RNA Localization and Signal Transduction

Vaughan Latham[1,2] and Robert H. Singer[1]
[1]*Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx, New York*
[2]*Dana-Farber Cancer Institute, Boston, Massachusetts*

Introduction

Sorting of mRNA to specific compartments of the cell determines cell asymmetry. This sorting occurs in oocytes and embryos as well as somatic cells such as fibroblasts and neurons (for reviews see [1–6]). Translation of localized mRNAs spatially directs protein synthesis. The cellular signals that direct specific RNA sequences to particular cellular compartments have recently been examined in fibroblasts, neurons, and *Drosophila* embryos. This chapter examines the regulation of mRNA localization through signal transduction pathways in organisms and their tissues.

Growth Factors Induce mRNA Localization

In chicken embryo fibroblasts (CEFs), β-actin mRNA has been shown to be localized toward the leading edge where it plays a role in cell motility and asymmetry [7,8]. A specific sequence in the 3′ untranslated region (UTR) of the β-actin mRNA, termed the *zipcode* [9], is necessary for the localization of the mRNA and specific *trans*-acting factors, termed *zipcode binding proteins* [10]. Growth factors can affect the site of synthesis of β-actin in the cytoplasm by inducing rapid localization of β-actin mRNA toward the leading edge in CEFs [11,12]. This localization can be induced after serum starvation by serum as well as growth factors such as LPA and PDGF and is inhibited by tyrosine kinase inhibitors herbimycin and genistein [11].

β-Actin mRNA localization can also be induced in growth cones of forebrain neurons by neurotrophin-3 (NT-3), forskolin, or db-cAMP [13]. Growth factors can act on growth cones to induce path finding in neuronal development [14]. In another study, NT-3 also induced localization of mRNA granules into dendrites and was inhibited by K252a, an inhibitor of tyrosine kinase receptors [15]. Brain-derived neurotrophic factor (BDNF) induced dendritic mRNA localization and translation of a reporter GFP with the 5′ and 3′UTR of CAMKII-α [16]. Growth factors such as NT-3 and BDNF may induce the movement of mRNAs into dendrites and induce the localized translation of these mRNAs at their target cellular compartment and affect changes in actin cytoskeletal reorganization within growth cones.

Tetanic stimulation of hippocampal neurons induces an increase in the concentration of CaMKII-α in the dendrites of postsynaptic neurons within 5 min after a tetanus [17]. This is the result of local synthesis on CaMKII mRNA within dendrites rather than the slower transport of CaMKII protein and was blocked by protein synthesis inhibitors [17–19]. In the case of *Arc* mRNA being targeted to active synapses, NMDA receptor activation is necessary for this localization [18,20]. The expression of *Arc* mRNA was induced by electroconvulsive seizure, and newly synthesized *Arc* mRNA was targeted to synapses in the dentate gyrus, but not when NMDA receptor antagonists were present [20]. Ca^{2+} signaling may be also be involved in localization and subsequent translation as binding of the growth factors to their receptors induced tyrosine phosphorylation [21] and subsequent increase in intracellular Ca^{2+} [22]. Increased $[Ca^{2+}]$ may induce stability of some mRNAs and subsequent translation [23,24].

Copyright © 2003, Elsevier Science (USA).
All rights reserved.

In *Drosophila* oogenesis the localization of mRNA when translated is a signal to surrounding cells. *Gurken* mRNA is localized in the posterior oocyte cortex and then translated into an EGF similar growth factor, which then induces the follicle cells to form the polarity of the egg chamber [25,26]. This localization of *Gurken* mRNA is necessary for signaling via the EGF receptor pathway, as *maelstrom* mutants that do not localize the mRNA develop follicle cells with anterior rather than posterior fates [27]. A similar localization of the mRNA of *wingless* determines the apical localization of the signaling protein [28,29].

Signaling from the Extracellular Matrix Induces mRNA Localization

Another cellular signaling system is that from the extracellular matrix, usually integrins [30]. A study using microbeads coated with focal adhesion complex (FAC) proteins such as β_1 integrin talin, vinculin, and the RGD peptide induced "halos" of poly $(A)^+$ RNA and ribosomes within a few minutes [31]. These are presumably the mRNAs of FAC proteins although this was not determined. These halos also formed when tension was exerted on adhered beads.

mRNAs Localized via the Cytoskeleton

Numerous investigations have examined which cytoskeletal components are involved in localizing mRNA. In fibroblasts it appears that the actin cytoskeleton is involved in localizing β-actin mRNA. Early work demonstrated that β-actin mRNA was not localized in the presence of cytochalasin D, but was localized with colchicine [32]. More recently the rapid (<5 min) localization induced by serum or growth factors was shown to be inhibited by cytochalasin D, but not nocodazole [33]. Conversely, in neurons, mRNA localization is predominantly microtubule based and is inhibited by colchicine but not cytochalasin D [4].

mRNA Granule Movement in Neurons

Movement of mRNA in neurons was first tracked with ^{3}H-uridine in pulse-chase experiments [34]. More recently a membrane-permeable nucleic acid stain, SYTO-14, has been used to track mRNA movement in living cells [35]. Messenger RNA granules move into neurites within 15 min after NT-3 stimulation [15]. These granules are enriched in the RNA binding protein Staufen [36,37] and are complexed with polyribosomes [38]. After depolarization, mRNAs shift to a less dense granule fraction but are not translationally competent [38]. The movement of mRNA granules is responsive to neurotrophins in neurons [39] and growth factors in fibroblasts [11,12].

Regulation of mRNA Localizing Proteins

Many proteins necessary for localization of mRNAs such as ZBP1 [10], ZBP2 [40] in fibroblasts and neurons, and Egalitarian (Egl) and BicaudalD (Bic D) in *Drosophila* [41] may be targets of signal transduction pathways regulating mRNA localization. These proteins may be directly phosphorylated through signaling pathways or through their interactions with motor molecules such as myosins, dyneins, or kinesins. One such protein, zipcode binding protein 1, is required for the localization of β-actin mRNA to growth cones [39]. Neurons transfected with EGFP-ZBP exhibited rapid bidirectional movements of granules that required ZBP1 binding to RNA.

GTPase Signals Regulating Actomyosin Interactions Are Involved in mRNA Localization

β-Actin mRNA localization near the leading edge in CEFs is dependent on the actin cytoskeleton and not the microtubule-based cytoskeleton [32]. Similarly the rapid induction of β-actin mRNA localization by serum and growth factors is also dependent on actin and not microtubules [33]. Consistent with the Rho GTPases regulation of the actin cytoskeleton [42,43], β-actin mRNA localization at the leading edge has been shown to be regulated by the Rho GTPase [33]. This signaling pathway goes through the Rho-associated kinases, because the specific inhibitorY-27632 inhibited localization and transfected Rho-kinase constructs induced localization. Because β-actin mRNA localization can lead to the development of cell polarity [7,44], this would allow for signaling regulation of cell polarity. Similarly in *Drosophila*, Rho-associated kinase (Drock) regulates planar cell polarity through the actin cytoskeleton [45]. Also in *Drosophila*, Rho regulatory proteins such as rhoGEF may play a role in the cytoskeletal changes during development [46] and may be involved in mRNA localization. The Rho signaling pathway may involve the formin protein p140mDia [47]. Because mDia can regulate the cytoskeleton through stabilization of microtubules [48] and the actin stress fibers [49,50] it could participate in mRNA localization on either actin or microtubules perhaps via anchoring of RNA by EF1α [51].

Cytoskeletal motors involved in mRNA localization have been demonstrated during development in *Xenopus* [52], and Drosophila and more recently a myosin V has been found to be involved in *ASH 1* mRNA localization in budding yeast [53–58]. Some studies using pharmacological inhibition of myosin showed inhibition of β-actin mRNA localization to the leading edge [33], and the inhibition of poly$(A)^+$ RNA movement into "halos" around integrin-coated beads with myosin ATPase inhibitor BDM [31] and the myosin light chain kinase inhibitor ML-7 [33]. Because Rho and Rho-kinase can regulate myosin phosphatase by inhibiting the phosphatase in the presence of ongoing myosin light chain kinase (MLCK) activity [59,60], the induction of mRNA localization by activation of this

pathway acts through myosin. Recently mouse embryo fibroblasts (MEFs) from myosin IIB knock-out mice were shown to be deficient in localizing β-actin mRNA to the leading edge [33]. MEFs from homozygous knock-out mice showed no movement of β-actin mRNA to the leading edge, whereas wild-type and the heterozygous MEFs responded within minutes [33]. The two-headed myosin II filaments can translocate on polarized bundles of actin filaments or stress fibers toward the leading edge associated with an mRNA complex that can bind the myosin. These actin bundles have a polarity with barbed ends increasingly directed toward the lamellipodium and thus could constrain activated myosin II-B movement only toward the leading edge [61,62]. Rho kinase can lead to phosphorylation of the light chains internally near the nucleus, where myosin filament assembly, stress fiber formation, and motility occur [63]. Growth cones from IIB knock-out mice neurons have been analyzed and showed differences in growth cone shape, actin organization, and reduced filipodial traction force [64], as does a localized knockout of myosin IIB using lazer inactivation.

Conclusion

There is no question now that motors move localized RNAs to their ultimate destination in the periphery of the polarized cell. This then allows proteins to be sorted asymmetrically to the specific region of the cell where they can exert their functionality. But this movement of the RNA is in response to the polarized nature of the asymmetric cell and is not causal for the asymmetry. The synthesis of specific protein components at the periphery enhances and stabilizes the polarity of the cell. How this polarity all gets established is a result of the signaling molecules impinging on the cell directionally from the outside. The transduction of these signals into polarity, through actin polymerization, for instance, provides a substrate for the trafficking of the RNA. In this way, the extracellular environment determines the distribution of specific protein synthesis within the cell. Figure 1 summarizes this process for asymmetric cells.

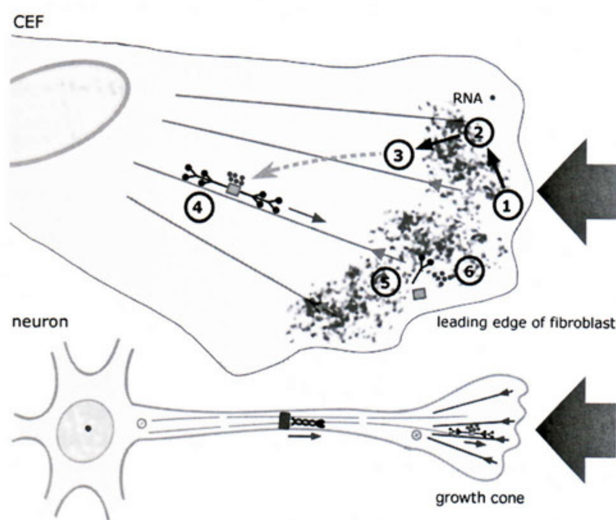

Figure 1 Model for how mRNA may move in response to a signal transduction mechanism on polarized actin bundles, associated with myosin II-B in fibroblasts (top) or with kinesin in neurons (bottom). Incoming signals (arrow) binding receptors (1) activate the RhoA cascade (2) through Rho-kinase (3), which activates myosin II-B light chains and myosin assembly with an mRNA complex. (4) The complex moves over polarized actin bundles to disassemble toward the leading edge as a result of heavy chain phosphorylation (5) and the RNA then anchors (6). The speed of myosin movement predicts that the mRNA would only be transported for 20 sec before it becomes anchored. Hence the steady-state distribution of the mRNA population would be at the lamella [33]. For neurons, an analogous cascade would be initiated by neurotrophins, possibly leading to the activation of the kinesin light chains. Kinesin would then move the ZBP1-RNA complex down the process and then onto microfilament bundles in the growth cone where myosin would take over [39].

References

1. Palacios, I. M. and Johnston, D. S. (2001). Getting the message across: The intracellular localization of mRNAs in higher eukaryotes. *Annu. Rev. Cell Dev. Biol.* **17**, 569–614.
2. Job, C. and Eberwine, J. (2001). Localization and translation of mRNA in dendrites and axons. *Nat. Rev. Neurosci.* **2**, 889–898.
3. Steward, O. and Schuman, E. M. (2001). Protein synthesis at synaptic sites on dendrites. *Annu. Rev. Neurosci.* **24**, 299–325.
4. Bassell, G. J. and Singer, R. H. (2001). Neuronal RNA localization and the cytoskeleton. *Results Probl. Cell Differ.* **34**, 41–56.
5. Hazelrigg, T. (1998). The destinies and destinations of RNAs. *Cell* **95**, 451–460.
6. Kloc, M., Zearfoss, N. R., and Etkin, L. D. (2002). Mechanisms of subcellular mRNA localization. *Cell* **108**, 533–544.
7. Kislauskis, E. H., Zhu, X., and Singer, R. H. (1997). β-Actin messenger RNA localization and protein synthesis augment cell motility. *J. Cell Biol.* **136**, 1263–1270.
8. Kislauskis, E. H., Zhu, X., and Singer, R. H. (1994). Sequences responsible for intracellular localization of beta-actin messenger RNA also affect cell phenotype. *J. Cell Biol.* **127**, 441–451.
9. Kislauskis, E. H. and Singer, R. H. (1992). Determinants of mRNA localization. *Curr. Opin. Cell Biol.* **4**, 975–978.
10. Ross, A. F., Oleynikov, Y., Kislauskis, E. H., Taneja, K. L., and Singer, R. H. (1997). Characterization of a beta-actin mRNA zipcode-binding protein. *Mol. Cell Biol.* **17**, 2158–2165.
11. Latham, V. M., Jr., 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.
12. 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**, 1221–1229.
13. Zhang, H. L., Singer, R. H., and Bassell, G. J. (1999). Neurotrophin regulation of beta-actin mRNA and protein localization within growth cones. *J. Cell Biol.* **147**, 59–70.
14. Song, H. J. and Poo, M. M. (1999). Signal transduction underlying growth cone guidance by diffusible factors. *Curr. Opin. Neurobiol.* **9**, 355–363.
15. Knowles, R. B. and Kosik, K. S. (1997). Neurotrophin-3 signals redistribute RNA in neurons. *Proc. Natl. Acad. Sci. USA* **94**, 14804–14808.
16. Aakalu, G., Smith, W. B., Nguyen, N., Jiang, C., and Schuman, E. M. (2001). Dynamic visualization of local protein synthesis in hippocampal neurons. *Neuron* **30**, 489–502.
17. Ouyang, Y., Rosenstein, A., Kreiman, G., Schuman, E. M., and Kennedy, M. B. (1999). Tetanic stimulation leads to increased accumulation of Ca(2+)/calmodulin-dependent protein kinase II via dendritic protein synthesis in hippocampal neurons. *J. Neurosci.* **19**, 7823–7833.
18. Steward, O. and Worley, P. F. (2001). A cellular mechanism for targeting newly synthesized mRNAs to synaptic sites on dendrites. *Proc. Natl. Acad. Sci. USA* **98**, 7062–7068.

19. Steward, O. and Worley, P. (2001). Localization of mRNAs at synaptic sites on dendrites. *Results Probl. Cell Differ.* **34**, 1–26.
20. Steward, O. and Worley, P. F. (2001). Selective targeting of newly synthesized Arc mRNA to active synapses requires NMDA receptor activation. *Neuron* **30**, 227–240.
21. Ip, N. Y., Li, Y., Yancopoulos, G. D., and Lindsay, R. M. (1993). Cultured hippocampal neurons show responses to BDNF, NT-3, and NT-4, but not NGF. *J. Neurosci.* **13**, 3394–3405.
22. Gao, F. B. (1998). Messenger RNAs in dendrites: Localization, stability, and implications for neuronal function. *Bioessays* **20**, 70–78.
23. Berninger, B., Garcia, D. E., Inagaki, N., Hahnel, C., and Lindholm, D. (1993). BDNF and NT-3 induce intracellular Ca2+ elevation in hippocampal neurones. *Neuroreport* **4**, 1303–1306.
24. Nikcevic, G., Perhonen, M., Boateng, S. Y., and Russell, B. (2000). Translation is regulated via the 3′ untranslated region of alpha-myosin heavy chain mRNA by calcium but not by its localization. *J. Muscle Res. Cell Motil.* **21**, 599–607.
25. Roth, S., Neuman-Silberberg, F. S., Barcelo, G., and Schupbach, T. (1995). cornichon and the EGF receptor signaling process are necessary for both anterior-posterior and dorsal-ventral pattern formation in *Drosophila. Cell* **81**, 967–978.
26. Gonzalez-Reyes, A., Elliott, H., and St. Johnston, D. (1995). Polarization of both major body axes in *Drosophila* by gurken-torpedo signalling. *Nature* **375**, 654–658.
27. Clegg, N. J., Frost, D. M., Larkin, M. K., Subrahmanyan, L., Bryant, Z., and Ruohola-Baker, H. (1997). Maelstrom is required for an early step in the establishment of *Drosophila* oocyte polarity: Posterior localization of grk mRNA. *Development* **124**, 4661–4671.
28. Simmonds, A. J., dosSantos, G., Livne-Bar, I., and Krause, H. M. (2001). Apical localization of wingless transcripts is required for wingless signaling. *Cell* **105**, 197–207.
29. Manseau, L. J. (2001). RNA localization meets wingless signaling. *Sci. STKE* **2001**, PE1.
30. Schwartz, M. A. (2001). Integrin signaling revisited. *Trends Cell Biol.* **11**, 466–470.
31. 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* **392**, 730–733.
32. Sundell, C. L. and Singer, R. H. (1991). Requirement of microfilaments in sorting of actin messenger RNA. *Science* **253**, 1275–1277.
33. Latham, V. M., Yu, E. H., Tullio, A. N., Adelstein, R. S., and Singer, R. H. (2001). A Rho-dependent signaling pathway operating through myosin localizes beta-actin mRNA in fibroblasts. *Curr. Biol.* **11**, 1010–1016.
34. Davis, L., Banker, G. A., and Steward, O. (1987). Selective dendritic transport of RNA in hippocampal neurons in culture. *Nature* **330**, 477–479.
35. 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.
36. Kiebler, M. A., Hemraj, I., Verkade, P., Kohrmann, M., Fortes, P., Marion, R. M., Ortin, J., and Dotti, C. G. (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.
37. Kohrmann, M., Luo, M., Kaether, C., DesGroseillers, L., Dotti, C. G., and Kiebler, M. A. (1999). Microtubule-dependent recruitment of Staufen-green fluorescent protein into large RNA-containing granules and subsequent dendritic transport in living hippocampal neurons. *Mol. Biol. Cell* **10**, 2945–2953.
38. Krichevsky, A. M. and Kosik, K. S. (2001). Neuronal RNA granules: A link between RNA localization and stimulation-dependent translation. *Neuron* **32**, 683–696.
39. Zhang, H. L., Eom, T., Oleynikov, Y., Shenoy, S. M., Liebelt, D. A., Dictenberg, J. B., Singer, R. H., and Bassell, G. J. (2001). Neurotrophin-induced transport of a beta-actin mRNP complex increases beta-actin levels and stimulates growth cone motility. *Neuron* **31**, 261–275.
40. Gu, W., Pan, F., Zhang, H., Bassell, G. J., and Singer, R. H. (2002). A predominantly nuclear protein affecting cytoplasmic localization of beta-actin mRNA in fibroblasts and neurons. *J. Cell Biol.* **156**, 41–51.
41. Bullock, S. L. and Ish-Horowicz, D. (2001). Conserved signals and machinery for RNA transport in *Drosophila* oogenesis and embryogenesis. *Nature* **414**, 611–616.
42. Hall, A. (1998). Rho GTPases and the actin cytoskeleton. *Science* **279**, 509–514.
43. Ridley, A. J. (2001). Rho family proteins: Coordinating cell responses. *Trends Cell Biol.* **11**, 471–477.
44. Shestakova, E. A., Singer, R. H., and Condeelis, J. (2001). The physiological significance of beta-actin mRNA localization in determining cell polarity and directional motility. *Proc. Natl. Acad. Sci. USA* **98**, 7045–7050.
45. Winter, C. G., Wang, B., Ballew, A., Royou, A., Karess, R., Axelrod, J. D., and Luo, L. (2001). *Drosophila* Rho-associated kinase (Drok) links Frizzled-mediated planar cell polarity signaling to the actin cytoskeleton. *Cell* **105**, 81–91.
46. Werner, L. A. and Manseau, L. J. (1997). A *Drosophila* gene with predicted rhoGEF, pleckstrin homology and SH3 domains is highly expressed in morphogenic tissues. *Gene* **187**, 107–114.
47. Watanabe, N., Madaule, P., Reid, T., Ishizaki, T., Watanabe, G., Kakizuka, A., Saito, Y., Nakao, K., Jockusch, B. M., and Narumiya, S. (1997). p140mDia, a mammalian homolog of *Drosophila* diaphanous, is a target protein for Rho small GTPase and is a ligand for profilin. *EMBO J.* **16**, 3044–3056.
48. Palazzo, A. F., Cook, T. A., Alberts, A. S., and Gundersen, G. G. (2001). mDia mediates Rho-regulated formation and orientation of stable microtubules. *Nat. Cell Biol.* **3**, 723–729.
49. Nakano, K., Takaishi, K., Kodama, A., Mammoto, A., Shiozaki, H., Monden, M., and Takai, Y. (1999). Distinct actions and cooperative roles of ROCK and mDia in Rho small G protein-induced reorganization of the actin cytoskeleton in Madin-Darby canine kidney cells. *Mol. Biol. Cell* **10**, 2481–2491.
50. Satoh, S. and Tominaga, T. (2001). mDia-interacting protein acts downstream of Rho-mDia and modifies Src activation and stress fiber formation. *J. Biol. Chem.* **276**, 39290–39294.
51. Liu, G., Grant, W. M., Persky, D., Latham, V. M., Jr., Singer, R. H., and Condeelis, J. (2002). Interactions of elongation factor 1alpha with F-actin and beta-actin mRNA: Implications for anchoring mRNA in cell protrusions. *Mol. Biol. Cell* **13**, 579–592.
52. Bashirullah, A., Cooperstock, R. L., and Lipshitz, H. D. (1998). RNA localization in development. *Annu. Rev. Biochem.* **67**, 335–394.
53. Kwon, S. and Schnapp, B. J. (2001). RNA localization: SHEdding light on the RNA-motor linkage. *Curr. Biol.* **11**, R166–R168.
54. 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.
55. Long, R. M., Gu, W., Lorimer, E., Singer, R. H., and Chartrand, P. (2000). She2p is a novel RNA-binding protein that recruits the Myo4p-She3p complex to ASH1 mRNA. *EMBO J.* **19**, 6592–6601.
56. Bertrand, E., Chartrand, P., Schaefer, M., Shenoy, S. M., Singer, R. H., and Long, R. M. (1998). Localization of ASH1 mRNA particles in living yeast. *Mol. Cell* **2**, 437–445.
57. Takizawa, P. A. and Vale, R. D. (2000). The myosin motor, Myo4p, binds Ash1 mRNA via the adapter protein, She3p. *Proc. Natl. Acad. Sci. USA* **97**, 5273–5278.
58. Bohl, F., Kruse, C., Frank, A., Ferring, D., and Jansen, R. P. (2000). She2p, a novel RNA-binding protein tethers ASH1 mRNA to the Myo4p myosin motor via She3p. *EMBO J.* **19**, 5514–5524.
59. Kimura, K., Ito, M., Amano, M., Chihara, K., Fukata, Y., Nakafuku, M., Yamamori, B., Feng, J., Nakano, T., Okawa, K., Iwamatsu, A., and Kaibuchi, K. (1996). Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). *Science* **273**, 245–248.
60. Somlyo, A. P. and Somlyo, A. V. (2000). Signal transduction by G-proteins, rho-kinase and protein phosphatase to smooth muscle and non-muscle myosin II. *J. Physiol.* **522** (Pt 2), 177–185.

61. Cramer, L. P., Siebert, M., and Mitchison, T. J. (1997). Identification of novel graded polarity actin filament bundles in locomoting heart fibroblasts: Implications for the generation of motile force. *J. Cell Biol.* **136**, 1287–1305.
62. Sellers, J. R. and Kachar, B. (1990). Polarity and velocity of sliding filaments: Control of direction by actin and of speed by myosin. *Science* **249**, 406–408.
63. Totsukawa, G., Yamakita, Y., Yamashiro, S., Hartshorne, D. J., Sasaki, Y., and Matsumura, F. (2000). Distinct roles of ROCK (Rho-kinase) and MLCK in spatial regulation of MLC phosphorylation for assembly of stress fibers and focal adhesions in 3T3 fibroblasts. *J. Cell Biol.* **150**, 797–806.
64. Bridgman, P. C., Dave, S., Asnes, C. F., Tullio, A. N., and Adelstein, R. S. (2001). Myosin IIB is required for growth cone motility. *J. Neurosci.* **21**, 6159–6169.
65. Diefenbach, T. J., Latham, V. M., Yimlamai, D., Liu, C. A., Herman, I. M., Vay, D. G. (2002). Myosin IC and myosin IIB serve opposing roles in lamellipodial dynamics of the neuronal growth cone. *J. Cell Biol.* **158**, 1207–1217.