

---

# Neuronal RNA Localization and the Cytoskeleton

Gary J. Bassell<sup>1</sup> and Robert H. Singer<sup>2</sup>

The ability of a neuronal process to grow and be properly directed depends upon the growth cone, whose shape and sensory capabilities are influenced by dynamic cytoskeletal filament systems. Microfilaments, which are composed of actin, can rapidly form bundles that affect filopodial protrusions, growth cone motility and process growth. An important area of basic research is to understand how the neuron targets cytoskeletal precursors over considerable distances to reach the growth cone for their utilization in filopodial protrusion formation and control of process outgrowth. The localization of mRNAs to distinct cellular compartments has been shown to be an important protein sorting mechanism in many cell types, which is used to generate cell polarity. It is likely that neurons also utilize mRNA sorting and localized synthesis as a means to influence cytoskeletal organization, neuronal polarity, and process outgrowth. This chapter will summarize recent advances in our understanding of mRNA targeting mechanisms in neurons with an emphasis on localized synthesis of cytoskeletal proteins in growth cones and neuronal processes. We will also discuss the role of cytoskeletal filament systems in the transport and localization of specific mRNAs in neuronal processes.

## 1 Localization of Cytoskeletal Proteins to Neuronal Processes and Growth Cones

Neurons are quintessential asymmetric cells, having two morphologically and functionally distinct types of processes, axons and dendrites. The development of neuronal shape and polarity involves the extension of neuronal processes from the cell body which must traverse long and complex paths to reach their appropriate targets. Growth cones are specialized motile structures at the termini of these processes that respond to extracellular cues and influence both the rate and direction of process outgrowth. Process elongation and growth cone motility depend upon a constant supply of unpolymerized actin and tubulin to assemble new cytoskeletal polymers that will induce membrane pro-

---

<sup>1</sup> Department of Neuroscience, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461, USA

<sup>2</sup> Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461, USA

trusion and promote process outgrowth (Forscher and Smith 1988; Okabe and Hirokawa 1991; Tanaka and Sabry 1995). Disruption of actin filaments in growth cone filopodia has been shown to result in abnormal pathfinding decisions *in vivo* (Bentley and Toroian-Raymond 1986). A major challenge of neuronal cell biology is to understand how cytoskeletal precursors are delivered to the growing axon and growth cone. Defects in the ability of the neuron to transport and localize cytoskeletal components to distal locations could have deleterious effects on neuronal polarity and cytoarchitecture and lead to abnormal development or degenerative processes.

Not only must the neuron be able to target cytoskeletal proteins to growth cones, it must also be able to target a distinct set of proteins whose identity, stoichiometry, and structural organization differ from that of the perikarya. Of fundamental interest is identification of how the cytoskeletal composition of the growth cone differs from the perikarya, and the identification of mechanisms involved in this sorting and assembly. One mechanism to provide axons and growth cones with specific cytoskeletal proteins is to actively transport them into processes and growth cones following their synthesis within the cell body. In contrast to the fast transport of membrane-bound organelles, the transport of cytoskeletal proteins and/or complexes occurs by a slow transport mechanism which has not been defined. Some studies have suggested the idea of polymer sliding, where the filaments themselves are transported down the axon (Lasek 1986). Others studies suggest that cytoskeletal proteins are synthesized in the cell body and are transported as monomers or possibly oligomers which then incorporate laterally into axonal filaments (Nixon 1987; Okabe and Hirokawa 1990; Sabry et al. 1995; Takeda and Hirokawa 1995). What also remains controversial is whether a slow transport mechanism from the cell body is sufficient to promote renewal of cytoskeletal proteins and maintain a large axonal mass (Koenig 1991).

It has been proposed that the transport of mRNAs into processes may provide an additional mechanism for the localization of newly synthesized cytoskeletal proteins (Crino and Eberwine 1997; Bassell et al. 1998; Zhang et al. 1999). Localized mRNAs could be translated at their site of localization, providing immediate assembly of structural components where they are needed. An mRNA localization mechanism would be more efficient than having to transport each protein molecule from the perikarya, as each localized mRNA could generate thousands of proteins locally. Contemporary models for cytoskeletal transport have not considered this alternative mechanism (Takeda and Hirokawa 1995; Tanaka et al. 1995). However, evidence continues to emerge which documents the localization and translation of mRNAs within neuronal processes. *In situ* hybridization analysis revealed that mRNAs for the microtubule associated protein, MAP2, were concentrated within dendrites of rat brain tissue sections (Garner et al. 1988). Similar dendritic mRNA distributions for MAP2 have also been observed in cultured hippocampal (Kleiman et al. 1990) and sympathetic neurons (Bruckenstein et al. 1990). Not all mRNAs encoding cytoskeletal filament proteins are localized to neuronal processes.

For example, tubulin appears to be restricted to the cell body, according to *in situ* hybridization analysis of rat brain and cultured hippocampal neurons (Garner et al. 1988; Kleiman et al. 1994).

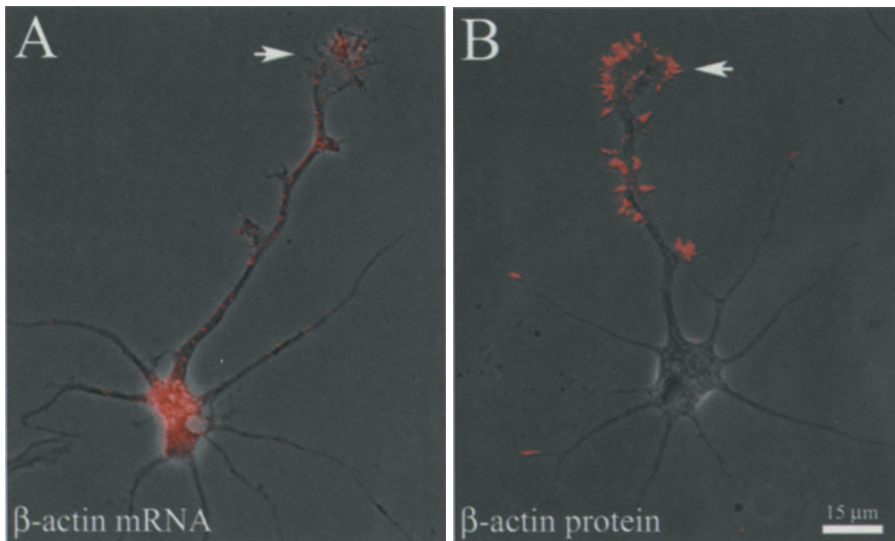
The localization of mRNAs into axons has also been demonstrated, although not all axons may utilize this mechanism, and many neurons may restrict protein synthetic machinery from entering the axon (Van Minnen 1994). Polyribosomes have also been detected in growing axons and growth cones *in vivo* (Tennyson 1970). The axonal distribution of tau protein (Binder et al. 1985; Peng et al. 1986; Ferreira et al. 1987; Brion et al. 1988) may be achieved by the localization of tau mRNAs to the proximal segment of growing axons (Litman et al. 1993). Tau also appears to be transported down axons by a slow transport mechanism which is post-translational (Mercken et al. 1995). Nonetheless, the delivery of tau mRNA to the axonal compartment may facilitate the transport of tau protein to more distal locations (Litman et al. 1993). Tropomyosin mRNA (Tm-5) extends into the proximal segment of growing axons and correlates with the axonal localization of Tm-5 protein (Hannan et al. 1995).

Other mRNAs may be targeted beyond the proximal segment of the axon. mRNAs encoding neurofilament proteins are localized to invertebrate axons and are associated with axonal polyribosomes morphologically and biochemically (Crispino et al. 1993a,b, 1997). Several reports have documented the presence of actin mRNA and actin protein synthesis within vertebrate and invertebrate axons. Actin was found in a cDNA library from squid axoplasm (Kaplan et al. 1992). Axonal preparations from rat sympathetic neurons were shown to be enriched for  $\beta$ -actin mRNAs, whereas tubulin mRNAs were confined to the cell body, suggesting a sequence-specific sorting mechanism (Olink-Coux and Hollenbeck 1996). The axonal synthesis of actin was demonstrated by analysis of proteins synthesized after radiolabeled amino acid incubation of axonal fractions from rat dorsal and ventral roots from rat spinal cord (Koenig 1989, 1991; Koenig and Adams 1982).

Specific mRNAs and translational components have also been localized within growth cones. Using a micropipette to sever the neurite and remove cytoplasm from growth cones, a heterogeneous population of mRNAs was isolated which included MAP2, along with intermediate filament proteins (Crino and Eberwine 1997). Translation of these mRNAs within growth cones was demonstrated by transfection of mRNA encoding an epitope tag and immunofluorescence localization. Dendritic growth cones have also been shown to contain translational machinery by their incorporation of radiolabeled amino acids into proteins following neurite transection (Davis et al. 1992). Ultrastructural analysis has revealed the presence of polyribosomes in growth cones of developing hippocampal neurons (Deitch and Banker 1993). We have shown that  $\beta$ -actin mRNAs extend into processes and growth cones of developing rat cortical neurons, whereas gamma-actin mRNAs were confined to the cell body (Bassell et al. 1998). We have also shown that  $\beta$ -actin mRNA, ribosomal proteins, and elongation factor 1 $\alpha$  are present as granules within growth cones

(Bassell et al. 1998). These growth cones contained polyribosomes which were morphologically identifiable by electron microscopy and probes to  $\beta$ -actin mRNA colocalized with translational components in growth cones (Bassell et al. 1998). Our hypothesis is that  $\beta$ -actin mRNA localization into dendritic and axonal processes provides a mechanism for the local enrichment of newly synthesized  $\beta$ -actin monomers within growth cones (Fig. 1).  $\beta$ -actin may be the preferred actin isoform for rearrangements of the actin cytoskeleton which occur in response to signaling events at the membrane. Locally elevated concentrations of the  $\beta$ -isoform may facilitate de novo nucleation of actin polymerization directly at the plasma membrane in response to physiological signals.

Actin is the most abundant protein within the peripheral region of growth cones, known as the leading edge. It is likely that active actin polymerization within the growth cone is supported by an anterograde flux of actin monomers that preferentially incorporates at the barbed end associated with the membrane (Okabe and Hirokawa 1991). Filopodial protrusion is generally followed by a retrograde flow of F-actin which is disassembled at the rear (Bamburg and Bray 1987; Okabe and Hirokawa 1991). Local synthesis of actin within the growth cone may play a role in actin polymerization, filopodial protrusion, and process outgrowth. In fibroblasts,  $\beta$ -actin mRNA localization has been shown



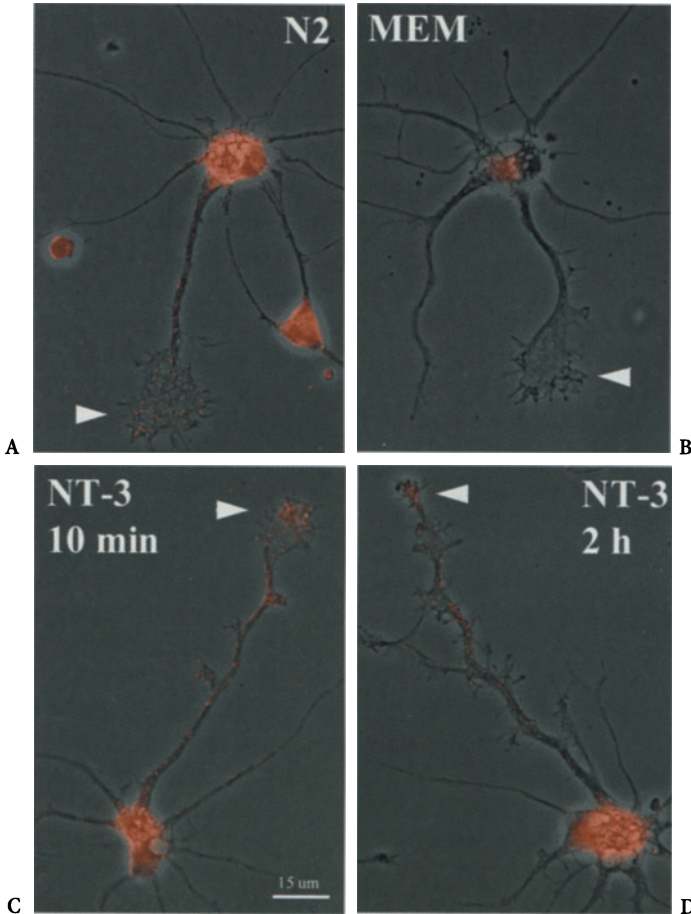
**Fig. 1A–B.** Localization of  $\beta$ -actin mRNA and protein. **A** Using in situ fluorescence hybridization and digital imaging microscopy,  $\beta$ -actin mRNA was localized in the form of spatially distinct granules, within neuronal processes and growth cones (*arrow*) of cultured chick forebrain neurons (see also Zhang et al. 1999). **B** Immunofluorescence with a monoclonal antibody, which was specific for the  $\beta$ -actin isoform, showed an enrichment of this isoform within growth cones and filopodia (*arrow*)

to enhance cell motility (Kislauskis et al. 1997). Fibroblast lamellae have certain structural and functional similarities to growth cones, hence it is reasonable to suggest that  $\beta$ -actin mRNA targeting also occurs in neuronal processes, yet the cytoskeletal transport mechanisms may differ (Bassell et al. 1998).

There has been an increasing consensus that actin isoforms in several cell types are sorted to different intracellular compartments and that  $\beta$ -actin has a specific role in regions of motile cytoplasm (Herman and D'Amore 1985; Otey et al. 1986; Shuster and Herman 1995; Von Arx et al. 1995; Yao and Forte 1995).  $\beta$ -Actin may be the predominant isoform at submembranous sites where it binds ezrin and thus may be involved in modulation of actin in response to extracellular signals (Shuster and Herman 1995). In cultured forebrain neurons, we have shown that  $\beta$ -actin protein is highly enriched within growth cones and filopodia (Fig. 1B). We have proposed that the asymmetric localization of  $\beta$ -actin protein within neurons is achieved by the localization of  $\beta$ -actin mRNA granules in processes (Fig. 1A) and the subsequent local synthesis of  $\beta$ -actin within growth cones (Bassell et al. 1998; Zhang et al. 1999).

## 2 Regulation of Neuronal mRNA Localization

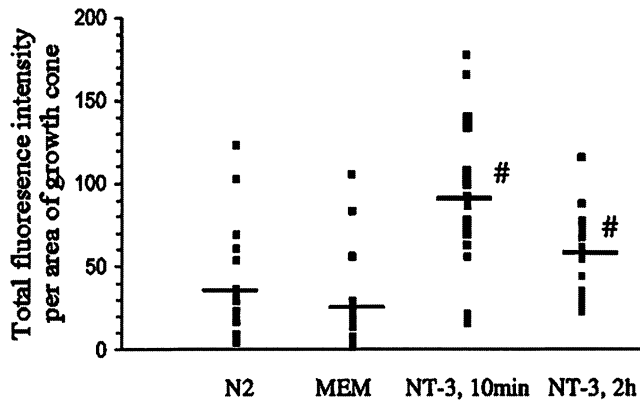
The elucidation of regulatory mechanisms for mRNA localization within neuronal growth cones would be important as it would strongly suggest that external cues encountered by the growth cone during pathfinding could directly affect protein synthetic activities locally rather than having to signal similar changes in protein synthesis within the perikarya. The active transport of  $\beta$ -actin mRNA to the fibroblast lamella is induced by serum and platelet-derived growth factor (PDGF); this induction is, in fact, required to obtain maximal rates of cell motility (Latham et al. 1994; Kislauskis et al. 1997). The regulated synthesis of mRNA, its localization, and actin polymerization within neurons could similarly influence 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 (Crino and Eberwine 1997). 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 (Crino and Eberwine 1997). We have observed that treatment of cultures with db-cAMP, an activator of adenylate cyclase, can induce the transport of  $\beta$ -actin mRNA, but not  $\gamma$ -actin mRNA, into processes and growth cones (Bassell et al. 1998), suggesting that activation of cAMP-dependent protein kinase A could be involved in the regulation of mRNA localization. The neurotrophin, NT-3, was shown to promote the anterograde localization of RNA granules within processes (Knowles and Kosik 1997). Recent work in our laboratory has shown that  $\beta$ -actin mRNAs are localized to growth cones following NT-3 treatment, and that this localization can be blocked by Rp-cAMP, an inhibitor of cAMP-dependent protein kinase A (Figs. 2, 3; see also Zhang et al. 1999). We also observed that NT-3 elicits a transient increase in cyclic APM-



**Fig. 2A–D.** Signaling of  $\beta$ -actin mRNA localization by neurotrophin-3 (NT-3). **A** In cells cultured under normal conditions, with N2 supplements and astrocyte coculture,  $\beta$ -actin mRNA was prominent in the cell body and localized within the axonal process and growth cone in the form of spatially distinct granules (*arrowhead*). **B** Cells which were starved in minimal essential medium (MEM) for 6 h showed hybridization within the cell body but growth cones did not reveal mRNA granules (*arrowhead*). **C, D** Cells which were starved for 6 h in minimum essential medium (MEM) and then stimulated with NT-3 for 10 min or 2 h were observed to re-localize  $\beta$ -actin mRNA granules within growth cones (*arrow*). *Bar* 15  $\mu$ m. (Figure modified with permission from Zhang et al. 1999)

dependent protein kinase A (PKA) activity, which precedes the localization of  $\beta$ -actin mRNA (Zhang et al. 1999). An increase in  $\beta$ -actin protein levels and actin polymerization was also observed following NT-3 treatment, which supports our model in which regulated mRNA localization and local synthesis can influence actin organization within growth cones. Further work will be needed to dissect out the downstream targets of trk receptor activation, which influence mRNA localization.

### NT-3 increases the density of $\beta$ -actin mRNA granules



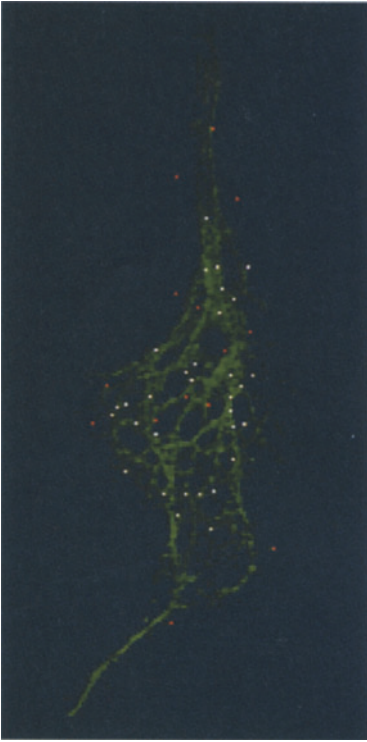
**Fig. 3.** Quantitative analysis of neurotrophin-stimulated  $\beta$ -actin mRNA localization. Neurons were fixed for in situ hybridization to  $\beta$ -actin mRNA. Twenty growth cones were imaged for each condition with identical exposure times. Data expressed as fluorescence density (total intensity/growth cone area). NT-3 was observed to increase the density of fluorescence signal for  $\beta$ -actin mRNA within growth cones. #,  $P < 0.01$  when MEM was compared with N2, or MEM was compared with NT-3, 10 min. or NT-3, 2 h. N2 normal culture medium, MEM starvation in minimum essential medium. (Modified with permission from Zhang et al. 1999)

**Table 1.** Effects of cytoskeletal perturbation on  $\beta$ -actin mRNA granule density. Fluorescence detection of actin mRNA granules. Data shown are the mean number of granules per 100  $\mu\text{m}$  of process length with standard deviation. For colchicine treatment, the top row indicates randomly selected cells and the bottom row represents those with altered microtubule distribution (74% of cells examined). A Student T-test was performed to measure differences between control and drug-treated cultures ( $*P < 0.01$ ,  $**P < 0.04$ ,  $***P < 0.005$ )

Untreated	$37.0 \pm 1.4$
Cytochalasin-D	$*53.0 \pm 1.4$
Cytochalasin/Actinomycin	$38.5 \pm 2.6$
Colchicine	$**23.6 \pm 8.4$
	$***17.0 \pm 0.5$

### 3 Mechanism of mRNA Localization

RNA localization is likely to be a multistep process having distinct transport and anchoring components (Bassell and Singer 1999). The interaction of mRNA localization sequences with specific cytoskeletal proteins and filament



**Fig. 4.** Colocalization of  $\beta$ -actin mRNA and microtubules in growth cones by 3-D digital imaging microscopy. To visualize  $\beta$ -actin mRNA and cytoskeletal filaments in cortical neurons with high resolution, a series of optical sections (100 nm) were taken from each cell and further processed using deconvolution algorithms and the application of a point spread function (Bassell et al. 1998).  $\beta$ -Actin mRNA was detected with rhodamine, tubulin protein was detected with fluorescein, and the two processed images were then superimposed. Pixels which contained both fluorochromes appeared *white* in optical sections, whereas *red* pixels denote probe that is not within the same pixel as anti-tubulin (*green*). The majority of  $\beta$ -actin mRNA granules colocalized with microtubules (*white pixels*). (Reprinted with permission from Bassell et al. 1998)

systems could provide a mechanism for localizing mRNAs to distinct intraneuronal regions. Evidence indicates that a nucleic acid-based recognition mechanism exists to sort mRNAs which code for cytoskeletal proteins (Kislauskis and Singer 1992). In many cell types, the presence of specific localization sequences within the 3' untranslated region (UTR) is a common occurrence. In cultured fibroblasts, a 54 nt (nucleotide) sequence or RNA zip code was found to be necessary and sufficient for localization of  $\beta$ -actin mRNA to the cell periphery. A downstream element, a 43 nt zip code, was shown to have weak localizing activity (Kislauskis et al. 1993, 1994). The targeting of  $\beta$ -actin mRNA to the lamellae is dependent upon microfilaments and not microtubules (Sundell and Singer 1991).

We have shown that the localization of  $\beta$ -actin mRNA in neuronal processes and growth cones is dependent on microtubules (Table 1, Figs. 4, 5). One explanation for these cell type-specific differences is that microtubules are the preferred filament system for long-distance translocation, and that  $\beta$ -actin mRNAs can shuttle between both filament systems. In fibroblasts, the *trans*-acting factor, Zip Code Binding Protein, binds the  $\beta$ -actin zip code and is involved in  $\beta$ -actin mRNA localization (Ross et al., 1997). The proteins binding



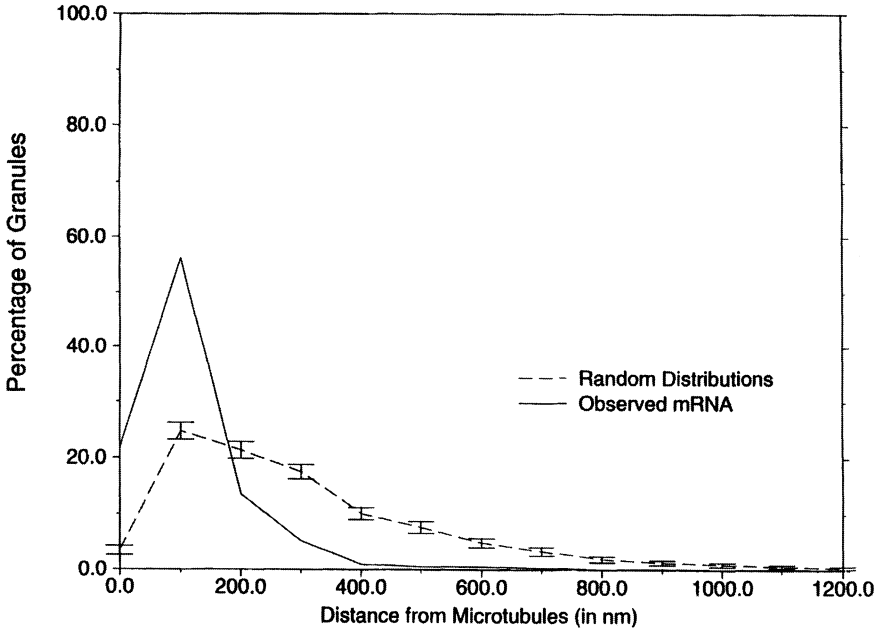


Fig. 5.  $\beta$ -Actin mRNA distribution is significantly nonrandom with respect to microtubules. The distance between  $\beta$ -actin mRNA (brightest voxels) and the nearest tubulin voxel was compared with a randomized distribution. This analysis was performed on a 3-D data set from 100-nm optical sections. The mean and standard deviations of the random distribution are shown. The observed distribution of  $\beta$ -actin mRNA is significantly closer to the microtubules than a random distribution. (Reprinted with permission from Bassell et al. 1998)

Vg1 mRNA in *Xenopus* oocytes were sequenced and their amino acid sequence identified them as a possible orthologue to ZBP (Vera/Vg1 RBP were discovered by different groups but are apparently the same protein) (Deshler et al. 1998; Havin et al. 1998). Thus Vera/Vg1 and ZBP-1 are almost identical proteins operating on different cytoskeletal elements in different cells and species. Further work is needed to determine whether the microtubule-dependent targeting of  $\beta$ -actin mRNA in neurons involves Zip Code Binding Protein and whether the targeting of  $\beta$ -actin mRNA in neurons uses the same *cis*-acting elements as in fibroblasts.

Discrete *cis*-acting localization elements have been identified for polarized cells. In oligodendrocyte processes, a 21-nucleotide sequence has been identified for myelin basic protein mRNA transport into processes (Ainger et al. 1997) which is also dependent on microtubules and kinesin (Carson et al. 1997). RNA localization zip codes have also been identified in oocytes that are involved in targeting proteins to distinct poles, and this sorting plays a fundamental role in the establishment of embryonic polarity (St. Johnston 1995). In *Xenopus* oocytes, Veg1 RNA is localized to the vegetal pole along

microtubules, and then is anchored to cortical actin filaments (Yisraeli et al. 1990). These multiple steps may be controlled by distinct *cis*-acting elements within a 340-nucleotide 3'UTR sequence (Mowry and Melton 1992). Two distinct pathways for RNA localization have been identified in *Xenopus*, both of which involve anchoring of RNA to actin filaments within the vegetal cortex, but only one pathway seems to involve microtubule transport of RNA to the cortex (Kloc and Etkin 1995). In *Drosophila* oocytes, the localization of Staufen-bicoid 3'UTR complexes is dependent on microtubules (Ferrandon et al. 1994).

RNA localization sequences have been identified in the translocation of mRNA into neuronal dendrites and axons. The dendritic targeting signal of calcium/calmodulin-dependent protein kinase II $\alpha$  mRNA is within the 3'UTR and may be required for the localization of CaMKII protein within dendritic spines (Mayford et al. 1996). A *cis*-acting targeting element is required for BC1 RNA localization to neuronal dendrites (Muslimov et al. 1997). The sorting of MAP2 mRNA into dendrites has been observed in hippocampal neurons in culture (Kleiman et al. 1990) and in vivo (Garner et al. 1988). MAP2 localization sequences for the high molecular weight isoform have been suggested to lie within the protein coding sequence (Kindler et al. 1996; Marsden et al. 1996); however, more recent evidence has shown that a 640-nucleotide sequence within the 3'UTR is both necessary and sufficient for dendritic targeting (Blichenberg et al. 1999). A short sequence within the coding region of MAP2 A,B is homologous to the 21-nucleotide RNA transport sequence for myelin basic protein mRNA, and can localize when injected into oligodendrocytes (Munro et al. 1999). It may be that there are redundant signals along the mRNA. The localization of tau mRNAs to the proximal segment of axons requires a 1300-nucleotide sequence within the 3'UTR. The targeting of tau mRNAs to this axonal compartment involves microtubules (Litman et al. 1993) and may be mediated by interactions between 3'UTR sequences and proteins which bind the mRNA to the microtubule (Behar et al. 1995). The mechanism of tau mRNA localization may share certain features with the localization of Vg1 RNA in *Xenopus* oocytes (Litman et al. 1996). Tau mRNA localization sequences injected into oocytes are localized to the vegetal cortex. Tau RNA sequences contain a binding site for Vg1 RNA binding protein and suggest conserved mechanisms of RNA localization between oocytes and neurons. Microtubules may be involved in long-distance translocation of RNA, a mechanism required by these highly polarized cells. Microfilaments may be involved in local movements or mRNA anchoring which could be a common mechanism used by many cell types.

#### 4 mRNA May Be Transported as Particles or Granules

RNAs may be packaged into transport particles or granules which contain multiple mRNA molecules and translational machinery (Bassell et al. 1999). These RNA particles may then be translocated along cytoskeletal filaments via inter-

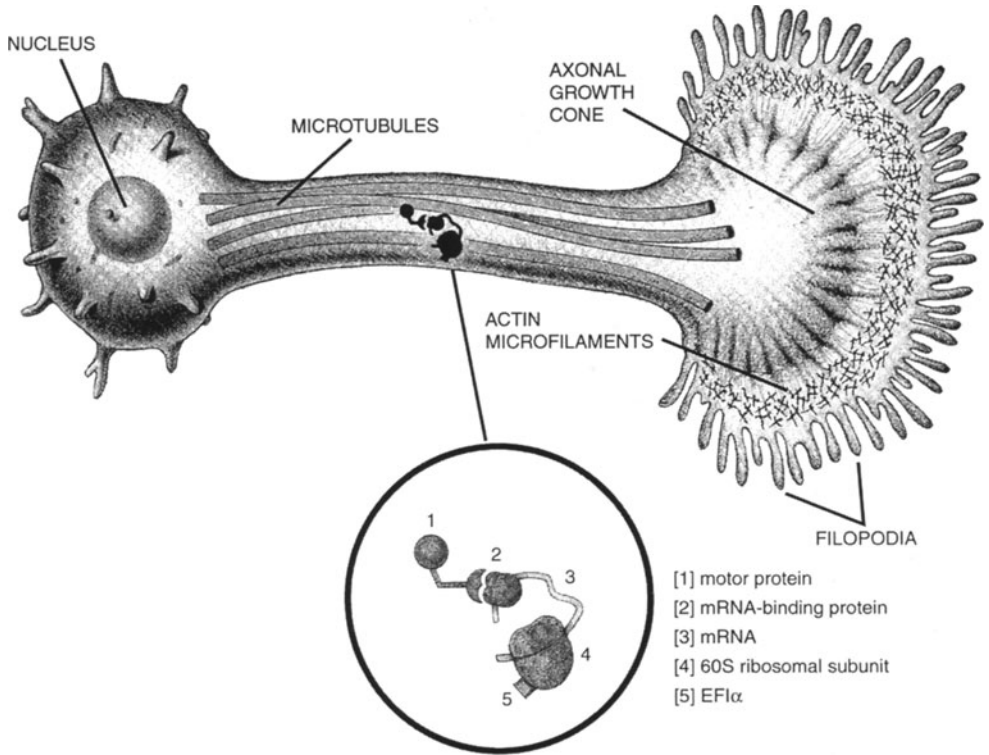
actions of *cis*-acting elements, motor molecules and accessory proteins. In situ hybridization studies have revealed particulate localization patterns for a variety of mRNAs; these patterns include 'island-like structures' of Xlsirt RNA (Kloc et al. 1993), formation of bicoid RNA 'particles' (Ferrandon et al. 1994), 'granules' of MBP mRNA (Ainger et al. 1993) and granules of  $\beta$ -actin mRNA within neuronal growth cones (Fig. 1; Bassell et al. 1998). 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 (Ainger et al. 1993). MBP mRNA granules colocalized with arginyl-tRNA synthetase, elongation factor  $1\alpha$  and rRNA, suggesting the presence of a translational unit (Barbarese et al. 1995). MBP RNA granules were estimated to have a radius of between 0.6 and 0.8  $\mu\text{m}$ , suggesting that RNA granules represent a supramolecular complex that could contain several hundred ribosomes (Barbarese et al. 1995). A complex of six proteins has been identified which interact with the RTS, the most abundant being the RNA binding protein, hnRNPA2 (Hoek et al. 1998).

mRNA transport into neuronal processes has been studied using the vital dye SYTO14; mRNA granules were observed which contained ribosomes and elongation factors (Knowles et al. 1996). RNA granules were translocated into processes at a rate of 0.1  $\mu\text{m}/\text{s}$  which is similar to rates reported for MBP RNA transport in oligodendrocytes (Ainger et al. 1993). Translocation of RNA granules was blocked by microtubule depolymerization. A subset of RNA granules contained  $\beta$ -actin mRNA, suggesting that the active transport of mRNA may play a role in the targeting of newly synthesized actin into neuronal processes. This mechanism is within the range reported for fast transport (Brady and Lasek 1982) and should be more efficient than the slow transport of newly synthesized actin proteins from the cell body, as each transported mRNA molecule could continuously generate new monomers once localized.

*Trans*-acting factors involved in localization of specific mRNA granules within neurons have not yet been elucidated. The mammalian homologue of *Drosophila* Staufen has been shown to be associated with large RNA-containing particles in dendrites (Kiebler et al. 1999). Recently, a Staufen GFP-fusion protein was expressed in cultured hippocampal neurons, allowing analysis of microtubule-dependent particle movements within dendrites (Kohrman et al. 1999).

## 5 Future Directions

Further work is needed to dissect out the molecular components involved in the assembly of  $\beta$ -actin mRNA granules, their transport along microtubules and translational control within growth cones (Fig. 6). Experiments are in progress to identify *cis*-acting localization elements and RNA-binding proteins which may tether an mRNP complex to microtubule-associated motor molecules. It will be interesting to know whether cell type-specific *cis*-acting elements and/or binding proteins exist for localization of  $\beta$ -actin mRNA over long



**Fig. 6.** Proposed model for targeting  $\beta$ -actin mRNA granules to growth cones.  $\beta$ -Actin mRNAs are targeted into developing dendrites and axons in the form of granules which contain translational machinery. These granules may contain multiple mRNA molecules, perhaps other mRNAs in addition to  $\beta$ -actin, which encode proteins involved in growth cone dynamics. RNA granules move and anchor to microtubules. Translation of  $\beta$ -actin mRNA may occur preferentially within the growth cone where it can incorporate into filopodial filaments during directed outgrowth. This targeting pathway may be regulated by neurotrophin signaling to influence actin polymerization and directed outgrowth

distances. Microtubules may represent the 'interstate' 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. Further work may reveal interactions of both actin and microtubule-based motor molecules at the surface of RNA granules. Once the mechanism for  $\beta$ -actin-mRNA targeting is defined, it will be important to perturb it and assess the consequences on growth cone dynamics and process outgrowth.

There has been seminal work in cell biology which documents the critical involvement of cytoskeletal proteins in growth cone motility and axonal growth. It is also apparent that the activation of intracellular signal transduc-

tion pathways by growth factors can have dramatic consequences on the organization of the cytoskeleton, which in turn promotes process outgrowth. Despite the obvious importance of this research and its implications for the development of the nervous system, the mechanism for targeting cytoskeletal machinery in processes remains ill-defined, and it is unclear whether the slow transport of proteins from the cell body is sufficient to respond to the changing needs of the growth cone. An mRNA localization mechanism would be more efficient than having to transport each protein molecule from the perikarya, as each localized mRNA could generate thousands of proteins directly within the growth cone. Localized mRNAs could be translated at their site of localization, providing immediate assembly of structural components where they are needed, at the site of membrane extrusion. RNA granules may represent a novel cytoplasmic organelle capable of localizing specific mRNAs and the necessary translational machinery to maintain an asymmetric distribution of cytoskeletal proteins.

**Acknowledgements.** We thank our colleagues who contributed to these studies: Honglai Zhang, Andrea Femino, Larry Lifshitz, Ira Herman, and Kenneth Kosik. These studies were supported, in part, by March of Dimes and Muscular Dystrophy Foundation awards (G.J.B.), NSF IBN9811384 (G.J.B.), NIH GM55599 (G.J.B.), and GM 54887 (R.H.S.).

## References

- Ainger K, Avossa D, Morgan F, Hill SJ, Barry C, Barbarese E, Carson JH (1993) Transport and localization of exogenous MBP mRNA microinjected into oligodendrocytes. *J. Cell Biol* 123:431–441
- Ainger KA, Avossa D, Diana AS, Barry C, Barbarese E, Carson JH (1997) Transport and localization elements in MBP mRNA. *J Cell Biol* 138:1077–1087
- Bamburg JR, Bray D (1987) Distribution and cellular localization of actin depolymerizing factor. *J Cell Biol* 105:2817–2825
- Barbarese E, Koppel DE, Deutscher MP, Smith CL, Ainger K, Morgan F, Carson JH (1995) Protein translation components are colocalized in granules in oligodendrocytes. *J Cell Sci* 108:2781–2790
- Bassell GJ, Zhang HL, Byrd AL, Femino AM, Singer RH, Taneja KL, Lifshitz LM, Herman IM, Kosik KS (1998) Sorting of beta actin mRNA and protein to neurites and growth cones in culture. *J Neurosci* 18:251–265
- Bassell GJ, Oleynikov Y, Singer RH (1999) The travels of mRNA through all cells large and small. *FASEB J* 13(3):447–454 [see Comment in *FASEB J* 13(3):419–420]
- Behar L, Marx R, Sadot E, Barg J, 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
- Bentley D, Toroian-Raymond A (1986) Disoriented pathfinding by pioneer neurone growth cones deprived of filopodia. *Nature* 323:712–715
- Binder LI, Frankfurter A, Rebhun LI (1985) The distribution of tau in the mammalian central nervous system. *J Cell Biol* 101:1371–1378
- Blichenberg A, Schwanke B, Rehbein M, Garner CC, Richter D, Kindler S (1999) Identification of a *cis*-acting dendritic targeting element in MAP2 mRNAs. *J Neurosci* 19:8818–8829
- Brady ST, Lasek RJ (1982) Axonal transport: a cell biological method for studying proteins that associate with the cytoskeleton. *Methods Cell Biol* 25:326–398

- Brion JP, Guillemot J, Couchie D, Flament J, Nunez J (1988) Both adult and juvenile tau proteins are axon specific in the developing and adult cerebellum. *Neuroscience* 25:139–146
- Bruckenstein DA, Lein PJ, Higgins D, Fremeau RT (1990) Distinct spatial localization of specific mRNAs in cultured sympathetic neurons. *Neuron* 5:809–819
- Carson JH, Worboys K, Ainger K, Barbarese E (1997) Translocation of MBP mRNA in oligodendrocytes requires microtubules and kinesin. *Cell Mot Cyto* 38:318–328
- Crino PB, Eberwine J (1997) Molecular characterization of the dendritic growth cone. *Neuron* 17:1173–1187
- Crispino M, Capano C, Kaplan B, Giuditta A (1993a) Neurofilament proteins are synthesized in nerve endings in squid brain. *J Neurochem* 61:1144–1146
- Crispino M, Martin R, Giuditta A (1993b) Protein synthesis in a synaptosomal fraction from squid brain. *Mol Cell Neurosci* 4:366–374
- Crispino M, Martin R, Giuditta A (1997) Active polysomes are present in presynaptic endings from squid brain. *J Neurosci* 17:7694–7702
- Davis L, Ping D, DeWitt M, Kater SB (1992) Protein synthesis within neuronal growth cones. *J Neurosci* 12:4867–4877
- Deitch JS, Banker GA (1993) An electron microscopic analysis of hippocampal neurons developing in culture: early stages in the emergence of polarity. *J Neurosci* 13:4301–4315
- Deshler JO, Hightett MI, Abramson T, Schnapp BJ (1998) A highly conserved RNA-binding protein for cytoplasmic mRNA localization in vertebrates. *Curr Biol* 8(9):489–96
- Elisha Z, Havin L, Ringel I, Yisraeli JK (1995) Vg1 RNA binding protein mediates the association of Vg1 RNA with microtubules in *Xenopus* oocytes. *EMBO J* 14:5109–5114
- Fay FS, Carrington W, Fogarty KE (1989) Three-dimensional molecular distribution in single cells analyzed using the digital imaging microscope. *J Microsc (Oxf)* 153:133–149
- Ferrandon D, Elphick L, Nusslein-Volhard C, St Johnston D (1994) Staufen protein associates with the 3'UTR of *bicoid* mRNA to form particles that move in a microtubule-dependent manner. *Cell* 79:1221–1232
- Ferreira A, Busciglio J, Caceres A (1987) Microtubule formation and neurite outgrowth in cerebellar macroneurons which develop in vitro: involvement of MAP1a, HMW-MAP2 and tau. *Dev Brain Res* 34:9–31
- Forscher P, Smith SJ (1988) Actions of cytochalasins on the organization of actin filaments and microtubules in a neuronal growth cone. *J Cell Biol* 107:1505–1516
- Garner CC, Tucker RP, Matus A (1988) Selective localization of mRNA for cytoskeletal protein MAP2 in dendrites. *Nature* 336:674–679
- Hannan AJ, Schevzov G, Gunning P, Jeffrey PL, Weinberger RP (1995) Intracellular localization of tropomyosin mRNA and protein is associated with development of neuronal polarity. *Mol Cell Neurosci* 6:397–412
- Havin L, Git A, Elisha Z, Oberman F, Yaniv K, Schwartz SP, Standart N, Yisraeli JK (1998) RNA-binding protein conserved in both microtubule- and microfilament-based RNA localization. *Genes Dev* 12(11):1593–8
- Hoek KS, Kidd GJ, Carson JH, Smith R (1998) hnRNP A2 selectively binds the cytoplasmic transport sequence of myelin basic protein mRNA. *Biochemistry* 37(19):7021–9
- Herman IM, D'Amore PA (1985) Microvascular pericytes contain muscle and nonmuscle actins. *J Cell Biol* 101:43–52
- Hook TC, Newcomb PM, Herman IM (1991) Beta-actin and its mRNA are localized at the plasma membrane and the regions of moving cytoplasm during the cellular response to injury. *J Cell Biol* 112:653–664
- Kaplan B, Gioio AE, Perrone-Capano C, Crispino M, Giuditta A (1992)  $\beta$ -actin and  $\beta$ -tubulin are components of a heterogeneous mRNA population present in the giant squid axon. *Mol Cell Neurosci* 3:133–144
- Kiebler MA, Hemraj I, Verkade P, Kohrman M, Fortes P, Marion RM, Ortin J, Dotti CG (1999) The mammalian Staufen protein localizes to the somatodendritic domain of cultured hippocampal neurons: implications for its involvement in mRNA transport. *J Neurosci* 19:288–297

- Kindler S, Muller R, Chung WJ, Garner CC (1996) Molecular characterization of dendritically localized transcripts encoding MAP2. *Mol Brain Res* 36:63–69
- Kislauskis EH, Singer RH (1992) Determinants of mRNA localization. *Curr Opin Cell Biol* 4:975–978
- Kislauskis EH, Li Z, Singer RH, Taneja KL (1993) Isoform-specific 3'-untranslated sequences sort  $\alpha$ -cardiac and  $\beta$ -cytoplasmic actin messenger RNAs to different cytoplasmic compartments. *J Cell Biol* 123:165–172
- Kislauskis EH, Zhu X, Singer RH (1994) Sequences responsible for intracellular localization of  $\beta$ -actin messenger RNA also affect cell phenotype. *J Cell Biol* 127:441–451
- Kislauskis E, Zhu X, Singer R (1997)  $\beta$ -actin messenger RNA localization and protein synthesis augment cell motility. *J Cell Biol* 136:1263–1270
- Kleiman R, Banker G, Steward O (1990) Differential subcellular localization of particular mRNAs in hippocampal neurons in culture. *Neuron* 5:821–830
- Kleiman R, Banker G, Steward O (1994) Development of subcellular mRNA compartmentation in hippocampal neurons in culture. *J Neurosci* 14:1130–1140
- Kloc M, Etkin LD (1995) Two distinct pathways for the localization of RNAs at the vegetal cortex in *Xenopus* oocytes. *Development* 121:289–297
- Kloc M, Spohr G, Etkin LD (1993) Translocation of repetitive RNA sequences with the germ plasm in *Xenopus* oocyte. *Science* 262:1712–1714
- Knowles RB, Kosik KS (1997) Neurotrophin-3 signals redistribute RNA in neurons. *Proc Natl Acad Sci USA* 94:14804–14808
- Knowles RB, Sabry JH, Martone MA, Ellisman M, Bassell GJ, Kosik KS (1996) Translocation of RNA granules in living neurons. *J Neurosci* 16:7812–7820
- Koenig E (1989) Cycloheximide sensitive methionine labeling of proteins in goldfish retinal ganglion cell axons in vitro. *Brain Res* 481:119–123
- Koenig E (1991) Evaluation of local synthesis of axonal proteins in the goldfish Mauthner cell axon and axons of dorsal and ventral roots of the rat in vitro. *Mol Cell Neurosci* 2:384–394
- Koenig E, Adams P (1982) Local protein synthesizing activity in axonal fields regenerating in vitro. *J Neurochem* 39:386–400
- Kohrman M, Lui M, Kaether C, DesGroseillers L, Dotti CG, Kiebler MA (1999) Microtubule dependent recruitment of Staufen-GFP into large RNA-containing granules and dendritic transport in living hippocampal neurons. *Mol Biol Cell* 10:2945–2953
- Lasek RJ (1986) Polymer sliding in axons. *J Cell Sci* 5:161–179
- Latham VM, Kislauskis EH, Singer RH, Ross AF (1994) Beta-actin mRNA localization is regulated by signal transduction mechanisms. *J Cell Biol* 126:1211–1219
- Litman P, Barg J, Rindzoonki L, Ginzburg I (1993) Subcellular localization of tau mRNA in differentiating neuronal cell culture: implications for neuronal polarity. *Neuron* 10:627–638
- Litman P, Behar L, Elisha Z, Yisraeli JK, Ginzburg I (1996) Exogenous tau RNA is localized in oocytes: possible evidence for evolutionary conservation of localization mechanisms. *Dev Biol* 176:86–94
- Marsden KM, Doll T, Ferralli J, Botteri F, Matus A (1996) Transgenic expression of embryonic MAP2 in adult mouse brain: implications for neuronal polarization. *J Neurosci* 16:3265–3273
- Mayford M, Baranes D, Podsypanina K, Kandel ER (1996) The 3'-untranslated region of CaMKII $\alpha$  is a *cis*-acting signal for the localization and translation of mRNA in dendrites. *Proc Natl Acad Sci USA* 93:13250–13255
- Mercken M, Fischer I, Kosik KS, Nixon RA (1995) Three distinct axonal transport rates for tau, tubulin and other MAPs. *J Neurosci* 15:8259–8267
- Mowry KL, Melton DA (1992) Veg-1 RNA localization directed by a 340 nt RNA sequence element in *Xenopus* oocytes. *Science* 255:991–994
- Munro TP, Magee RJ, Kidd GJ, Carson JH, Barbarese E, Smith LM, Smith R (1999) Mutational analysis of a heterogeneous nuclear ribonucleoprotein A2 response element for RNA trafficking. *J Biol Chem* 274:34389–34395
- Muslimov IA, Santi I, Perini S, Higgins D, Tiedge H (1997) RNA transport in dendrites: a *cis*-acting signal within BC1 RNA. *J Neurosci* 17:4722–4733

- Nixon RA (1987) The axonal transport of cytoskeletal proteins: a reappraisal. In: Bisby MA, Smith RS (eds) Axonal transport. Liss, New York, pp 175–200
- Okabe S, Hirokawa N (1990) Turnover of fluorescently labeled tubulin and actin in the axon. *Nature* 343:479–482
- Okabe S, Hirokawa N (1991) Actin dynamics in growth cones. *J Neurosci* 11:1918–1929
- Olink-Coux M, Hollenbeck PJ (1996) Localization and active transport of mRNA in axons of sympathetic neurons. *J Neurosci* 16:1346–1358
- Otey CA, Lessard JL, Bulinski JC (1986) Immunolocalization of the gamma isoform of nonmuscle actin. *J Cell Biol* 102:1732–1737
- Paves H, Saarma M (1997) Neurotrophins as in vitro guidance molecules for embryonic sensory neurons. *Cell Tissue Res* 290:285–297
- Peng A, Binder L, Black MM (1986) Biochemical and immunological analysis of cytoskeletal domains of neurons. *J Cell Biol* 102:252–262
- Pilkis SJ, Steiner DF, Heinrikson RL (1980) Phosphorylation of rat pyruvate kinase. *JBC* 255:2770–2775
- Ross A, Oleynikov Y, Kislauskis E, Taneja K, Singer R (1997) Characterization of a  $\beta$ -actin mRNA zipcode-binding protein. *Mol Cell Biol* 17:2158–2165
- Sabry J, O'Connor TP, Kirschner MW (1995) Axonal transport of tubulin in T11 pioneer neurons. *Neuron* 14:1247–1256
- Shuster CB, Herman IM (1995) Indirect association of ezrin with F-actin: isoform specificity and calcium sensitivity. *J Cell Biol* 128:837–848
- St Johnston D (1995) The intracellular localization of messenger RNAs. *Cell* 81:161–170
- Sundell CL, Singer RH (1991) Requirement of microfilaments in sorting of actin mRNAs. *Science* 253:1275–1277
- Takeda S, Hirokawa N (1995) Tubulin dynamics in neuronal axons. *Neuron* 14:1257–1264
- Tanaka E, Sabry J (1995) Cytoskeletal rearrangements during growth cone guidance. *Cell* 83:171–176
- Tanaka E, Ho T, Kirschner M (1995) The role of microtubule dynamics in growth cone motility and axonal growth. *J Cell Biol* 128:139–155
- Tennyson VM (1970) The fine structure of the axon and growth cone of the dorsal root neuroblast of the rabbit embryo. *J Cell Biol* 44:62–78
- Van Minnen J (1994) RNA in the axonal domain: a new dimension in neuronal functioning? *Histochem J* 26:377–391
- Von Arx P, Bantle S, Soldati T, Perriard J (1995) Dominant negative effect of cytoplasmic actin isoproteins on cardiomyocyte cytoarchitecture and function. *J Cell Biol* 131:1759–1773
- Walsh DA, Van Patten SM (1994) Multiple pathway signal transduction by cAMP dependent protein kinase. *FASEB J* 8:1227–1236
- Yao X, Forte JG (1995) Polarized distribution of actin isoforms in gastric parietal cells. *Mol Biol Cell* 6:541–557
- Yisraeli JK, Sokol S, Melton DA (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
- Zhang HL, Singer RH, Bassell GJ (1999) Neurotrophin regulation of beta-actin mRNA and protein localization to neuronal growth cones. *J Cell Biol* 147:59–70