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Control of Cell Migration Through Mrna Localization and Local Translation

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Abstract

Cell migration plays an important role in many normal and pathological functions such as development, wound healing, immune defense and tumor metastasis. Polarized migrating cells exhibit asymmetric distribution of many cytoskeletal proteins which is believed to be critical for establishing and maintaining cell polarity and directional cell migration. To target these proteins to the site of function, cells use a variety of mechanisms such as protein transport and mRNA localization-mediated local protein synthesis. In contrast to the former which is intensively investigated and relatively well understood, the latter has been under-studied and relatively poorly understood. However, recent advances in the study of mRNA localization and local translation have demonstrated that mRNA localization and local translation are specific and effective ways for protein localization and are crucial for embryo development, neuronal function and many other cellular processes. There are excellent reviews on mRNA localization, transport and translation during development and other cellular processes. This review will focus on mRNA localization-mediated local protein biogenesis and its impact on somatic cell migration.

Keywords

cell migration; invasion; mRNA localization; local translation; cytoskeleton; actin; formin; Dia1; Arp2/3; zipcode; ZBP; IMP; mRNP; APC; Rho; myosin; cytoplasm streaming; force gradient; adhesion; ER; Signaling

Cell migration and protein localization

Cell migration plays an important role in many normal and pathological cellular functions such as development^{1–5}, immune defense^{6–8}, wound healing^{9–11} and tumor metastasis^{12–16}. It is a well-orchestrated cyclic process that includes formation of protrusions and focal adhesions at the leading edge, cell polarization, nucleus movement, detachment at the rear, and finally body translocation. These highly localized events are regulated by specific factors, which often exist at high concentrations at the site of function. Indeed, in polarized migrating cells there is an asymmetric distribution of many cytoskeletal and signaling proteins, which is crucial for establishing and maintaining cell polarity and directional cell migration^{17–26}. For example, the actin polymerization nucleator Arp2/3 complex is localized

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at the leading edge of migrating cells for efficient migration and chemotaxis^{27–29}. Additionally, myosin X, which induces the formation of filopodia and is present at the tip of filopodia³⁰, interacts with the β -1 integrin and PIP3 to aid in the localization of these proteins to the filopodia, and therefore plays a crucial role in the regulation of tumor cell migration and invasion³¹. As a matter of fact, in addition to its role in cell migration, protein localization has long been recognized as an evolutionary advantage for eukaryotes that have sequestered intracellular compartments for specialized functions. Given the critical role of protein localization, much work has been done to gain an understanding of the functional significance and mechanisms by which proteins are sorted and transported to the site of function in different compartments^{32–37}.

mRNA localization and local translation

In addition to the relatively well studied mechanisms for protein sorting and transport, an increasing volume of evidence has demonstrated that mRNA localization-mediated, local protein biogenesis is an important mechanism for intracellular protein targeting^{38–43}. Since 1983, when Jeffery et al. first reported that β -actin mRNA was asymmetrically distributed in ascidian eggs and embryos⁴⁴, many studies have demonstrated that mRNA localization is a general phenomenon which exists in oocytes, developing embryos as well as differentiated somatic cells^{45–50}. Even in prokaryotes, recent studies in *E. coli* reveal that some mRNAs are targeted to cytoplasm, poles, or inner membrane in a translation-independent manner with functional significance^{51–54}. In most of these cases, there is a close spatial correlation of the mRNAs with their protein products. This strongly argues for a role of mRNA localization in protein targeting. Unique advantages of targeting mRNAs to the site of function may include: 1) It is an energy-saving process as it costs less energy to transport an mRNA molecule that can subsequently be translated into thousands of protein molecules; 2) It minimizes inappropriate protein-protein interactions; and 3) It facilitates specific protein interactions for protein modification and complex assembly. These advantages underscore the non-redundant role of local protein biogenesis in cellular function as shown by many studies in which alteration of mRNA localization, but not the level of the corresponding protein, often resulted in functional defects in many models^{38–43, 55–59}.

Mechanisms of mRNA localization

Results from decades of studies indicate that cells use a variety of mechanisms to target mRNAs. These mechanisms can be categorized into several models: **a) Signal recognition particle (SRP) and translation mediated mRNA localization and local translation on the ER.** This mechanism ensures that transcripts encoding membrane and secreted proteins are synthesized and undergo unique post-translational modifications in defined compartments^{60–63}. **b) SRP-independent mRNA localization on the ER.** Recent studies in several model systems reveal that a subset of transcripts which encode the secreted, membrane and cytoplasmic proteins can be targeted to the ER, independent of the SRP^{64–78} and/or translation^{66–79}. **(c) RNA localization sequence (zipcode) and transacting factor (TAF)-mediated mRNA localization.** For most of the localizing mRNAs which encode cytoplasmic proteins, their intracellular localization is mediated by zipcode(s) within the RNA molecules and TAFs^{38–41, 43, 80–82}. The zipcodes are often found in the 3' untranslated

region (**3'UTR**) of the mRNAs but can also be found in the 5'UTR and coding regions. TAFs are mostly RNA binding proteins that bind to the corresponding zipcodes and regulate the transport, anchorage and translation of the mRNA. **d) Other mechanisms.** These include the diffuse/transport-trap model^{83–86} and global degradation with differential stability^{87–89}. For detailed discussions on these mechanisms, mRNA localization and local translation in development and neurons, we refer the readers to several recent excellent reviews^{42, 58, 90–92}.

mRNA localization and local translation in cell protrusions

Extending protrusions at the leading front of a migrating cell has been proposed as a crucial step for directional cell migration¹⁸. Many signaling and cytoskeletal proteins are known to localize at the leading protrusions where they often play important roles in the regulation of protrusion dynamics^{17–26}. To study the role of mRNA localization in protein targeting to the protrusions, in situ hybridization has been used to detect intracellular distribution of mRNA and only a limited number of mRNAs have been identified as localized in the protrusions⁵⁸. Since the use of a modified Boyden chamber to separate the cell pseudopodia from cell body in 2006, more than 700 protrusion-localized mRNAs have been identified in cultured neuron, cancer cells, fibroblasts and astrocytes^{93–97}. However, most of these localizing mRNAs remained uncharacterized for function and mechanism of localization. Among the mRNAs whose mechanisms of localization have been studied, most use the zipcode-mediated model^{58, 80, 94, 96, 98–100}. Consistent with the prediction that localized mRNAs are locally translated, components of the mRNA translation machinery and newly synthesized proteins are co-localized with mRNAs at the leading edge of migrating cells. mRNA localization is often regulated by the same signaling pathways that regulate protein activity and localization for cell polarity and migration. To date, β -actin mRNA is the most studied and relatively well understood transcript for localization and local translation in migrating cells. It is localized in the protrusions of fibroblasts and myoblasts, as well as in the growth cone of neurons^{48, 101}. Its localization requires serum or individual stimulus such as PDGF and LPA, intact actin cytoskeleton, activation of GTPase Rho and Rho downstream effector myosin IIB. This suggests a Rho-regulated, myosin II motor mediated transport of translationally silent β -actin mRNA along the actin filament tracks^{48, 98, 102–106}. At a molecular level, a fragment of 54 nucleotides in the 3'UTR has been identified as the zipcode for β -actin mRNA localization⁹⁸. A transacting factor, zipcode binding protein 1 (ZBP1, also called IMP1, IGF2BP1 or CRD/BP)^{105, 107}, binds to the zipcode sequence through its two KH domains^{98, 108, 109}. ZBP1/IMP1 binds to the β -actin mRNA in the nucleus, escorts its transport and suppresses its translation^{106, 110, 111, 112}. At the destination, β -actin mRNA is anchored on actin filaments through EF1 α which is a translation elongation factor and an actin binding protein¹¹³. The association of mRNAs and components of translation machinery on the cytoskeleton has been known to significantly enhance protein translation efficiency^{114, 115}. For the localizing β -actin mRNA, its translation at the cell protrusion is regulated by Src kinase which is locally activated and phosphorylates ZBP1/IMP1, leading to dissociation of ZBP1/IMP1 from β -actin mRNA and initiation of β -actin mRNA translation¹¹². Local translation of β -actin mRNA at cell contact sites has been demonstrated in live cells¹¹⁶.

Another example of protrusion localized mRNAs is the transcripts encoding the actin polymerization nucleator Arp2/3 complex. The Arp2/3 protein complex is an important regulator of the actin cytoskeleton and is localized at the leading edge of migrating cells^{27–29}. All the mRNAs encoding the seven subunits of the Arp2/3 complex are localized in the protrusions of primary chicken embryo fibroblasts (CEFs) and primary human foreskin fibroblasts (HFFs)¹⁰³. This is the first evidence that all the mRNAs encoding the subunits of a protein complex are localized to the same intracellular location, suggesting a mechanism for facilitating protein complex assembly through local translation or even co-translational assembly^{103, 117}. Similar to β -actin mRNA, the protrusion localization of Arp2/3 complex mRNAs is dependent on serum, PDGF and LPA, actin and microtubule cytoskeleton, activation of Rho and myosin II, and is independent of translation^{103, 118} (Mingle, Liu, unpublished). Interestingly, global activation of Rho by expressing constitutively active Rho causes delocalization of Arp2/3 mRNA. Further studies indicate that localized Rho activity is the key for Arp2 mRNA localization and Arp2 mRNA and active Rho are mutually exclusive in space¹¹⁸. ZBP1/IMP1 may be involved in the localization of the Arp2/3 mRNAs as mRNAs encoding the subunit of the complex bind to ZBP1/IMP1¹¹⁹. Because cells on 2D culture often show different morphology from those in tissues, the localization of Arp2/3 mRNAs was confirmed in tissue sample (see Fig. 1, Mingle, Van de Water, Liu, unpublished). It is noteworthy that only a small percent of the cells (30%) showed strong protrusion localization of β -actin and the Arp2/3 mRNAs at any moment because the cells are at different stages of migration. This is better demonstrated by following the dynamic Arp2 mRNA movement in a live cell as shown in Figure 2 (Mingle and Liu, unpublished). The rapid change of mRNA localization from one end to another following the switch of the direction of cell movement, as shown in Figure 2, also suggests an efficient process for translocation of mRNAs within a cell.

In addition to the ZBP1/IMP1-mediated targeting mRNA to the protrusions, a novel pathway for targeting mRNA to the protrusions of migrating cells was reported in 2008⁹⁴. About 50 transcripts are found significantly enriched in the pseudopodia fraction isolated from NIH/3T3 fibroblasts using the modified Boyden Chamber. By testing two of the localizing mRNAs encoding Rab13 and plakophilin 4, the 3'UTR of these mRNAs are revealed as the sequences for localization, suggesting a zipcode mediated mRNA targeting pathway. No consensus motif at the level of primary sequence was found among the localizing transcripts, suggesting possible higher order complex RNA structures which are common among the known zipcodes¹²⁰. The transacting factor for these localizing mRNAs turned out to be the tumor suppressor protein adenomatous polyposis coli (APC) which was known to play a role in cell migration and invasion^{121, 122}. It preferentially associates with the transcripts enriched in the protrusions, suggesting it is a major transacting factor for this group of mRNAs. APC and the messenger ribonucleoproteins (mRNPs) colocalize on the tips of stable microtubules, which is independent of actin cytoskeleton and myosin activity^{94, 100}. The translation of APC-associated mRNAs is regulated by an RNA-binding protein Fus/TLS which recruits APC-mRNP granules and preferentially promotes translation in the protrusions¹²³. These features indicate that APC mediates a different mechanism from the ZBP-mediated pathway for mRNA targeting to the protrusions. As for the relationship of these two pathways, it is suggested that these two pathways are not simultaneously

operational in a cell¹⁰⁰. For more detailed discussions about these two pathways, we refer the readers to this excellent review¹⁰⁰.

Apart from the ZBP and APC pathways in targeting mRNAs to the cell protrusions, there are limited studies on the mechanism for other protrusion localizing transcripts isolated using the modified Boyden chamber. In MSV-MDCK-INV tumor cells, 261 transcripts were found enriched in the protrusions in a Rho GTPase and Rho kinase ROCK dependent manner⁹⁵. These transcripts include the β -actin and Arp2/3 mRNAs that are known to localize in the protrusions of cells cultured on 2D substrates. Among these transcripts, *M-Ras* and *Shp2* are unique to this tumor cell line in comparison to the protrusion enriched transcripts isolated from neuronal processes. Using similar method to isolate protrusion and cell body from MDA-MB-231 cancer cell line, Jakobsen et al. identified 709 protrusion-enriched transcripts using direct RNA sequencing⁹⁶. In addition to the transcripts which were previously identified by other laboratories^{94, 95}, a few new transcripts were also identified. Transcript *p0071* (*Plakophilin-1/PKP4*), which was previously characterized by Mili et al as a protrusion localizing mRNA in the APC pathway⁹⁴, was selected for further study. In comparison to MCF7, a non-metastatic cell line, *p0071* mRNA and protein are specifically localized to the protrusions of metastatic MDA-MB-231 cells. Furthermore, some alternative splicing isoform of *p0071* mRNAs showed protrusion localization whereas others are not, indicating isoform-specific localization. Using the same approach, this group also studied protrusion-enriched transcripts in astrocytes⁹⁷. ~ 250 transcripts were identified as protrusion-enriched. *Nestin* mRNAs, which encodes an intermediate filament protein, was selected for further study. Its localization is dependent on its 3'UTR and requires Fragile X mental retardation syndrome proteins Fmrp and Fxr1.

It is worth noting that in most of the protrusion samples, mRNAs encoding β -actin and the Arp2/3 complex were not enriched in the protrusions isolated using the modified Boyden chamber^{94, 96, 97}. It is unclear what the causes of the discrepancy are. One possible explanation is that this reflects different regulatory mechanisms at work as ZBP and APC are supposed to be mutually exclusive in regulation of mRNA localization¹⁰⁰. Another possible explanation is that these differences are due to different cell types or treatments of cells.

A potential role of mRNA localization-mediated local protein synthesis on focal adhesion dynamics

Focal adhesion dynamics plays a crucial role in cell migration^{124–127}. Intensive studies have revealed that the formation, maturation and disassembly of focal adhesions must be well orchestrated in time and space for proper cell migration^{128–132}. To date, the protein factors and mechanisms by which adhesion dynamics is regulated are relatively well understood. In contrary, only a limited number of reports have suggested that mRNA localization-mediated local protein synthesis may regulate adhesion dynamics. Substrate adhesion triggered rapid increase in protein translation was reported in the 70's¹³³. However, it appears that there has been no report on the localization of mRNA at the easily visualized, matured focal adhesions in normally cultured cells. Our tests did not show significant co-localization of mRNAs encoding β -actin and the Arp2/3 complex with adhesion marker paxillin or vinculin at the

focal adhesions in normally cultured fibroblasts (Mingle, Liu, unpublished). Interestingly, it was reported that mRNAs and ribosomes were rapidly and specifically localized to focal adhesions that formed when cells binds to extracellular matrix-coated microbeads in an integrin and actin cytoskeleton dependent manner¹³⁴. In another experimental setting, soon after the cells were plated, localization of numerous RNA binding proteins (RBPs), ribosomal RNAs and other RNAs was observed at the newly formed focal complexes (named as spreading initiation center, SIC)¹³⁵. In both cases, the recruitment of the cellular components is rapid and transient. These experimental conditions likely synchronize and enhance the formation of newly formed focal complexes, which makes the detection of the localization of ribosomes, RBPs and RNAs possible. Interfering with the RBPs reduces cell spreading, suggesting a role of RNA and RBP in the initiation of cell spreading¹³⁵. These data point to a possibility that local protein synthesis occurs at the focal complex to regulate the dynamics of the focal adhesions. This prediction gains supports from several recent studies. It is observed that ZBP1/IMP1 transports β -actin mRNA to the focal adhesions, where it dwells for minutes, suggesting a connection between adhesions and newly polymerized actin filaments from local synthesis. Using the newly modified MS2 system for targeting RNA, β -actin mRNA was tethered to the focal adhesions, which leads to slow adhesion turnover and cell migration¹³⁶. These results suggest that the location and dynamics of β -actin mRNA localization-mediated local protein synthesis are crucial for proper cell functions. The timing, duration and location for the local supply of the newly synthesized β -actin must be correctly controlled. It is worth noting that in addition to β -actin mRNA, ZBP1/IMP1 binds specifically to the mRNAs encoding some cytoskeletal proteins and adhesion protein such as Arp2/3 complex, α -actinin and E-cadherin¹³⁷. Arp2/3 complex has been known to be recruited to the newly formed focal adhesions and disruption of this recruitment results in diminished lamellipodial protrusion and cell spreading¹³⁸. It is tempting to speculate that, like in the case of β -actin, ZBP1/IMP1 may mediate Arp2/3 local synthesis at the focal adhesions to initiate the polymerization of locally synthesized β -actin monomers. Manipulation of ZBP1/IMP1 in several cell types has resulted in altered cell migration, adhesion and invasion^{119, 137, 139, 140}, and some of these effects could be attributed to ZBP1/IMP1-mediated mRNA localization and local translation. In addition to the mRNAs encoding these cytoplasmic proteins, mRNA encoding membrane protein integrin $\alpha 3$ is localized to adhesion complexes, which is mediated by Muscleblind-like protein MLP1¹⁴¹. Knockdown of MLP1 resulted in loss of focal adhesions and changed cell shape. Interestingly, ACACC, the MLP1 binding motif in the 3'UTR of *integrin* $\alpha 3$ mRNA is part of the zipcode of β -actin mRNA. Whether ZBP1/IMP1 and MLP1 can cross interact with *integrin* $\alpha 3$ mRNA and β -actin mRNA has yet to be tested.

A potential role of cytoplasm streaming (or force gradient) in asymmetric mRNA localization in migrating cells

As a common feature in leukocytes and amoebae, protoplasmic flow is responsible for driving cytoplasmic materials toward the pseudopods of a migrating cell^{142–144}. Although it may appear as a less selective way for intracellular transport, it could be a very efficient way to move a group of factors to a cellular compartment where they work cooperatively to define a cellular event. In other cell types, similar acts have been observed although they are

defined differently^{145–147}. In NIH3T3 fibroblasts, when high molecular weight linear polyacrylamide (PAA), which was used as a passive force sensor, was injected into the posterior region of migrating cell, it moved toward the front. This preferential forward movement could be blocked by inhibiting myosin II activity, suggesting a myosin II-dependent force gradient in migrating cells¹⁴⁵. Similarly, in an independent study, injected dextrans localize to the protrusions of CEFs in a size-dependent manner¹⁴⁷. In fish epithelial keratocytes into which inert quantum dots and dextran were introduced by electroporation and microinjection, respectively, fluid flow was shown to be directed from the cell body towards the leading edge in a myosin II activity dependent manner¹⁴⁶. Furthermore, the dots and dextran are enriched at the leading edge and the localization is positively correlated with the size of these probes. Because Rho GTPase is a well-known regulator for myosin II activation¹⁴⁸, these results point to a Rho-myosin signaling pathway in the generation of the force gradient or fluid flow similar to the cytoplasm streaming defined earlier for leukocytes and amoebae. These observations raise an interesting question whether the cells also use this mechanism to transport mRNPs for localization. Intriguingly, it was reported that MS2 labeled full length and truncate forms of β -actin mRNA, without the zipcode for leading edge localization, localized at the leading edge of CEFs in a size-dependent manner¹⁴⁷. It is unclear why the zipcode is dispensable in their experimental setting. It is possible that the incorporation of the MS2 repeats in the tested transcripts increase the size of the mRNPs, which could mask the contribution of the zipcode in mRNA localization. It is also possible that there are kinetic differences, which were not analyzed. Regardless the cause of the discrepancy, it is noteworthy that in freshly isolated (< 48 hours) embryonic fibroblast from a transgenic mouse in which all β -actin mRNA was fluorescently labeled, β -actin mRNA localized predominantly by diffusion and trapping as single mRNAs.¹⁴⁹ It is possible that in some cell types, cytoplasm streaming/force gradient influences the diffusion-trapping process. It is tempting to speculate a scenario in which cytoplasm streaming (or force gradient) facilitate zipcode-transacting factor mediated mRNA localization. In this speculative model, Rho-mediated activation of myosin IIB which is localized at the rear of migrating cells¹⁵⁰, generates a contractile force for cytoplasm streaming to the front. Diffusing mRNPs are carried forward with the flow. In the process, zipcode-dependent and motor-mediated transport on the cytoskeleton track is still at work as an mRNP transporting on the track drops off (due to limit of motor processivity), moves along with the flow and then gets on the track for another short length of directed movement. The trapping (or anchoring) of the mRNPs at the destination is likely zipcode dependent although physical/spatial confinement by the cytoskeletal structures at the leading edge is also possible¹⁴⁵. The cooperation between cytoplasm streaming and zipcode-mediated direct transport may account for the rapid translocation of *Arp2* mRNA as shown in Figure 2.

Manipulation of mRNA localization for mechanism and functional studies in cell migration

In comparison to the studies for signaling and protein localization in cell migration, there are several unique challenges for the studies of mechanism and functions of mRNA-localization mediated local protein biogenesis. Widely used approaches such as knockdown/knockout become irrelevant and mutagenesis of the mRNA is not appropriate if it leads to changes in

protein sequence. Furthermore, the highly dynamic nature of RNA secondary and tertiary structures often makes identification of a definitive zipcode and its corresponding transacting factor a difficult task as alteration of fragment of the RNA could lead to change of the overall profiles of the RNA structures and the RNA binding proteins. In addition, it is possible that a combination of multiple zipcodes and transacting factors determines the specific location and translation of a localizing mRNA. Nevertheless, through mRNA truncation studies, some zipcodes and their corresponding transacting factors have been identified^{98, 120}. Expression of exogenous mRNA with either deleted or mutated zipcode(s) in cells, where the endogenous gene is knockdown/knockout, have been used for functional studies¹²⁰. In addition, antisense oligonucleotides (**Oligos**) have been used to block the zipcode in the endogenous mRNA from binding to corresponding transacting factor^{55, 110}. However, due to the aforementioned challenge in identifying zipcode and transacting factor, only few of the asymmetrically localized mRNAs in motile cells are with defined zipcodes. To date, no consensus zipcode has been identified for a group of mRNAs that are targeted to the same location in the cell. Such deficiency in our knowledge about the mechanism of localization for these mRNAs has hindered the study of their functional significance in cell migration. Furthermore, as a transacting factor could interact with many mRNAs, the interpretation of readouts in functional tests from manipulation of a transacting factor has an issue of specificity⁸⁰. To accelerate the functional studies of mRNA localization-mediated local protein biogenesis, active and guided targeting techniques without the prior knowledge of mechanism of mRNA localization are much needed. A few years ago, taking advantage of a novel mechanism for targeting the *Dial1* mRNA, which encodes the actin polymerization nucleator, Diaph1, to the perinuclear ER⁷² (Fig 3), the normally protrusion localized *Arp2* mRNA was mis-localized to the perinuclear region. This mis-targeting was achieved by expressing *Arp2* as a bicistronic mRNA with *Dial1* mRNA⁵⁷. The advantage of this approach is that there is no mutation in the mRNA or the protein product of interest hence eliminating concerns as to whether a mutation could cause unintended changes in structures and binding specificity outside the mutation point. Using the MS2 system, which has been widely used in the RNA field for tracking RNA movement in fixed and live cells and organisms¹⁵¹, a new RNA targeting system was recently created¹³⁶. This method uses the MS2 binding protein that is fused to vinculin which localizes at the focal adhesions in combination with β -actin mRNA containing 24 copies of MS2 stem-loops in its 3'UTR. Through vinculin-MS2 binding protein, β -actin mRNA is tethered to the focal adhesions. A potential useful technique for the manipulation of intracellular mRNA localization is the magnetic nanoparticles mediated manipulation of intracellular localization of protein of interest¹⁵². In this method, magnetic force is used to move nanoparticles which are associated with the GTPases and to investigate the effects of local activity of the GTPases in live cells. It is a promising technique which allows the users to manipulate intracellular mRNA localization without the knowledge of the zipcode and transacting factors. Furthermore, it gives the user more control over localizing mRNAs in time and space in the cell.

Physiological significance of mRNA localization and local translation in cell migration and invasion

Despite many reports on which the important role of mRNA localization during development and other cellular processes has been well demonstrated, there are still skeptical views regarding the importance of local protein synthesis in the cells as proteins diffuse quickly and mRNA localization is not always correlated with protein localization. Furthermore, it is difficult to comprehend how critical local synthesis is when it supplies only a small fraction of the total protein concentration that is required a function. A case in point is the β -actin. As newly synthesized β -actin molecules can only supply about 7% of the actin for polymerization at the leading edge^{55, 111, 153}, why are there observed defects in cell with altered β -actin mRNA localization or local translation? In a previous review¹¹¹, Condeelis and Singer argued: 1) if the mRNA is targeted to a single location, and the β -actin protein is translated in this restricted volume, the local contribution of newly synthesized actin to polymerization could be substantial; 2) if the locally synthesized actin is of a single isoform that has isoform-specific interactions with actin-binding proteins, then the effects of mRNA targeting and local translation could have profound effects on the actin cytoskeleton; 3) newly synthesized actin could have a faster rate of polymerization due to its post-translational modifications; and 4) the synthesis of β -actin away from proteins that may prevent its polymerization. Indeed, some of these arguments are supported by independent results that nascent β -actin is favorably arginylated over nascent γ -actin^{154, 155}. More importantly, arginylated actin is more active for polymerization due to its reduced affinity for capping and severing proteins in comparison to non-arginylated actin¹⁵⁶. Therefore, it is likely that the newly synthesized β -actin contributes more to the polymerization than the “old” actin which may have different modifications and hence be less active for polymerization. This example points to an often neglected role of local protein biogenesis for supplying fully competent proteins to the site of function, which may not be completely compensated by an apparently sufficient supply of total protein through protein transport or diffusion. With this in mind, we will discuss the functional role of mRNA localization and local protein biogenesis in cell migration and invasion.

Because of the relatively well characterized mechanism of mRNA localization, the zipcode of β -actin mRNA has been manipulated for functional studies to determine the role of β -actin mRNA localization and local translation^{55, 56, 157}. Blocking the zipcode with antisense oligos in CEFs has led to compromised cell migration without altering the β -actin protein level⁵⁵. Further study indicates that delocalization of β -actin mRNA results in delocalization of nucleation sites for actin polymerization and β -actin protein from the leading edge, leading to loss of cell polarity and directional movement^{55, 56, 110}. Similarly, antisense oligos against the β -actin zipcode in rat smooth muscle cells blocked peripheral localization and inhibited cell migration¹⁵⁷. Furthermore β -actin mRNA is targeted to the focal adhesion in a very dynamic manner as the mRNP dwells there for minutes¹³⁶. To test if this dynamic localization links to motility through the formation of stable connections between adhesions and newly synthesized actin filaments, β -actin mRNA was tethered to the focal adhesions with the modified MS2 system. This led to reduced turnover of the focal adhesions and migration. These results demonstrated an important role of β -actin local synthesis in cell

migration. Different from the β -actin mRNA, the functional importance of the *Arp2/3* mRNA localization was tested through a novel approach. Using the perinuclear ER localizing *Dial1* mRNA as a carrier, *Arp2* mRNA, one of the seven mRNAs encoding the subunits of the *Arp2/3* complex, was mis-localized to the perinuclear region in fibroblasts by expressing it as a bicistronic mRNA with the *Dial1* mRNA in cell with depleted endogenous *Arp2*⁵⁷. The cells exhibited poor directionality of movement with slow speed even though the overall level of *Arp2* protein is similar to the wild type control⁵⁷. These defects are likely the consequence of the low efficiency of *Arp2/3* complex assembly caused by synthesis of a member of the complex at a distant location. These results prove the concept that mRNA localization mediated local protein biogenesis has a unique functional role which cannot be compensated by protein transport. As for the mRNAs regulated by the APC pathway, the functional significance of their mRNA localization has yet to be directly tested. Nonetheless, earlier studies showed that depletion of APC resulted in defects of cell polarization and migration^{121, 158, 159}. These results suggest that at least some of these defects may be related to the ability of APC binding to the mRNAs and *Fus/TLS* for local translation at the cell protrusions.

Because cell migration and invasion are crucial processes in cancer metastasis¹⁶⁰, it is of great interest to understand the functional significance of local protein biogenesis on these processes. However, probably due to the limited understanding of the mechanisms and lack of tools for specific manipulation of local protein synthesis, there has been limited definitive information for the role of local protein synthesis in tumor cell migration and invasion. We could not find any report in which antisense oligos were used to specifically block a defined zipcode of a localizing mRNA for impacts on cancer cell migration or invasion. There are a substantial number of reports in which the ZBP/IMP family proteins were manipulated for cancer cell proliferation, survival, migration or invasion^{107, 119, 137, 140, 161–174}. The approach to manipulate ZBP/IMP protein is likely less specific than the antisense oligos because the ZBP/IMP family proteins can interact with many other signaling proteins and RNAs^{107, 169, 175, 176}. On the other hand, some of the defects in cancer cell migration and invasion after manipulation of the ZBP/IMP family proteins could be attributed to alterations of mRNA localization and local translation. With this in mind, we will briefly describe several cases in which tumor cell migration and invasion was altered after manipulation of ZBP1/IMP1, the relatively well characterized member of the family for its role in β -actin mRNA localization and translation. Expression of ZBP1/IMP1 is correlated with poor prognosis in ovarian cancer¹⁷⁷. This is at least in part because ZBP1/IMP1 which, in complex with at least four protein, stabilizes c-MYC mRNA to enhance tumor cell proliferation¹⁶⁶. In another case, ZBP1/IMP1 was shown to promote in tumor-derived cells the formation of invadopodia by preventing degradation of the CD44 mRNA, supporting its role as a pro-metastatic factor¹⁴⁰. Using shRNA to knockdown all three isoforms of the ZBP/IMP protein or expressing a dominant-negative mutant of ZBP1/IMP1 in human carcinoma cells resulted in dramatic drop in lamellipodia and ruffle formation and inhibition of cell movement, respectively, as reported by the Yisraeli laboratory^{162, 165}. Several recent reports support the role of ZBP1/IMP1 in promoting tumor cell migration and/or invasion. In one report, ZBP1/IMP1 promotes choriocarcinoma cell migration and invasion through two effectors, RSK2 and PPME1¹⁷². In another report, ZBP1/IMP1 promotes tumor cell

migration by enhancing the expression of LEF1 and SNAIL (SLUG)¹⁷¹. In a report which may be more relevant to explain the role of ZBP1/IMP1 in regulation of actin dynamics, ZBP1/IMP1 was shown to promote tumor cell migration. This is achieved by inhibiting MAPK4 mRNA translation to antagonize MK5 activation and prevent phosphorylation of HSP27, which sequesters actin monomers. At the same time, ZBP1/IMP1 also stabilizes PTEN mRNA to enhance PTEN protein level, leading to enhanced directional cell migration¹⁷⁰. In contrast to the above reports in which ZBP1/IMP1 promotes tumor cell migration and/or invasion, ZBP1/IMP1 was also shown to inhibit cancer cell migration in culture and in vivo. In an isolated invasive sub-population of cancer cells, ZBP1/IMP1 expressed level was suppressed¹⁷⁸. Further study indicates that repression of ZBP1/IMP1 expression led to increased proliferation and migration of metastatic breast-cancer cells¹³⁷. Detailed analysis of cell migration behavior in culture and in tumor of live animals revealed that ZBP1/IMP1 enhances cell polarity and reduces chemotaxis¹⁶³. This finding raises an interesting question as how to connect the link between polarized cell migration and chemotactic capability.

Prospective

Significant progress has been made recently in the study of regulation of mRNA localization and local translation in motile cells. However, it remains a relatively unexplored territory and many questions remain unanswered. Precise mapping of the zipcodes and identifying their specific transacting factors remain a big challenge because of the complexity of the system. A multiple disciplinary approach is much needed to tackle these complexities through a combination of expertise in cell and molecular biology, structural biology and bioinformatics. Recent advances in imaging technology and the manipulation of intracellular localization of RNA are expected to accelerate the studies for the functional importance of mRNA localization and local translation in cell migration. Although it is not covered in this article, non-coding RNAs are known to regulate mRNA translation and may turn out to be important regulators for mRNA localization in the cell.

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Figure 1. Arp3 protein and mRNA are localized at the cell leading protrusions in wound tissue
 Day 4 rat skin wound tissue cryo-sections were processed for protein using immunofluorescence staining (A) or for mRNA using fluorescence in situ hybridization with tyramide signal amplification (B). To present a whole 10 μm cryo-section, 10 digital images of optical Z-sections (1 μm each) were deconvolved to remove out-focus fluorescence and then compressed to generate a 2-D projection image. Note in A that the stationary keratinocytes over the wound bed (above the dotted line) exhibit a relatively uniform distribution of Arp3 protein. The Arp2/3 is a very stable protein complex, and detection of

one member is indicative of the location of this protein complex¹⁷⁹. Arrows point to the localized protein (red in A) and arrowheads point to localized mRNA (green in B). Unpublished data of Mingle, Van De Water and Liu.

Figure 2. Visualization of *Arp2* mRNA movement in live fibroblasts

A. Illustration of the MS2 system for visualization of RNA in live cells (model adapted from Bertrand et al.¹⁵¹). The phage MS2 coat protein (MCP) is fused with GFP, and a nuclear localization signal (NLS) tag that sequesters the MCP-GFP in the nucleus if it is not bound to the mRNA. The MS2 stem-loop repeat is inserted into the 3'-UTR of *Arp2* mRNA. The MCP-GFP protein binds to the MS2 RNA repeat with high affinity so the location of the mRNA can be detected by the GFP fluorescence. **B.** Still images from a one-hour time lapse show the dynamic movement of the *Arp2* mRNA in a fibroblast cell. Arrows point to the protrusions and arrowheads point to the nucleus in which the unbound MCP-GFP is sequestered. Unpublished data of Mingle and Liu.

Figure 3. Differential intracellular distribution of the *Arp2/3* mRNA and *Dia1* mRNA in fibroblasts

Representative intracellular distribution of *Arp2* mRNA (green) and *Dia1* mRNA (red) in the CEFs. Dash lines indicate cell border and blue for nucleus. *Arp2* mRNA (green) is concentrated at the leading lamella while the *Dia1* mRNA (red) is perinuclear. Similar differential distribution of the *Arp2/3* complex mRNAs and *Dia1* mRNA were also observed in human foreskin fibroblasts (not shown). Adapted from⁷².