Insights into mRNA transport in neurons

Fabrice Roegiers*

Departments of Physiology and Biochemistry, Howard Hughes Medical Institute, University of California, 533 Parnassus Avenue, San Francisco, CA 94122

Many large or morphologically complex cells compartmentalize information by targeting either mRNAs or proteins to specific domains and maintain the localization of these molecules over time. These distinct cellular domains can function to determine cell polarity, define embryonic axes, or contribute to cellular memory. Although much is understood about how proteins move and are targeted in cells, our understanding of the mechanisms of RNA transport and localization are at an early stage.

Localized mRNAs have been observed in numerous cell types from yeast to humans (reviewed in ref. 1). In large polarized cells such as oocytes and early embryos, mRNA localization is an important mechanism for establishing embryonic axes and functioning as tissue determinants (2). In *Drosophila*, embryonic axis specification is determined by two localized mRNAs: *bicoid*, which is localized to the anterior pole, and *nanos*, which is localized to posterior pole (3). These localized RNAs set up opposing protein gradients that define the anterior and posterior axes of the embryo. VegT mRNA, a determinant for endoderm formation, must be localized to the vegetal pole during oogenesis in *Xenopus* to ensure normal establishment of embryonic germ layers (4). A striking example of RNA localization is found in the Ascidian *Ciona intestinalis*, where an mRNA, *macho-1,* which has been identified as a muscle determinant, is tightly localized to a region of the egg before fertilization and is asymmetrically segregated during early cleavage stages (5). Other examples of asymmetric segregation of mRNAs during cell division can be found in yeast and *Drosophila*. In yeast, asymmetrically segregated *ASH1* mRNA determines mating type in the daughter cell during division (reviewed in ref. 6). In proliferating *Drosophila* neuroblasts, the homeobox transcription factor Prospero is asymmetrically localized to the basal side of the cell and segregated to the ganglion mother cell (7–9). *prospero* mRNA is also asymmetrically localized in these cells, and its localization depends on Staufen (10–14), a conserved mRNA-binding protein. In addition to Staufen's role in localizing *prospero* mRNA in *Drosophila* neuroblasts, Staufen is also an integral component of the localization machinery of both *oskar* mRNA (15–17), a crucial step in establishing the Nanos protein gradient, and *bicoid* mRNA during axis formation in the *Drosophila* oocyte and early embryo (15, 18). In this issue of PNAS, Mallardo *et al.* (19) have, for the first time, isolated ribonucleoprotein particles containing both the mammalian homologue of Staufen and localized mRNAs from rat brain, which may provide an important tool for determining the components of the machinery of mRNA localization in neurons.

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The *staufen* gene was initially identified in a screen of maternal effect embryonic anterior–posterior patterning mutants in *Drosophila* (20). Staufen's interaction with specific mRNAs is mediated through several double-stranded mRNA-binding domains (dsRBDs) (15); both *Caenorhabditis elegans* and *Drosophila* Staufen contain five of these domains (reviewed in ref. 21). Recently, two homologues of Staufen have been identified in mammals: Staufen 1 (22–26) and Staufen 2 (27–29). Both Staufen 1 and 2 are expressed in the nervous system; both proteins contain several dsRBDs and have a tubulin-binding domain (22–29). What role could Staufen be playing in the mammalian nervous system?

In neurons, local translation of specific mRNAs at the synapse has been proposed as a mechanism to create distinct postsynaptic domains (reviewed in ref. 30). If these localized domains play an important role in either synaptic development or synaptic plasticity, then how are specific mRNAs, which are transcribed in the nucleus, transported along the dendrite and targeted to the synapse? There are four proposed models for localization of mRNAs in cells (extensively reviewed in ref. 1): diffusion and local anchoring, localized degradation, localized mRNA synthesis, and active transport.

Early evidence for active transport of mRNAs in neurons came from injection of myelin basic protein (MBP) mRNA into oligodendrocytes (31). MBP mRNA forms large granules in these cells that are transported to the periphery. What could the role of Staufen be in this process? In cultured hippocampal neurons, both Staufen 1 and 2 are localized to the somatodendritic domain and are excluded from axons (24, 28, 29). Staufen–GFP colocalizes with particles enriched in mRNA labeled by the vital dye SYTO-14 (25), indicating that Staufen could be involved in either the transport or anchoring of mRNAs in neurons. The formation of Staufen–GFP/mRNA particles depends on microtubules, and these particles move from the soma into the dendrite at up to 24.3 μm/min, suggesting an active transport of particles by a microtubule motor (25). Interestingly, Staufen 1 and 2 may have nonredundant functions in trafficking of mRNAs to the dendrites, because, although they are both observed in particles in neurons, they do not colocalize (28). Evidence for Staufen's direct role in mRNA targeting in mammalian neurons comes from a study showing that overexpression of full-length Staufen 2 protein in neurons leads to an increase in bulk polyA mRNA in the dendrites, whereas overexpression of a truncated form of Staufen lacking the domain responsible for the dendritic localization of Staufen 2 has the opposite effect (29). Finally, the isolation of Staufen-containing particles by Mallardo *et al.* (19) has confirmed the previous suggestion that there are two pools of both Staufen 1 and 2 in mammalian neurons (24, 25). One pool fractionates with endoplasmic reticulum (ER) in their biochemical experiments and may represent Staufen observed in large granules that colocalize with the rough ER within the soma or at synaptic terminals (22, 26, 28, 32, 33). The other Staufen pool cofractionates with Kinesin heavy chain and several dendritically targeted mRNAs. The cofractionation of Staufen and Kinesin suggests that this fraction may represent the particles transported down the dendrite in a microtubule-dependent manner.

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*E-mail: fabrice@itsa.ucsf.edu.

The composition and behavior of mRNA-containing particles in neurons have some striking similarities to those observed in *Drosophila.* Injection of *bicoid* mRNA into living embryos causes the formation of particles containing both *bicoid* mRNA and Staufen, which are transported to the embryonic periphery at the cellular blastoderm stage (34). In *Drosophila* oocytes, Staufen's localization at the posterior pole also depends on microtubules (18). In addition, the microtubule plus end-directed motor Kinesin 1 is required for the localization of both Staufen and *oskar* mRNA to the posterior pole in *Drosophila* oocytes (35). Fluorescently labeled pair-rule mRNAs, such as *wingless*, have been visualized to form particles that are transported to an apical compartment of blastoderm embryos in a dynein-dependent manner (36). Taken together, these results suggest that there may be a conserved mechanism for mRNA transport along microtubules involving formation of a particle containing mRNAs, Staufen, and Kinesin in both insects and mammals. By isolating these particles biochemically, Mallardo *et al.* (19) have provided a tool to analyze the composition of ribonucleoprotein complexes at the protein and mRNA level.

Although the interaction of *Drosophila* Staufen with mRNA, specifically for *oskar* and *bicoid,* has been studied in detail both *in vivo* and *in vitro* (34, 37–39), a comparable analysis of mammalian Staufen is only just beginning. Given that mammalian Staufen has a role transporting mRNAs in neurons, and homologues of *bicoid* and *oskar* have not been found in mammals, it is certain that mammalian Staufen is interacting with different mRNAs, but which ones? Mallardo *et al.* (19), by isolating particles that contain both Staufen and mRNA from rat brain, have found that several mRNAs, such as *BC1* and the α subunit of *CaMKII*, are enriched in these fractions. *BC1* is a noncoding mRNA that is dendritically targeted (40), and *BC1* transcript level is activity-dependent in neurons (41). In the case of *CaMKII*, which is also localized to both the soma and dendrites in neurons, induction of tetanic stimulation in hippocampal slices cause a significant increase in the concentration of *CaMKII* mRNA recruited to the dendrites, indicating that *CaMKII* may have a role synaptic plasticity (42). Further evidence for the role of *CamKII* in plasticity comes from a recent study in mice showing that deletion mutants of the 3′UTR of *CaMKII* mRNA drastically reduces the amount of transcript in dendrites and, as a consequence, leads to significant reduction in learning and memory in knockout mice (43). Biochemical isolation of ribonuclear particles by Mallardo *et al.* (19) is likely to reveal other mRNAs that associate with Staufen and are trafficked to the distal dendrite to participate in localized transcription at the synapse.

The identification of the transport unit of dendritically targeted mRNAs, the Staufen ribonucleoprotein particle, may provide a new avenue for exploration of the mechanism of neuronal mRNA transport. Although these studies may produce a list of potential binding partners or components of Staufen-containing ribonucleoprotein particles, a careful biochemical analysis of proteins that interact directly with Staufen may provide additional clues to some of the factors necessary for correct targeting of mRNAs to the dendrites. Identification of other mRNAs contained in these particles may yield valuable clues in our quest to understand the role of localized mRNAs in synaptic plasticity and memory. Nevertheless, in addition to these studies, cell biological approaches and *in vivo* imaging will continue to contribute to our understanding of the dynamics of mRNA transport and may allow us to refine our knowledge of the path of mRNA, from its synthesis in the nucleus to translation at local sites at the synapse. Finally, loss-of-function genetic approaches can provide a more systemic view of the importance of mRNA transport in the brain function, for instance by the analysis of *staufen* knockout mice.

1. Palacios, I. M. & Johnston, D. S. (2001) *Annu. Rev. Cell Dev. Biol.* **17,** 569–614.
2. Gavis, E. (1997) *Trends Cell Biol.* **7,** 485–492.
3. van Eeden, F. & St Johnston, D. (1999) *Curr. Opin. Genet. Dev.* **9,** 396–404.
4. Zhang, J. & King, M. L. (1996) *Development (Cambridge, U.K.)* **122,** 4119–4129.
5. Nishida, H. & Sawada, K. (2001) *Nature* **409,** 724–729.
6. Chartrand, P., Singer, R. H. & Long, R. M. (2001) *Annu. Rev. Cell Dev. Biol.* **17,** 297–310.
7. Ikeshima-Kataoka, H., Skeath, J. B., Nabeshima, Y., Doe, C. Q. & Matsuzaki, F. (1997) *Nature* **390,** 625–629.
8. Hirata, J., Nakagoshi, H., Nabeshima, Y. & Matsuzaki, F. (1995) *Nature* **377,** 627–630.
9. Shen, C.-P., Jan, L. & Jan, Y. (1997) *Cell* **90,** 449–458.
10. Broadus, J., Fuerstenberg, S. & Doe, C. Q. (1998) *Nature* **391,** 792–795.
11. Li, P., Yang, X., Wasser, M., Cai, Y. & Chia, W. (1997) *Cell* **90,** 437–447.
12. Schuldt, A. J., Adams, J. H., Davidson, C. M., Micklem, D. R., Haseloff, J., St Johnston, D. & Brand, A. H. (1998) *Genes Dev.* **12,** 1847–1857.
13. Matsuzaki, F., Ohshiro, T., Ikeshima-Kataoka, H. & Izumi, H. (1998) *Development (Cambridge, U.K.)* **125,** 4089–4098.
14. Shen, C.-P., Knoblich, J., Chan, Y.-M., Jiang, M.-M., Jan, L. & Jan, Y. (1998) *Genes Dev.* **12,** 1837–1846.
15. St Johnston, D., Beuchle, D. & Nusslein-Volhard, C. (1991) *Cell* **66,** 51–63.
16. Kim-Ha, J., Smith, J. & MacDonald, P. (1991) *Cell* **66,** 23–35.
17. Ephrussi, A., Dickinson, L. & Lehmann, R. (1991) *Cell* **66,** 37–50.
18. Ferrandon, D., Elphick, L., Nusslein-Volhard, C. & St Johnston, D. (1994) *Cell* **79,** 1221–1232.
19. Mallardo, M., Deitinghoff, A., Müller, J., Goetze, B., Macchi, P., Peters, C. & Kiebler, M. A. (2003) *Proc. Natl. Acad. Sci. USA* **100,** 2100–2105.
20. Schupbach, T. & Wieschaus, E. (1986) *Dev. Biol.* **113,** 443–448.
21. Roegiers, F. & Jan, Y. N. (2000) *Trends Cell Biol.* **10,** 220–224.
22. Marion, R. M., Fortes, P., Beloso, A., Dotti, C. & Ortin, J. (1999) *Mol. Cell. Biol.* **19,** 2212–2219.
23. DesGroseillers, L. & Lemieux, N. (1996) *Genomics* **36,** 527–529.
24. Kiebler, M. A., Hemraj, I., Verkade, P., Kohrmann, M., Fortes, P., Marion, R. M., Ortin, J. & Dotti, C. G. (1999) *J. Neurosci.* **19,** 288–297.
25. Kohrmann, M., Luo, M., Kaether, C., DesGroseillers, L., Dotti, C. & Kiebler, M. (1999) *Mol. Biol. Cell* **10,** 2945–2953.
26. Wickham, L., Duchaine, T., Luo, M., Nabi, I. R. & DesGroseillers, L. (1999) *Mol. Cell. Biol.* **19,** 2220–2230.
27. Buchner, G., Bassi, M. T., Andolfi, G., Ballabio, A. & Franco, B. (1999) *Genomics* **62,** 113–118.
28. Duchaine, T. F., Hemraj, I., Furic, L., Deitinghoff, A., Kiebler, M. A. & DesGroseillers, L. (2002) *J. Cell Sci.* **115,** 3285–3295.
29. Tang, S. J., Meulemans, D., Vazquez, L., Colaco, N. & Schuman, E. (2001) *Neuron* **32,** 463–475.
30. Steward, O. & Schuman, E. M. (2001) *Annu. Rev. Neurosci.* **24,** 299–325.
31. Ainger, K., Avossa, D., Morgan, F., Hill, S. J., Barry, C., Barbarese, E. & Carson, J. H. (1993) *J. Cell Biol.* **123,** 431–441.
32. Duchaine, T., Wang, H. J., Luo, M., Steinberg, S. V., Nabi, I. R. & DesGroseillers, L. (2000) *Mol. Cell. Biol.* **20,** 5592–5601.
33. Krichevsky, A. M. & Kosik, K. S. (2001) *Neuron* **32,** 683–696.
34. Ferrandon, D., Koch, I., Westhof, E. & Nusslein-Volhard, C. (1997) *EMBO J.* **16,** 1751–1758.
35. Brendza, R. P., Serbus, L. R., Duffy, J. B. & Saxton, W. M. (2000) *Science* **289,** 2120–2122.
36. Wilkie, G. S. & Davis, I. (2001) *Cell* **105,** 209–219.
37. Micklem, D. R., Adams, J., Grunert, S. & St Johnston, D. (2000) *EMBO J.* **19,** 1366–1377.
38. Macdonald, P. M. & Struhl, G. (1988) *Nature* **336,** 595–598.
39. Macdonald, P. M., Kerr, K., Smith, J. L. & Leask, A. (1993) *Development (Cambridge, U.K.)* **118,** 1233–1243.
40. Tiedge, H., Fremeau, R. T., Jr., Weinstock, P. H., Arancio, O. & Brosius, J. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 2093–2097.
41. Muslimov, I. A., Banker, G., Brosius, J. & Tiedge, H. (1998) *J. Cell Biol.* **141,** 1601–1611.
42. Ouyang, Y., Rosenstein, A., Kreiman, G., Schuman, E. M. & Kennedy, M. B. (1999) *J. Neurosci.* **19,** 7823–7833.
43. Miller, S., Yasuda, M., Coats, J. K., Jones, Y., Martone, M. E. & Mayford, M. (2002) *Neuron* **36,** 507–519.