

## mRNA localization and the cytoskeleton

Jolanta Bogucka Glotzer and Anne Ephrussi



*All cytoskeletal systems including actin, microtubules and intermediate filaments are thought to participate in the localization of messenger RNA (mRNA) in cells. The cytoskeleton is believed to function in both the transport and the maintenance of mRNA at specific sites within the cell. In this review we summarize the evidence for the involvement of the cytoskeleton in mRNA localization and discuss several potential mechanisms for the transport of mRNAs.*

**Key words:** cytoplasmic flow / cytoskeleton / mRNA / transport

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THE POLARIZATION OF A CELL is both the cause and the consequence of the non-uniform or asymmetric distribution of intracellular molecules and organelles. In 1983 Jeffery and colleagues showed that actin mRNA accumulates preferentially in a region of the ascidian egg called the myoplasm, providing the first evidence for polarized mRNA distribution within a cell.<sup>1</sup> This finding has been followed by numerous reports of mRNAs localized in oocytes, eggs, embryos and somatic cells (reviewed in refs 2,3). Localization of mRNA allows the spatial control of gene expression since the mRNA serves as a localized template for protein synthesis (reviewed in ref 4).

Translocation of mRNA molecules within cells could be achieved in several ways (Figure 1). After export from the nucleus (Figure 1a; reviewed in ref 5), the mRNA could randomly diffuse in the cytoplasm (Figure 1b). Alternately, the mRNA could be specifically transported along polarized cytoskeletal elements (Figure 1c), in a manner analogous to cytoskeleton-dependent vesicle transport. Finally, mRNA translocation could be achieved by non-specific cytoskeleton-dependent cytoplasmic flow (Figure 1d). At its destination, the mRNA could be anchored by associating with pre-localized receptors (Figure 1e), many of which could be associated with

the cytoskeleton. In this review we will discuss recent experiments that reveal the role of the cytoskeleton in the movement and anchoring of mRNAs. The results are principally derived from four experimental systems: *Drosophila* and *Xenopus* oocytes, neurons and fibroblasts. However, specific localization of mRNA is likely to be important for the function of many cell types.

### Mechanisms of mRNA translocation

#### *Simple diffusion*

Translocation of mRNA within cells could in principle be achieved by simple diffusion (Figure 1b). Although systematic measurements of mRNA diffusion have not been reported, the diffusion of fluorescent dextrans has been measured in live fibroblasts.<sup>6</sup> These data predict that cytoplasm would significantly impair the diffusion of mRNA molecules larger than 1.6 kb. In fact, mRNAs of 1 kb (or longer) injected into *Drosophila* embryos barely diffuse, as determined by in-situ hybridization performed 2 hours after injection.<sup>7</sup> Moreover, in the cytoplasm, mRNAs are likely to form complexes with multiple proteins (ribonucleoprotein particles or RNPs), and these particles may also associate with ribosomes. An RNA that is incorporated into an RNP would most likely diffuse more slowly than naked RNA.

In conclusion, although localization of mRNA by simple diffusion seems unlikely for most mRNAs, shorter RNAs may utilize such means to reach their destination.

#### *Directional transport*

Intracellular movement of vesicles relies on directional transport along actin filaments or microtubules.<sup>8</sup> The molecular motors of the dynein family and many members of the myosin and kinesin superfamilies provide the mechanical force that propels the vesicles.<sup>8</sup> Directional, cytoskeleton-dependent transport has also been postulated to

*From the Developmental Biology Program, European Molecular Biology Laboratory, Meyerhofstrasse 1, Heidelberg, D-69117, Germany*

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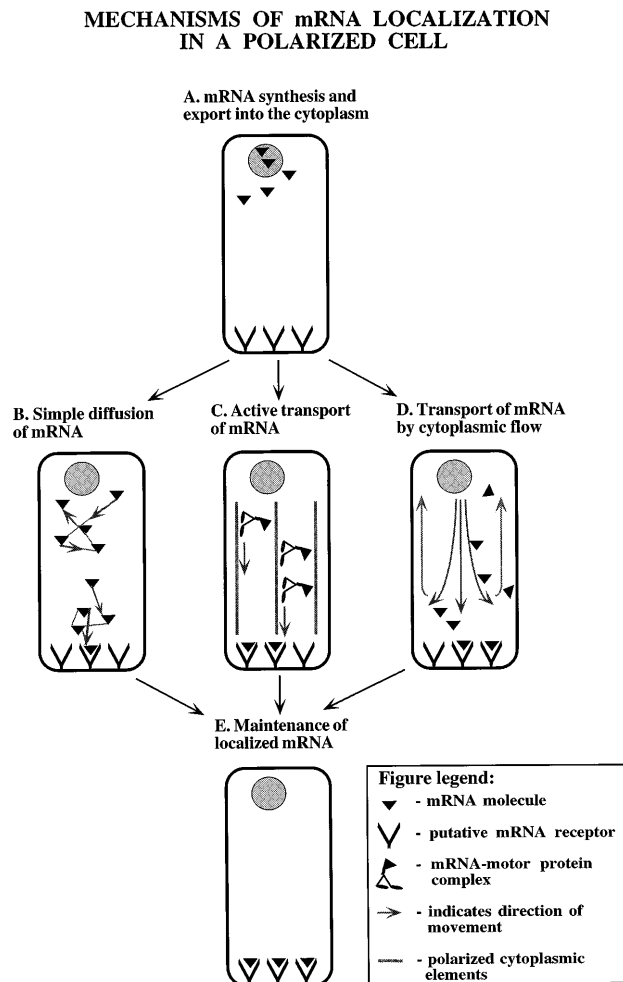
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mediate mRNA translocation in polarized cells including neurons, epithelial cells, and oocytes (Figure 1c). By analogy to vesicle transport, directional transport of mRNAs would require an organized cytoskeletal network to serve as a track for the translocation, and motor proteins to propel the mRNA along these

cytoskeletal elements. A transport complex could consist of one or more mRNA molecules, the proteins that bind the RNA to form an RNP particle, and one or more motor proteins. cytoplasmic flow

A spatio-temporal correlation between the rearrangements of the microtubule network and the redistribution of mRNAs during oogenesis in *Drosophila* strongly suggests that microtubules play a role in the transport of mRNAs. *Drosophila* oocytes develop in strings of progressively maturing egg chambers (for a review of oogenesis see refs 9,10). An egg chamber contains an oocyte connected at its anterior by cytoplasmic bridges (or ring canals) to a complex of 15 nurse cells (Figure 2). The ring canals allow the intercellular exchange of cytoplasmic components. In early egg chambers (stages 1-6; Figure 2a) microtubules are nucleated in the oocyte and pass through the ring canals into the nurse cells.<sup>11</sup> In these egg chambers several mRNAs including *Bicaudal-D* (*Bic D*), *bicoid* (*bcd*), *female sterile (1)* (*K10*), *gurken* (*grk*), *oo18 RNA-binding* (*orb*), and *oskar* (*osk*) are transcribed in the nurse cells and accumulate in the cytoplasm of the developing oocyte.<sup>12-20</sup> During mid-oogenesis (stages 7-9; Figure 2b), the distribution of microtubules in the egg chambers undergoes a dramatic rearrangement: microtubules are nucleated at the anterior of the oocyte with the plus ends pointing towards the oocyte posterior pole.<sup>11</sup> During these stages, mRNAs that have accumulated within the oocyte become redistributed: *Bic-D*, *bcd*, *K10* and *orb* mRNAs are selectively enriched at the anterior margin, *grk* mRNA at the antero-dorsal 'corner', and *osk* mRNA at the posterior pole. Based on the observed correlation between the organization of the polarized microtubule network and the distribution of mRNAs in the egg chambers, the transfer of these mRNAs from the nurse cells into the oocyte and the subsequent redistribution of these mRNAs within the oocyte have been proposed to occur by microtubule-dependent active transport.<sup>11</sup>

The translocation of mRNAs from the nurse cells into the oocyte during the early stages of oogenesis would require a minus end-directed motor, and indeed, dynein heavy chain protein DHC64C accumulates in the oocyte in early egg chambers.<sup>21</sup> In mid-oogenesis, a plus end-directed motor would be required to transport *osk* mRNA from the anterior to the posterior of the oocyte. Consistent with this requirement, a kinesin- $\beta$ -galactosidase fusion protein (plus end-directed motor) accumulates at the posterior pole of the oocyte.<sup>22</sup> A minus end-directed motor would be required to transport other mRNAs in the opposite direction, but dynein also accumulates at the



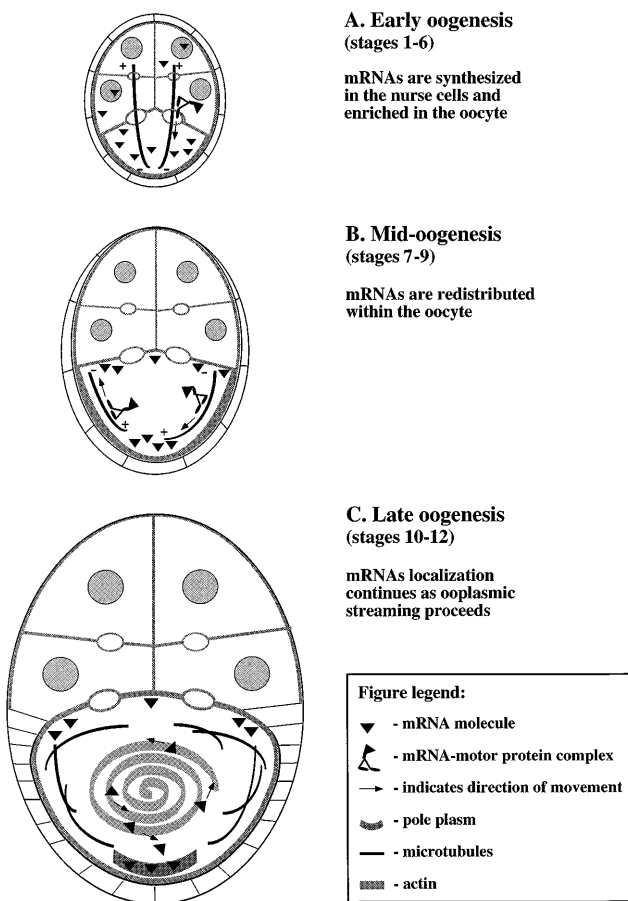
**Figure 1.** Alternative mechanisms of localization of mRNA in a polarized cell. The figure shows a diagram of a polarized cell with the nucleus (grey filled circle) at the top and putative mRNA receptors at the bottom. (A) mRNA is synthesized in the nucleus and exported into the cytoplasm. mRNA molecule may reach its final destination by one of the following mechanisms: (B) mRNA could diffuse randomly within the cytoplasm; (C) mRNA in complex with cytoskeleton-dependent motor proteins could be actively transported along polarized cytoskeletal elements; (D) mRNA migration in the cytoplasm could be mediated by concurrent cytoplasmic flow. Each of the proposed mechanisms would result in localization of a portion of the mRNA in a destined cellular region where it would become anchored and maintained (E).

oocyte posterior during mid-oogenesis<sup>21</sup> raising the possibility that the oocyte contains microtubules of opposite polarities, or that the posterior accumulation of dynein reflects a function in anchoring microtubules to the oocyte cortex.<sup>23</sup>

These first results indicating a correlation between the distribution of mRNAs and motor proteins, and the arrangement of microtubules led to the suggestion that mRNA transport in *Drosophila* egg chambers is microtubule-dependent. Several recent observations lend more support to this proposal. In protein kinase A (PKA) mutant egg chambers, reorganization of the microtubule network fails in mid-oogenesis and microtubules are nucleated at both the anterior and posterior poles.<sup>24</sup> In this mutant, both *osk* mRNA and kinesin- $\beta$ -galactosidase are absent from the posterior pole and instead are localized to the center of the oocyte, the presumed position of the microtubule plus ends (dynein localization has not yet been reported). In contrast, *bcd* mRNA, which is normally localized at the anterior pole, is found at both anterior and posterior in PKA mutant oocytes. Sim-

ilarly, in Notch mutants, *osk* mRNA, kinesin- $\beta$ -galactosidase and dynein localize to the middle of the oocyte, and *bcd* mRNA localizes to both the anterior and the posterior poles.<sup>21,25</sup> In this mutant the distribution of microtubules has not been examined, but it is likely to resemble the distribution of microtubules in the PKA mutant. In conclusion, although there is evidence suggesting that mRNAs are directly transported along microtubules, demonstration that mutations in dynein or kinesin-like genes can affect mRNA localization during *Drosophila* oogenesis, would greatly strengthen this hypothesis.

The *Xenopus* oocyte provides another example in which the dynamic reorganization of microtubules correlates with the redistribution of mRNAs. During early oogenesis (stages III/IV) a polarized microtubule network is established along the animal-vegetal axis.<sup>26</sup> At that time, initially dispersed *Vg1* mRNA



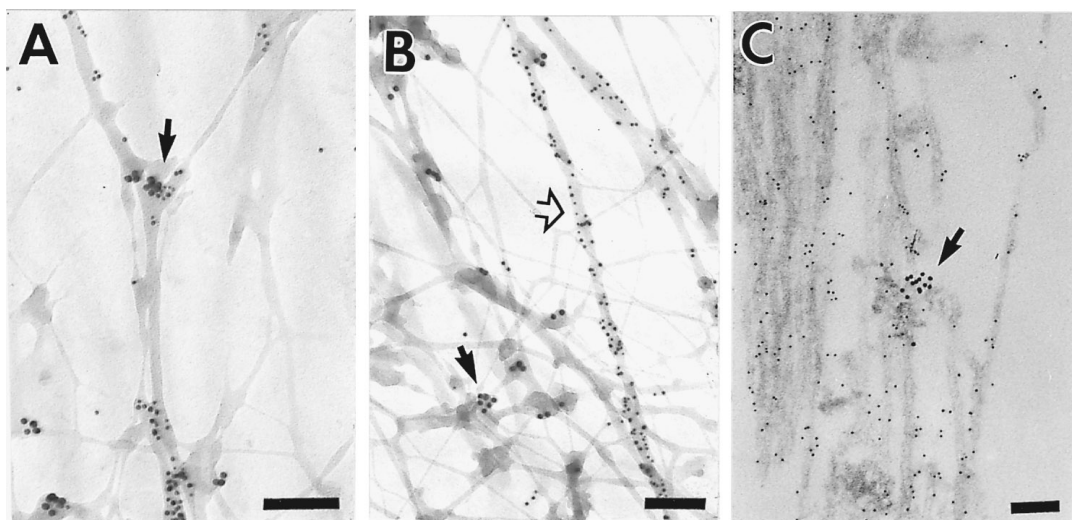
**Figure 2.** mRNA localization during oogenesis in *Drosophila*. The figure shows three egg chambers representative of the stages indicated. Each egg chamber consists of 15 nurse cells (four of which are diagrammed at the top of each egg chamber) and an oocyte (the cell at the bottom containing thick actin-rich cortex). The nurse cells are interconnected with each other and with the oocyte at its anterior margin (facing the nurse cells) by cytoplasmic bridges (surrounded by actin-rich ring canals, diagrammed as grey empty ovals). The bridges allow cytoplasmic exchange in the syncytium-like egg chamber. (A) In early oogenesis several mRNAs including *bcd*, *Bic-D*, *grk*, *K10*, *orb* and *osk*, are transcribed in the nurse cells and become enriched in the oocyte. At that time, microtubules are nucleated in the oocyte, pass through the ring canals, and may mediate active transport of mRNAs from the nurse cells into the oocyte. (B) In mid-oogenesis mRNAs become redistributed within the oocyte: *bcd*, *Bic-D*, *K10*, and *orb* mRNAs localize to the anterior, and *osk* mRNA to the posterior pole of the oocyte. *grk* mRNA localizes to the anterior-dorsal region of the oocyte, close to the oocyte nucleus (not shown). During these stages microtubules reorganize within the oocyte and are nucleated at the oocyte anterior, with the plus ends pointing towards the oocyte posterior. These polarized microtubules could transport different mRNAs, associated with minus or plus end-directed microtubule-dependent motors, to opposite ends of the oocyte. (C) In late oogenesis, another group of mRNAs (*nos*, *gcl* and *cycB*) become localized to the oocyte posterior pole. This region of the oocyte cytoplasm is called pole plasm, or germ plasm, since it contains germ line inducing activity.<sup>69</sup> At these stages no microtubules with antero-posterior polarity have been found in the oocyte. Rather, microtubules form thick bundles at the oocyte cortex and mediate the process of 'ooplasmic streaming' which rapidly mixes the oocyte cytoplasm. Such streaming is likely to facilitate translocation of mRNAs within the oocyte cytoplasm, allowing them to reach the pole plasm where they become anchored.

becomes localized exclusively to the vegetal hemisphere,<sup>27</sup> whereas other mRNAs including *An1*, *An2* and *An3*, become localized to the animal hemisphere.<sup>28,29</sup> In late stage IV oocytes, microtubules nucleate at the vegetal cortex of the oocyte, as indicated by the enrichment of  $\gamma$ -tubulin,<sup>30</sup> a marker of microtubule organizing centers. This microtubule reorganization is accompanied by the relocation of *Vg1* mRNA to the vegetal cortex.<sup>27</sup> These observations suggest that *Vg1* mRNA travels towards the minus ends of microtubules. However, as in *Drosophila*, no motor proteins have been shown to participate in the transport.

Although a strong correlation exists between the polarized distribution of microtubules and mRNA translocation in both *Drosophila* and *Xenopus* oocytes, in neither case has the colocalization of mRNAs with microtubules been demonstrated. However, in somatic cells, electron microscopy studies have revealed that mRNAs colocalize with all major cytoskeletal systems: actin filaments, microtubules, and intermediate filaments.<sup>31,32</sup> In fibroblasts, approximately 70% of poly (A<sup>+</sup>) mRNA (including specific transcripts such as actin, vimentin and tubulin mRNAs) associates with actin filaments (Figure 3A), 30% with

intermediate filaments (vimentin) and less than 10% with microtubules (Figure 3B).<sup>31,33</sup> In contrast, in neuronal processes the majority of poly (A<sup>+</sup>) mRNA (55%) including a neural-specific  $\beta$ -tubulin mRNA colocalizes with microtubules (Figure 3C).<sup>32</sup> Similarly, myelin basic protein (MBP) mRNA colocalizes with microtubules in oligodendrocyte processes.<sup>34</sup> The observed colocalization of mRNAs with cytoskeletal elements strongly supports the role of the cytoskeleton in mRNA localization. However, since these observations are the result of steady-state analyses, they do not allow one to determine whether the colocalization of mRNA with the cytoskeleton reflects intermediate steps in mRNA transport along cytoskeletal tracks, or whether it indicates local retention of mRNAs.

If microtubules mediate active transport of mRNA, then depolymerization of microtubules should interfere with the intracellular distribution of mRNA. In cultured cells, microtubule depolymerizing drugs cause an approximately four-fold reduction in the number of oligodendrocytes containing MBP mRNA in their processes, suggesting that intact microtubule polymers are involved in MBP mRNA transport (Carson, manuscript in preparation). In *Xenopus*



**Figure 3.** Association of poly(A) mRNA with cytoskeletal filament systems visualized using ultrastructural in-situ hybridization and immunogold. Oligo-dT probes end-labeled with digoxigenin-dUTP were detected using antibodies to digoxigenin and 10 nm gold conjugated secondary antibodies. Cytoskeletal proteins were detected using 5 nm gold conjugated secondary antibodies. (A) In human fibroblasts, the majority of poly(A<sup>+</sup>) mRNA is associated with isotropic networks of F-actin. Hybridization colocalizes within 10 nm of the actin binding protein filamin (ABP 280; arrow). (B) The majority of poly(A<sup>+</sup>) mRNA (arrow) is not associated with microtubules labelled with anti-tubulin (open arrow). (C) By contrast, the majority of poly(A<sup>+</sup>) mRNA in dendrites of cortical neurons colocalizes with microtubules (arrow). Bar, 100 nm. (Electronmicrographs courtesy of Gary J. Bassell).

oocytes, depolymerization of microtubules prevents translocation of *Vg1* mRNA from the vegetal hemisphere to the vegetal cortex.<sup>35</sup> In *Drosophila*, feeding flies with colchicine, an inhibitor of microtubule polymerization, impedes both the oocyte-specific accumulation of mRNA in early oogenesis and the subsequent mRNA redistribution within the oocyte in mid-oogenesis.<sup>22,36,37</sup> In the latter experiments it was found that a 3.5 hour drug treatment was sufficient to depolymerize microtubules within egg chambers, but 10 hour incubations with the drugs were required to abolish *osk* mRNA localization to the posterior pole of the oocyte.<sup>22</sup> This suggests that microtubules are involved both in active transport of mRNA and in other aspects of mRNA translocation and/or maintenance (see later).

If actin filaments serve as tracks for the directional transport of mRNA, depolymerization of these filaments should affect its transport. In chicken embryo fibroblasts, actin mRNA translocation to the cell periphery is inhibited by cytochalasin, a drug which prevents actin polymerization, suggesting that in these cells dynamic actin filaments play a role in mRNA transport.<sup>38</sup>

In squid axon cytoplasm, vesicles switch between microtubules and actin filaments during transport.<sup>39</sup> These two cytoskeletal systems also cooperate in mRNA localization. Although microtubules appear to be involved in mRNA localization in *Drosophila* oocytes, a mutation in the actin-binding protein, cytoplasmic tropomyosin II (cTm) affects *osk* mRNA redistribution within the oocyte during mid-oogenesis and dramatically reduces *osk* mRNA localization to the posterior pole.<sup>40</sup> cTm could function in the retention of *osk* RNA at the oocyte posterior cortex, which is actin-rich.<sup>41</sup> The recently reported localization of p150 Glued, a component of the dynactin complex, to the posterior pole of the *Drosophila* oocyte<sup>42</sup> could reflect a structural link between cortical actin and microtubules, as has been hypothesized in yeast and *C. elegans* (reviewed in ref 23). Alternatively, cTm could in some way mediate an interaction of *osk* mRNA with a microtubule-dependent motor, or, cTm could be directly involved in actin-dependent *osk* mRNA transport, by analogy to its proposed function in the movement of cytoplasmic particles in fibroblasts.<sup>43</sup> In wild type egg chambers, at the time when *osk* mRNA begins to accumulate at the posterior pole of the oocyte, it is also transiently localized at the anterior pole.<sup>19,20</sup> In cTm mutant egg chambers, this anterior accumulation is enhanced relative to the wild

type. Thus cTm could be required for the release of *osk* mRNA from its initial anterior localization sites.

Directional transport of a molecule on a cytoskeletal system is expected to be linear, unidirectional and saltatory, and characterized by a steady rate during the uninterrupted phase of movement. If mRNAs were translocated along microtubules for example, one would expect to find rates of movement compatible with those of known motors. Motor proteins move at rates ranging from 0.5 to 600  $\mu\text{m}/\text{min}$ .<sup>44-46</sup> Few data are available on the kinetics of mRNA migration in cells. In cultured hippocampal neurons, newly synthesized mRNA is translocated from the nucleus into the dendrites at a speed of 0.3  $\mu\text{m}/\text{min}$ .<sup>47</sup> The rate of translocation of *Vg1* mRNA injected into *Xenopus* oocytes is even slower (0.07  $\mu\text{m}/\text{min}$ ).<sup>35</sup> However, since mRNA could move and pause, these measurements may not in fact reveal the actual rate of uninterrupted movement. Hence, it is likely that the calculated rates represent an underestimate.

Only in a single case has the rate of mRNA translocation been measured directly in living cells. In this case, fluorescently-labelled MBP mRNA was injected into oligodendrocytes.<sup>34</sup> Within minutes, the microinjected RNA assembled into particles of 0.3  $\mu\text{m}$  in diameter. Although most particles were stationary at a given time, some particles were observed to migrate unidirectionally in the processes towards the cell periphery at a speed of 12  $\mu\text{m}/\text{min}$ , highly suggestive of active transport (Figure 4).

mRNA-containing particles may constitute a common transport 'vehicle'. MBP mRNA- and other mRNA-containing particles also contain aminoacyl-transferase, translation elongation factor EF1- $\alpha$ , and ribosomes,<sup>31,48</sup> suggesting that ribosome-bound mRNAs may be transported in translationally competent complexes. Endogenous RNA-containing particles labelled with the vital dye SYTO12 specific to RNA, have been observed to move in oligodendrocyte processes in both anterograde and retrograde directions at a speed similar to MBP mRNA-containing particles (Carson, personal communication). RNA-containing particles, revealed by the vital RNA dye, have been also observed to move unidirectionally in axons (Bassell, personal communication). Other mRNAs, such as *Vg1* mRNA, also form particles in the cytoplasm of the vegetal hemisphere of the *Xenopus* oocyte,<sup>49</sup> but whether or not these particles move has not yet been determined. In *Drosophila*, Staufen (STAU) and Exuperantia (EXU) proteins are required for localization of *bcd* mRNA to the anterior pole of the oocyte and embryo.<sup>14,50</sup> *bcd* mRNA

injected into *Drosophila* embryos recruits the RNA-binding protein STAU into particles which may represent transport particles.<sup>51</sup> EXU-green fluorescent protein (EXU-GFP) fusion protein forms particles that move from the nurse cells into the oocyte.<sup>52</sup> It will be interesting to see whether *bcd* mRNA is transported in the EXU-containing particles.

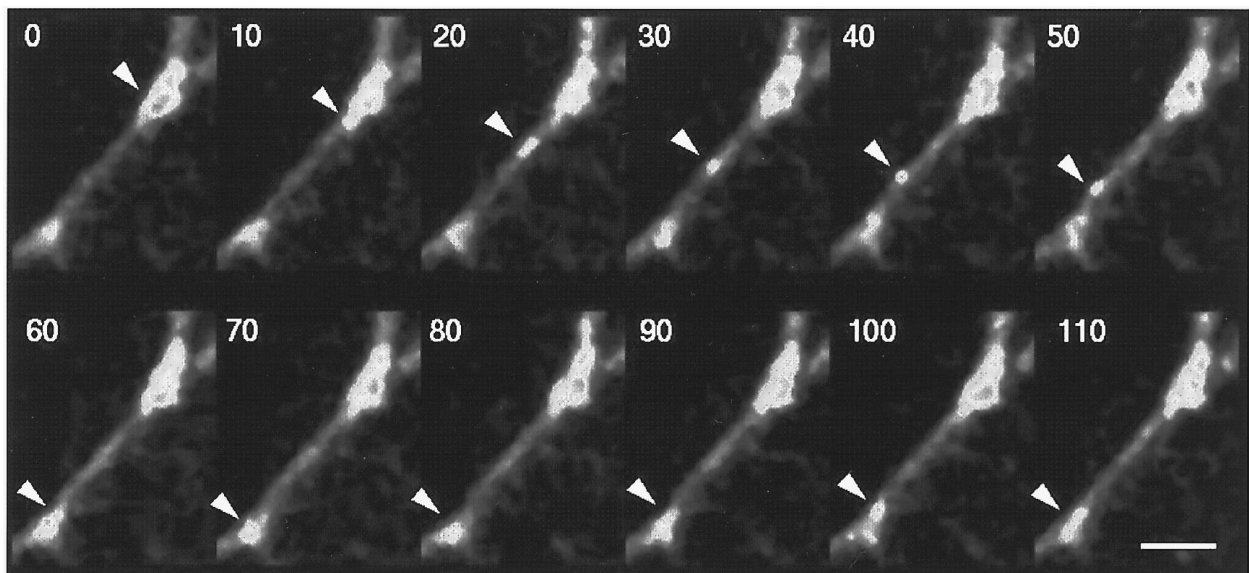
#### Transport by cytoplasmic flow

While the cytoskeleton may serve as tracks for the directional transport of mRNA, it could also participate in mRNA translocation in a less selective manner. In many cells the cytoskeleton plays a role in generating rapid cytoplasmic flows that may facilitate the distribution of intracellular molecules and organelles. A directional actin-dependent cytoplasmic flow (4-7  $\mu\text{m}/\text{min}$ ) has been postulated to provide the motive force in the transport of P granules to the posterior pole of the *C. elegans* first cleavage embryo.<sup>53,54</sup> P granules are particles 1-2  $\mu\text{m}$  in diameter containing both mRNA and protein, and are correlated with germ line formation in the *C. elegans* embryo.<sup>55,56</sup>

An even more rapid cytoplasmic flow (20-25  $\mu\text{m}/\text{min}$ ), ooplasmic streaming, occurs in *Drosophila* oocytes during late oogenesis (stages 10b-12;

Figure 2c).<sup>57,58</sup> This streaming can be followed by direct observation of autofluorescent yolk granules that move as a spiral wave within the oocyte, mixing its entire content. Ooplasmic streaming is microtubule-dependent, and it has been suggested that it is driven by translocation of vesicles along the subcortical microtubules.<sup>36</sup> *nanos (nos)*, *germ cell-less (gcl)* and *cyclin B (cycB)* mRNAs localize to the posterior pole of the oocyte following the onset of ooplasmic streaming.<sup>59-61</sup> The rapid cytoplasmic flow is likely to facilitate the diffusion of these and other mRNAs, allowing them to reach the posterior pole of the oocyte, where they are retained. Experiments following the translocation of injected fluorescent mRNA indicate that *osk* mRNA localization to the posterior pole continues while ooplasmic streaming proceeds (Glotzer, manuscript in preparation). Taking these data together with the previous data on *osk* mRNA localization during the earlier phases, we conclude that in the *Drosophila* oocyte multiple mRNA localization mechanisms operate at different times. Alternatively, localization of all mRNAs in *Drosophila* oocyte could occur by facilitated diffusion, since the ooplasm is also in constant motion during the stages preceding ooplasmic streaming.<sup>58</sup>

As both directional transport and cytoplasmic flow require organized cytoskeletal networks, distinguish-



**Figure 4.** Time lapse analysis of RNA granule motion in an oligodendrocyte process. Fluorescein-labelled MBP mRNA was microinjected into an oligodendrocyte cell body. A series of sequential confocal microscopic images were collected at 10sec intervals. A small portion of an oligodendrocyte process containing a mobile granule is shown. The position of the granule within the process is indicated by an arrowhead. Bar, 5  $\mu\text{m}$ . Reproduced from J Cell Biol (1993) 123:431-441, by copyright permission of The Rockefeller University Press (ref 34).

ing between these two mechanisms of mRNA translocation is difficult. Both mechanisms require integrity of the cytoskeleton and may rely on cooperation between different filament systems. Direct observation of the movement of mRNA should allow one to distinguish between these mechanisms. Alternatively, inhibition of specific motors could disrupt localization of specific mRNAs without affecting cytoplasmic flow, which would demonstrate directed transport. Finally, in-vitro reconstitution of the association and movement of mRNAs along isolated cytoskeletal elements would allow deeper analysis of this mechanism.

### Retention of mRNA

A function of the cytoskeleton in the maintenance of mRNA at a site has been inferred from experiments in which depolymerization of a given cytoskeletal system causes the dispersion of an already localized mRNA. In *Xenopus*, treatment of stage IV–VI oocytes with cytochalasins releases *Vg1* mRNA from its association with the vegetal cortex, indicating that actin filaments are required for the retention of these transcripts.<sup>35</sup> Cytochalasin treatment also releases actin mRNA from its association with actin filaments in the leading edge of fibroblasts.<sup>38</sup> Treatment of *Drosophila* egg chambers with cytochalasins has no effect on mRNA distribution, but prolonged treatment with microtubule-depolymerizing drugs releases *bcd* and *osk* mRNAs from their respective anterior and posterior cortical sites.<sup>37,62</sup> This argues that in the *Drosophila* oocyte microtubules may mediate mRNA retention. It also raises the possibility that RNA retention could be a dynamic process and depend on a continuous association and dissociation of mRNA with the cytoskeleton.

The nature of the association between mRNA and the cytoskeleton is unknown. In fibroblasts, poly(A<sup>+</sup>) mRNA, together with the actin binding proteins filamin and  $\alpha$ -actinin, are enriched at actin filament intersections (Figure 3A), suggesting that these actin-binding proteins may participate in anchoring of the RNA.<sup>31</sup> In the case of the actin-*Vg1* mRNA interaction, a class of untranslated RNAs, the Xsirts, are required for the association of *Vg1* mRNA with the vegetal cortex.<sup>63</sup> Thus a novel function of untranslated, but localized RNAs may be to mediate the association between other mRNAs and an anchoring system, possibly the cytoskeleton. Finally, mRNAs, ribosomes and EF-1 $\alpha$ , colocalize with the cytoskeleton in fibro-

blasts and oligodendrocytes indicating that they may be a part of cytoskeleton-associated translation complexes.<sup>31,48,64</sup> Co-purification of ribosomes with tubulin through multiple cycles of assembly and disassembly of the tubulin polymer indicates that there is a link between the cytoskeleton and the translation machinery.<sup>65</sup> Electron microscopy of the purified complexes indicates that the ribosomes are connected to microtubules by a stalk. Salt extraction of the complexes eliminates the interaction and yields a 77 kD protein.<sup>66</sup> Further purification and identification of the specific factors mediating the RNA-cytoskeletal association will reveal the complexity of the interactions.

### Concluding remarks

In this review we have discussed three mechanisms of mRNA translocation in polarized cells: simple diffusion, directional transport and non-selective transport by cytoplasmic flow. There is clear experimental evidence supporting the latter two mechanisms. It is possible that in certain cases where mRNA localization has been exclusively attributed to active transport, cytoplasmic flow may play a heretofore unsuspected role. A distinguishing feature of RNA localization by cytoplasmic flow is the requirement for an effective mRNA retention system at the localization site, to prevent the diffusion of mRNA by cytoplasmic counterflows. In contrast, RNA localization by directional transport may not need a receptor-mediated retention, provided that the RNA unloaded from the cytoskeletal tracks cannot diffuse, due to the local structure of the cytoplasm. In conclusion, the mechanisms underlying mRNA retention are important subjects for future research.

Another point that should receive more attention in the future concerns the role of degradation and protection of mRNAs as means to achieve their localization. For example, *Hsp 83* mRNA which is initially uniformly dispersed throughout the *Drosophila* egg, once embryogenesis starts, is found only at the posterior pole of the embryo.<sup>67</sup> The observed distribution of *Hsp 83* mRNA may be achieved by general degradation with selective protection of the mRNA at the localization site.

Localization of mRNA facilitates the localized translation of intracellular proteins. It is interesting that even the mRNA encoding GRK, a secreted protein, has been found to be localized within the *Drosophila* oocyte.<sup>17</sup> Translation and secretion of GRK may be

facilitated by ribosomes and the fragmented Golgi apparatus that are also found distributed along the cortex of the *Drosophila* egg.<sup>68</sup> and presumably the oocyte. It will be interesting to see whether mRNA localization targets localized secretion of proteins in other polarized systems.

## Acknowledgements

We thank Drs William Brook, John Carson, Suzanne Eaton, Michael Glotzer, Eric Karsenti, Iain Mattaj, and the members of Ephrussi laboratory for advice on the manuscript. We also thank Drs Gary Bassell and John Carson for providing photographs included in this review and for sharing unpublished results. Finally, we thank all other members of the 'RNA community' for sending us manuscripts describing their most recent results.

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