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Mechanistic insights into the basis of widespread RNA localization

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The importance of subcellular mRNA localization is well established, but the underlying mechanisms mostly remain an enigma. Early studies suggested that specific mRNA sequences recruit RNA-binding proteins (RBPs) to regulate mRNA localization. However, despite the observation of thousands of localized mRNAs, only a handful of these sequences and RBPs have been identified. This suggests the existence of alternative, and possibly predominant, mechanisms for mRNA localization. Here I re-examine currently described mRNA localization mechanisms and explore alternative models that could account for its widespread occurrence.

In many cell types, mRNAs are transported to specific subcellular locations, creating localized protein pools with diverse functions. This was first observed 40 years ago, when asymmetric mRNA distribution was found in ascidian eggs[1]. Recent genome-wide studies have underscored the importance of this process, revealing thousands of RNAs that are localized to specific sites within cells[2–22]. This phenomenon has been observed in various organisms, including yeasts, plants, insects and vertebrates (reviewed in refs. 23,24), as well as in a multitude of cell types, including 523 human cell lines[22,25], emphasizing its widespread nature. It is especially prominent in highly polarized cells, such as oocytes, migrating cells, and neurons. For instance, the development of the embryonic body axes in *Drosophila* depends on the asymmetric localization of the maternal mRNAs *gurken*, *bicoid*, *oskar* and *nanos* (reviewed in ref. 26). As highly polarized cells, neurons rely on specific mRNA localization patterns within their cell bodies (soma) and extensions (neurites) for their proper functioning. For instance, in developing neurons, the localization of mRNA encoding β-actin (*ACTB*; called '*β-actin*' here) to growth cones is crucial for axon guidance[27,28]. Synaptic plasticity, which is crucial for learning and memory, is also dependent on mRNA localization. Here, synaptic localization of activity-regulated cytoskeleton-associated protein (*Arc*) mRNA is required for regulation of the trafficking of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors that mediate synaptic transmission[29,30]. Although the importance and extensive scale of mRNA localization are well established, the mechanisms driving this process mostly remain elusive. In this Review I revisit the current models of asymmetric mRNA localization and explore alternative mechanisms that might explain the widespread occurrence of this phenomenon.

Zipcode model of mRNA localization

In the early 1990s it was postulated that mRNAs undergo localization due to specific *cis*-acting elements in their 3′ untranslated region (UTR)—so-called 'zipcodes' or 'localizers'[31]. The localization of *β-actin* mRNA to lamellipodia in fibroblasts was used as a model to map the localization determinants[32]. To achieve this, localization of fusion constructs between the coding sequence of β-galactosidase and segments of the *β-actin* 3′ UTR was analysed. This approach identified two key regions (54 and 43 nucleotides in length, respectively) within the *β-actin* 3′ UTR that were responsible for its localization. The 54-nucleotide segment was more effective in mediating mRNA localization and was termed the 'mRNA zipcode'. That study also reported that these localization determinants do not impact mRNA stability or protein production, but only mediate mRNA transport within the cell. Based on this, the authors postulated that zipcodes recruit RNA-binding proteins (RBPs) that are involved in transport, such as cytoskeleton-associated proteins[32] (see Box 1 for details of the role of the cytoskeleton). In support of this, zipcode-binding protein 1 (ZBP1, also called IGF2BP1 and IMP1) was subsequently identified as binding to the *β-actin* zipcode[33]. ZBP1 forms ribonucleoprotein (RNP) granules that have been suggested to move along cytoskeleton fibres in a motor-dependent manner to locations such as the edge in fibroblasts[34], or to neuronal growth cones[28] and dendrites[35]. Further work[36] showed that the *β-actin* zipcode consists of two short motifs separated by a spacer of 10–25 nucleotides in length. This sequence has been found to be conserved in 114 other mRNAs, suggesting that it may play a role in the localization of multiple mRNAs. The localization of *β-actin* mRNA has thus become the foundation for the understanding of mRNA localization within cells (Fig. 1a and Box 2 provide more details on the *β-actin* mRNA zipcode).

A well-studied example of mRNA localization that is mediated by a zipcode, as validated with in vitro reconstitution experiments, is the transport of *ASH1* mRNA in yeast, which is required for the proper control of mating-type switching (reviewed in ref. 37; Table 1). The zipcode of *ASH1* mRNA is composed of four stem–loop structures,

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BOX 1

Role of the cytoskeleton in mRNA localization

Localization of mRNAs within the cell is expected to rely heavily on the organization and orientation of the cytoskeleton, as well as on the specificity of motor proteins (reviewed in ref. 144). Myosin motors move along polar actin filaments, and microtubules act as tracks for kinesins and dynein, which travel towards their plus- and minus-ends, respectively. For instance, axonal microtubules exhibit a consistent orientation with their plus-ends facing outward, enabling kinesin-mediated cargo transport into axons (reviewed in ref. 144). Conversely, dendrites in mammalian neurons have mixed polarity, with dynamic tyrosinated microtubules mainly oriented with their plus-ends outward, and stable untyrosinated microtubules oriented in the opposite direction[145]. These different groups of microtubules are preferred by different motor proteins, mediating transport in opposite directions. The so-called 'sushi belt' model[146] suggests that neuronal RNAs are transported with different motor proteins bidirectionally—outward from the soma to dendrites and inward from dendrites to the soma—resembling a conveyor belt. Neuronal synapses then selectively retrieve the necessary components from passing cargo. In most animal cells, long actin filaments with uniform polarity are absent (reviewed in ref. 144), which points to myosin-mediated short-distance transport along actin filaments, such as for instance the delivery of post-synaptic proteins to synapses within dendritic spines.

with three located within its coding sequence and one within the 3′ UTR (reviewed in ref. 37). These stem–loop structures are bound by the RBPs She2p and She3p and transported by the myosin motor Myo4p along actin filaments to the tip of the daughter cells (reviewed in ref. 37).

Another example of zipcode-mediated RNA transport that has been reconstituted in vitro involves the Bicaudal-D (BicD) dynein adaptor protein and the RBP Egalitarian (Egl), which mediate mRNA transport in *Drosophila*[38]. Egl specifically interacts with the stem–loop structures found in the mRNAs of *gurken*, *oskar*, *K10*, *hairy* and the *I factor retrotransposon*[38–41]. This binding facilitates their interaction with BicD and the recruitment of dynein, along with its accessory complex dynactin[38]. The resulting complex moves towards the minus-ends of microtubules, helping to establish cell polarity.

Alongside zipcode-mediated recruitment of RNA to motor proteins for transport, other mechanisms also play a part in RNA localization within cells, such as diffusion, anchoring and selective RNA degradation in specific cellular regions, as discussed in the following (Table 1). The scientific community has not reached a consensus on whether the term 'zipcode' should apply to all *cis*-acting elements responsible for RNA localization, or if it should be reserved only for those elements linking mRNA to motor proteins through specific RBPs for mRNA transport. In some recent studies, *cis*-acting elements that mediate RNA localization have been referred to more generally as localization elements or localization signals[37,42,43]. For clarity, I will use the term 'localization element' to include all elements that mediate the local accumulation of transcripts, irrespective of the specific underlying mechanism.

RNA anchoring

As mentioned above, mRNA transport not only involves motor-dependent mechanisms. For instance, the localization of *nanos* mRNA in *Drosophila* oocytes is achieved by a diffusion-driven mechanism coupled with the anchoring of *nanos* within the germ plasm, a specialized cytoplasm at the posterior pole, through its association with the actin cytoskeleton[44] (Fig. 1b and Table 1). Although this does not directly involve motor-mediated transport of the mRNA, its diffusion is accelerated by motor-induced movements in the cytoplasm that result in cytoplasmic streaming[45]. Similarly, during *Xenopus* oogenesis, *nanos1* mRNA is localized through a process of diffusion and subsequent entrapment in the Balbiani body[46].

Translation-dependent mRNA anchoring has long been recognized as a mechanism to direct mRNAs that encode membrane and secreted proteins to the endoplasmic reticulum (ER) (reviewed in ref. 47). In this process, the emerging peptide acts as a signal, which is bound by the signal recognition particle and its corresponding receptor on the ER. This interaction anchors mRNA that is being translated to the ER through the nascent peptide. Recent studies have revealed that co-translational mRNA targeting is more prevalent than previously thought and that it occurs at various intracellular locations, including mitochondria[22], centrosomes[48], cytoplasmic protrusions, endosomes, the Golgi apparatus and the nuclear envelope[25]. These findings suggest that the nascent protein chains may anchor mRNAs that encode them at specific subcellular locations. It remains an open question whether such translation-dependent anchoring contributes to mRNA localization to more distant sites, such as within neurons. For example, fragile X mental retardation protein (FMRP) has been suggested to play a part in the transport of mRNAs that are stalled in translation to distal sites in neurons[49]. An intriguing possibility is that mRNAs may become anchored through their nascent peptides to membrane organelles, thereby enabling the mRNAs to 'hitchhike' on the organelles.

mRNA degradation

Another means of mRNA localization is through localization-dependent mRNA degradation, which differs fundamentally from active transport of mRNA from one subcellular region to another without altering the overall mRNA levels within the cell. However, in this mechanism, enrichment of an mRNA in certain cellular regions is achieved by decreasing its levels in other areas. One such example of localization-dependent degradation, already described in the early 1990s[50], is the localization of *Hsp83* mRNA to the posterior pole of *Drosophila* embryos. This is achieved by *Hsp38* degradation throughout most of the embryo, whereas it is protected from degradation at the posterior pole[50] (Fig. 1c and Table 1). Here, the local concentration of *Hsp83* mRNA at the posterior pole remained constant, whereas the total amount of *Hsp83* decreased as the localization pattern was established, ruling out mRNA transport as a possible mechanism[50]. Subsequent research revealed that *Hsp83* mRNA degradation involves recruitment of the CCR4-NOT deadenylation complex, which is mediated by the RNA-binding protein Smaug[51]. Smaug also binds to the 3′ UTR of *nanos* mRNA in *Drosophila* embryos, which targets it for degradation, whereas *nanos* is shielded from degradation at the posterior pole through interaction with Oskar protein[52].

Our work[53] has shown that in neurons, mRNAs with binding sites for the microRNA let-7 or those containing $(AU)_n$ repeats ($n > 5$) are preferentially degraded in the cell body, resulting in an enrichment of these mRNAs in neurites. Let-7 is the most abundantly expressed microRNA in the mammalian brain and has crucial roles in neuronal differentiation[54], regeneration[55,56] and synapse formation[57,58]. Interestingly, the protein components of the microRNA machinery are depleted from neurites, which leads to a preferential degradation of let-7 targets within cell bodies[53].

Insights from transcriptome-wide localization datasets and assays

The advancement of next-generation sequencing technologies has led to the creation of multiple datasets that report mRNA localization on a

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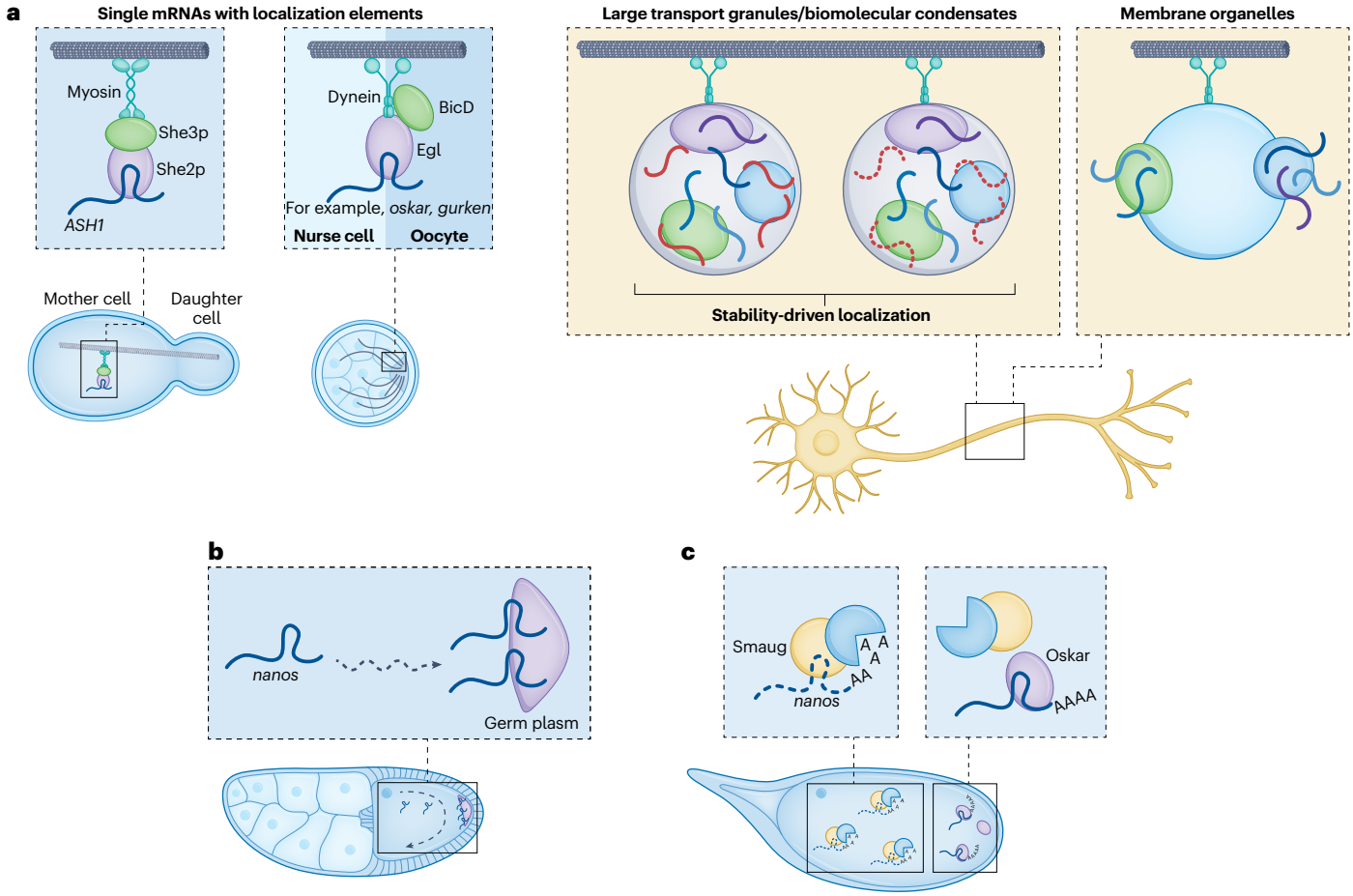

Fig. 1 | Schematic illustration of the different mechanisms of subcellular RNA localization. a, Motor-mediated transport of mRNA. Transport over longer distances (for example, in neurons) is achieved mainly through motor proteins. Recruitment of an mRNA to a motor protein can occur via a specific localization element (left). The image illustrates examples of *ASH1* transport in yeast and the loading of multiple mRNAs from nurse cells into the oocyte in *Drosophila*. In addition, less specific interactions bring numerous RNAs into large localization granules, which may form via biomolecular condensation and are then transported by motors along microtubules (middle). For instance, stable housekeeping transcripts (shown in blue), such as those involved in translation, have been shown to localize to distal cellular sites due to their high stability, without relying on specific sequence elements. Such transcripts may be recruited to motor proteins through degenerate RBP motifs or through non-specific RNA-binding properties of motor-bound RBPs, and may remain in these transport complexes owing to their high stability. Any less stable mRNAs (shown in red) are likely to be degraded before arriving at the cell periphery. Furthermore, RNAs can also hitchhike on membrane organelles for their transport (right). **b**, Diffusion and anchoring. Diffusion is a means of mRNA transport over shorter distances, but it can also be involved in long-distance transport in cases where cytoplasmic streaming (dotted arrow) occurs, such as in oocytes. mRNA remains localized due to anchoring at a specific subcellular region. The image illustrates a specific example of *nanos* localization and anchoring at the germ plasm in a *Drosophila* oocyte. **c**, Localization-dependent degradation. mRNA can be localized due to being protected from degradation in a specific region, while it is degraded elsewhere. Shown is an example of *nanos* localization in a *Drosophila* embryo. Throughout the embryo, *nanos* is degraded via the recruitment of the Smaug protein. However, it is shielded from degradation at the posterior pole through its interaction with the Oskar protein. For further details, see the main text and Table 1.

transcriptome-wide scale[2–15,17–19,59–64]. An integrative analysis of 20 such datasets, spanning different species and types of neuron, has identified a conserved set of mRNAs that were consistently found to localize to neurites in multiple studies[21]. This set includes mRNAs that encode for ribosomal proteins (RPs), components of translation machinery, mitochondrial proteins, cytoskeletal elements and proteins involved in neurite formation[21].

An interesting finding from these comprehensive datasets is the identification of thousands of localized mRNAs, with between 5% and 15% of the cellular transcriptome being at least twofold enriched in neurites compared to cell bodies. However, localization elements have been identified only for a few of them (Table 1). Therefore, we and others have employed massively parallel reporter assays (MPRAs) to map the localization elements within the mRNA that are localized to neurites[53,65,66]. In these studies, a pool of oligonucleotides, representing fragments of 3′ UTRs from neurite-localized transcripts, were cloned into the 3′ UTR of a reporter gene library, which was then introduced into neurons. Subsequently, the neurons were divided into subcellular compartments—cell bodies and neurites—and the enrichment of individual fragments was analysed through sequencing.

Two of the studies found shared localization elements, including $(AU)_n$ motifs and binding motifs for the CELF/BRUNOL (GU-rich motif) and PCBP (C-rich motif) protein families[53,66]. Notably, CELF/BRUNOL plays a part in localized translation in *Drosophila* oocytes[67,68]. PCBP2 is involved in *Mapt* splicing, which is critical for neuron survival and function[69], and its C-rich motif was identified in our study of neurite-enriched RNAs[12]. Interesting, a role for let-7 binding sites in RNA localization was only observed in the study that used primary cortical neurons[53], but not in the one using two different neuroblastoma cell lines[66], probably due to differences in let-7 expression in these cells. Some of the previously known localization elements, such as the cytoplasmic polyadenylation element, were also identified[53].

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BOX 2

A refined view of *β-actin* mRNA localization

Understanding of the localization of mRNAs with canonical zipcodes, such as *β-actin* mRNA, has evolved considerably since their initial description. Live-imaging studies using fluorescently labelled *β-actin* mRNA indicated that its localization in fibroblasts depends mainly on diffusion and local anchoring, rather than on motor-driven transport[87]. Although the neuronal localization of *β-actin* requires motor transport, ZBP1-knockout studies revealed that ZBP1 is not essential for the transport of *β-actin* to dendrites but has a role in mRNA anchoring[118]. Indeed, co-immunoprecipitation experiments have identified kinesin family member 11 (KIF11) as a ZBP1 interactor[147], but there is currently no evidence supporting the importance of this interaction for neuronal mRNA transport. Furthermore, KIF11 is unable to enter dendrites[148], raising the question of how *β-actin* mRNA is transported into dendrites without ZBP1. Interestingly, in vitro reconstitution experiments have suggested that its transport may be mediated by other motifs and proteins[117]. Specifically, *β-actin* mRNA and mRNA encoding β2B-tubulin (*TUBB2B*; called '*β2B-tubulin*' here) have been shown to utilize their G-rich motifs to associate with kinesin-2 through the kinesin adaptor KAP3 and the APC protein, and to travel distances spanning tens of micrometres[117]. Additionally, disruption of the binding of APC to *β2B-tubulin* led to the loss of dynamic microtubules and impaired the migration of cortical neurons in vivo[94]. Furthermore, localization determinants can also affect translation, allowing for protein production from already localized transcripts, which adds yet another layer of gene regulation. For instance, the association of ZBP1 with the *β-actin* zipcode has been shown to prevent its premature translation[149]. Once *β-actin* mRNA is localized at distal sites of the cell, the protein kinase Src activates translation by phosphorylating a crucial tyrosine residue in ZBP1, which results in a release of *β-actin* and its translation. Therefore, the asymmetric distribution of the β-actin protein is achieved through a combination of localization of its mRNA and regulated translation.

The third MPRA study[65] identified GA-rich sequences similar to those that regulate localization to projections of mesenchymal cells[70]. However, as it focused on the dissection of only eight neurite-localized transcripts, its overlap with other datasets was not informative.

Remarkably, only a fraction of the analysed transcripts in these assays showed identifiable localization elements. For example, in primary cortical neurons, asymmetrically localized fragments were found for one-third of the 99 analysed transcripts[53], whereas in N2A and CAD neuroblastoma lines, localized fragments were identified for one-tenth and one-fifth of analysed transcripts, respectively[66]. These findings raise the question of how the majority of transcripts achieve their localization. One explanation is the limited capability of MPRA-based assays to detect localization elements due to their design constraints. MPRA-based assays detect relatively short localization elements, shorter than the library fragments used (ranging from 150 to 198 nucleotides). For instance, the localization element of *Arc* mRNA, which includes a 350-nucleotide region[71], exceeds the mapping capability of MPRA-based assays. Localization elements that comprise multiple motifs spread across the 3′ UTR or the coding sequence, as well as those that are splicing dependent[72], are also undetectable with the current versions of the MPRA-based assay. A further limitation of these assays is that they examine the activity of localization elements within a fixed vector backbone. Different backbones, with variations in their promotors, splicing status and GC content, can influence the activity of the embedded fragments differently. Additionally, the effectiveness of individual localization elements may vary depending on the developmental stage of neurons and on neuronal activity.

An alternative explanation for the inability of MPRA-based assays to detect localization elements in most mRNAs could be the existence of other mechanisms for mRNA localization that do not depend on specific sequences for their recruitment to the localization machinery (for example, motor or anchoring proteins). Instead, these may be driven by mRNA stability, as discussed next.

Stability-driven localization

A plausible factor that could influence mRNA localization is its stability, as mRNAs that are prone to rapid degradation are less likely to reach the cell periphery. mRNA labelling and modelling experiments have estimated that it takes ~4.8 h for an mRNA to cover a distance of 100 µm (refs. 73,74). With the average half-life of neuronal mRNAs being ~3.7 h (ref. 75), the importance of mRNA stability in its transport to distal sites is evident. Our recent work[75] performed a transcriptome-wide quantification of mRNA degradation rates in subcellular neuronal compartments and assessed how differential mRNA stability influences mRNA localization in neurons. This study demonstrated that high mRNA stability is a reliable predictor for mRNA localization to neurites. The stable, neurite-localized transcripts are linked with housekeeping functions such as translation, for example, RP-encoding transcripts. The stability of such RP transcripts is maintained through the binding of the LARP1 protein to 5′ terminal oligopyrimidine (5′ TOP) tracts in these mRNAs[76]. Crucially, experiments that destabilized these transcripts via LARP1 depletion also interfered with their localization to neurites, local translation and neuronal activity[75], suggesting that high mRNA stability is necessary to localize these mRNAs to distant parts of the cell, and this mechanism is essential for neuronal function. Consistently, previous studies have shown that RPs are not only translated locally in neurites[21] but are also incorporated into axonal ribosomes and are required for ribosome function[77,78]. Furthermore, mRNAs that encode for RPs have been reported to localize in various other cell types. This includes the protrusions of mesenchymal-like migrating cells[79] and the basal surface of epithelial cells[80], indicating conservation of this mechanism across diverse cell types.

Destabilizing specific mRNAs disrupted their localization to neurites, whereas stabilizing them reinforced it[75]. For example, when mRNAs were globally stabilized by interfering with the function of the deadenylase CAF1, the amounts of RNA localized to neurites increased approximately twofold (ref. 75). Furthermore, perturbing specific mRNA decay pathways confirmed the role of mRNA stability in localization. For example, m^{6}A (N^6-methyladenosine) modifications in mRNAs trigger their degradation through the recruitment of YTH domain-containing family (YTHDF) proteins that recognize these modified mRNAs and attract deadenylases[81,82]. Depletion of YTHDF proteins or interfering with the protein that deposits m^{6}A modifications on mRNA leads to a stronger accumulation of these mRNAs in neurites[75]. These data suggest that high mRNA stability is not only necessary but also sufficient to localize an mRNA to distant parts of the cell, and that stable mRNAs might localize to neurites mainly because they remain intact long enough to reach the cell periphery (Fig. 1). I propose the term 'stability-driven localization' to describe the idea that stable housekeeping transcripts, which are continuously required in remote locations, localize to distant areas due to their high stability, without relying on specific sequences to recruit them to a localization machinery.

The differential stability of mRNAs is largely determined by *cis*-acting elements within the mRNA. High mRNA stability is typically a consequence of the absence of destabilizing elements in the mRNA (such as m^{6}A or AU-rich elements) or the presence of stabilizing elements (for example, 5′ TOP or an optimal choice of

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Table 1 | RNA localization elements

RNA	Mechanistic details	Organism/cell type	Selected references
Localization elements mediating recruitment to a transport machinery			
ASH1	Localization element: four elements in coding region and 3′ UTR—E1, E2A, E2B and E3; RBPs: She2p and She3p; motor: myosin Myo4p	Yeast, bud tip	119
Hairy, *I-factor retrotransposon, K10*, *gurken*, *oskar* (loading into oocyte)	Localization element: stem–loop RNA localization elements, OES; RBP: Egl; adaptor: BicD; motor: dynein; accessory complex: dynactin	*Drosophila*	38–41
oskar	Localization element: dimerized *oskar* 3′ UTR mediates motor loading, SOLE/EJC mediate motor activation; RBP: DmTropomyosin1-I/C; motor: kinesin-1 or KHC	Posterior pole of *Drosophila* oocyte stages 8–10	120–123
β-actin, *β2B-tubulin*	Localization element: G-rich motif, RBP: APC; adaptor: KAP3; motor: kinesin-2	Mammalian neurons	117
MBP (myelin basic protein)	Localization element: 11-nt A2 response element (A2RE11); RBP: hnRNP A2; motor: Kif1b	Mouse and zebrafish oligodendrocytes, processes; rat hippocampal neurons, dendrites	124–126
Rab13, *Kif1c*, *Net1*	Localization element: GA-rich motif RGAAGRR (R=purine), RBP: APC; motor: KIF1C	Protrusions of mesenchymal cells, endothelial cells, cancer cell lines, neuroblastoma cell lines, fibroblasts, HeLa and basal pole of epithelial cells	70,80,127–130
Anchoring elements			
β-actin	Anchoring element: ‘RNA zipcode’/RBP: ZBP1 (also known as IGF2BP1 and IMP1)	Mouse cortical neurons, dendrites	118
bicoid	Anterior anchoring independent of microtubule and actin cytoskeleton	*Drosophila* oocyte, anterior pole	131
nanos	Posterior anchoring/RBP: Oskar	*Drosophila* oocyte, posterior pole	44,52
oskar	Long Oskar protein	*Drosophila* oocyte, posterior pole	132
gurken	Static dynein/squid anchoring within sponge bodies	*Drosophila* oocyte, dorsal anterior corner	133
Localization-dependent degradation elements			
Hsp83	Degradation element: SREs in ORF/Smaug/CCR4-NOT	*Drosophila* embryo	51,134
nanos	Degradation element: SREs 3′ UTR/Smaug/CCR4-NOT	*Drosophila* embryo	52
let-7 targets	Degradation element: let-7 binding sites/AGO2&TNRC6/CCR4-NOT	Mouse primary cortical neurons	75
$(AU)_n$-containing mRNAs	Degradation element: $(AU)_n$ with $n>5$/HBS1L	Mouse primary cortical neurons	75
Stability-driven localization			
Stable housekeeping mRNAs, for example, transcripts encoding ribosomal proteins	Stabilizing *cis*- and *trans*-acting elements (5′ TOP/LARP1, ELAVLs, optimal codons) act as positive regulators of localization to a distant location, and destabilizing elements (m^6A/YTHDF/METTL3, AREs) act as negative regulators of localization	Mouse primary cortical neurons, mouse forebrains (m^6A), intestinal epithelial cells and fibroblasts (5′ TOP/LARP1 or LARP6)	75,79,135,136
Localization elements with unknown effector			
Bc1 (non-coding)	Localization element: 75 nt (stem–loop); RBP: hnRNP A2	Rat neurons, dendrites	137
Map2, *Bdnf*, *cyclinB*	Localization element: CPE and its binding protein CPEB	Rat hippocampal neurons, dendrites; *Xenopus* embryos	138–140
Camk2α, *neurogranin*	Localization element: 30 nt	Rat hippocampal neurons, dendrites	141
Arc	Localization element: 350 nt	Rat hippocampal neurons, dendrites	71
Tau	Localization element: U-rich; RBP: HuD	P19 cells, axons	142
GlyRα2 (glycine receptor α2 subunit)	Localization element: $(YCAY)_4$ element; RBP: Nova	Neuroblastoma N2A, neurites	143
G-quadruplex-containing RNAs	G-quadruplex; RBP: FMRP	Neuroblastoma CAD, neurites	80

Different types of localization element and their associated cofactors are described. These cofactors comprise adaptor proteins that bind to localization elements and recruit effectors, for example, motor proteins, proteins that regulate RNA stability, or mediate its anchoring. If no effector protein is identified, the localization element is categorized as an unknown type. OES, oocyte entry signal; Egl, Egalitarian; BicD, Bicaudal-D; SOLE, spliced *oskar* localization element; EJC, exon junction complex; KHC, kinesin heavy chain; APC, adenomatous polyposis coli; SRE, Smaug recognition element; CPE, cytoplasmic polyadenylation element; ORF, open reading frame.

codons)[75]. Interestingly, ZBP1, which binds to the *β-actin* zipcode, has recently been shown to bind to the methylated consensus GG(m^{6}A)C sequence and stabilize bound mRNAs[83]. Such a consensus sequence is also present in the *β-actin* zipcode[36]. Additionally, Staufen2, orthologues of which have roles in RNA transport across various species (reviewed in ref. 84), has been found to stabilize its target mRNAs in primary neurons[85]. This suggests there is potential overlap between these mechanisms and that some of the previously described localization elements might also function by stabilizing their mRNA substrates.

Reconciling current data with models for asymmetric RNA localization

As discussed, transcriptome-wide analyses of mRNA localization have revealed several key points: (1) thousands of mRNA molecules are localized within cells; (2) for the majority of these localized mRNAs, no specific localization elements could be identified; (3) high mRNA stability appears sufficient to achieve its localization to neurites. These observations raise the question of how mRNAs that lack localization elements are transported to distal parts of the cell. In vertebrates, axons can span up to a metre, and dendrites can extend hundreds of micrometres from a cell body typically measuring 10–25 µm in diameter[86]. Although diffusion followed by anchoring might account for RNA localization in cells where cytoplasmic streaming occurs (as seen in oocytes[44]) or over short distances (for example, in fibroblasts[87]), it cannot explain the asymmetric mRNA distribution in thin and long neurites[74,75]. Here, mRNAs are thought to be localized by motor-driven transport, mediated by interactions between adaptor RBPs and localization elements.

However, recent findings have challenged this view, suggesting that specific RBP–RNA sequence interactions may not always be required for recruitment to motor proteins. In fact, a substantial number of RBPs display only low sequence-specificity or non-specific RNA-binding properties (reviewed in ref. 88). Indeed, of over 2,000 known human RBPs[89], RNA-binding specificity has been determined for only 223 proteins[90]. Furthermore, RNA Bind-n-Seq experiments designed to determine specific binding motifs identified multiple interacting sequences in more than half of the analysed RBPs (41 of 78)[91]. This suggests that many RBPs tolerate a high degree of variation in their RNA-binding sites. Consistent with this, RBPs found in transported RNA–protein complexes (referred to as transport complexes or granules) often bind hundreds of RNAs. For example, 1,206 RNAs were significantly enriched in immunoprecipitates of Staufen2, a key RBP in transport granules in the rat brain[85]. Additionally, an analysis of FMRP immunoprecipitates from mouse brains identified more than 400 associated mRNAs[92]. Similarly, crosslinking and immunoprecipitation (CLIP) of survival motor neuron protein 1 (SMN1) identified more than 200 associated mRNAs in NSC-34 motor neuron-like cells[93]. In addition, CLIP of adenomatous polyposis coli (APC), which has been implicated in RNA transport, revealed 260 mRNA targets[94]. These findings indicate that multiple mRNAs might be recruited to the transport machinery (that is, RBPs and motor proteins) in a less specific manner than previously thought (Fig. 1a).

However, several studies employing imaging techniques have provided experimental support that transport complexes contain only either a single RNA or only a few RNA molecules[87,95–97]. A limitation of these approaches is that they can only monitor one or a few transcripts at a time. Therefore, it cannot be excluded that such complexes contain additional mRNA species.

Indeed, recent studies suggest that RNAs are transported within larger, complex granules that comprise a mix of different RNAs and RBPs. For instance, *CamkIIα*, *Neurogranin* and *Arc* mRNAs were found to co-assemble into the same RNP and are transported together along microtubules by kinesin motors[98]. The transport of multiple RNAs in the same transport unit makes the process more energy-efficient.

In line with this, recent findings have suggested that mRNA transport may involve the assembly of higher-order mRNP transport granules through phase separation (reviewed in ref. 43). Phase separation describes a process in which untranslated RNA and proteins with intrinsically disordered regions segregate from the bulk cytoplasm or nucleoplasm and create so-called biomolecular condensates (reviewed in ref. 43). Examples include P-granules, stress granules, germ granules, processing bodies and the nucleolus (reviewed in refs. 99,100). An example of the transport of biomolecular condensates are the L-bodies (localization bodies) in *Xenopus* oocyte, which orchestrate the transport of over 450 RNAs, including *Vg1*, and 86 proteins[101]. Another example are *oskar*-containing RNP granules in *Drosophila* oocytes, which form condensates with solid-like physical properties[102] and, at later stages of oogenesis, can encompass tens to hundreds of *oskar* molecules[103]. Neuronal RNP transport granules reach hundreds of nanometres in diameter[104,105] and can also form through phase separation (reviewed in ref. 106). For instance, FMRP exhibits phase separation in vitro with RNA, forming liquid droplets due to its C-terminal low-complexity disordered region[107]. Similarly, TDP-43 RNP granules found in the axons of rodent primary cortical neurons show liquid-like properties[108]. The low-complexity domains in FUS prompt its reversible transition into liquid droplets and hydrogels[109]. These large transport granules, comprising multiple RNAs and proteins, may allow for numerous mRNAs and RBPs to be co-transported within cells, while only requiring a limited number of motor and adaptor proteins.

Hitchhiking on membrane organelles, such as the ER, mitochondria and endosomes, has emerged as an alternative mode of RNA transport (reviewed in ref. 42) and is observed in fungi[110,111], plants[112] and animals[113–115] (Fig. 1a). For instance, the fungus *Ustilago maydis* bidirectionally transports RNA bound to endosomes, facilitated by both kinesin and dynein, along its growing hyphal structures[110]. As mentioned above, yeast *ASH1* mRNA has been demonstrated to co-migrate with the ER to the yeast bud[111]. RNA granules in human induced pluripotent stem cell (hiPSC)-derived neurons have been observed to use lysosomes for movement, with annexin A11 as an adaptor[113]. Moreover, RNA granules have been reported to associate with both Rab7a-labelled late endosomes[116] and Rab5-marked early endosomes[115]. Additionally, neuronal mitochondria have been found to transport the *Pink1* mRNA using synaptojanin 2 as adaptor[114]. Organelle-mediated RNA transport may involve various adaptors and RBPs, offering an additional mechanism for the co-transport of numerous RNAs.

It is important to note that the same mRNA can be transported by different means. For instance, *β-actin*, one of the most extensively studied mRNAs, has been reported to be asymmetrically localized by kinesin-2 motor-dependent transport, assisted by the proteins APC and KAP3A, as well as its G-rich motif, in in vitro reconstitution experiments[117], by diffusion and local entrapment in fibroblasts[87], by anchoring via ZBP1 in dendrites of mouse cortical neurons[118] and, finally, by hitchhiking on lysosomes, with annexin A11 acting as an adaptor in hiPSC-derived neurons[113]. The participation of multiple mRNA elements and different means to reach its destination site may be important for ensuring proper localization of an mRNA.

Conclusions and future perspectives

Multiple studies have shown the widespread nature of RNA localization, with thousands of RNAs localized within cells. However, the mechanisms that regulate localization for the vast majority of these RNAs remain unknown. Regardless of the exact mechanism for recruitment of the mRNA to the motor proteins, that is, directly through RBPs or indirectly through large biomolecular condensates or membrane organelles, it is evident that, with thousands of mRNAs requiring asymmetric localization, the underlying mechanisms are likely to be less selective than initially thought. Moreover, it is clear that high stability of an mRNA is crucial in ensuring that it can localize to distant sites. Such stability-driven mRNA localization is especially important for

housekeeping transcripts, such as those associated with translation[75]. The notion that localization can depend on mRNA stability rather than only the presence of a specific sequence for recruitment to transport machinery aligns with Occam's razor, the principle suggesting that the simplest explanation is often correct, and thus offers a simple and efficient mechanism to localize housekeeping transcripts to remote locations where they are continuously needed.

Future research is clearly needed to better understand the factors that regulate the incorporation of RNAs and proteins into transport granules, as well as to understand how their localization impacts cell function. The binding of RBPs to motor proteins probably depends on specific protein–protein interactions, whereas the recruitment of RNAs and other RBPs may be less specific. Addressing these questions will become possible with advances in methodologies for high-resolution single-molecule imaging and spatial omics, along with the development of tools to analyse and manipulate RNP granules.

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