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RNA Localization and Polarity: From A(PC) to Z(BP)

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Abstract

Cell polarization relies on the asymmetric organization of cellular structures and activities, and is fundamentally important during development as well as for the proper function of most somatic cells. Asymmetries in the distribution and activity of proteins can be achieved through localization of RNA molecules that usually give rise to proteins at specific subcellular sites. It is increasingly appreciated that this is a widely used mechanism affecting protein function at multiple levels. The description of a new RNA localization pathway involving the tumor-suppressor protein APC raises questions regarding coordination between distinct localization pathways and their effects on protein function and cell polarity.

Introduction

Establishment and maintenance of cellular polarity is fundamental for the correct execution of developmental programs and the specification of cell fates, as well as for the proper function of somatic cells. A mechanism widely used to achieve such polarized phenotypes involves the localization of ribonucleic acid transcripts, which usually give rise to proteins at specific subcellular sites. Strikingly, ~70% of mRNAs in oocytes and early embryos of *Drosophila* are localized, in dozens of distinct patterns [1]. RNA localization also exerts a pivotal role in a variety of somatic cells of diverse organisms. For example, many RNAs are enriched in dendrites and axons of *Drosophila*, *Xenopus* and mammalian neurons [2,3]. Localized RNAs are also found in fibroblasts and epithelial cells [4–7], suggesting that RNA localization is a widespread property of polarized cells.

Localized RNAs exist in large ribonucleoprotein (RNP) complexes together with proteins that control every step during their life cycle (Box 1). Several reviews have discussed mechanisms and factors involved in the formation, transport, and regulation of translation of localized RNPs [8–11]. An important pathway for RNA localization in migrating cells involves the RNA-binding protein ZBP1 (Zipcode-Binding protein 1, also known as IMP in humans and CRD/BP in mice). In addition, however, recent work has uncovered, in migrating mammalian cells, the existence of a distinct RNA localization pathway that involves the tumor-suppressor protein APC [5]. We discuss here evidence pointing to potential coordinate control of these two pathways, likely consequences of RNA localization on the function of the encoded proteins, and the impact of RNA localization on cell polarity.

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RNA localization pathways in migrating cells

ZBP1-mediated RNA localization

Migrating cells provide a useful system in which to study the dynamics of cell polarization. In response to a migratory stimulus, cells polarize so that, at the leading edge, actin polymerization and formation of new adhesions drive the extension of protrusions in the direction of migration, while at the rear, contractile forces and adhesion disassembly serve to pull the cell forward [12]. Localized RNAs appear to play an important role in this process. The mRNA for β -actin is concentrated at the leading edge and is important for polarized, persistent cell movement [4,13]. A similar localization is exhibited by mRNAs encoding all seven subunits of the Arp2/3 actin-nucleating complex [14]. Additionally, mRNAs of unknown identity accumulate at sites of new integrin engagement [15] as well as at spreading initiation centers, structures formed at early stages of cell spreading [16].

The mechanisms underlying localization of the β -actin RNA have been studied in some detail. A key factor is the RNA-binding protein ZBP1, which associates with β -actin RNA during transcription in the nucleus and accompanies it to the cytoplasm [17] (Fig. 1A). ZBP1 is important for the transport of the RNA to the leading edge [18] as well as for its translational repression, which is relieved at the cell periphery by local phosphorylation via the Src kinase [19] (Fig 1A). While ZBP1 and β -actin mRNA can be transported on both microtubule and actin filaments [17,20], transport of the RNA to the leading edge is mostly dependent on an intact actin cytoskeleton and requires the function of myosin II-B [21]. Additionally, anchoring of the RNA at the leading edge has been proposed to rely on interaction of the RNA with the actin cytoskeleton, mediated by the translation elongation factor EF1 α [22] (Fig 1A).

Studies of ZBP1 orthologues in different organisms have shown that, apart from β -actin, ZBP1 family members bind to several other RNA targets and can regulate their localization, translation or stability [23–25]. Such targets include the c-myc and insulin-like growth factor II (Igf-II) mRNAs as well as various mRNAs encoding components of the secretory pathway, extracellular matrix and adhesion proteins. Cells in which expression of the human ZBP1 homologues (IMP1-3) have been knocked-down exhibit fewer cellular extensions and are defective in forming invadopodia and cell-cell contacts, likely because of deregulation of its multiple RNA targets [25].

APC-mediated RNA localization

A recent study of migrating mammalian cells has revealed a novel localization pathway that is in several respects distinct from that involving ZBP1 [5]. Specifically, a genome-wide approach showed that in response to different migratory stimuli, more than 50 transcripts accumulate in protrusions of fibroblasts. These transcripts encode proteins involved in functions such as membrane trafficking, cytoskeleton regulation and signaling. However, they do not include the β -actin or Arp2/3 complex mRNAs and in further contrast, they require an intact microtubule (MT) cytoskeleton for their accumulation at protrusions but are not affected by disruption of the actin cytoskeleton or inhibition of myosin light chain kinase activity ([5] and SM and IGM, unpublished observations). Furthermore, at the tips of protrusions, the RNAs are stably anchored in granules at the plus ends of de-tyrosinated MTs (Glu-MTs), a subset of MTs marked by removal of the C-terminal tyrosine of α -tubulin (Fig. 1A). Interestingly, anchoring requires the Adenomatous Polyposis Coli (APC) tumor-suppressor protein, loss of function of which is a main initiating event in colorectal cancer progression. APC is present in the RNA granules and, given its ability to bind directly to MTs could potentially mediate the RNA-MT interaction [5] (Fig. 1A). It is currently unknown whether MTs are used solely for anchoring or whether they also serve to transport RNAs at protrusions. However, in this context, it may be relevant that Glu-MTs are preferentially used by the kinesin motor Kif5c

[26], the cargoes of which include RNA granules in neurons and the Myelin Basic Protein RNA in oligodendrocytes [27,28]. Additionally, Kif5c forms clusters at the ends of MTs that appear to overlap extensively with APC peripheral clusters [26]. Possibly, therefore, Glu-MTs provide the specific tracks on which APC-RNA granules are transported prior to their anchoring at the plus ends (Fig. 1A). APC might also be transported independently of the RNAs since, in epithelial cells, APC associates with and depends on the kinesins Kif3a-Kif3b for its accumulation in peripheral clusters [29].

Anchoring of RNAs at the tips of MTs provides a novel function for the APC protein, the most studied role of which is the regulation of the transcription factor β -catenin, a key effector of the Wnt signaling pathway [30]. APC negatively regulates β -catenin by participating in the 'destruction complex', which targets β -catenin for degradation. This complex contains β -catenin, GSK3 β and the scaffolding proteins APC and axin (Fig 1B). As mentioned above, APC also associates with the plus ends of microtubules at the tips of cellular extensions and regulates MT stability and dynamics [31]. Loss of APC leads to multiple defects, which include defects in cell migration. Specifically, in cultured cells, APC knockdown reduces cell migration and protrusion formation, while *in vivo* disruption of APC function prevents migration of intestinal cells [32–34]. It is currently hypothesized that the MT-associated function of APC is, at least partly, responsible for these effects on cell migration [31]. In light of the above-mentioned involvement of MT-bound APC in RNA localization, the APC-associated RNAs could provide attractive candidates for mediating the effects of APC on cell motility. However, given the fact that APC also associates with other cytoskeletal regulators, such as the Rac1 and Cdc42 effector IQGAP and the guanine nucleotide exchange factor Asef [31], further studies will be needed to delineate the relative contribution of each individual APC function on cell motility and more broadly on tumor-suppression.

The multitude of complexes in which APC participates raises the question of whether these interactions occur concurrently or whether distinct complexes exist. β -catenin partially localizes at the leading edge of migrating astrocytes [35] and can be observed in some cases in peripheral APC clusters [36,37]. Biochemically, however, APC exists in two discrete complexes, one that can bind to MTs and one corresponding to the β -catenin destruction complex [38]. *In vivo*, therefore, the MT-bound and the β -catenin-bound APC likely reside, at least partially, in distinct complexes (Fig 1B). Notably, these distinct APC complexes appear to be coordinately regulated by components of the Wnt pathway. Wnt3a decreases the amount of APC at MT-plus ends in the periphery of growth cones [39]. Mutant β -catenin that cannot be degraded accumulates in APC clusters at MT-ends and inhibits APC function in neurite extension. This inhibitory function of β -catenin does not depend on its ability to activate transcription, suggesting that β -catenin directly blocks or competes away the MT-bound fraction of APC [37] (Fig. 1B). The effect of another Wnt-pathway component, GSK3 β , is not as well defined, with different studies reporting contrasting effects of GSK3 β on the ability of APC to associate with MTs [39–42]. It has been proposed that different levels of GSK3 β inhibition can elicit different cellular responses, thus confounding the interpretation of the results [39]. Nevertheless, the above data suggest that Wnt-signaling activation and the subsequent increase in β -catenin levels, inhibit the association of APC with MT-plus ends and thus likely prevent correct localization of RNAs utilizing the APC-mediated pathway (Fig. 1B).

Coordinate control of ZBP1- and APC-mediated localization pathways in migrating cells?

What is the functional significance of the presence of two distinct RNA localization pathways (one involving ZBP1 and the other APC) in migrating cells? An intriguing possibility is that the two pathways are not simultaneously operational in a cell. ZBP1 is transcriptionally upregulated by activated β -catenin and in turn participates in a positive feedback loop by

binding and stabilizing the β -catenin mRNA [43,44]. Taken together with the inhibitory effect of β -catenin on the MT-bound fraction of APC, described above, a reasonable prediction is that upregulation of ZBP1 or activation of β -catenin (and initiation of such a feedback loop) would on the one hand lead to enhanced localization of ZBP1-target RNAs but would also concurrently inhibit/mislocalize APC-bound mRNAs. In accord with such a mutually exclusive function of the two localization pathways, the expression patterns of APC and ZBP1 are markedly distinct. APC is expressed in wild type somatic cells and its loss of function leads to the development of colorectal tumors and other types of neoplasias [31]. By contrast, ZBP1 is mostly absent from somatic cells but its expression is significantly upregulated in colorectal and various other types of cancer (reviewed in [23]).

Another indication of functional diversity of the two pathways comes from studies of neuronal cells. ZBP1 and its target β -actin mRNA are found in growth cones of extending neurites [45–47]. Similarly, APC localizes at axonal growth cones [42,48] and several APC-associated RNAs are enriched in growing neurites (SM, H. Zhang and IGM, unpublished observations). However, each pathway appears to have distinct functional effects. Interference with the β -actin RNA/ZBP1 interaction or with translation of β -actin RNA does not affect growth cone extension, but inhibits its ability to turn in response to gradients of guidance cues [46,47]. On the other hand, dominant negative APC fragments inhibit axon growth [42] and local inactivation of APC through chromophore-assisted laser inactivation (CALI) leads to collapse of the treated side of the growth cone [48]. Further studies will be needed to establish whether the effect of APC is mediated through its bound RNAs and whether this is specific for mammalian cells [49]. Nevertheless, these results again point towards a functional difference between the ZBP1- and APC-mediated pathways. Understanding how each RNA localization pathway mediates its specific functional consequences will require knowledge of the RNAs targeted in each particular cellular context. It will additionally be important to understand how targeting of these RNAs affects the function of the encoded proteins, which ultimately mediate cellular responses. In the following section, drawing from studies of localized RNAs in various systems, we discuss ways in which localization of an RNA can impact on protein function.

Consequences of RNA localization on protein function

Most localized transcripts that have been studied to date are mRNAs with protein-coding capacity. Such mRNAs can encode proteins that fall into a wide variety of classes and include structural cytoskeletal proteins (e.g. β -actin [4]), cytoskeletal regulators (RhoA, cofilin [50, 51]), transmembrane receptors (integrin $\alpha 3$, EphA2 receptor [52,53]) or cytoplasmic scaffolding proteins (stardust [7]). Additionally, ribosomes are found in neuronal dendrites and growth cones [2,54] and ribosomal RNAs become enriched at sites of integrin engagement and at spreading initiation centers, presumably participating in translation of mRNAs localized there [15,16]. Interestingly, studies of ribosomal proteins encoded by duplicated genes in yeast, showed that translation of the localized ASH1 mRNA requires a specific subset of ribosomal protein paralogs [55], raising the intriguing possibility that ribosomes translating localized mRNAs might exhibit distinct features compared to the general translation machinery. Translation of localized mRNAs can also be regulated by other non-coding RNAs such as the BC1 RNA and the small miRNAs [8]. We focus below on localized messenger RNAs, studied in various systems, and discuss ways through which their translation can affect the function of the locally encoded proteins (Fig. 2).

Targeting of membrane proteins

Localized RNAs can encode transmembrane or secreted proteins, and targeting of the RNA may be required for delivery of the protein to the correct membrane. For example, localization of *crumbs* RNA is required for targeting of Crumbs (a transmembrane protein) to the apical membrane of *Drosophila* follicle epithelial cells [6]. Similarly, RNA localization is required

for targeting of integrin $\alpha 3$ to peripheral adhesion complexes in epithelial cells [53]. Generally, sorting of proteins to plasma membrane domains in polarized cells relies on intrinsic protein-sorting codes that are recognized and segregated by cytoplasmic adaptor complexes [56]. However it would appear that in the cases mentioned above additional information contributed by the localized RNA impacts on correct protein sorting.

Perhaps relevant to these observations is the fact that some RNAs, such as the yeast ASH1 mRNA are co-transported in association with the endoplasmic reticulum to the yeast bud [57]. Furthermore, localization of another yeast mRNA, the IST2 mRNA, to the daughter cortex, is required for delivery of the Ist2 transmembrane protein to the plasma membrane of the daughter cell. In this case, RNA targeting appears to participate in directing the Ist2 protein to a distinct type of secretory pathway that does not require the classical sorting machinery [58]. Such a non-canonical secretory pathway might also be present in neuronal axons. In contrast to dendrites, axons do not rely for membrane supply and outgrowth on known regulators of the secretory pathway [59]. Consistently, ER-exit sites and Golgi outposts are readily found along dendrites, but are absent in axons [60].

Distinct properties of locally synthesized proteins

Translation of a localized transcript can serve to accumulate a protein to one particular location or to form a concentration gradient that helps polarize the cell. Such cases include the morphogen gradients created through localized RNAs (e.g. *bicoid*, *oskar*) in *Drosophila* oocytes, and the local production of cell fate determinants encoded by pair-rule mRNAs during early *Drosophila* embryogenesis [61]. In other cases, however, localized RNAs encode proteins that are already abundant throughout the cell. For example, β -actin protein locally produced in axons corresponds to <1% of the actin synthesized in cell bodies and transported into the axons [62]. Similarly, treatment of axons with NGF leads to local translation of cAMP response element binding protein (CREB) mRNA, but the CREB protein concentration in axons is substantially less than that in the nucleus [63]. Based on these observations, it has been suggested that newly synthesized proteins translated from localized mRNAs have unique properties that distinguish them from pre-existing proteins.

In support of this idea, axonally synthesized CREB accounts for the majority of phosphorylated CREB that appears in the nucleus after stimulation of axons with NGF [63]. Axonal CREB co-localizes with the NGF receptor that mediates CREB phosphorylation, and this association could render axonal CREB more susceptible to modification than cell body CREB [63]. Another transcription factor, Elk-1, when dendritically synthesized can mediate neuronal cell death, but not when it is translated in the cell soma, presumably because it acquires distinct post-translational modifications in the dendritic environment [64]. For β -actin, newly synthesized molecules might lack modifications such as arginylation or glutathionylation which affect their polymerization into filaments [46,65]. Directly testing these ideas will require the ability to isolate locally translated proteins from pre-existing ones, and to biochemically characterize them. Methods that preferentially tag nascent proteins and permit affinity purification would be useful in such approaches [66,67].

Local translation could additionally provide proteins with unique properties by creating a high local concentration, which would increase reaction rates for colocalized substrates, or favor association with specific binding partners. Alternatively, unique complexes might be formed if partners are translated in close proximity, so they can interact cotranslationally, as opposed to post-translational binding. Indeed, in a process termed “dynamic cotranslation”, peripherin intermediate filaments were shown to assemble cotranslationally from mRNPs containing multiple peripherin mRNAs [68]. Distinct mRNAs can also be co-assembled and co-transported into common RNP transport particles both in yeast cells and mammalian neurons [69,70]. However, it is currently not known how many distinct RNAs can be contained in such

particles and how assembly affects the translation and function/associations of the encoded proteins.

Retrograde signaling

Translation of localized RNAs can generate proteins that will function near the translation site. Nonetheless, a few examples suggest that locally translated proteins can also be trafficked away from the site of synthesis, perhaps as a means to allow communication between cellular compartments. In one such case, injury of dorsal root ganglion neurons triggers local translation of importin- β , RanBP1, and vimentin mRNAs [71–73]. RanBP1 stimulates RanGTP dissociation from importins, permitting newly synthesized importin- β to bind importin- α [73]. The importin- α/β heterodimer in turn binds to the retrograde motor dynein [71]. In parallel, vimentin binds phosphorylated Erk (pErk) and through concomitant binding to importin- β links the activated kinase to the retrograde transport system. Formation of this complex allows translocation of pErk to the cell body and activation of the transcription factor Elk1 [72]. This process enables coupling of axonal injury to specific transcriptional responses.

In a similar manner, axons contain mRNA for the transcription factor CREB. In response to NGF, the CREB mRNA is translated and the newly synthesized protein, which as mentioned above is preferentially phosphorylated, is trafficked back to the cell body in a microtubule-dependent manner [63]. This CREB subpopulation is responsible for mediating transcription in response to NGF, since selective depletion of axonal CREB transcripts prevents CRE-mediated transcription and NGF-induced neuronal survival [63].

Several transcripts localized at MT-ends in fibroblasts (see above) encode proteins with known nuclear functions (such as TAT SF1, a transcription elongation factor; Bmi1, a member of the Polycomb group of transcriptional regulators; and the centromere-binding protein CENP-B) [5]. It would be interesting to test whether these proteins, once translated in actively growing protrusions, are also transported back into the nucleus, perhaps as a means of communicating peripheral signals such as the formation of new adhesions or exposure to environmental cues.

Translation-independent roles

Finally, in addition to the protein-coding capacity of localized RNAs, some transcripts might also exert structural and regulatory functions that are translation-independent. Indeed, such roles have been suggested for mRNAs on the mitotic spindle [74], as well as for the *oskar* mRNA at early stages of *Drosophila* oogenesis [75] and the VegT mRNA in the vegetal cortex of *Xenopus* oocytes [76]. Little is known about the roles fulfilled by these RNAs, but a structural role has been proposed for VegT RNA, because it is integrated into the cytoskeleton and its depletion leads to disruption of the cytoskeleton [76].

RNAs have the potential to organize large protein complexes, as exemplified in the case of ribosomes, spliceosomes, and the signal recognition particle. In this context, it is interesting that the p68 RNA helicase is required for displacing axin from β -catenin, during PDGF-induced epithelial-mesenchymal transition [77]. Axin serves as a scaffolding protein that together with APC holds β -catenin in the ‘destruction’ complex (Fig 1B). The involvement of the p68 RNA helicase in disrupting this interaction raises the possibility that RNA(s) might serve as structural component(s) of the β -catenin destruction complex. While the presence of specific RNA(s) in the axin/ β -catenin complex was not tested, β -catenin can bind RNA both *in vitro* and in cell extracts [78]. It would be interesting to determine the identity of such potential RNA components of the β -catenin destruction complex and how they are related to the APC-associated RNAs localized at MT-plus ends discussed above.

Localized RNAs: changing or maintaining cell polarity

In some polarized cells, localized RNAs appear to be required during particular stages or for specific cellular responses. For example, RNAs and translation machinery are found in axons of neurons during axon outgrowth and pathfinding but, at later stages, when mature connections are established the axons are largely devoid of RNAs [3]. Additionally, growth cones contain β -actin and other RNAs, and translation is required for the ability of the growth cone to turn in response to guidance cues, but not for growth cone extension [54]. One model consistent with these observations is that translation of these localized RNAs is not necessary for the maintenance of an established polarized state (such as the maintenance of mature connections or the persistent extension of a growth cone), but is specifically required for changes in polarity in response to environmental cues.

In accord with this idea, the mRNA for the polarity protein Stardust (Std) is apically localized only in dividing, developing *Drosophila* epithelia [7]. At later stages of development, coinciding with the presence of mature epithelia, *sdt* mRNA is not localized, due to an alternative splicing-based mechanism which removes the localization determinants [7]. Therefore, again in this case, RNA localization appears to be utilized at stages when polarity is labile but not when a polarized state has been already generated.

Maintenance of polarity is thought to rely on positive feedback loops that sustain an asymmetric distribution of signaling and structural molecules [79–81]. Such feedback loops can be initiated by changes in the concentration or dynamics of proteins either in response to asymmetric cues or from stochastic fluctuations of local concentrations [79–81]. Translation of localized RNAs might provide a way to direct such changes in concentration in a non-random manner. We envision that local translation, in response to specific cues, might play an important role in breaking feedback loops that already exist, and establishing new directions of polarity.

Growth cone steering provides an illustration of this idea. Turning in response to a guidance cue requires localization and translation of β -actin mRNA [46,47]. Additionally, dynamic MTs (on which β -actin mRNA is transported in neurons [45]) are required for growth cone steering [82] and active Src kinase (which activates translation of β -actin mRNA [19]) is enriched at sites of interaction that induce growth cone turning [46,82] (Fig. 3). Importantly, inhibition of β -actin translation blocks Ca^{+2} -dependent growth cone turning but does not impair random turning [46]. Therefore, asymmetric translation of β -actin is not required *per se* for the ability of the growth cone to turn. Turning and new polarity axis formation can still occur randomly, perhaps as a result of stochastic fluctuations in spatial actin dynamics. We hypothesize that local translation of actin (and potentially of other ZBP1-targeted mRNAs) serves to alter the concentrations and dynamics at a specified location so as to reorient the axis of polarity in a deterministic manner towards the direction of the external cue (Fig. 3).

Other localized RNAs can instead participate in feedback loops that stably maintain polarized states. In the *Drosophila* oocyte, *oskar* mRNA participates in a positive feedback loop, in which Oskar recruits Par-1 to the posterior to increase polarization of the MT cytoskeleton which in turn directs the localization of more *oskar* RNA, thus maintaining the posterior identity of the oocyte [83]. In mammalian neuronal cells, APC also participates in maintenance of polarity, since interference with APC function inhibits axon growth and leads to defects in axon specification and polarity [39,42,48,84]. APC function is required for microtubule organization and localization of the polarity protein Par-3 at axon tips [39,84]. Whether APC-localized RNAs are involved in these processes is currently not known.

Yeast offers another paradigm for cell polarization, in which at the G1-S phase transition the cell polarizes to form a bud at a site adjacent to the one formed in the previous cell cycle. A central player is the Cdc42 GTPase. Active Cdc42 stimulates actin cable formation, which in

turn allows Cdc42 to be delivered to the same site on the plasma membrane through type V myosin, thus completing a positive feedback loop [85]. Interestingly, yeast cells localize the mRNA for Cdc42 at the future bud site and this localization precedes Cdc42 protein enrichment and bud site appearance [86]. Local Cdc42 synthesis might promote polarity establishment and act dominantly over spontaneous, random bud site emergence.

The ideas presented above are rather speculative at the moment. Testing them will require the identification of components participating in each feedback loop and determination of how localization of RNAs affects their spatial distributions and activities.

Conclusions

Polarized migrating cells utilize at least two distinct pathways, mediated through ZBP1 or APC, in order to localize RNAs at the leading edge. The available evidence and the known regulatory interactions between components of the two pathways suggest that they likely mediate different functional effects and are utilized in a spatially and/or temporally distinct manner. This functional specialization of each pathway is likely mediated by their ability to localize distinct groups of RNAs, which in turn produce proteins with diverse properties, or exert translation-independent roles. Because of their ability to concurrently regulate a large group of RNAs, components of these pathways are likely to be central players during polarized cellular responses. Elucidating the mechanisms underlying their control and coordination will thus lead to a more complete understanding of how cellular polarity is established and maintained and how it is perturbed during processes such as tumor progression and metastasis.

Box 1

Assembly, transport and regulation of localized RNPs

Localized RNAs exist as large RNP complexes. Several of the proteins important for RNA localization and regulation join these complexes already in the nucleus while others associate upon remodeling in the cytoplasm [87,88]. Correct localization and assembly of a localized RNP is directed by specific sequences within the RNA that serve as localization determinants and recruit the necessary trans-acting factors. Such targeting elements can be simple, short nucleotide sequences, however they are most commonly defined by a more complex combination of secondary and tertiary structure [89]. Asymmetric distribution of an RNA can be achieved through several mechanisms including localized protection from degradation, passive diffusion coupled with local entrapment, continuous active transport or active transport followed by local anchoring, which appears to be the most commonly used mechanism [11]. Depending on the RNA and the particular cellular context, active transport can occur on actin filaments using myosin motors or on microtubules using kinesins or dynein (depending on the direction of transport) [9,11]. Actin, microtubules, or motor proteins are also involved in anchoring RNAs once they reach their final subcellular location [5,90]. During their transport, localized RNAs are thought to be translationally silenced by repressor RNA-binding proteins that block translation at several different steps, or by non-coding miRNAs [8,10]. Inactivation of such repressors can be achieved through the spatially and temporally controlled action of conserved signaling pathways, leading to local protein production [8,10].

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Figure 1.

A. ZBP1- and APC-mediated RNA localization pathways in a migrating cell. See text for details. **B.** Association of APC with distinct cellular complexes. APC participates, together with β -catenin, axin and GSK3 β , in the ‘destruction complex’. In the absence of Wnt signaling (left panel), β -catenin is phosphorylated and targeted for degradation by the 26S proteasome. An apparently separate pool of APC is found at the plus ends of Glu-MTs in association with several mRNAs. Upon Wnt signaling activation (right panel), inactivation of GSK3 β leads to stabilization of β -catenin, which enters the nucleus and activates transcription of Wnt-target genes. Stabilized β -catenin can also directly block APC function at MT-ends.

Figure 2.

Potential functional roles of localized RNAs, discussed in the text. Localized RNAs can be translated into proteins that (a) are targeted to specific membrane compartments, (b) carry post-translational modifications (*) distinct from those carried by pre-existing protein or protein found at other cellular locations, (c) form specific complexes with other binding-partners or (d) are trafficked to other locations to mediate communication between cellular compartments. Localized RNAs can also exert translation-independent roles, such as participating as structural components of ribonucleoprotein complexes (e).

Figure 3.

Schematic depicting the proposed role for localized RNAs in establishing new directions of polarity during growth cone turning. A positive feedback loop between components A and B maintains a stable axis of polarity so that outgrowth persists towards a specific direction (maintenance of polarity also likely requires the action of lateral inhibitors, which are not depicted here for simplicity). In response to a guidance cue, dynamic MTs transport β -actin mRNA and likely other localized RNPs. Additionally, a local increase in Src kinase activity relieves the translational repression of β -actin mRNA mediated by ZBP1. This leads to a local increase in the amount of β -actin (and likely of other proteins produced by localized RNAs). We hypothesize that this local increase in concentrations and activities plays an important role in re-orienting the polarity axis towards a new direction. Other proteins translated from (or affected by) such localized RNAs could correspond to factors A or B or to regulators of them.