarly, this regulatory mechanism was shown to be necessary for neuron growth cone turning toward a chemotactic cue (15, 94, 96–98).

Although work until now has focused largely on the β-actin mRNA-ZBP1 complex, it is worthwhile to mention that this interaction is not exclusive. β-Actin mRNA may be bound and regulated by many other proteins, and in turn, ZBP1 can bind and regulate at least 116 other mRNAs (22, 99, 100). Post-transcriptional regulation requires the proper interaction of multiple RBPs along with the mRNA. The present challenge involves solving the intricate network of associations of RBPs with motors and multiple mRNA targets. This will allow a better understanding of the molecular mechanisms that govern mRNA movement and localization in live cells.

Conclusions

In recent decades, it has become evident that localization of mRNAs within cells is a widespread and evolutionarily conserved strategy for asymmetric distribution and concentration of mRNP complexes at specific sites. A vast number of mRNAs show subcellular distribution. RNA movement and localization are cell-specific. Local environmental conditions, cytoskeletal constraints, and specific docking sites are important for diffusion-based localization. In situations where active transport is necessary, regulation is exerted on cytoskeletal orientation and the association of RBPs with motors. In all of the aforementioned examples, mRNA movement is probabilistic, with biases introduced by zipcodes and RBPs. Currently, efforts are focused on understanding similarities and differences between diverse mRNA subtypes that localize in the same manner and the molecular mechanisms that govern their targeting to specific subcompartments within the cell as well as the biological significance associated with the localization event. The field of mRNA imaging was previously hindered by technological limitations; however, it is now at a turning point, where we are able to visualize with accurate precision the methods of mRNA localization. The dynamics and stoichiometry of mRNP compositions need to be revealed. Combining high-throughput biochemical, bioinformatic, and imaging methodologies with functional analyses will provide answers to the questions of how a specific mRNA moves and localizes within cells and its phenotypic function.

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