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Cup-ling *oskar* RNA localization and translational control

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RNA localization and spatially restricted translational control can serve to deploy specific proteins to particular places within a cell. *oskar* (*osk*) RNA is a key initiatior of posterior patterning and germ cell specification in *Drosophila,* and its localization and translation are under elaborate control. In this issue, Wilhelm et al. (2003) show that the protein Cup both promotes *osk* localization and participates in repressing translation of unlocalized *osk*.

RNA localization and translational control are coupled processes that cooperate to target proteins to specific locations within the *Drosophila* oocyte (Cooperstock and Lipshitz, 2001; Johnstone and Lasko, 2001). This is particularly important in establishing the pole plasm, a specialized region of oocyte cytoplasm located at the posterior pole that is necessary after fertilization for the establishment of germ cells and for posterior somatic patterning. *oskar* (*osk*) RNA is the first molecule to be recruited to the pole plasm, and mislocalization of *osk* to the anterior of the oocyte can lead to ectopic assembly of pole plasm and formation of ectopic functional germ cells (Ephrussi et al., 1991; Kim-Ha et al., 1991; Ephrussi and Lehmann, 1992). As *osk* can catalyze the recruitment of all essential downstream pole plasm components, it is of great interest to understand the mechanisms by which *osk* RNA is localized. *osk* is also under translational control, such that unlocalized *osk* is silent, but *osk* in the pole plasm is translationally derepressed and active (Kim-Ha et al., 1995). Thus, translational regulation and RNA localization both ensure that Osk protein is restricted to the posterior pole of the oocyte.

osk RNA accumulates in the oocyte shortly after the specification of that cell. Approximately when yolk uptake commences (stage 8), it localizes transiently to the anterior pole and then becomes enriched in the posterior pole plasm. As *osk* is unlocalized in null mutants for *kinesin heavy chain* (*khc*), kinesin I–driven microtubule-dependent plus end–directed transport is implicated in posterior localization of *osk* (Brendza et al., 2000; Tekotte and Davis, 2002). A key molecule involved in coupling *osk* to kinesin I is the protein Barenstz (Btz; van Eeden et al., 2001). Although many mutations impact upon RNA localization within the oocyte, most affect multiple localization processes, and encode proteins involved in RNA metabolism (*mago nashi*, *tsunagi*, and *staufen*) or that interact with the microtubule and/or microfilament cytoskeleton (*cappuccino*, *spire*, *chickadee*, *TropomyosinII*, and *par-1*). However, *btz*-null mutations completely block posterior accumulation of *osk* without affecting other localized RNAs or cytoskeletal polarization. Furthermore, Btz colocalizes to the posterior with *osk* mRNA, in a manner dependent upon *osk* mRNA.

osk is also under complex translational regulation. Bruno (Bru) and an unidentified protein called p50 interacts with specific sequences in the *osk* 3′UTR, and an *osk* transgene deleted for the response elements is prematurely translated before the RNA localizes to the posterior (Kim-Ha et al., 1995). Thus, Bru is involved in repressing translation of unlocalized osk. Yet even when the Bru–*osk* interaction is abrogated, translation is still silenced until stage 7. Other gene products such as Bicaudal-C and ME31B also contribute to translational repression of *osk* (Saffman et al. 1998; Nakamura et al. 2001). Relief of *osk* translational repression also involves several factors, in particular, Orb, Staufen, and Aubergine (Chang et al., 1999; Micklem et al., 2000; Harris and Macdonald, 2001).

In previous work, Wilhelm and his coworkers purified ribonucleoprotein (RNP) complexes containing *osk* RNA (Wilhelm et al., 2000). They recovered eight distinct protein species. Two corresponded to Exuperantia (Exu) and Ypsilon Schachtel (Yps). *exu* is required for anterior localization of *bicoid* (*bcd*) RNA (St. Johnston et al., 1989), and Wilhelm et al. demonstrated a modest effect of *exu* mutations on *osk* localization as well. Yps is a Y-box protein related to *Xenopus* FRGY2, an oocyte-specific protein implicated in translational silencing (Matsumoto et al., 1996). This work led to the conclusion that *osk* RNA exists in cytoplasmic RNP complexes, associated with proteins involved in its localization and translational control. Now, Wilhelm et al. (2003) have taken a significant step forward toward development of a more complete understanding of *osk* localization and translational control, and of how these two processes are linked. They report that another component of the *osk* RNPs is Cup. Existing *cup* mutants implicate it in the transfer of nurse cell cytoplasm to the oocyte in late oogenesis (Keyes and Spradling, 1997). Wilhelm et al. found that *osk* localiza-

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Abbreviations used in this paper: 4E-T, eIF4E transporter; Bru, Bruno; Btz, Barenstz; Exu, Exuperantia; Osk, Oskar; Yps, Ypsilon Schachtel.

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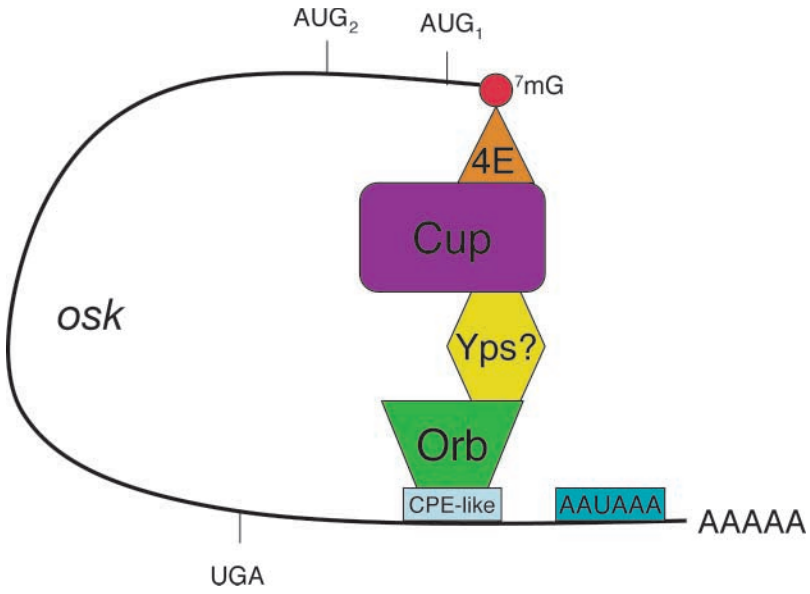

Figure 1. **A hypothetical model explaining Cup-mediated translational repression of *osk*.** The model is based on that proposed by Stebbings-Boaz et al. (1999) for maskin. See text for details.

tion, and transport of Btz to the oocyte, were also abrogated in *cup* mutant ovaries. Thus, Cup is required to recruit Btz to *osk* RNA, and thus for its localization.

Mutations in most genes required for *osk* localization also block *osk* translation, since unlocalized *osk* is translationally repressed. In *cup* mutants, however, *osk* translation is derepressed, indicating that unlike these other gene products, Cup functions in translational regulation of *osk*. Cup shares some sequence similarity with eIF4E transporter (4E-T), a mammalian protein implicated in nuclear import of the 5′ mRNA cap binding protein eIF4E (Dostie et al., 2000). The nuclear function of eIF4E is not known. However, overexpression of 4E-T in cultured cells strongly represses translation of a luciferase reporter construct, presumably by reducing the level of cytoplasmic eIF4E. Wilhelm et al. showed that like 4E-T, Cup binds eIF4E. Moreover, they observed a dynamic localization of eIF4E within the oocyte that corresponds remarkably with those of Cup and *osk* RNA.

Based on their results, Wilhelm et al. propose an attractive model whereby Cup is required in early oogenesis to recruit Btz to the *osk* RNP. Cup also binds the eIF4E component of the *osk* RNP, thus inhibiting translation. Later in oogenesis, the *osk* RNP rearranges allowing Btz to recruit kinesin. Finally, after posterior localization of *osk*, the *osk* RNP is remodeled so that the Cup–eIF4E association is broken, and *osk* translation is derepressed.

A key test of this model would be to determine whether ovaries expressing only a mutant form of Cup that is specifically abrogated for interaction with eIF4E would show premature *osk* translation. Such a mutation would be straightforward to generate as a canonical eIF4E binding site is present in Cup. Other studies suggest that control of poly(A) tail length is critical to the translational regulation of *osk* (Chang et al., 1999). Interestingly, Yps, another component of the *osk* RNP that contains Cup, represses *osk* translation by acting antagonistically to Orb in regulating its poly(A) tail (Mansfield et al., 2002). Cup's role could be analogous to that of maskin (Stebbings-Boaz et al., 1999), a translational repressor in *Xenopus* oocytes that bridges CPEB (Orb in flies) and eIF4E. Although Cup is not the most similar gene in the *Drosophila* genome to maskin, both proteins bind eIF4E and negatively regulate translation of maternal mRNAs. If Cup and maskin share some functionality, then an association between Cup and Orb must exist. Such an association has not been demonstrated, but perhaps could occur indirectly through Yps (Fig. 1), which operates antagonistically to Orb in *osk* regulation (Mansfield et al., 2002).

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