Cell, Vol. 107, 707–710, December 14, 2001, Copyright ©2001 by Cell Press

Microtubules, Motors, and mRNA Localization Mechanisms: Watching Fluorescent Messages Move

Minireview

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Proper spatial and temporal localization of specific mRNAs is pivotal in the early stages of development. To dissect the mechanisms of localization, several groups are employing advanced fluorescence microscopy to track RNA movements in live oocytes and embryos.

The asymmetric placement of cytoplasmic machinery is critical in the cells of higher organisms. An elegant example of this is found in following the developmental path that leads from a symmetric *Drosophila* germline stem cell to an embryo imprinted with a body pattern for the future fly. The stem cell initiates a series of 4 mitotic cycles with incomplete cytokinesis to produce a cyst of 16 cells interconnected by cytoplasmic bridges called ring canals. One of the cells becomes the oocyte, while the remaining 15 become nurse cells (Figure 1A). During oogenesis, proteins, mRNAs, and organelles synthesized in nurse cells are delivered to the oocyte through the ring canals. The developing oocyte asymmetrically localizes many components, including specific mRNAs that determine the major body axes (Figure 1B) (Riechmann and Ephrussi, 2001). In the syncytial blastoderm embryo, those maternal mRNAs produce broad gradients of factors that create smaller expression domains for gap gene products. The gap proteins then coordinate the transcription of pair-rule and segment polarity genes in narrow repeating stripes of blastoderm nuclei (Pankratz and Jackle, 1993). Tight localization of those transcripts helps generate different developmental fates in neighboring sets of nuclei (Figure 1C) (Davis and Ish-Horowicz, 1991; Simmonds et al., 2001).

The importance of mRNA localization in *Drosophila* development, as well as in neurons and other specialized cell types, has stimulated great interest in the localization mechanisms. In eukaryotic cells, most cytoplasmic transport processes depend on cytoskeletal filaments. This is well established for the active transport of chromosomes, membraneous organelles, and some large protein complexes. Force-producing ATPases (motor proteins) attach to the object to be moved and then walk along a filament, overcoming the resistance to movement imposed on large objects by the gel-like nature of cytoplasm. When compared to those classic cargoes of active transport, the relatively small size of an mRNA suggests that random diffusion and specific anchoring to the cytoskeleton in a target area might suffice for localization. In cells however, mRNAs can complex with many proteins to form large ribonucleoprotein particles (RNPs). Perhaps because of RNP size and/or requirements for efficiency, the localization of some mRNAs requires motor proteins, suggesting that the cytoskeletal filaments are actually used as tracks for active transport (Ainger et al., 1997; Bohl et al., 2000; Brendza et al., 2000).

Fluorescent Transcripts Show the Way

Investigation of a dynamic process can benefit tremendously from the development of methods for observing it in live cells. Following the pioneering work of Glotzer et al. with microinjection of fluorescently labeled transcripts (Glotzer et al., 1997), Wilkie and Davis reported earlier this year in *Cell* meticulous studies of mRNA localization in *Drosophila* embryos (Wilkie and Davis, 2001). At the end of the 14th mitotic cycle, a *Drosophila* embryo is syncytial with a single layer of nuclei positioned near the surface, each draped with a set of microtubules that are probably arranged with minus ends in the apical cytoplasm and plus ends in the basal cytoplasm or yolk (Figure 1C). Pair-rule and segment polarity mRNAs are expressed in those syncytial nuclei, exported, and localized apically (Davis and Ish-Horowicz, 1991; Simmonds et al., 2001). To study the localization mechanism, bright fluorescent mRNA analogs were created, purified, and injected beneath the layer of blastoderm nuclei. Widefield deconvolution microscopy revealed that pair-rule transcripts formed particles that moved directly to the apical cytoplasm with a net velocity of $\sim$0.5 μm/s (Wilkie and Davis, 2001; see movies at http://www.cell.com/cgi/content/full/105/2/209/DC1). The transport was blocked by preinjection with colcemid, a microtubule destabilizing drug, and restored by inactivation of the drug with a pulse of UV light. These results provide a compelling argument that the pair-rule transcript localization mechanism includes active transport along microtubules.

To probe the mechanism further, Wilkie and Davis tested the possibility that cytoplasmic dynein, a minus-end-directed microtubule motor, provides the force for moving pair-rule transcript particles along microtubules. Apical transport was blocked by preinjecting the embryos with antibodies that specifically bind cytoplasmic dynein. In addition, particle transport was slowed 2- to 3-fold by partial-loss-of-function mutations in *Dhc64C*, which encodes the force-producing subunit of cytoplasmic dynein. Based on these and other results, the mechanism for apical localization of pair-rule mRNAs appears to include: (1) transcription and nondirectional export from blastoderm nuclei and (2) dynein-mediated RNP transport along microtubules into the apical cytoplasm. A particularly important remaining question regards how dynein is linked to the RNA particles.

Shared mRNA Transport Machinery in Egg Chambers and Embryos

A new report in *Nature* by Bullock and Ish-Horowicz describes work that probes RNP transport mechanisms extensively, revealing mRNA structural requirements and critical proteins (Bullock and Ish-Horowicz, 2001). Fluorescent transcripts corresponding to a number of well-known mRNAs that are synthesized in nurse cells then localized to the early oocyte (*bicoid, oskar, gurken,*

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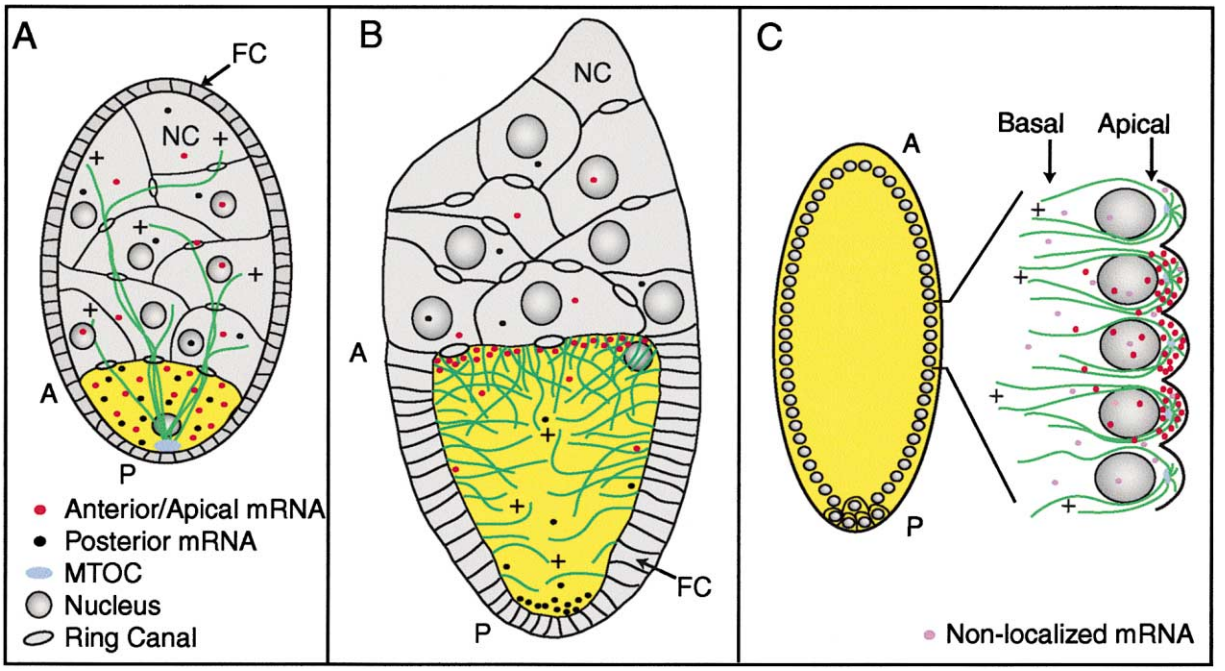

Figure 1. Localization of mRNAs during *Drosophila* Oogenesis and Early Embryogenesis

Cartoons highlight the organization of (A) an egg chamber in early oogenesis, (B) an egg chamber in mid-oogenesis, and (C) a syncytial blastoderm embryo. Oocytes and embryo are yellow. Nurse cells (NC) and follicle cells (FC) are gray. Microtubules are green and their plus ends are marked (+). The scale for each panel is arbitrary.

nanos, K10) were injected beneath the layer of nuclei in embryos. With the exception of *oskar*, the maternal transcripts showed efficient apical localization within 10 min, and the localization was dramatically inhibited by preinjection with colcemid. As discussed above, apical transport in blastoderm embryos is almost certainly toward the minus ends of microtubules (Figure 1C). In the early oocyte, a microtubule organizing center (MTOC) containing numerous centrioles resides near the posterior cortex (Figure 1A). Microtubules emanate from it and extend through ring canals into the nurse cells. Thus, microtubule-based transport into the early oocyte from nurse cells is again probably minus-end-directed. Although Bullock and Ish-Horowicz did not report function disruption tests for cytoplasmic dynein, it would not be surprising to find, as they suggest, that dynein provides the force for both apical transport and nurse cell-to-oocyte transport of most maternal mRNAs.

Comparison of the 3′ sequence requirements for mRNA localization in embryos and in the early oocyte showed remarkable parallels. A stem-loop structure in the 3′ UTR of *K10* mRNA is known to be required for localization to the early oocyte (Serano and Cohen, 1995). To test the influence of the stem-forming structure in embryos, Bullock and Ish-Horowicz used a classical approach: testing mutations that cause stem disruption and compensatory restoration. Fluorescent *K10* transcripts with stem nucleotides on one side converted to their complements showed no appreciable apical localization. Transcripts with stem nucleotides on both sides converted to their complements restored apical localization, although it was less robust. Furthermore, the *K10* stem-loop sequence was sufficient for apical concentration of two reporter transcripts that otherwise showed no apical localization. This indicates that transcript sequences required for nurse cell-to-oocyte transport can successfully engage the apical localization machinery of the blastoderm embryo. The reverse is also true. Pair-rule transcripts (*hairy*, *fushi tarazu*, *runt*) expressed ectopically in nurse cells localized to the early oocyte. Deletion analysis of *fushi tarazu* suggests that similar 3′ UTR sequences are required in both localization processes. Clearly, 3′ UTR sequences have a fundamental role in transport, perhaps in linking mRNAs to RNPs that can bind microtubule motors.

In search of protein components of such RNP complexes, Bullock and Ish-Horowicz capitalized on the wealth of information on factors that influence oogenesis. Egalitarian and Bicaudal D are required for normal mRNA localization in oocytes. However, they also influence the organization of microtubules and oocyte specification early in oogenesis, making their specific role in mRNA transport difficult to define (Mach and Lehmann, 1997; Swan and Suter, 1996). When apically targeted pair-rule or maternal transcripts were injected into the basal cytoplasm of blastoderm embryos, maternal Egalitarian and Bicaudal D first concentrated at the injection site, then localized apically in parallel with the transcripts. The proteins did not concentrate at the site of injection or localize apically when transcripts with mutated localization sequences were injected. Although the presence of Egalitarian and Bicaudal D in single RNA particles was not demonstrated, the other results argue that they are specific components of apically transported RNPs.

Do Egalitarian and Bicaudal D contribute to the trans-

port process or are they simply hitchhikers? Preinjection of embryos with antibodies to either protein greatly inhibited the apical localization of *hairy* transcripts. Partial loss-of-function *Bicaudal D* mutations mildly inhibited *hairy* localization and sensitized embryos to preinjection with dilute Bicaudal D antibody solutions, suggesting specific effects on Bicaudal D function. Similar results were seen for Egalitarian. Although important gaps remain, one can imagine that Bicaudal D and Egalitarian help link specific RNPs to dynein in both blastoderm embryos and early egg chambers. At the very least, Bicaudal D and Egalitarian are important components of some transcript localization/targeting processes.

Specific mRNA Localization within the Oocyte

During the middle stages of oogenesis (stages 7–10), *bicoid* and *oskar* mRNAs are localized to opposite ends of the oocyte, marking the anterior and posterior poles, respectively. Both localizations are sensitive to microtubule disrupting agents, raising the possibility of active transport by microtubule motors (Riechmann and Ephrussi, 2001). Consistent with this, germline mutations that disrupt kinesin I, a plus-end-directed microtubule motor, inhibit the posterior localization of *oskar* mRNA (Brendza et al., 2000). The mutations also inhibit posterior localization of Staufen, an RNA binding protein required for posterior *oskar* localization (Micklem et al., 2000). Does kinesin I bind *oskar*-Staufen RNPs and move them to the posterior pole? Glotzer et al. studied the localization of fluorescent *oskar* transcripts injected into mid-stage oocytes. Their results indicate that microtubules are not absolutely required for posterior *oskar* localization. In colchicine-treated oocytes, fluorescent *oskar* transcripts injected far from the posterior pole did not localize, but those injected close to the posterior pole did (Glotzer et al., 1997). This result and others suggest that a specific posterior anchoring system for *oskar* RNPs contributes to localization. It may be that kinesin I does not drive *oskar* directly to the posterior pole along well-oriented microtubules (see below). Instead, it may drive less oriented, saltatory movements, perhaps biased toward the posterior pole, that help *oskar* RNPs find their posterior anchors.

Cha, Koppetsch, and Theurkauf recently reported in *Cell* an exciting study of the mechanism of anterior *bicoid* mRNA localization during mid-oogenesis (Cha et al., 2001). Purified fluorescent *bicoid* transcripts, when injected into oocytes that had intact microtubules, localized promiscuously to any nearby part of the oocyte cortex, except at the posterior pole (see movies at http://www.cell.com/cgi/content/full/106/1/35/DC1). However, after injection into the nucleus of a nurse cell, *bicoid* transcripts localized specifically to the anterior cortex. To determine if anterior specificity requires nuclear processing, they injected fluorescent transcripts into nurse cell cytoplasm. The RNA formed particles and large aggregates that displayed rapid, microtubule-dependent saltatory movements. Small particles moved to ring canals, then into the oocyte and concentrated along the anterior cortex. Since particles were not apparent after nuclear injection, those seen after cytoplasmic injection may not be entirely normal. However, their clear anterior localization suggests that anterior specificity does not require nuclear processing.

Perhaps the anterior specificity is simply due to the fact that oocyte ring canals are at the anterior cortex. When *bicoid* mRNA enters, it might engage with the first cortical binding sites it encounters and remain anterior. Cha et al. used a clever approach to demonstrate that this is not the whole story. Fluorescent *bicoid* transcripts were injected into a nurse cell for approximately 30 s, then nurse cell cytoplasm, containing newly formed fluorescent particles, was drawn back into the microneedle and injected into the oocyte of a second egg chamber. Exhibiting rapid saltatory movements, some of the "conditioned" fluorescent transcripts localized to the lateral cortex nearest the injection site, but there was a significant bias for anterior localization. This suggests that there is more to anterior targeting than just local binding after entry through the ring canals.

It is known that Exuperentia protein is important for anterior *bicoid* localization. Cha et al. showed that an Exuperentia::green fluorescent protein fusion concentrated in the *bicoid* particles that form in nurse cells. Furthermore, when fluorescent *bicoid* transcripts were injected into the nurse cells of *exuperentia* mutant egg chambers, fluorescent particles could form and move into the mutant oocyte. However they then showed little active movement and eventually dispersed without localizing to the cortex. Where is Exuperentia needed? Preinjection of fluorescent *bicoid* transcripts into an *exuperentia* mutant nurse cell followed by withdrawal and injection into the wild-type oocyte of a second egg chamber produced promiscuous cortical localization. Thus, while exposure of *bicoid* transcripts to Exuperentia just in the oocyte allows cortical localization, it is not sufficient for anterior specificity. Further tests revealed that anterior specificity requires exposure of *bicoid* transcripts to Exuperentia in the nurse cells. Fluorescent *bicoid* transcripts preinjected into a wild-type nurse cell, withdrawn and injected into an *exuperentia* mutant oocyte showed a clear bias toward anterior cortical localization. This requirement for Exuperentia activity specifically in nurse cells suggests there are additional factors that influence the anterior specificity of *bicoid* RNPs.

Specific Localization on Tangled Tracks?

Microtubules are important in the mRNA localization mechanisms of all three *Drosophila* scenarios discussed here. In the syncytial blastoderm embryo and the early egg chamber, cases can be made for reasonably ordered microtubule arrays, based on the locations of MTOCs and the trajectories of microtubules (Figures 1A and 1C). Hence, apical transport in the embryo and transport to oocytes in early egg chambers are probably minus-end-directed. However, in the middle stages of oogenesis, when *oskar* and *bicoid* mRNAs are localized to opposite ends of the oocyte, microtubule organization is complex (Figure 1B). During stage 7, the posterior MTOC disappears and most of the oocyte cortex takes on the capacity to nucleate microtubules. That capacity appears strongest at the anterior cortex and weakest at the posterior cortex, but the trajectories of microtubules emanating from the cortex appear random (Cha et al., 2001).

Despite the complex organization, microtubules of mid-stage oocytes can obviously support polarized localization of mRNAs. Fluorescent *bicoid* transcripts pretreated with nurse cell cytoplasm accumulate preferentially at the anterior cortex. With minus ends apparently

abundant along the lateral cortex as well, this presents a mechanistic puzzle. Cha et al. (2001) raise the possibility that all oocyte microtubules may not be functionally equivalent. Rather, there could be a subset of microtubules emanating from the anterior cortex that are modified to favor transport by *bicoid* RNPs. This could be through distinct microtubule surface lattice structures, variation in tubulin isoforms, and posttranslational modification. Perhaps *bicoid* RNPs, after proper assembly in nurse cells with Exuperentia, can influence the function of a minus-end-directed motor (cytoplasmic dynein?), differentially altering its processivity on microtubules originating from lateral and anterior cortical sites. Such directed movement of fluorescent *bicoid* particles is not obvious in the movies from Cha et al., but perhaps quantification of the directions and velocities of particle saltations will reveal an anterior bias. A mild anterior bias might stem simply from the elevated density of microtubule minus ends at the anterior cortex of the oocyte: e.g., a minus-end-directed motor like cytoplasmic dynein could naturally concentrate its cargoes toward the anterior. It is also worth considering that Exuperentia could alter *bicoid* RNPs to enhance anterior as opposed to lateral anchorage, rather than altering interactions with specific microtubule subsets.

What Next?

The work described here focuses attention on a number of key questions. What are the physical links between motors and the mRNAs that they move? Can specific binding partners be identified for transcript localization sequences? What are the components of localized RNPs and how are they assembled? How do RNPs find their way when filament tracks are tangled, e.g., can distinct subclasses of microtubules mark a preferred path? Then finally, what mechanisms keep mRNAs localized at their proper destinations? Certainly, tracking the movements of fluorescent transcripts in live cells has helped open the door to answering these questions. Combined with genetic, biochemical, and biophysical approaches (Bohl et al., 2000; Gross et al., 2000; Wilhelm et al., 2000), we should see exciting progress in the next few years.

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