The road not taken: new destinations for yeast mRNAs on the move

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Highly specific and precise mechanisms have evolved for transport of mRNAs to subcellular regions in polarized eukaryotic cells. RNA localization is largely thought to be driven by RNA structures that are recognized by RNA-binding proteins. In this issue of *The EMBO Journal*, Kilchert and Spang (2011) show that yeast ABP140 mRNA is localized through a translation-dependent mechanism. The first 17 amino acids of the nascent ABP140 peptide chain confer this activity. Much attention has been directed to mRNAs moving into the budding daughter cell of yeast. ABP140 mRNA goes the wrong way, localizing the mother cell's pole opposite to the bud. Moreover, the cotranslational localization motif in

the ABP140 N terminus is able to overcome RNA structure-driven transport. This provides a novel mechanism to target mRNAs to subcellular sites that may be shared by other mRNAs that pave routes to previously unrecognized destinations.

mRNA transport with subsequent localized protein synthesis is a mechanism that evolved in eukaryotes for spatially and temporally modulating protein levels (Martin and Ephrussi, 2009). The locally translated protein products help to establish and maintain cellular polarity and allow subcellular domains to respond to stimuli autonomous from other regions of the cell. Asymmetric distribution of *bicoid* and *oskar* mRNAs to the opposite poles in *Drosophila*

Figure 1 Schematic of cotranslational mRNA targeting of ABP140 mRNA (dark blue) to distal pole versus traditional RNA element-dependent targeting of ASH1 mRNA to the bud tip. ASH1 mRNA (light blue) is linked to myosin motor (orange) through She proteins (red and pink), whereas ABP140's nascent N terminus (purple) links to retrograde actin cable flow through an ABD. In the ABP140/ASH1 chimeric mRNA shown at the lower left, ABP140 mRNA's cotranslational-dependent transport dominates over the ASH1 RNA UTR localization element.

embryos supports anterior–posterior polarization, and antero-dorsal localization of *gurken* mRNA helps to establish the embryo's dorso-ventral axis (Wilhelm *et al*, 2005). Localization of *Vg1* and *VegT* mRNAs to the vegetal pole of *Xenopus* oocytes contributes to patterning of mesodermal and endodermal tissues (Melton *et al*, 1989). In neurons, protein products of localized mRNAs contribute to growth and function of neuronal processes. Although most early analyses of mRNA transport focused on single or small numbers of mRNAs, profiling studies of transported mRNAs have shown surprisingly large populations of cellular mRNAs with subcellular localization. Indeed, a high throughput *in situ* hybridization study in *Drosophila* showed that two-thirds of more than 3000 tested mRNAs exhibited subcellular localization (Lecuyer *et al*, 2007). With such increasingly complex populations of mRNAs now recognized to undergo transport, eukaryotes must have multiple mechanisms to target mRNAs to these sites. However, our current knowledge of the mechanisms underlying this targeting is quite limited. Work by Kilchert and Spang (2011) appearing in this issue of *The EMBO Journal* uncovers two novel aspects of mRNA targeting in budding yeast.

In budding yeast, targeting of ASH1 mRNA to the budding daughter cell enables the subsequent suppression of mating-type switching (Long *et al*, 1997). At least 24 different mRNAs are transported to the daughter cell in budding yeast (Shepard *et al*, 2003). However, there has not been evidence for mRNAs selectively targeted to the pole of the mother cell that is located opposite to the bud. Kilchert and Spang (2011) report that ABP140 mRNA is targeted to this distal pole. This selective targeting of ABP140 mRNA is seen even in yeast mutants, with random and bipolar budding patterns. Effectively, one could refer to ABP140 mRNA as 'going the wrong way' (Figure 1). Transporting mRNAs the wrong way is somewhat analogous to neuronal systems, where mRNAs and translational machineries were initially thought to localize only to postsynaptic and not to presynaptic processes. However, closer inspection showed that mRNAs can also be selectively targeted to the presynaptic process (Yoon *et al*, 2009). In neurons, similar mechanistic themes are used to target mRNAs into either process. This is not the case for yeast ABP140 versus the bud-targeted mRNAs. Moreover, transport of ABP140 is translation dependent in contrast to the mRNAs targeted to the budding daughter cell, whose transport occurs in the absence of their translation.

In most cases where transport mechanisms of mRNAs have been defined, targeting initiates through RNA-binding proteins (RBPs) recognizing specific RNA structures. Such RNA elements or 'zipcodes' have typically been restricted to untranslated regions (UTRs) of the mRNAs and are only rarely seen in the open reading frames (Andreassi and Riccio, 2009). This RNA–RBP complex, likely accompanied by other proteins in the form of a large ribonucleoprotein

complex, links the mRNA to molecular motors for delivery to subcellular sites. This transport occurs along microfilaments in yeast, but microtubules are used for RNA movement in many larger cell types (e.g., neurons). For yeast ASH1 mRNA, She2p protein binds to the mRNA and is then linked to the Myo4p myosin motor via the She3p adapter for transport along actin cables to the bud (Long *et al*, 2000). Other 'bud-targeted' mRNAs have been identified based on their interaction with She proteins (Shepard *et al*, 2003). In contrast, ABP140 mRNA localizes in a She protein-independent manner. This opens up the possibility that other distal-pole-targeted mRNAs may share transport mechanisms with ABP140.

A general scheme for mRNA trafficking is that the mRNA is translationally repressed *en route* (Martin and Ephrussi, 2009). Kilchert and Spang (2011) show that trafficking ABP140 is not translationally repressed and it does not use RNA structure for targeting. It is the initial N terminus of the nascent ABP140 polypeptide chain that sends the mRNA to the distal pole of the mother cell. Although it is not clear what proteins are needed to transport ABP140 mRNA, it is clear that these are not RBPs or at least RNA-binding domains are not necessarily involved. For this cotranslational transport model, the first 17 residues in the ABP140's actin-binding domain (ABD) ride on actin cables in retrograde flow to the distal pole as a ternary complex of the mRNA, translating ribosomes and nascent N-terminal ABD. This certainly raises the complexity for how mRNAs can move about cells. Surprisingly, the N-terminal ABD was able to overcome the UTR targeting motifs, sending a chimeric mRNA with ABP140's ABD and ASH1 mRNA's 3'UTR zipcode towards the distal pole (Figure 1). Thus, the cotranslational targeting mechanism wins out over the traditional mechanisms for moving mRNAs about cells.

It should be noted that similar to many other locally generated proteins, the function(s) of the locally translated ABP140 remain unknown. Intuitively, translational repression of trafficking mRNAs provides a means to restrict expression of the encoded proteins to where and when they are most needed. For ABP140, this would concentrate the protein at the distal pole of the mother cell. Proteins that are targeted for cotranslational secretion provide a model for temporarily arresting elongation, where the signal recognition particle recognizes a nascent N-terminal signal peptide and arrests continued translation of the mRNA until it docks with the rough endoplasmic reticulum. Whether an analogous mechanism exists for ABP140 mRNA remains to be tested. A factor beyond the above ABP140 ternary complex could provide a mechanism generalizable to other cotranslationally transported mRNAs.

Conflict of interest

The authors declare that they have no conflict of interest.

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