The travels of mRNAs through all cells large and small

G. J. BASSELL,*[,1] Y. OLEYNIKOV,[†] AND R. H. SINGER[†]

*Departments of Neuroscience and of [†]Anatomy and Structural Biology, Albert Einstein College of Medicine, New York 10461, USA

The determination of asymmetry is one of the most important events in cell differentiation and development. Cellular asymmetry in many cases is essential for normal cellular function, as in the polarity of neurons that transmit electrical and chemical signals or the polarity of morphogenetic gradients in oocytes required for patterning in the embryo. The asymmetric localization of specific proteins is a distinct feature of morphological asymmetry. An important mechanism to achieve nonuniform protein distributions is to synthesize specific proteins at or near their sites of function. Observations that RNAs can be localized to distinct regions within cells provided a direct link between gene expression and cellular asymmetry (reviewed in ref 1). The mechanisms involved in mRNA localization have been studied over the last decade and have demonstrated that many localized mRNAs contain noncoding '*cis*-acting elements' or 'zip codes' in the 3′ UTR that confer localization of the mRNA (2). Many of these noncoding sequences have been shown to be necessary and sufficient for mRNA localization, appear to be unique in each mRNA, and are often present as multipartite elements that vary in length. Proteins that bind the *cis*-acting elements have been identified which may be involved in distinct aspects of the mRNA localization pathway (2–4). It has been proposed that these proteins act as *trans*-acting factors and are involved in mRNA transport, anchoring, localized translation, and stability. This review will highlight recent advances in understanding these mechanisms, with an emphasis on somatic cells and conserved features with oocytes.

ASYMMETRIC CELL DIVISION

One newly discovered role for mRNA localization is the specification of cell fate through asymmetric cell division. The asymmetric localization of Prospero is required for differentiation of the early neuroblast lineage in the development of the nervous system in *Drosophila* (5). This sorting is dependent on both mRNA and protein localization mechanisms (6). Prospero is a homeodomain transcription factor and its mRNA is sorted differentially into the nuclei of the ganglion mother cells (GMC).[2] Staufen has recently been shown to play a role in the asymmetric distribution of *prospero* mRNA, identifying a new role for this protein in specifying lineage in somatic cells (7). Staufen interacts with the 3′ UTR of *prospero* mRNA and is necessary for its transport during mitosis. Staufen-dependent localization of *prospero* mRNA requires intact actin filaments, in contrast to the requirement for microtubules in the localization of *bcd* RNA in *Drosophila* oocytes, which also involves Staufen (8). Miranda is another protein involved in the asymmetric localization of Prospero, and appears to be a complex with Staufen and Prospero proteins that forms on the apical side of the sensory organ precursor cell and is translocated into the GMC during mitosis (6). The redundant localization machinery in polarized cells, where both mRNA and protein localization are active, ensures asymmetric distribution of Prospero protein. This feature will be noted again in mammalian cells in a later section on protein sorting to neuronal growth cones.

A similar system of cell fate determination through asymmetric cell division has been observed in budding yeast, where Ash1 protein, the determinant for mating type, is localized to the daughter cell (9, 10). Mating type switching is prevented by the sorting of Ash1p, which represses transcription of the HO endonuclease in the daughter cell. Localization of Ash1p to the daughter cell is achieved by localization of its mRNA from the mother cell nucleus to the bud tip (11, 12). This mechanism is distinct from the asymmetric localization of Prospero protein, which also occurs by a protein localization mechanism (7). The mechanism by which *ASH1* mRNA moves to the bud tip involves sequences in the 3′ UTR of *ASH1* mRNA and five *SHE* genes that are known to be required for the asymmetric repression of mating type switching. She1/Myo4, a type V myosin, and an intact microfilament were required for *ASH1* mRNA localization (11, 12).

[1] Correspondence: Department of Neuroscience, Albert Einstein College of Medicine, 1300 Morris Park, Bronx, New York 10461, USA. E-mail:bassell@aecom.yu.edu

[2] Abbreviations: CaMKIIα, calcium/calmodulin-dependent protein kinase II; EF1α, elongation factor 1α; GFP, green fluorescent protein; GMC, ganglion mother cell; MAP, microtubule-associated protein; MBP, myelin basic protein; NT, neurotrophin; RTS, RNA transport sequence; ZBP-1, zip code binding protein 1.

0892-6638/99/0013-0447/$02.25 © FASEB

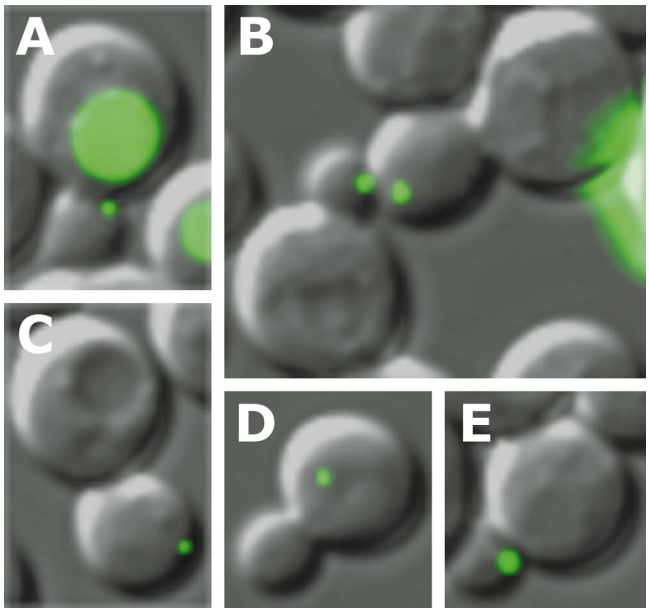

Figure 1. Localization of *ASH1* 3′ UTR/MS2 fusion mRNAs. Examples of the reporter mRNA localizing to the bud tip of living yeast. The mRNA was detected by a GFP-MS2 chimera that is bound to MS2 sites on LacZ mRNA fused to the *ASH1* 3′ UTR. A 'particle' is observed in the mother (*D*) or daughter (*A–C, E*) localizing to the bud tip. Copyright permission from Cell Press (4).

Myosin could be involved either directly in RNA localization on microfilaments or indirectly by transporting other proteins that would anchor the *ASH1* RNA to the bud cortex (11, 12). The ability to distinguish between these mechanisms required an approach to visualize RNA movements in living cells. Fluorescent in situ hybridization was not adequate to address mechanisms of RNA movement because it lacked the temporal resolution to obtain precise kinetic measurements. Real time RNA movement in living yeast cells was monitored by coexpressing a GFP-MS2 fusion protein and an *ASH1* RNA reporter fused to MS2 binding sequences (13). Since the *ASH1* RNA sequences were capable of interacting with the localization machinery, it was possible to visualize these events by the binding of the GFP-MS2 protein, which merely provided a fluorescent tag. The directionality and speed of particle localization suggested that the reporter mRNA was transported by a myosin motor and that localization from the mother to the bud tip occurred within a few minutes (**Fig. 1**). One conclusion from this analysis was that the transport events occurred via a macromolecular complex, a particle whose existence and localization was dependent on specific nucleotides in a stem-loop region of the *ASH1* 3′ UTR. Localization of the *ASH1* RNA particle was consistent with a mechanism of active transport by motor.

Several proteins were identified that may be involved in the assembly and localization of the *ASH1* RNA particle. She3p may be involved in the association of the particle with a myosin motor. This was suggested by the observation that She3p and She1p/Myo4p colocalized with the *ASH1* RNA particle. She3p and She1p also colocalized during anaphase to the bud tip (9), but this association was only transient and may involve delivery of the mRNA to the bud tip. She5p was localized to the bud tip and may have a role in polarizing the actin cytoskeleton (14). She2p and She4p were suggested to be required for the assembly of *ASH1* RNA particles. She1, a specific myosin motor (15), was believed to transport the *ASH1* mRNA reporter, as the rate and direction was consistent with an actin-based motor (16). The reporter was not localized into the bud in mutations in She1/Myo4, suggesting that SHE1p/Myo4p was directly involved in the active transport of the *ASH1* reporter. Another interpretation is that She1 was indirectly involved in *ASH1* RNA localization through transport of other components to the bud tip, which could anchor the mRNA. However, it would be difficult to envision a mechanism that would be selective for the *ASH1* RNA particle movement without direct interaction. Analysis of particle movements suggests that the polarity of the actin filaments on which She1 transported *ASH1* RNA were with the barbed end pointed toward the bud tip. The specific interaction of the *ASH1* RNA localization sequences with proteins required for particle assembly and transport suggests a structural entity specialized for this function, termed the locusome. It is analogous to the 'ribosome' and 'spliceosome', which also contain RNA within a supramolecular RNA–protein that is specialized for a particular function.

RNA PARTICLES IN OTHER SYSTEMS

The particle described here in yeast may have some similarity to the particles observed in other systems. In the localization of *bcd* mRNA in *Drosophila* (8), the 3′ UTR of the mRNA induces the formation of a particle containing Staufen protein by forming a multimolecular complex through RNA–RNA interactions. Staufen protein is a dsRNA binding protein required for the anchoring of the RNA late in localization at the anterior pole of the oocyte, presumably on the spindle microtubule (8). The early phase of *bcd* mRNA localization from the nurse cell into the oocyte may also involve particles (17). In the first application of green fluorescent protein (GFP) technology to study mRNA localization, Hazelrigg (17) has shown that GFP-Exu (exuperantia) forms particles that are moving through the ring canals. More recently, Hazelrigg and Therkauf (18) have identified three types of movements, two of which are microtubule dependent. The first example of

RNA particle movements in somatic cells was from the Carson lab (19) showing that fluorescently labeled MBP (myelin basic protein) mRNA microinjected into oligodendrocytes formed granules that were translocated into processes at approximately 0.2 μm/s. The intensity of fluorescence within granules formed by microinjection of MBP RNA labeled with a single fluorochrome suggested that the granules contained multiple mRNA molecules (19). MBP mRNA granules colocalized with arginyl-tRNA synthetase, elongation factor 1α(EF1α), and rRNA, suggesting the presence of a translational unit (20). MBP RNA granules were estimated to have a radius between 0.6 and 0.8 μm, suggesting that RNA granules represent a supramolecular complex that could contain several hundred ribosomes (20). These granules colocalized with microtubules (19), and microtubule-disrupting drugs inhibited MBP RNA localization (21). Kinesin or kinesin-like proteins may be the motor involved, as antisense oligonucleotides to kinesin resulted in decreased localization of MBP mRNA (21). The transport of MBP mRNA granules requires a 21 nt sequence within the 3′ UTR called the RTS (RNA transport sequence) (22). This sequence was sufficient to transport MBP mRNA into processes; however, an additional element was responsible for localization of MBP mRNA within the myelin compartment (22). A complex of six proteins has been identified which interact with the RTS, the most abundant being the RNA binding protein hnRNPA2 (23).

mRNA transport into neuronal processes has been studied by the Kosik lab (24) using the vital dye SYTO14. RNA granules were observed that contained ribosomes and elongation factors. RNA granules were translocated into processes at a rate of 0.1 μm/s, which was blocked by microtubule depolymerization. A subset of RNA granules contained β-actin mRNA, suggesting that the active transport of mRNA may play a role in the targeting of newly synthesized actin into neuronal processes. Correlative fluorescence and electron microscopic observations of RNA granules labeled with SYTO14 dye have shown that granules correspond ultrastructurally to large clusters of ribosomes (24). RNA granules have now been identified in a number of cell types such as neurons, oligodendrocytes and oocytes and are proposed to represent a supramolecular complex that is transported directly with translational components (20, 24, 25).

CONSERVED MECHANISMS ACROSS SPECIES

During the early characterization of RNA–cytoskeletal interactions in different systems, it became apparent that microtubules were involved predominantly in the mechanism of mRNA localization in oocytes, whereas somatic cells such as fibroblasts used microfilaments for mRNA localization (26). For example, *bcd* RNA localization in *Drosophila* oocytes and *Vg1* RNA localization in *Xenopus* oocytes could be disrupted by microtubule-depolymerizing drugs (27, 28). In contrast, β-actin mRNA localization to the leading edge of chick fibroblasts required actin filaments (29). However, observations that neurons also used microtubules to localize mRNAs suggested similarities between transport mechanisms in oocytes and highly polarized somatic cells (25, 30). A dual role for microfilaments and microtubules in the RNA localization pathway within a single cell was suggested by observations that *Vg1* RNAs might also interact with actin filaments at the vegetal cortex once they were localized (28). Recent evidence indicates that some of the protein components involved in mRNA localization can participate in both microtubule and actin-dependent localization pathways. Isolation of proteins that binds the β-actin zip code in chick fibroblasts (zip code binding protein 1, or ZBP-1) and *Vg1* RNA localization sequences in *Xenopus* oocytes revealed an identity in their amino acid sequence and structural organization of KH domains and RNA recognition motifs (32, 33). The proteins binding *Vg1* mRNA (Vg RBP or Vera) in *Xenopus* oocytes were believed to be a possible orthologue to ZBP. Since Vera/Vg RBP and ZBP-1 act in very different mRNA localization pathways in different cellular milieu, these results suggested a conserved protein which may mediate interactions between microfilaments and microtubules.

Evidence to support the idea that a specific mRNA can interact with both microfilaments and microtubules is noted by observations that the localization of β-actin mRNA to the fibroblast leading edge requires microfilaments (29), yet β-actin mRNA localization to neuronal growth cones requires microtubules (see **Fig. 2**). The protein responsible for β-actin mRNA localization in fibroblasts, ZBP-1 (31), may be involved in the localization of β-actin mRNA particles between microtubules and microfilaments. Cell type-specific proteins that interact with ZBP could promote the preferential usage of one filament system over another (**Fig. 3**).

Further support for the idea that an RNA binding protein involved in mRNA localization may interact with different filaments is noted by observations that Staufen, which is involved in the microtubule-dependent localization of mRNAs in oocytes (8), is also required for the actin-dependent localization of *prospero* RNA (7). It therefore seems likely that the RNA binding proteins recognize different zip codes and transport the RNAs, perhaps switching from one cytoskeletal element system to the other in the process. These proteins may be bifunctional, having

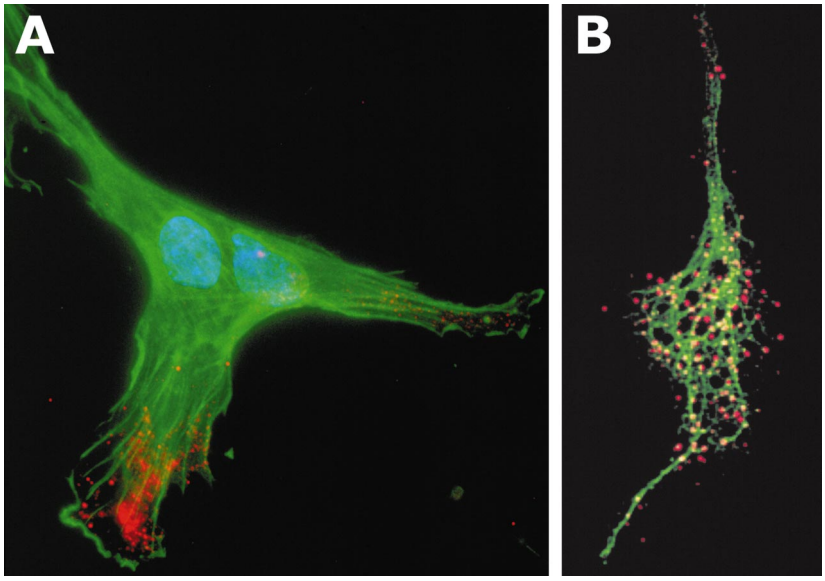

Figure 2. Localization of β-actin mRNA in fibroblast lamellae and neuronal growth cones. *A*) β-Actin mRNA (red), detected by in situ hybridization, localizes to the leading edge of fibroblasts as granules. F-actin is labeled by fluorescein-phalloidin (green). Nuclei are stained with DAPI. Disruption of microfilaments, but not microtubules, resulted in delocalization of the mRNA (not shown). Figure supplied by Edward Kisluaskis. Copyright permission obtained from Cell Press (4). *B*) β-Actin mRNA (red) localized to neuronal growth cones were present as granules that were aligned along microtubules (see also ref 25). Disruption of microtubules, but not microfilaments, resulted in delocalization of the mRNA (not shown). Copyright permission from Cell Press (4).

distinct binding motifs for either actin- or microtubule-based motors that allow the cargo or 'granule' to move on either cytoskeletal filament system.

RNA LOCALIZATION TO CELLULAR PROCESSES

Besides serving to segregate determinants during cell division, mRNA localization may also provide a means of generating asymmetry in terminally differentiated cells (22, 34–37). A classic example of this can be found in oligodendrocytes, which are involved in myelination of neurons. Myelin basic protein mRNA is localized to the peripheral processes in oligodendrocytes (19) where myelination occurs. However, it remains to be proved that the localization of the mRNA is necessary for MBP protein accumulation and myelination. It also remains to be established that loss of mRNA localization will disrupt MBP localization and result in defective myelination. So, while it has been well established in oocytes that mRNA localization is in fact essential for cell polarity, direct evidence in somatic cells has been lacking.

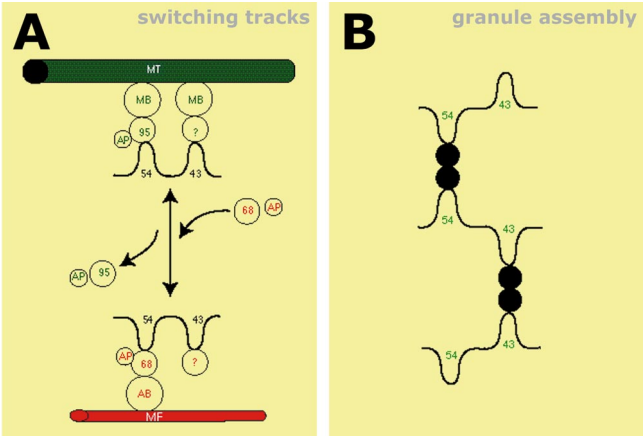

Figure 3. Schematic illustration of how mRNA granules may switch filament systems (*A*) and how RNA granules may assemble (*B*). *A*) β-Actin mRNA is localized by two zip code sequences (54 and 43 nt sequences), which may form stem-loops. Zip code binding proteins (ZBPs) may be involved in conferring binding of the mRNA to either microtubules or microfilaments. It is unclear whether ZBPs bind the filament directly or indirectly. Both ZBP-1 (69 kDa) and ZBP-2 (95 kDa) bind the 54 nt zip code. We speculate that there may be adaptor proteins (AP) that bind ZBPs and influence filament binding. MT, microtubule; MF, microfilament; MB, protein binding to microtubule; AB, protein binding to actin. *B*) Hypothetical model showing how dimerization of ZBPs at two zip codes could result in cross-linking of multiple mRNAs within a particle.

Neurons, like oligodendrocytes, also differentiate long processes. An additional challenge faced by neurons is that they elaborate two types of processes, axons and dendrites, which differ in protein composition. Neurons must localize proteins specifically to either axons or dendrites as well as to spatially distinct compartments within the process, i.e., growth cone and synapse. It is unknown whether mRNA localization mechanisms are an essential feature of neuronal polarity and function. One of the problems in identifying a function for mRNA localization in neuronal processes has been that protein localization mechanisms also seem to be involved, hence perturbation of the mRNA localization might not completely abolish protein localization and produce an obvious cellular phenotype. The dual role for both protein and mRNA localization mechanisms is appreciated in lower systems, such as the localization of *prospero* mRNA and protein in dividing neuroblasts in *Drosophila* (7). In differentiated neurons, it has been well documented that cytoskeletal proteins can be transported into processes posttranslationally by a slow transport mechanism (38). How-

ever, this mechanism remains ill defined, and it is unclear whether the transport of proteins from the cell body is sufficient to respond to the changing needs of the growing process.

It has been proposed that transport of mRNAs into processes and growth cones may provide an additional mechanism for the localization of newly synthesized cytoskeletal proteins (25, 39). Localized mRNAs could be translated at their site of localization, which would be more efficient than having to transport each protein molecule from the perikarya. Contemporary models for cytoskeletal transport have not considered this alternative mechanism (40, 41). However, evidence continues to emerge that documents the localization and translation of mRNAs within neuronal processes. The microtubule-associated protein MAP2 has its mRNAs within dendrites (42, 43); tau mRNA, which encodes an axonal MAP, has its mRNA localized to the proximal segment of the axon (44). The localization of tau mRNAs requires a 1300 nt sequence within the 3′ UTR. The targeting of tau mRNAs to this axonal compartment involves microtubules (44) and may be mediated by interactions between the 3′ UTR sequences and proteins that bind the mRNA to the microtubule (45). The mechanism of tau mRNA localization may share certain features with the localization of *Vg1* RNA in *Xenopus* oocytes (46). Tau mRNA localization sequences injected into oocytes are localized to the vegetal cortex. *Vg1* RNA transport to the vegetal cortex may involve a 69 kDa microtubule-associated protein (47). Tau RNA sequences contain a binding site for Vg1 RNA binding protein and suggest conserved mechanisms of RNA localization between oocytes and neurons.

Specific mRNAs and translational components are localized within growth cones. Using a micropipet to sever the neurite and remove cytoplasm from growth cones, a heterogeneous population of mRNAs was identified that included MAP2, along with intermediate filament proteins (48). Translation of these mRNAs within growth cones was demonstrated by transfection of mRNA encoding an epitope tag and immunofluorescence localization. β-actin (25) and tropomyosin mRNAs (49, 50) are also localized to developing processes and growth cones. Axonal preparations from rat sympathetic neurons were shown to be enriched for β-actin mRNAs, whereas tubulin mRNAs were confined to the cell body, suggesting a sequence specific sorting mechanism (51). We have also shown that β-actin mRNAs extend into processes and growth cones of developing rat cortical neurons, whereas γ-actin mRNAs were confined to the cell body (25). β-Actin mRNA, ribosomal proteins, and EF1α were present as granules within growth cones (25). These growth cones contained morphologically identifiable polyribosomes by electron microscopy and probes to β-actin mRNA colocalized with translational components in growth cones (25). These studies support the presence of an mRNA localization mechanism in axons, but further work is needed to understand its function in neuronal polarity and process outgrowth (49).

REGULATION OF mRNA LOCALIZATION

The regulated localization of mRNA can influence cellular morphogenesis during development by providing locally elevated concentrations of proteins that are needed in the assembly of protein complexes specialized for a particular function. The active transport of β-actin mRNA to the fibroblast lamella is induced by serum and platelet-derived growth factor (52, 53), and this induction is in fact required to obtain maximal rates of cell motility (53, 54). This is strong evidence for a function of mRNA localization in a somatic cell, i.e., the assembly of protein complexes involved in polymerization and motility. Signaling of mRNA localization between integrin receptors and the extracellular matrix may also be involved in the assembly of focal adhesion complexes (55).

The regulated synthesis of mRNA localization within neurons could similarly influence the assembly of macromolecular complexes involved in process outgrowth. Evidence in support of regulation was suggested by the observation that the amount of mRNA within growth cones was dependent on the stage of development and varied for each mRNA species (48). Collapse of growth cones with the calcium ionophore A23187 promoted transport of mRNAs encoding intermediate filaments into growth cones, further suggesting that local synthesis may be regulated (48). The specific signal transduction pathways involved in the regulation of mRNA localization to growth cones are unclear. We have observed that treatment of cultures with db-cAMP can induce the transport of β-actin mRNA into processes and growth cones (25), which suggests that activation of cAMP dependent protein kinase A could be involved in the regulation of mRNA localization. Recent evidence indicates that the neurotrophin NT-3 can promote the anterograde localization of RNA granules within processes (56), and we have observed that β-actin mRNAs are transported to growth cones after NT-3 (57).

mRNA LOCALIZATION AND SYNAPTIC PLASTICITY

It has been known for many years that polyribosomes are localized beneath synapses, and more

recent quantitative ultrastructural analysis has shown that >80% of dendritic polyribosomes are localized within or at the base of spines (58). This observation led to a hypothesis of synapse-specific gene expression in which local synthesis would provide a mechanism to target gene products to the proper spines, allowing for precise spatial and temporal control of synaptic structure and strength (59). Several studies have shown that new protein synthesis is required for long-lasting modifications of synaptic strength (60, 61) and appears to be essential to maintain a late phase of long-term potentiation (62). Local synthesis of synaptic proteins could be achieved by the translational regulation of mRNAs already present at synapses and/or the transport of specific mRNAs to subsynaptic locations.

Several mRNAs have been localized within dendrites, including the microtubule-associated protein MAP2 (42, 43), the α subunit of calcium/calmodulin-dependent protein kinase II (CaMKIIα) (63), the activity-related cytoskeletal protein ArC (64, 65), glutamate receptor subunits (66, 67), cAMP response element binding protein (39), and the RNA polymerase III transcript BC1 (68). Glycine receptor mRNAs have been localized to postsynaptic specializations within dendrites of spinal neurons (69). Poly(A) mRNAs are concentrated at the base of dendritic spines in rat hippocampus (70), yet it is unknown whether these mRNAs encode proteins involved in synaptic plasticity and memory. An attractive candidate is CaMKII, a neuronal serine-threonine protein kinase that is a major component of the postsynaptic density at glutamatergic synapses (71) and is required for long-term potentiation and spatial learning (72). CaMKIIα mRNA localization into dendrites is increased after induction of long-term potentiation (73).

Cis-acting elements within the mRNA sequence may be involved in dendritic mRNA localization. The dendritic targeting signal of calcium/calmodulin-dependent protein kinase II mRNA is within the 3′ UTR and may be required for the localization of CaMKII protein within dendritic spines (74). A *cis*-acting targeting element is required for BC1 RNA localization to neuronal dendrites (75). The transport of Arc mRNA into dendrites and activated synapses is also dependent on the mRNA sequence (76, 77). Synaptic activity is required for the dendritic localization of BC1 RNA localization during development, suggesting a mechanism to regulate localized protein synthesis and synaptic function (78). Future work may reveal *trans*-acting factors involved in dendritic mRNA localization that may be essential for localized protein synthesis and synaptic function.

FUTURE DIRECTIONS

A number of laboratories have identified the presence of RNA particles or granules, which may represent a novel cytoplasmic organelle capable of localizing specific mRNAs and the necessary translational machinery along cytoskeletal filament systems so as to maintain an asymmetric distribution of proteins. In cells that localize mRNAs by actin filaments (11, 29) or microtubules (24, 25, 30), granules appear to be a universal and conserved feature for transporting and/or anchoring these mRNAs (20, 24).

Direct proof that any of the particles or granules contain multiple copies of mRNA is still lacking, and further technological development is needed to determine the stoichiometry of RNA within these particles. Although the data suggest that mRNAs are packaged into larger particles, it is not yet known whether single mRNAs can be transported individually. Single mRNAs have been identified within the nucleus (79); hence, it may be possible to use this technology to address the number of mRNAs within a cytoplasmic particle. Also, it is not known how mRNAs are held together within a granule, though RNA binding proteins and cytoskeletal associated proteins are likely candidates. Additional work is needed to define the molecular components involved in the assembly of mRNA particles or granules, their transport and anchoring on cytoskeletal filaments, and translational regulation (Fig. 3).

Work is in progress to identify RNA binding proteins that may bridge the mRNA sequence to microtubule or microfilament associated motor molecules. It would be interesting to know whether cell type-specific *cis*-acting elements and/or binding proteins exist for localization of mRNA over long distances. Microtubules may represent the 'highways' for mRNA localization over long distances, whereas microfilaments are used for movement of mRNAs over shorter distances. In some cell types or compartments, it is also possible that mRNA granules may switch filament systems (Fig. 3). Future work may reveal interactions of both actin and microtubule-based motor molecules at the surface of RNA granules. Once the mechanisms for mRNA targeting are defined, it will be important to perturb it and assess the consequences on cell polarity and function. FJ

We thank Tulle Hazelrigg for providing unpublished work and for offering helpful discussions. We thank Shailesh Shenoy for his expertise with image analysis and for preparation of the figures. We thank Roy Long, Edward Bertrand, and Edward Kislauskis for providing images for figures.

REFERENCES

1. St. Johnston, D. (1995) The intracellular localization of messenger RNAs. Cell 81, 161–170

2. Oleynikov, Y., and Singer, R. H. (1998) RNA localization: different zipcodes, same postman. Trends Cell Biol. 8, 381–383
3. Arn, E. A., and MacDonald, P. M. (1998) Motors driving mRNA localization: new insights from in vivo imaging. Cell 95, 151–154
4. Hazelrigg, T. (1998) The destinies and destination of RNAs. Cell 95, 451–460
5. Doe, C. Q., Chu-LaGraff, Q., Wright, D. M., and Scott, M. P. (1991) The prospero gene specifies cell fates in the *Drosophila* central nervous system. Cell 65, 451–464
6. Schuldt, A. J., Adams, J. H., Davidson, C. M., Micklem, D. R., Haseloff,J., St. Johnson, D., and Brand, A. H. (1998) Miranda mediates asymmetric protein and RNA localization in the developing nervous system. Genes Dev. 12, 1847–1857
7. Broadus, J., Fuerstenberg, S., and Doe, C. Q. (1998) Staufen dependent localization of prospero mRNA contributes to neuroblast daughter cell fate. Nature (London) 391, 792–795
8. Ferrandon, D., Elphick, L., Nusslein-Volhard, C., and St. Johnston, D. (1994) Staufen associates with the 3′UTR of bicoid RNA to form particles that move in a microtubule dependent manner. Cell 79, 1221–1232
9. Jansen, R. P., C, D., Michaelis, C., Galova, M., and Nasmyth, K. (1996) Mother cell specific HO expression in budding yeast depends on myo4p. Cell 84, 687–697
10. Sil, A., and Herskowitz, I. (1996) Identification of asymmetrically localized Ash1p for lineage specific transcription of the yeast HO gene. Cell 84, 711–722
11. Long, R. M., Singer, R. H., Meng, X., Gonzalez, I., Nasmyth, K., and Jansen, R. P. (1997) Mating type switching in yeast controlled by asymmetric localization of *ASH1* mRNA. Science 277, 383–387
12. Takizawa, P. A., Sil, A., Herskowitz, I., and Vale, R. D. (1997) Actin dependent localization of an RNA encoding a cell fate determinant in yeast. Nature (London) 389, 90–93
13. Bertrand, E., Chartrand, P., Schaefer, M., Shenoy, S. M., Singer, R. H., and Long, R. M. (1998) Localization of *ASH1* RNA particles in living yeast. Mol. Cell 2, 437–445
14. Evangelista, M., Blundell, K., Longtime, M. S., Chow, C. J., Adams, N., and Boone, C. (1997) Bni1p, a yeast formin linking cdc42p and the actin cytoskeleton. Science 276, 118–122
15. Haarer, B. K., Petzold, A., Lillie, S. H., and Brown, S. S. (1994) Identification of MYO4, a second class V myosin gene in yeast. J. Cell Sci., 1055–1064
16. Cheney, R. E., O'Shea, M. K., Heuser, J. E., Forscher, P., and Mooseker, M. S. (1993) Brain myosin V is a two headed unconventional myosin with motor activity. Cell 75, 13–23
17. Wang, S., and Hazelrigg, T. (1994) Implications for bcd mRNA localization from spatial distribution of exu protein in *Drosophila* oogenesis. Nature (London) 369, 400–403
18. Theurkauf, W. E., and Hazelrigg, T. I. (1998) In vivo analysis of cytoplasmic transport and cytoskeletal organization during *Drosophila* oogenesis: characterization of a multi step anterior localization pathway. Development 125, 3655–3666
19. Ainger, K., Avossa, D., Morgan, F., Hill, S. J., Barry, C., Barbarese, E., and Carson, J. H. (1993) Transport and localization of exogenous MBP mRNA microinjected into oligodendrocytes. J. Cell Biol. 123, 431–441
20. Barbarese, E., Koppel, D. E., Deutscher, M. P., Smith, C. L., Ainger, K., Morgan, F., and Carson, J. H. (1995) Protein translation components are colocalized in granules in oligodendrocytes. J. Cell Sci. 108, 2781–2790
21. Carson, J. H., Worboys, K., Ainger, K., and Barbarese, E. (1997) Translocation of myelin basic protein mRNA in oligodendrocytes requires microtubules and kinesin. Cell Motil. Cytol. 38, 318–328
22. Ainger, K. A., Avossa, D., Diana, A. S., Barry, C., Barbarese, E., and Carson, J. H. (1997) Transport and localization elements in MBP mRNA. J. Cell Biol. 138, 1077–1087
23. Hoek, K. S., Kidd, G. J., Carson, J. H., and Smith, R. (1998) hnRNPA2 selectively binds the cytoplasmic transport sequence of MBP mRNA. Biochemistry 37, 7021–7029
24. Knowles, R. B., Sabry, J. H., Martone, M. E., Deerinck, T. J., Ellisman, M. H., Bassell, G. J., and Kosik, K. S. (1996) Translocation of RNA granules in living neurons. J. Neurosci. 16, 7812–7820
25. Bassell, G. J., Zhang, H. L., Byrd, A. L., Femino, A. M., Singer, R. H., Taneja, K. L., Lifshitz, L. M., Herman, I. M., and Kosik, K. S. (1998) Sorting of beta actin mRNA and protein to neurites and growth cones in culture. J. Neurosci. 18, 251–265
26. Bassell, G. J., and Singer, R. H. (1997) mRNA and cytoskeletal filaments. Curr. Opin. Cell Biol.
27. Pokrywka, N.J. and Stephenson, E. C. (1995) Microtubules are a general component of mRNA localization systems in *Drosophila* oocytes. Dev. Biol. 167:363–370
28. Yisraeli, J. K., Sokol, S. and Melton, D. A. (1990) A two-step model for the localization of maternal mRNA in *Xenopus* oocytes: Involvement of microtubules and microfilaments in the translocation and anchoring of Vg1 mRNA. Development 108, 289–298
29. Sundell, C. L., and Singer, R. H. (1991) Requirement of microfilaments in sorting of actin mRNAs. Science 253, 1275–1277
30. Bassell, G. J., Singer, R. H., and Kosik, K. S. (1994) Association of poly(A) mRNA with microtubules in cultured neurons. Neuron 12, 571–582
31. Ross, A., Oleynikov, Y., Kislauskis, E., Taneja, K., and Singer, R. (1997) Characterization of a β-actin mRNA zipcode-binding protein. Mol. Cell. Biol. 17, 2158–2165
32. Deshler, J. O., Highett, M. I., and Schnapp, B. J. (1998) A highly conserved RNA binding protein for cytoplasmic mRNA localization in vertebrates. Curr. Biol. 8, 489–496
33. Havin, L., Git, A., Elisha, Z., Oberman, F., Yaniv, K., Schwartz, S. P., Standart, N., and Yisraeli, J. K. (1998) RNA binding protein conserved in both microtubule and microfilament based RNA localization. Genes Dev. 12, 1593–1598
34. Kislauskis, E. H., Li, Z., Singer, R. H., and Taneja, K. L. (1993) Isoform-specific 3′-untranslated sequences sort α-cardiac and β-cytoplasmic actin messenger RNAs to different cytoplasmic compartment. J. Cell Biol. 123, 165–172
35. Kislauskis, E. H., Zhu, X., and Singer, R. H. (1994) Sequences responsible for intracellular localization of β-actin messenger RNA also affect cell phenotype. J. Cell Biol. 127, 441–451
36. Wiseman, J. W., Glover, L. A., and Hesketh, J. E. (1997) Evidence for a localization signal in the 3′UTR from vimentin mRNA. Int. J. Biochem. Cell Biol. 29, 1013–1020
37. Wiseman, J. W., Glover, L. A., and Hesketh, J. E. (1997) Evidence for a localization signal in the 3′UTR of myosin heavy chain mRNA. Cell Biol. Int. 21, 243–248
38. Nixon, R. A. (1987) The axonal transport of cytoskeletal proteins: a reappraisal. In: Axonal Transport (Bisby, M. A., and Smith, R. S., eds) pp. 175–200, Alan R. Liss, New York
39. Crino, P., Khodakhah, K., Becker, K., Ginsberg, S., Hemby, S., and Eberwine, J. (1998) Presence and phosphorylation of transcription factors in developing dendrites. Proc. Natl. Acad. Sci. USA 95, 2313–2318
40. Takeda, S., and Hirokawa, N. (1995) Tubulin dynamics in neuronal axons. Neuron 14, 1257–1264
41. Tanaka, E., Ho, T., and Kirschner, M. (1995) The role of microtubule dynamics in growth cone motility and axonal growth. J. Cell Biol. 128, 139–155
42. Garner, C. C., Tucker, R. P., and Matus, A. (1988) Selective localization of mRNA for cytoskeletal protein MAP2 in dendrites. Nature (London) 336, 674–679
43. Kleiman, R., Banker, G., and Steward, O. (1990) Differential subcellular localization of particular mRNAs in hippocampal neurons in culture. Neuron 5, 821–830
44. Litman, P., Barg, J., Rindzoonski, L., and Ginzburg, I. (1993) Subcellular localization of tau mRNA in differentiating neuronal cell culture: implications for neuronal polarity. Neuron 10, 627–638
45. Behar, L., Marx, R., Sadot, E., Barg, J., and Ginzburg, I. (1995) *cis*-Acting signals and *trans*-acting proteins are involved in tau mRNA targeting into neurites of differentiating neuronal cells. Int. J. Dev. Neurosci. 13, 113–127
46. Litman, P., Behar, L., Elisha, Z., Yisraeli, J. K., and Ginzburg, I. (1996) Exogenous tau RNA is localized in oocytes: possible evidence for evolutionary conservation of localization mechanisms. Dev. Biol. 176, 86–94
47. Elisha, Z., Havin, L., Ringel, I., and Yisraeli, J. K. (1995) Vg1 RNA binding protein mediates the association of Vg1 RNA with microtubules in *Xenopus* oocytes. EMBO J. 14, 5109–5114
48. Crino, P. B., and Eberwine, J. (1997) Molecular characterization of the dendritic growth cone. Neuron 17, 1173–1187
49. Hannan, A. J., Gunning, P., Jeffrey, P. L., and Weinberger, R. P.

(1998) Structural compartments within neurons: developmentally regulated organization of microfilament isoform mRNA and protein. Mol. Cell Neurosci. 11, 289–304
50. Hannan, A. J., Schevzov, G., Gunning, P., Jeffrey, P. L., and Weinberger, R. P. (1995) Intracellular localization of tropomyosin mRNA and protein is associated with development of neuronal polarity. Mol. Cell Neurosci. 6, 397–412
51. Olink-Coux, M., and Hollenbeck, P. J. (1996) Localization and active transport of mRNA in axons of sympathetic neurons. J. Neurosci. 16, 1346–1358
52. Hill, M. A., Schedlich, L., and Gunning, P. (1994) Serum-induced signal transduction determines the peripheral location of beta-actin mRNA within the cell. J. Cell Biol. 126, 1221–1230
53. Latham, V. M., Kislauskis, E. H., Singer, R. H., and Ross, A. F. (1994) Beta actin mRNA localization is regulated by signal transduction mechanisms. J. Cell Biol. 126, 1211–1219
54. Kislauskis, E., Zhu, X., and Singer, R. (1997) β-Actin messenger RNA localization and protein synthesis augment cell motility. J. Cell Biol. 136, 1263–1270
55. Chicurel, M. E., Singer, R. H., Meyer, C. J., and Ingber, D. E. (1998)Integrin binding and mechanical tension induce movement of mRNA and ribosomes to focal adhesions. Nature (London) 392, 730–733
56. Knowles, R. B., and Kosik, K. S. (1997) Neurotrophin-3 signals redistribute RNA in neurons. Proc. Natl. Acad. Sci. USA 94, 14804–14808
57. Zhang, H. L., Singer, R. H., and Bassell, G. J. (1997) Neurotrophin signaling of β-actin mRNA localization to neuronal growth cones. ASCB Abstr. 1239
58. Steward, O., Falk, P. M., and Torre, E. R. (1996) Ultrastructural basis for gene expression at the synapse. J. Neurocytol. 25, 717–734
59. Steward, O. S., and Banker, G. A. (1992) Getting the message from the gene to the synapse: sorting and intracellular transport of RNA in neurons. Trends Neurosci. 15, 180–186
60. Bailey, C. H., Montarolo, P., Chen, M., Kandel, E. R., and Schacher, S. (1992) Inhibitors of protein and RNA synthesis block structural changes that accompany long term heterosynaptic plasticity in *Aplysia*. Neuron 9, 749–758
61. Martin, K. C., Casadio, A., Zhu, H., Yaping, E., Rose, J. C., Chen, M., Bailey, C. H., and Kandel, E. R. (1997) Synapse specific long term facilitation of Aplysia sensory to motor synapses: a function for local protein synthesis in memory storage. Cell 91, 927–938
62. Frey, U., Krug, M., Reymann, K. G., and Matthies, H. (1988) Anisomycin blocks late phases of LTP in the hippocampal CA1 region. Brain Res. 452, 57–65
63. Burgin, K. E., Waxham, M. N., Rickling, S., Westgate, S. A., Mobley, W. C., and Kelly, P. T. (1990) *In situ* hybridization histochemistry of Ca^{2+}/calmodulin-dependent protein kinase in developing rat brain. J. Neurosci. 10, 1788–1798
64. Link, W., Konietzko, U., Kauselmann, G., Krug, M., Schwanke, B., Frey, U., and Kuhl, D. (1995) Somatodendritic expression of an immediate early gene is regulated by synaptic activity. Proc. Natl. Acad. Sci. USA 92, 5734–5738
65. Lyford, G. L., Yamagata, K., Kaufman, W. E., Barnes, C. A., Sanders, L. K., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., Lanahan, A. A., and Worley, P. F. (1995) ArC, a growth factor and activity related gene encodes a novel cytoskeletal protein that is enriched in neuronal dendrites. Neuron 14, 433–445
66. Gazzaley, A., Benson, D., Huntley, G., and Morrison, J. (1997) Differential subcellular regulation of NMDAR1 protein and mRNA in dendrites of dentate. J. Neurosci. 17, 2006–2017
67. Miyashiro, K., Dichter, M., and Eberwine, J. (1994) On the nature and differential distribution of mRNAs in hippocampal neurites: implications for neuronal functioning. Proc. Natl. Acad. Sci. USA 91, 10800–10804
68. Tiedge, H., Fremeau, J., R T, Weinstock, P. H., and Arancio, O. (1991)Dendritic location of neural BC1 RNA. Pro. Natl. Acad. Sci. USA 88,2093–2097
69. Racca, C., Gardiol, A., and Triller, A. (1997) Dendritic and postsynaptic localization of glycine receptor RNA. J. Neurosci. 17, 1691–1700
70. Martone, M. E., Pollack, J. A., Jones, Y. Z., and Ellisman, M. H. (1996)Ultrastructural localization of dendritic mRNA in adult rat hippocampus. J. Neurosci. 16, 7437–7446
71. Kennedy, M. B. (1997) The postsynaptic density at glutamatergic synapses. Trends Neurosci. 20, 264–268
72. Silva, A., Stevens, C., Tonegawa, S., and Wang, Y. (1992) Deficient hippocampal LTP in CaMKII mutant mice. Science 257, 201–206
73. Thomas, K. L., Laroche, S., Errington, M. L., Bliss, T. V., and Hunt, S. P. (1994) Spatial and temporal changes in signal transduction pathways during LTP. Neuron 13, 737–745
74. Mayford, M., Baranes, D., Podsypanina, K., and Kandel, E. R. (1996) The 3′-untranslated region of CaMKIIa is a *cis* acting signal for the localization and translation of mRNA in dendrites. Proc. Natl. Acad. Sci. USA 93, 13250–13255
75. Muslimov, I. A., Santi, I., Perini, S., Higgins, D., and Tiedge, H. (1997) RNA transport in dendrites: a *cis* acting signal within BC1 RNA. J. Neurosci. 17, 4722–4733
76. Steward, O., Wallace, C. S., Lyford, G. L., and Worley, P. F. (1998)Synaptic activation causes the mRNA for Arc to localize selectively near activated postsynaptic sites on dendrites. Neuron 21, 1–20
77. Wallace, C. S., Lyford, G. L., Worley, P. F., and Steward, O. (1998)Differential intracellular sorting of immediate early gene mRNAs depends on signals in the mRNA sequence. J. Neurosci. 18, 26–35
78. Muslimov, I. A., Banker, G., Brosius, J., and Tiedge, H. (1998) Activity dependent regulation of dendritic BC1 RNA in hippocampal neurons in culture. J. Cell Biol. 141, 1601–1611
79. Femino, A. M., Fay, F. S., Fogarty, K., and Singer, R. H. (1998) Visualization of single RNA transcripts in situ. Science 280, 585–590