Highways for mRNA Transport

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Two new studies reveal the role of microtubule polarity in the asymmetric localization of mRNAs. In this issue of *Cell*, Zimyanin et al. (2008) show that the asymmetric localization of *oskar* mRNA in fruit fly oocytes results from a slight bias in the direction of its transport. Meanwhile, Messitt et al. (2008) reporting in *Developmental Cell* find a subpopulation of microtubules that is critical for the asymmetric distribution of *Vg1* mRNA in frog oocytes.

Messenger RNAs (mRNAs) can diffuse efficiently within the confined volume of the nucleus (Shav-Tal et al., 2004), whereas the localization of mRNAs in distinct regions of the cell periphery requires a dedicated transport mechanism given the much greater volume of the cytoplasm. Well-studied examples of asymmetrically localized mRNAs include *oskar* and *Vg1* in oocytes of the fruit fly and frog, respectively (reviewed in Shav-Tal and Singer, 2005). Although it is generally accepted that motor proteins moving along polarized microtubules are involved in the cytoplasmic transport of localized mRNAs, assessing the role of microtubule-based transport in mRNA localization has been unexpectedly difficult. Work published in this issue (Zimyanin et al., 2008) and recently in *Developmental Cell* (Messitt et al., 2008) provides new insight into mechanisms that establish asymmetry in the cytoplasmic localization of mRNAs. Zimyanin et al. (2008) show that *oskar* mRNA is actively transported toward both poles of the *Drosophila* oocyte, but its eventual localization to the posterior pole is promoted by a slight bias in the direction of its transport. In related work, Messitt et al. (2008) show that *Vg1* mRNA is directed to the vegetal pole in *Xenopus* oocytes by at least two kinesins that move along a subpopulation of microtubules.

Although the orientation of microtubules in somatic cells is relatively well established, the orientation of microtubules in oocytes has been a source of controversy. There are various hypotheses for how microtubules are oriented in *Drosophila* oocytes. Evidence indicates that a substantial proportion of microtubules in *Xenopus* oocytes are apparently pointing in the wrong direction for transport driven by the molecular motor kinesin. Furthermore, the localization of mRNAs takes orders of magnitude longer than would be expected if the mRNAs were simply whisked along on microtubules, like automobiles on the Autobahn. To confound the situation further, at a particular stage in their development *Drosophila* oocytes are subject to kinesin-dependent cytoplasmic streaming, in which the ooplasm acts like an intracellular "washing machine," carrying everything along with it including mRNAs. Thus, mRNAs could be captured passively at the posterior pole or could be moved selectively away from the anterior and lateral cortex.

One of the problems of the experimental approaches used so far is that they are end-point analyses, that is, the individual steps of mRNA localization have not yet been defined. Observing endogenously transcribed mRNAs moving in living cells is one of the ways to define the steps of mRNA localization. This technique was developed originally in yeast (Bertrand et al., 1998) and has since been applied to mammalian cells (Fusco et al., 2003) and *Drosophila* embryos (Forrest and Gavis, 2003). It involves inserting a cassette of stem loops from the RNA phage MS2 that bind tightly to MS2 capsid protein, which can be fused to a fluorescent marker. The new work by Zimyanin et al. (2008) refines this technique to observe what may be individual *oskar* mRNA "particles" moving in oocytes from both wild-type and mutant flies. The authors find that the mRNAs move in both directions because the orientation of microtubules is mixed, an observation long known in neurons and most recently described in Dictenberg et al. (2008). However, the major contribution of this work comes from the combination of detailed measurements with genetic mutations. They reveal a slight bias (14%) in transport of *oskar* mRNA toward the posterior pole of the oocyte. Over 6–10 hr, this bias leads to the asymmetric localization of *oskar* mRNA. Importantly, these movements correlate with the known effects of various mutant kinesin motors and are not affected by mutant dynein. Hence, this work provides direct evidence that *oskar* mRNA is associated with kinesin motors. It has been observed that *oskar* mRNA mislocalizes to the anterior pole in some mutant fly strains, such as *mago*, *barentsz*, and *Tropomyosin II.* The analysis of particle movement demonstrated that the bias is indeed reversed in these mutant strains. It is unclear what determines this reversal; the authors suggest that the minus-end-directed motor dynein might also be part of the *oskar* ribonucleoprotein complex, and that the activity of dynein is unleashed by the loss of the plus-end-directed kinesin. Supporting this notion is the observation that in flies carrying mutations in dynein the particles move slightly faster, as though the dynein was acting as a brake. Clearly, the anchoring of the mRNAs at the posterior pole is also important for the establishment and maintenance of mRNA polarity. For instance, in flies lacking the anchoring protein Staufen, the localization of the mRNA to the posterior

pole is transient and weak. The efforts of Zimyanin et al. working with *oskar* mRNA could be extended to determine the point where movement stops and anchoring and translation begin. Using similar technology, Weil et al. (2008) have recently shown that the asymmetric localization of *bicoid* mRNA to the anterior pole requires microtubules for movement and actin filaments for anchoring.

Messitt et al. also reveal a direct role for kinesin in the transport of a localized mRNA, in this case *Vg1* mRNA to the vegetal pole of *Xenopus* oocytes. Although most microtubules in *Xenopus* oocytes have their minus ends pointed toward the vegetal pole, the authors find that there is a minor population of microtubules oriented in the opposite direction. This resolves the conundrum of how kinesin-mediated transport could be involved in *Vg1* mRNA localization. Interestingly, they find two kinesins involved in the transport (kinesins 1 and 2). These kinesins are not redundant in that reduction of either by RNA interference blocks transport. However, overexpression of either kinesin can overcome this block, indicating that they may supplement each other and that the availability of motors for transport may be limiting. This is not the whole story, however, as the mRNA can move halfway toward the pole without either of the kinesins, suggesting that yet another mystery player is involved in the transport of the mRNA. Given that *Vg1* mRNA can accumulate at this halfway point the authors suggest that bidirectional transport takes place. Unlike Zimyanin et al., they propose that the bias to a particular pole is driven by a mechanism involving anchoring of the *Vg1* mRNA at the cortex. Cortical anchoring may be a mechanism conserved between *Xenopus* and *Drosophila* and could function for *oskar* mRNA as well, although there is no evidence for this as yet (Figure 1).

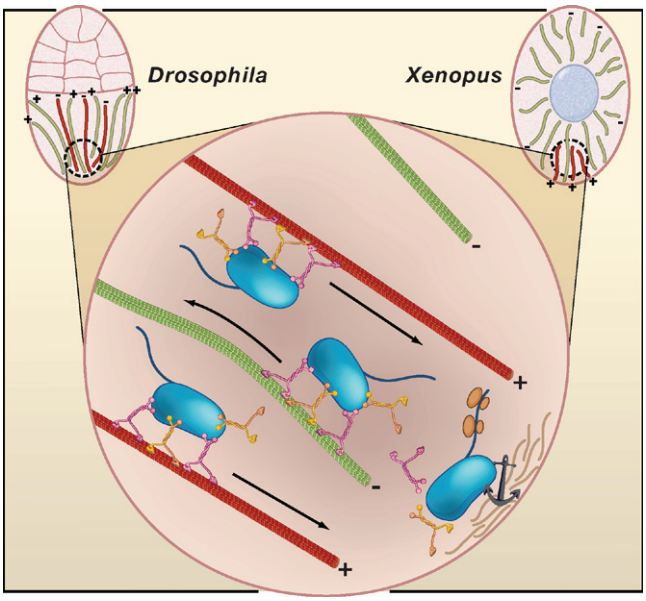

Figure 1. Conserved Mechanisms for mRNA Localization in Oocytes
Microtubules are oriented in both directions in oocytes from the fruit fly *Drosophila* (upper left) and the frog *Xenopus* (upper right). Dual polarity of microtubules is a feature of neuronal dendrites as well. In such cases, granules containing mRNAs (turquoise) can move in either direction, but the predominant orientation of the microtubules determines the overall directional bias (see expanded view). In cases where the microtubule orientation that promotes the eventual mRNA localization is in the minority, an anchoring mechanism must remove mRNAs from the mobile pool. Several kinesins are responsible for this plus-end-directed movement (shown in pink and gold). It is likely that this anchoring mechanism (indicated by anchor icon) includes cortical actin (shown as filaments) and may also include Tropomyosin and Staufen. Translation then occurs after anchoring (ribosomes indicated).

These findings bring to mind a set of related observations concerning another prominent microtubule structure in oocytes, the meiotic spindle. A rapidly expanding field has shown that many mRNAs, some of them important for spindle assembly and chromosome segregation, are physically associated with spindle microtubules and centrosomes. This has been most recently detailed by Eliscovich et al. (2008), who show that mRNAs associated with the spindle appear to be poised, upon translational activation by polyadenylation, to initiate synthesis of proteins needed during meiotic progression (or cell-cycle control in the case of the mitotic spindle). Spindle-associated mRNAs may stably associate with motor proteins, which are possibly in a nonmotile state. How some mRNAs distinguish between various microtubule compartments in oocytes or in somatic cells will likely be a subject of further exciting research.

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