mRNA and cytoskeletal filaments

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The localization of some mRNAs to distinct intracellular regions is achieved through interactions of the mRNA with cytoskeletal filaments. RNA–cytoskeletal interactions exist that influence the transport, anchoring and translation of mRNA. Recent analysis of RNA movements in living cells suggests the formation of RNA granules and their active transport along microtubules. The anchoring and translation of mRNA may be mediated by interactions with orthogonal networks of F-actin and elongation factor 1α.

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Current Opinion in Cell Biology 1997, **9**:109–115

Electronic identifier: 0955-0674-009-00109

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Abbreviations
EF1α elongation factor 1α
MBP myelin basic protein
RNP ribonucleoprotein
UTR untranslated terminal repeat

Introduction

Since this subject was last reviewed in *Current Opinion in Cell Biology* [1], some significant progress has been made in clarifying the role of cytoplasmic filaments in mRNA regulation, that is, in mRNA localization and translational control. The function of RNA localization within cells is to influence sites of protein synthesis and regulate localized translation. RNA-localization mechanisms are used by cytosolic, membrane-associated and cytoskeletal proteins [2].

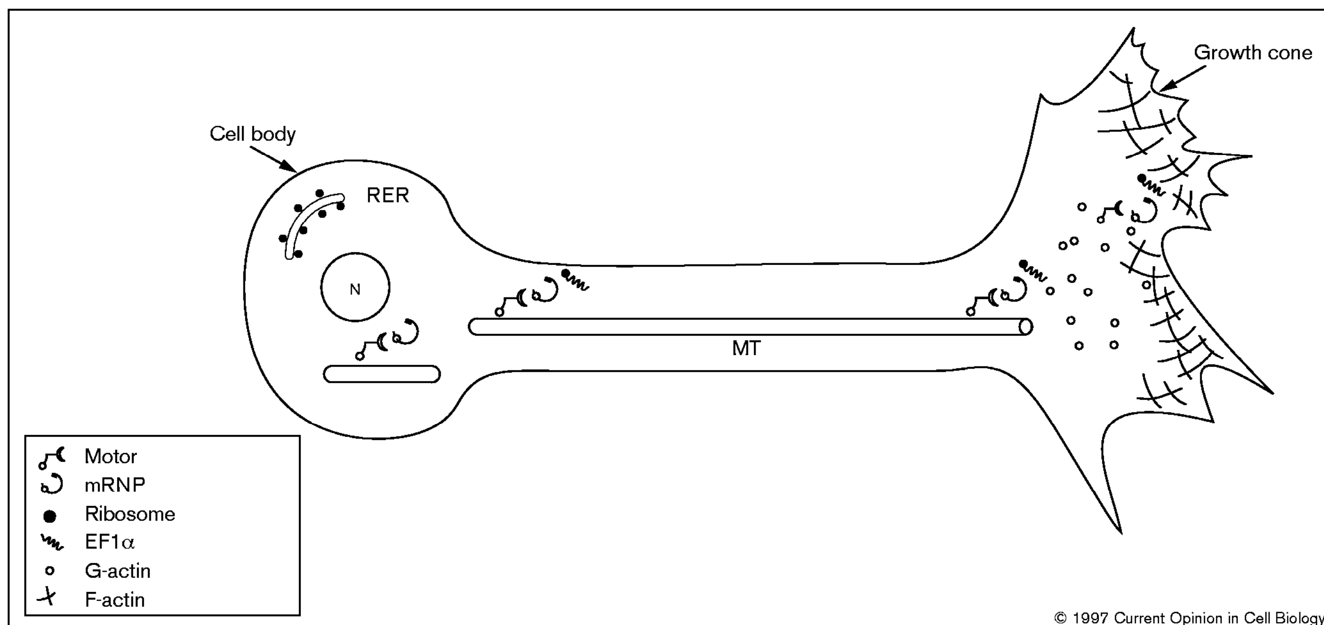
It is now accepted that mRNA localization in regions of the ooplasm of germ cells, or in the cytoplasm of somatic cells, requires the cytoskeleton to transport the mRNA and also to anchor it at its destination. The three major cytoskeletal filament systems, microtubules, microfilaments and intermediate filaments, have been implicated in mRNA localization in a variety of cell types and organisms [3,4]. Generally, microtubules appear to be involved in long-distance travel, in oocytes and in neurons (see Fig. 1). In those cells, microtubule-disrupting drugs, such as colcemid, arrest the localization process, and cytochalasin, an actin-disrupting drug, has no effect while the mRNA is localizing. In fibroblasts, actin filaments play the major role and the opposite effect is seen with cytoskeleton disruption: colcemid has no effect and cytochalasin disrupts localization [5]. It is possible that mRNA associates with various filament systems through a common mechanism, and that these filaments, whether they be microtubules, actin, or even intermediate filaments, could provide a solid structure for RNA binding in order to sequester it, preventing random movement of RNA within the cytoplasm and bringing it into contact with regulatory factors. Most of the current work in this field continues to be phenomenological, but efforts to characterize the nature of the RNA–cytoskeletal interactions continue and proteins are currently being identified that may bridge the gap from the RNA to the filament. In this review, we will focus primarily on work that elucidates RNA–filament interactions, and on the functional significance of these interactions for RNA regulation.

Localized synthesis of cytoskeletal proteins

The localization of mRNAs encoding cytoskeletal proteins may facilitate the sorting of cytoskeletal proteins and their assembly into macromolecular complexes. Through localized synthesis, cytoskeletal proteins may be made near their sites of function and this allows the spatial coupling of gene expression and cellular structure. Vimentin and desmin mRNAs are highly localized to costameres and localized synthesis may be involved in the assembly of myofibrillar structure [6]. RNA localization may also be involved in the assembly of the motility apparatus at the leading edges of myoblasts and fibroblasts. The best studied example is the localization of β-actin mRNA to the cell periphery of motile cells [7]. The sorting of β-actin mRNA to the cell periphery is isoform-specific [8,9] and regulated by signal transduction mechanisms that influence the cytoskeletal organization of the leading edge [10,11]. A polarized distribution of β-actin mRNA also exists in epithelia [12] and early embryos [13].

Several mRNAs encoding cytoskeletal proteins have been localized to neuronal processes. Transcripts encoding high molecular weight microtubule-associated protein 2 (MAP2) have been localized to dendrites and the mechanism of mRNA targeting appears to be contained within the protein coding sequence [14•,15]. Actin, tubulin and neurofilament mRNAs are all found within squid or Mauthner axons and the targeting of mRNAs may provide an additional mechanism for the transport of cytoskeletal proteins over considerable distances [16]. During neuronal development, tropomyosin mRNA is targeted to the axonal process and this localization correlates with the appearance of the encoded protein [17]. Tau mRNAs are also localized to growing axons; their specific targeting to the proximal segment is dependent on interactions with microtubules [18]. Both tau mRNAs and MAP2 mRNAs are contained within the detergent-insoluble cytoskeletal fraction, whereas mRNAs localized to the cell body are extracted by this treatment [18].

Figure 1



In neurons, the transport of RNAs to distal locations, for example the growth cone, occurs mainly via microtubules (MT) and may involve a motor protein. Once at the end of the neurite, the RNA may be translocated to actin filaments prior to translation. Translation factors may move with the RNA in a granule. N, nucleus; RER, rough endoplasmic reticulum.

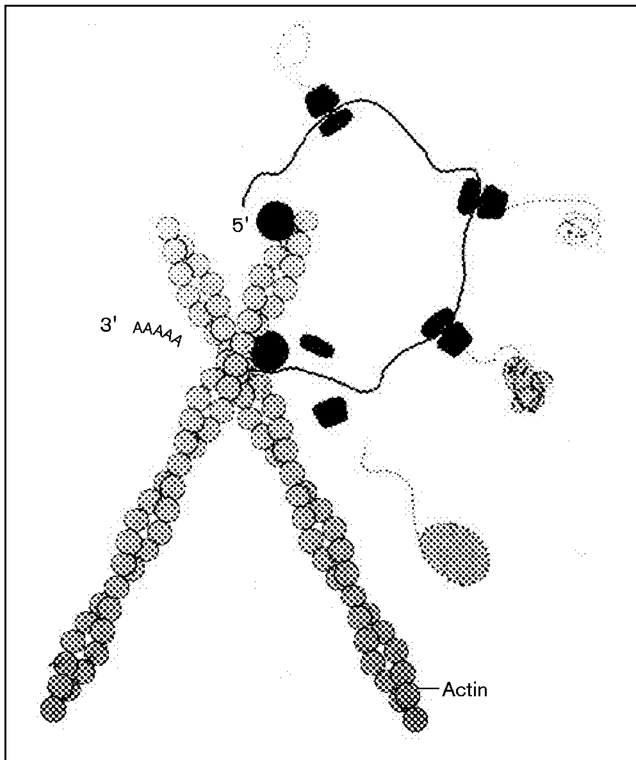
RNA localization along filaments

RNA localization is likely to be a multistep process having the following distinct transport and anchoring components: first, the transport of newly synthesized mRNA along filaments, perhaps by a motor molecule, to its region of localization; and second, the subsequent anchoring of that mRNA at sites for translation [19]. An alternative view would be that mRNAs first diffuse throughout the cytoplasm and are subsequently trapped by an interaction with a localized receptor or anchoring protein. The requirement for active transport seems, however, to be obligated by diffusional constraints that would restrict the movement of large RNA particles within cells [20,21]. Moreover, the active transport of mRNA has been directly visualized [22,23••].

Regardless of which mRNA-translocation mechanism is operative in a particular system, the anchoring of mRNA to the cytoskeleton appears to be a necessary step in its functional expression. Using electron microscopic *in situ* hybridization, the majority of poly(A) mRNA in fibroblasts is localized to actin-filament intersections which also contain ribosomes and elongation factor 1α (EF1α) [4]. The vertex may represent a site for sequestration of mRNAs and translational machinery (Fig. 2). The presence of specific docking sites for mRNA within the cytoskeleton is further suggested by the observations that EF1α is an actin-binding protein whose involvement in translation is regulated by its ability to dissociate from actin following changes in pH, releasing EF1α for interaction with the translational apparatus [24••].

Multiple filament systems may participate in an mRNA-localization pathway. The initial targeting of Vg1 RNA to the vegetal pole of *Xenopus* oocytes appears to be dependent upon microtubules, but Vg1 RNA is later anchored to cortical actin filaments [19]. Two distinct pathways for RNA localization have been identified, both of which involve anchoring to actin filaments within the vegetal cortex, but only one of which is dependent on microtubules [25•]. In *Drosophila* oocytes, the localization of Staufen–bcd 3′ untranslated terminal repeat (UTR) complexes is dependent on microtubules [26], consistent with work on a general role of microtubules in the bicoid mRNA localization pathway [27]. Oskar RNA localization to the posterior pole of *Drosophila* oocytes requires microtubules [28–30]. The possible dual involvement of microfilaments and microtubules in oskar RNA localization was suggested by the requirement of the actin-binding protein tropomyosin [31•,32•]. In *Drosophila* mutants that lack the cytoplasmic form of tropomyosin II (cTmII), oskar RNA fails to accumulate at the posterior pole of the embryo [31•,32•]. Tropomyosin may facilitate mRNA anchoring within the posterior cortex [2]. The localization of oskar RNA at the posterior pole is required for its translation [33–35]. It is currently unknown whether actin filaments participate in the mechanism of localization-dependent translation. Components of the dynactin complex are localized to the posterior pole, a fact that is also suggestive of a link between microtubules and microfilaments in this region [36]. Further work may reveal whether RNAs ‘switch tracks’ as do membrane-bound vesicles [37]. Microtubules may serve as the tracks for

Figure 2



Sequestration of mRNA to actin-filament vertices, where the transported RNA comes into contact with translation factors. EF1α is one of the translation factors that is known to be attached to the actin filaments and that interacts with the RNA, possibly upon RNA–cytoskeleton interaction. EF1α (and possibly other translation factors) is represented by the black circles at the vertex and near the translation-intiation site at the 5′ end of the mRNA. The mRNA is proposed to circularize during translation, according to electron microscopic studies [4]. Polypeptide chains are represented by dotted lines; the grey areas represent folded protein; and the black irregular shapes represent ribosomes.

long-distance translocation, and actin filaments could be involved in local mRNA movements, mRNA anchoring and translation.

Microfilaments solely are involved in the polarized distribution of β-actin mRNA, at least in fibroblasts [5] and *Fucus* embryos [13]. β-actin mRNA is also localized within neuronal processes and growth cones, although microtubules are involved in this sorting ([23••]; G Bassell *et al.*, unpublished data). It is possible that β-actin mRNA can interact with both microfilaments and microtubules and that the preferential usage of a particular filament system is characteristic of the specific cell type. The tendency for actin mRNA to associate with microtubules in neurons may represent the dominance of long-distance translocation (Fig. 1). This mechanism could involve *cis*-acting elements within the actin mRNA 3′UTR that interact with a set of cell type specific RNA-binding proteins and/or motor proteins that promote preferential usage of one filament system. Previous analysis of the association of poly(A) mRNA with the cytoskeleton indicated a predominant role for microfilaments in fibroblasts, but for microtubules in neurons [3,4,23••,38]. In sea urchin embryos specific mRNAs and polyribosomes copurify with microtubules, and individual ribosomes are observed to be bound to microtubules via stalk-like projections [39], suggesting that translation may occur on microtubules. These observations from a number of different systems suggest the presence of cell-type- and cell-compartment-specific differences in mRNA localization and anchoring mechanisms.

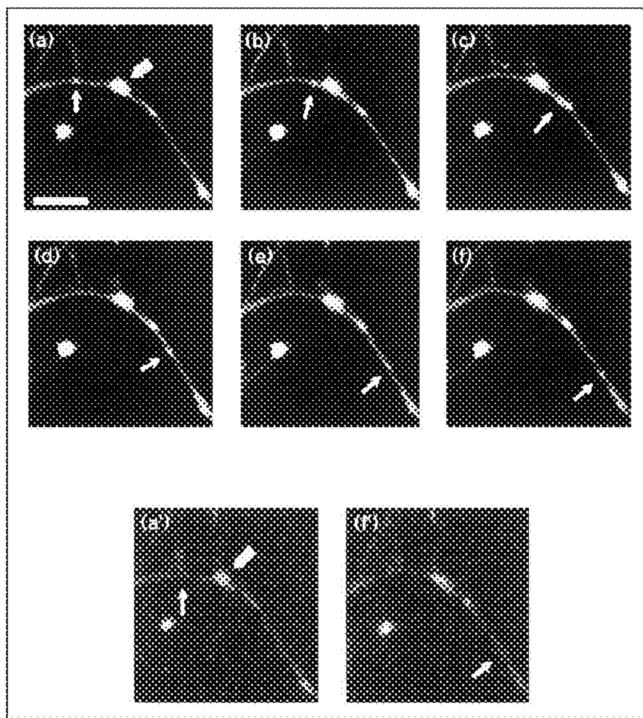
RNA granules and active transport

RNAs may be packaged into transport particles or ‘granules’ which contain multiple mRNA molecules and translational machinery. These RNA particles may then be translocated along cytoskeletal filaments via interactions of *cis*-acting elements, motor molecules and/or accessory proteins. *In situ* hybridization studies have revealed particulate RNA localization patterns for a variety of mRNAs; these patterns include ‘punctate’ actin mRNA [40], ‘island-like structures’ of Xlsirt, XwntII and Xcat-2 RNA [25•,41•], formation of bicoid RNA ‘particles’ [26], and ‘granules’ of myelin basic protein (MBP) mRNA [22]. The intensity of fluorescence within granules formed by microinjection of MBP RNA labelled with a single fluorochrome suggested that the granules contained multiple mRNA molecules [22]. MBP mRNA granules colocalized with arginyl-tRNA synthetase, EF1α and rRNA, suggesting the presence of a translational unit [42••]. Using dual-channel cross-correlation analysis of confocal images, MBP RNA granules were estimated to have a radius of between 0.6 and 0.8 μm, suggesting that RNA granules represent a supramolecular complex that could contain several hundred ribosomes [42••].

Analysis of mRNA granule transport into neuronal processes using the vital dye SYTO 14 indicates that mRNA forms granules which contain translational components and translocate along microtubules at a rate of 0.1 $\mu m\ sec^{-1}$ [23••] (see also Fig. 3). This rate was similar to the rate of the directed translocation of MBP RNA along microtubules in oligodendrocyte processes [22]. These observations of RNA particle movement in living cells further support the hypothesis that the process is driven by a motor. A kinesin could be a candidate for the transport of mRNAs into developing neurites [23••], as the microtubules are of uniform polarity with plus ends oriented distal to the cell body [43].

A kinesin or member of the kinesin superfamily may also be involved in RNA localization to the posterior pole of *Drosophila* embryos. A kinesin fusion protein transiently localizes to the posterior pole during mid-oogenesis at the time that oskar RNA and Staufen protein localize there. Colchicine treatment disrupts the localization of both the kinesin protein and the oskar RNA to the posterior pole [44]. Mutations that affect oskar RNA localization simi-

Figure 3



Time-lapse analysis of RNA granule movement in minor neurites of living neurons. **(a–f)** RNA granule locations (shown by small arrows) at one-minute intervals. Mitochrondria were stationary during this period (see a′, f′, and large arrow), as determined by a mitochondrial-specific dye. The vital dye SYTO 14 (Molecular Probes Inc) labeled RNA granules, which translocated at an average rate of 0.1 μm sec^{-1}. Movement was blocked by colchicine. Reproduced with permission from [23••].

larly compromise the kinesin fusion protein localization, suggesting the involvement of a member of the kinesin family in oskar mRNA localization [28]. Protein kinase A mutations result in a reorganization of microtubules, such that the plus ends are now found in the center of the oocyte [45]. In this mutant, oskar RNA and kinesin become localized to the center, whereas bicoid RNA is now found at both poles [45].

The synthesis of dynein within nurse cells and its subsequent accumulation within the oocyte suggest that RNP (ribonucleoprotein) particles that move from the nurse cell into the oocyte could be directed towards the minus ends of microtubules [46]. EXU protein, a factor involved in bicoid RNA localization, was observed to form particles that localized at ring canals which bridge the nurse cell and oocyte [47]. The localization of these particles was disrupted by colchicine, suggesting the movement of RNP particles along microtubules into the oocyte [47].

The above studies indicate an association of RNAs with microtubules having a distinct polarity and hence imply the involvement of specific types of motor molecules; however, the direct involvement of a motor in RNA transport has not been proven. Although this mechanism is likely to be used for RNA granule movement in oligodendrocytes and neurons, oocytes also use cytoplasmic flow or facilitated diffusion of RNA (reviewed in [2]). In this model, the mRNA may not be directed along filaments by active transport, but is instead displaced within the oocyte by a dramatic reorganization of cytoskeletal filaments. Microtubule-dependent cytoplasmic streaming, which precedes the localization of some RNAs during late oogenesis, may bring RNAs into contact with anchoring filaments in the cortex because the cytoplasm circulates as a result of cytoplasmic streaming. The RNA in this case would be carried passively [29].

RNA-binding proteins associated with the cytoskeleton

The best characterized RNA-binding protein associated with cytoskeletal binding as well as function is the maternal protein Staufen [48]. Staufen has been implicated in the localization of maternal mRNAs, including both anterior mRNAs such as bicoid and posterior mRNAs such as oskar. Deletions in this gene result in partial mislocalization of the mRNAs. Therefore, Staufen has the characteristics of an anchoring protein. Staufen also binds to astral microtubules, as evidenced by immunofluorescence studies [48]. Genetic studies also implicate its binding, in addition to that of oskar mRNA, to the actin cytoskeleton [49]. Microinjection of the bicoid 3′UTR sequences resulted in the formation of granules of Staufen being coalesced onto the RNA [26]. The protein has some of the characteristics of an RNA helicase [50]. Although it is not clear what role Staufen plays in its interaction with microtubules or actin and with RNA, it remains the sole protein characterized to date that links the cytoskeleton to mRNA localization in *Drosophila*.

Several other putative localization proteins are in various states of characterization. A 70 kDa protein binds specifically to the β-actin zipcode sequences, but not to nonlocalizing β-actin variants. The sequence of this protein has similarities to a heterogeneous (hn) RNP. Evidence suggests that it interacts with actin-binding proteins, such as tropomyosin (A Ross, Y Oleynikov, E Kislauskis, K Taneja, R Singer, unpublished data). A protein of 36 kDa binds to the transport sequence of MBP and peptide fragments from this protein correspond to hnRNPA2 (K Hoek, R Smith, personal communication). A *Drosophila* protein, EXL, appears to bind bicoid mRNA directly [51]. An additional protein, EXU, which is important in the transport of bicoid mRNA out of nurse cells, binds this mRNA. Its binding is nonspecific, however, because the RNA-binding sites are not required for bicoid localization [47].

The tau mRNA localization sequence is contained within its 3′UTR; this sequence demonstrates an affinity for proteins enriched by their association with microtubules

[52]. The mechanisms of tau mRNA localization may share certain features with the localization of Vg1 RNA in *Xenopus* oocytes [53•]. Tau RNA localization sequences injected into oocytes are localized to the vegetal cortex, as is endogenous Vg1 RNA, whereas tau RNA sequences from coding regions were not localized [53•]. Vg1 RNA transport to the vegetal cortex involves microtubules and possibly a 69 kDa microtubule-associated protein [54••]. Tau RNA sequences contain a binding site for Vg1 RNA binding protein and suggest conserved mechanisms of RNA localization between oocytes and neurons [53•].

Proteins are being characterized that bind to the cytoskeleton and are involved in translational control. An RNA-binding protein localized to cytoplasmic microtubules appears to be involved in spermatid RNA transport and/or activation or repression of translation [55]. A testis/brain RNA-binding protein attaches repressed and transported RNAs to microtubules, and has sequence elements conserved among other localized mRNAs [56]. In one case of a localized RNA, the protein product interacts with another localized mRNA to repress the translation of the second mRNA in a concentration-dependent manner [57].

Conclusions

The association of RNA with the cytoskeleton was observed over 20 years ago; triton extraction of the cell did not release cellular mRNAs [58]. The functional significance of this interaction is just beginning to be appreciated, however. Regulation of mRNA in the cytoplasm is an important component of gene expression, and cellular mechanisms are being elucidated that control mRNA translation, stability and localization. The cytoskeleton is one of the fundamental components of this mechanism. First, it provides a solid state interface for the interaction and concentration of cellular components, such as translation factors, with RNA, presumably circumventing diffusion-mediated reactions. Second, it allows a way to spatially position RNAs stably within the cytoplasm so that their cognate proteins are localized appropriately. Furthermore, these proteins can interact with other identically localized proteins to form supramolecular complexes. Conversely, proteins that should not interact can be sequestered away from each other. Third, the interaction of RNA with an addressable system in the cytoplasm allows a means for feedback regulation of RNA; for instance, when subcellular sites are saturated, there may be a mechanism for degradation and/or translational repression of RNAs not attached to the cytoskeleton. Fourth, a mechanism must exist which prevents mRNAs from being translated while they are being transported; the 'granules' may serve this purpose. Anchoring points on the cytoskeleton may sequester some translation factors away from the mRNAs until they are spatially in place. Hence, it can be predicted that translation, stability, association with the cytoskeleton and localization are all synergistic events mediated primarily through the mRNA's 3′UTR.

Acknowledgements

We thank Ken Kosik for providing data included in this review. We thank Ken Kosik and John Condeelis for helpful discussions. We also thank the members of RH Singer's laboratory for their work and critical input. RH Singer is supported by NIH (National Institutes of Health) grants GM54887 and AR41480, and G Bassell is supported by a Basal O'Connor award, the March of Dimes.

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