Cup-ling oskar RNA localization and translational control

Paul Lasko

Department of Biology, McGill University, Montréal, Québec, Canada

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 initiator 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 Barentsz (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 sunfish) 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 FGRY2, 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 exu 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-

Address correspondence to Paul Lasko, Department of Biology, McGill University, 115 West University Parkway, Montréal, Québec, Canada. Tel.: (514) 398-6401. Fax: (514) 398-5069. Email: Paul.Lasko@mcgill.ca

Abbreviations used in this paper: 4E-T, eIF4E transporter; Bru, Bruno; Btz, Barentsz; Exu, Exuperantia; Osk, Oskar; Yps, Ypsilon Schachtel.
Cup functions in translational regulation of osk. Other studies suggest that control of osk translation is derepressed. In osk 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 elf4E transporter (4E-T), a mammalian protein implicated in nuclear import of the 5′ mRNA cap binding protein elf4E (Dostie et al., 2000). The nuclear function of elf4E 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 elf4E. Wilhelm et al. showed that like 4E-T, Cup binds elf4E. Moreover, they observed a dynamic localization of elf4E 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 elf4E 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–elf4E 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 elf4E would show premature osk translation. Such a mutation would be straightforward to generate as a canonical elf4E 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 elf4E. Although Cup is not the most similar gene in the Drosophila genome to maskin, both proteins bind elf4E 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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