mRNA localization: motile RNA, asymmetric anchors

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Techniques to label mRNA with green fluorescent protein (GFP) have provided the first real-time images of RNA motility in live yeast cells. Genetic screens for factors responsible for mRNA asymmetry (e.g. *SHE* genes) in yeast identified type V myosin among other proteins. Analysis of mRNA movement in various *she* mutants revealed the role of motor proteins in long-range transport, factors for particle formation, and cortical anchors for docking the mRNA.

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Abbreviations

3′UTR	3′ untranslated region
ER	endoplasmic reticulum
GFP	green fluorescent protein
KH	HnRNP K homology
MBP	myelin basic protein
RRM	RNA recognition motif
Vera	Vg1 ER associated
Vg1 RBP	Vg1-RNA-binding protein
ZBP-1	zipcode-binding protein-1

Introduction

RNA localization is an important and conserved mechanism producing cellular asymmetries in a variety of organisms. The first observation of RNA localized to restricted regions of a cell was reported about 15 years ago. There are now over 75 spatially restricted transcripts identified in eggs, embryos, somatic cells, and single cell organisms. How these RNAs are transported and localized has been the subject of intensive investigation and several comprehensive literature reviews [1,2••,3–7]. Most recently *in vivo* imaging techniques have enabled visualization of RNA dynamics in live cells [8••,9••]. These techniques reveal a cytoskeletal based transport mechanism that facilitate RNA interactions with asymmetrically localized binding sites, which in turn determine RNA position. A summary of the yeast work will be discussed within the context of information derived from larger cells, highlighting the dynamic aspects of mRNA transport.

The players

3′UTR: localization determinants and translational control

The primary determinant for RNA localization is in the 3′ untranslated region (3′UTR). 3′UTRs are able to direct reporter gene constructs to restricted regions of a cell. There is not a single consensus sequence that determines the spatial fate of the RNA, rather a complex set of dispersed and often functionally redundant domains. Sequence elements within the 3′UTR directing RNA localization are typically larger than 44 nucleotides. These elements may provide a recognized structure (conservation of stem-loop structures rather than specific nucleotides), nucleotide specificity, or both ([10]; reviewed in [2••]). Dissection of the 3′UTR of *ASH1* mRNA from yeast revealed additional features required for the formation of an RNA–protein particle [11•,12•]. Finally, specificity elements have been localized within the coding region of *ASH1* mRNA [12•].

The 3′UTR is also capable of repressing translation until the RNA reaches its destination [13]. In *Drosophila* oocytes, both the *oskar* and *nanos* mRNAs are translated at the site of mRNA localization. Malpositioning of Oskar and Nanos proteins is sufficient to disrupt normal body-patterning, resulting in extreme developmental defects [14]. Sequences within the 3′UTR have been identified that restrict translation of the de-localized transcripts (i.e. in *osk*, BRE: bruno response elements [14]; in *nanos*, SRE: smaug recognition elements [15]). Like the localization elements, these sequences contain discrete, repeated elements that are largely functionally redundant [2••]. Positioning of translational regulation, localization determinants and RNA–protein complex formation within the 3′UTR emphasizes the complex nature of this noncoding region.

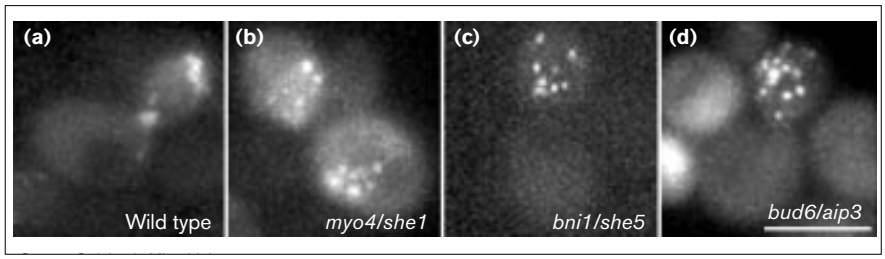
Translational products that control mRNA localization

The translation product of spatially restricted RNAs can also contribute to mRNA localization. Translation of yeast *ASH1* is required for tight anchoring of *ASH1* mRNA to the tip of budded cells [12•]. A reporter gene fused to full length *ASH1* coding sequence including the 3′UTR is correctly localized. A stop codon placed between the fusion moiety and *ASH1*, decreases the stringency of localization. Interestingly, co-expression of wild-type *ASH1* did not suppress the delocalization, indicating that Ash1p promotes anchoring only of mRNA from which it has been, or is being translated. Hence, Ash1p is a cis-acting regulator of *ASH1* mRNA localization. In *Drosophila*, maintenance of *osk* mRNA localization requires the production of Oskar protein [13]. Elements present in the 3′UTR are sufficient to direct and maintain *osk* mRNA localization in the presence of full length Osk protein. In contrast to Ash1p, however, Oskar protein is required in trans for maintenance of a reporter gene that contains solely *osk* 3′UTR. Thus translation of both *ASH1* and *oskar* mRNAs are required for their respective mRNA localization, but presumably via distinct mechanisms.

RNA-binding proteins

The 3′UTR forms an intricate stem-loop structure that is recognized by specific RNA-binding proteins. Proteins binding localized mRNAs have been identified in

Figure 1



Time composites showing $gRNA_{ASH1}$ particle movement [8••]. Ten sequential time-lapse images for **(a)** wild-type, **(b)** *myo4/she1*, **(c)** *bni1/she5*, and **(d)** *bud6/aip3* cells were combined into a single composite image. Each image illustrates the dynamic movements of the gRNA particle through the time series. (a) Wild-type cells maintained $gRNA_{ASH1}$ at the bud tip until migration to the bud neck prior to cytokinesis (elapsed time 20 minutes). (b) In *myo4/she1* cells, the $gRNA_{ASH1}$ remains motile within the mother domain of a small budded cell (lower right; a small bud extends upward from the mother cell body), and an unbudded cell (upper left) (elapsed time 10 minutes). In (c) *bni1/she5* and (d) *bud6/aip3* cells, $gRNA_{ASH1}$ was restricted to the bud but was not anchored at the bud tip enabling the particle to move throughout the bud (elapsed time 10 minutes). The scale bar represents 5 μm.

Drosophila (i.e. staufen, exl, bruno, smaug) and *Xenopus* — i.e. Vg1-RNA-binding protein (Vg1 RBP) also independently identified as Vera (Vg1 ER associated [16•]) and chick, zipcode-binding protein-1 (ZBP-1). These proteins share several motifs including HnRNP K homology (KH) and RNA recognition motif (RRM) domains. Vg1 RBP has five of these RNA-binding motifs: four KH and one RRM domain [16•,17•]. Vg1 RBP, ZBP-1, and the human protein KOC (KH-domain containing protein overexpressed in cancer) are over 75% identical throughout their peptide sequences. The high degree of homology between these proteins was not predicted from studies of protein function. First, Vg1 RBP and ZBP-1 recognize different motifs. ZBP-1 binds a six nucleotide repeat ACACCC [18], whereas Vg1 RBP/Vera binds a U-rich sequence (Vera UUCAC [16•]; Vg1 RBP UUUCUA [17•]). Second, these proteins have very different cytoskeletal associations. ZBP-1 fractionates from an RNA affinity column with actin, tropomyosin and gelsolin [18], whereas Vg1 RBP associates with microtubules both *in vitro* and *in vivo* [19]. These homologies are indicative either of conserved features within the different cytoskeletal networks, or that a given RNA interacts with both microtubules and the actin cytoskeleton towards its final destination (see below).

Cytoskeleton and cytoskeletal-based motors

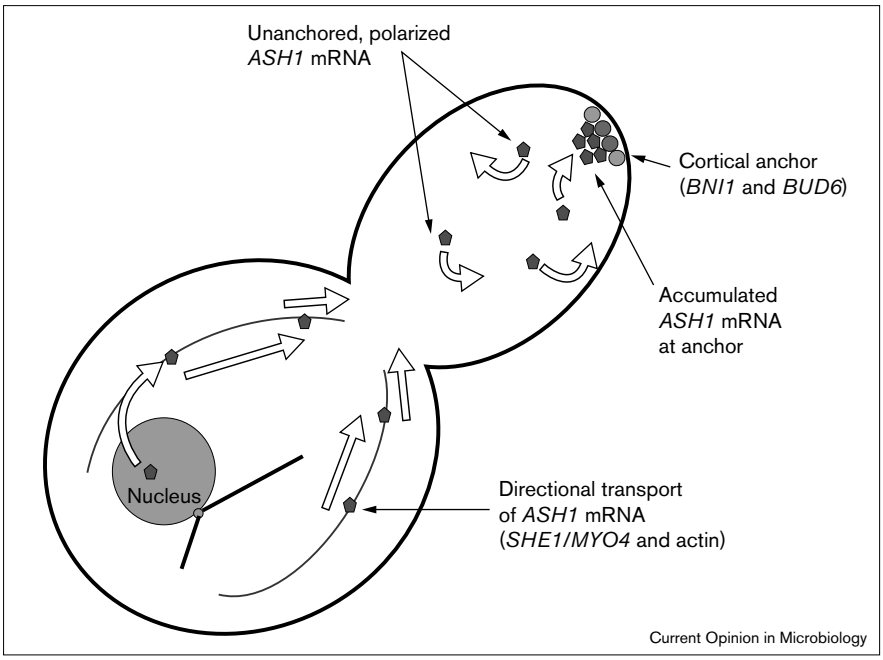
The role of the cytoskeleton is evident from biochemical interactions of RNA-binding proteins, and has been alluded to from the earliest experiments in which cytoskeletal inhibitors were found to disrupt RNA localization [20]. In early drug studies, both microtubule as well as actin depolymerizing drugs (nocodazole and cytochalasin, respectively) were effective in delocalizing positioned RNAs. Treatment with these drugs is likely to inhibit transport as well as promoting release of RNAs from cortical sites. These early indications have been borne out in studies from a variety of systems and current research is focused on identifying particular cytoskeletal based motors that mediate the interactions.

A clear example for the role of a specific motor protein in localizing RNA comes from studies on yeast. The localization of Ash1p is determined via a mRNA localization mechanism, defined in part by the *SHE* genes [9••,21,22]. Five *SHE* genes were identified in a genetic screen for defects in the expression of the HO endonuclease (*SHE*, symmetric HO expression) [23]. These mutants are defective in localizing a repressor of HO transcription, Ash1p, to the daughter cell nucleus. *SHE1* is identical to a previously identified gene, *MYO4*, which encodes an unconventional type V myosin. The absence of *she1/myo4* prevents *ASH1* mRNA translocation into the bud [8••,9••].

The case for microtubule-based motors in localizing mRNA has been made from several observations. Localization of the *bicoid* (*bcd*) mRNA is required for proper anterior patterning including head and thorax development. *bcd* mRNA in association with staufen is localized to the anterior pole of *Drosophila* oocytes in a microtubule-dependent fashion [24]. Staufen also forms a complex with *osk* RNA, which is deposited to the posterior pole. Kinesin–β-galactosidase fusion protein localizes to the posterior of *Drosophila* stage 8–9 oocytes, coincident with *osk* mRNA localization at this time [25]. These data are indicative of microtubule arrays organized with their plus-ends at the posterior and minus-ends at the anterior axis of the oocyte. However, cytoplasmic dynein, a minus end directed microtubule-based motor, also localizes to the posterior of the oocyte at stages 9 and 10 [26], indicating that microtubule organization and motor protein function/localization are not necessarily spatially correlated *in vivo*. Direct evidence for the role of kinesin in RNA localization derives from studies in oligodendrocytes [27]. Injection of kinesin anti-sense oligonucleotides prevents myelin basic protein (MBP) mRNA translocation.

Interaction of mRNA with microtubules may also be indirect. In *Xenopus*, Vg1 RNA is associated with endoplasmic reticulum (ER) membranes via Vera [28]. Since the ER is transported on microtubules [29,30], the dependence of Vg1 mRNA on the microtubule cytoskeleton may well be explained via this association. Thus, although the molecular nature of mRNA–cytoskeletal interactions may be varied, there is little question that both microtubule and

Figure 2



A general mechanism for mRNA localization in yeast and larger cells. This model depicts a multistep pathway composed of directed long-range movements and shorter stochastic movements. In yeast the long-range movements of *ASH1* mRNA (pentagons) from mother to bud are actin-based (thin, curved lines in mother cell), but are likely to be microtubule-based in *Drosophila*, *Xenopus* and mammalian cells (the yeast nucleus is shown in the mother cell with microtubules extending from the spindle pole body embedded in the nuclear envelope). Transport from the mother compartment into the bud requires the actin-based motor, *SHE1/MYO4*. Once mRNA reaches the vicinity of its final destination, cytoplasmic flow or passive diffusion may enable mRNA accumulation at that site (the bud tip in yeast). The anchoring complex in yeast is composed in part of *SHE5/BNI1* and *BUD6/AIP3*. Cortically localized proteins like inscuteable may provide this function in *Drosophila*.

actin cytoskeletal systems are requisite for the fidelity of RNA localization.

Where the action is

An inherent difficulty in ascribing the role of the cytoskeleton in mRNA localization is incorporating the dynamics of the network with the mechanism of transport. The advent of several techniques to image RNA or RNA-associated proteins in live cells has been invaluable in these efforts. Initially, injection of fluorescently labeled mRNAs was utilized. This technique is limited to large cells, and transient detection of mRNA. The advent of green fluorescent protein (GFP) enabled real-time images of endogenous proteins associated with their mRNAs (with the caveat that the protein may or may not be bound to the RNA). The construction of indirect labeling techniques to visualize mRNA itself bypass previous limitations on visualizing mRNAs through their RNA-binding protein. Targeted RNA imaging provides the opportunity for real-time measurements and brings dynamic views to bear on the transport and anchoring processes in living cells.

Microinjection

MBP mRNA was conjugated to fluoroscein and injected into oligodendrocytes to visualize mRNA in live cells. The injected mRNA becomes organized into granules that co-localize with cytoplasmic microtubules then translocate into oligodendritic processes [31]. While the movements are varied and complex, sustained movements with velocities of 0.2 μm/s (12 μm/min) are entirely consistent with microtubule-based kinesin driven transport. In addition, MBP mRNA granules align in tracks throughout the cytoplasm and undergo oscillatory motion at branch points in the network. These tracks correlate with microtubules extending through the processes.

Injection of fluorescently labeled *oskar* mRNA into *Drosophila* stage 9, 10 and 11 oocytes also revealed the importance of microtubule-based transport mechanisms [32]. Initial dispersion of the RNA was followed by accumulation at the posterior pole at these stages of oogenesis. The surprising result was that the microtubule system was required for long-range transport, but not for short-range events. RNA injected from a site distal to the posterior pole was sensitive to colchicine (a microtubule destabilizing drug), whereas RNA injected within 50 μm of the posterior pole was localized in a microtubule insensitive fashion.

GFP-labeled protein

An alternative strategy allows visualization of GFP-labeled proteins that interact with RNA. *Exuperentia* (*exu*) protein is required for *Bicoid* (*Bcd*) mRNA localization to the anterior pole of the *Drosophila* oocyte [33,34••]. GFP–*Exu* particles were examined in nurse cells, through ring canals and within the developing oocyte. The particles show a wide range of velocities (15–145 μm/min) in the nurse cell cytoplasm, yet had no clear polarity of movement. Diverse populations of dynamic microtubules mediate cytoplasmic GFP–*Exu* movement. Transport through the ring canals occurred with an average velocity of 6–8 μm/min and was independent of microtubule and actin cytoskeletal arrays. Final delivery of GFP–*Exu* within the oocyte was again dependent upon microtubules, while accumulation at the anterior pole required cortical docking factors. The requirement for specific cortical factors that specify the final destination is consistent with identification of the cortical anchors for *ASH1* mRNA in yeast (see below).

Green RNA

The latest breakthrough in RNA imaging was development of techniques for indirect visualization of mRNA itself. The system exploits the MS2 bacteriophage coat protein fused to GFP (CP–GFP) and the cognate RNA-binding site [35] introduced into a 3′UTR. Co-expression of the CP–GFP fusion protein and *ASH1* mRNA containing the coat-protein's binding site results in an mRNA–protein complex (henceforth denoted as $gRNA_{ASH1}$). Together with the development of techniques to perform extended time-lapse imaging [36], $gRNA_{ASH1}$ movements have been examined throughout the cell cycle. Although these techniques were developed for yeast, they are currently being adapted to other cell types. In the first iteration, six tandem CP–GFP binding sites were introduced together with a nuclear localization signal (included to reduce the levels of unbound cytoplasmic CP–GFP) [9••]. A subsequent method used a regulated promoter to restrict the levels of cytoplasmic CP–GFP [8••]. In both cases RNA movements in live cells were observed. Bertrand *et al.* [9••] measured rates of mRNA movement in live cells to be 0.33–0.73 μm/min, consistent with myosin V velocities *in vitro*. Beach *et al.* [8••] determined an average velocity of long-range movement to be 0.3 μm/min with wide variation, and short-range movements of 0.1 μm/min.

Transport of $gRNA_{ASH1}$ particles into the bud required *she1/myo4*, consistent with the notion that this motor protein is involved in RNA transport. A direct role for Myo4p in *ASH1* mRNA transport comes from recent studies demonstrating an association between *ASH1* mRNA and Myo4p via the activity of *SHE2* and *SHE3* [37]. The behavior of $gRNA_{ASH1}$ in the *SHE5* mutant was also illuminating. Beach *et al.* [8••] combined ten sequential time-lapse images into a single frame to create a time-based montage of RNA movement (Figure 1). In the absence of *she1/myo4*, *ASH1* mRNA was motile but restricted to the mother cell. In *she5/bni1* and *bud6/aip3* mutants, $gRNA_{ASH1}$ was transported into the bud, but not localized to the bud tip indicating that transport and anchorage are separable events.

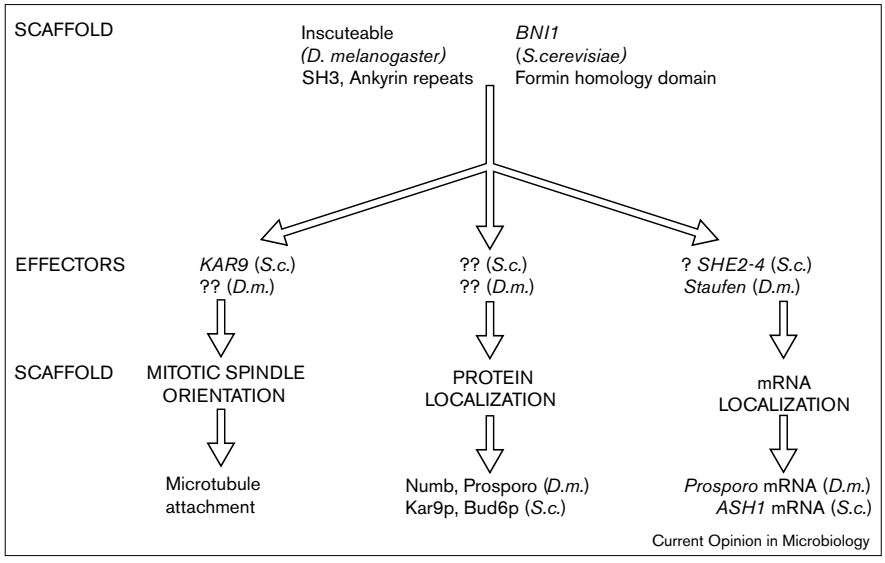
Studies of injected *osk* mRNA revealed that the cytoskeletal network itself was not necessary for mRNA accumulation to the posterior pole, rather an anchoring system was postulated to account for mRNA accumulation at the posterior pole [32]. Furthermore, *osk* mRNA localization to the posterior pole was shown to be dependent upon tropomyosin (encoded by *TmII*), an actin-binding protein that stabilizes actin filaments [38]. These results are reminiscent of early experiments with Vg1 in which it was demonstrated that Vg1 RNA expanded from a tight cortical cap into a broad band upon cytochalasin treatment [20]. In contrast, nocodazole treatment resulted in the complete dispersion of Vg1 mRNA. Thus microtubules may provide long-range transport (important in big cells), whereas actin and actin-binding proteins may facilitate local interactions (Figure 2). In yeast, the actin cytoskeleton may serve both long-range and local functions. Tracking *ASH1* mRNA movements in live cells revealed directed movements from mother to daughter, highly irregular (random) movements once in the bud [9••], and stable associations upon reaching the bud tip ([8••] and Figure 1a). The time-lapse montages (Figure 1c) of *ASH1* mRNA in *she5/bni1* mutants provide the first molecular characterization of anchoring molecules involved in the asymmetric RNA localization pathway.

Anchors away

The identification of *SHE5* as *BNI1* extends the analysis of one specialized pathway, namely mRNA localization, toward understanding the generalized mechanism that determines subcellular asymmetries. Just as the 3′UTR of

Figure 3

A hierarchy of proteins involved in the formation of a cortical scaffold complex. Proteins bound at the cell cortex form a platform to support localization of specific effector molecules. Each effector molecule facilitates a unique process including mitotic spindle orientation, specific protein and mRNA localization. *D.m.*, *D. melanogaster*; *S.c.*, *S. cerevisiae*.



a localized RNA has multiple, functionally redundant elements, it is likely that the localization mechanism features multiple, functionally redundant pathways. This view is borne out in examining the hierarchy of interactions required for establishing asymmetry in budding yeast and developing *Drosophila* neuroblasts (Figure 3).

The yeast *SHE5*/*BNI1* gene is a member of the formin gene family that has a role in providing spatial information for mitotic spindle orientation [39], protein localization (Kar9p) [40], mRNA localization (*ASH1*) [8••], and actin organization [41]. Bni1p contains a proline-rich formin homology domain (FH) that mediates interactions with profilin and is critical in regulating actin polymerization at cortical sites. Bni1p interacts with the actin-binding protein Bud6p/Aip3p. Bud6p/Aip3p contains SH3 domains and is also required for positioning *ASH1* mRNA [8••]. The *Drosophila* inscuteable protein is also required for mitotic spindle orientation, protein localization (Numb and prospero) and mRNA localization (*prospero* mRNA through staufen) [42,43]. Inscuteable contains predicted SH3 and ankyrin domains, which like the domains in Bni1p, are also implicated in cytoskeletal interactions.

The tight correlation of mitotic spindle orientation, protein, and mRNA localization in *Drosophila* neuroblasts can be decoupled through loss of *inscuteable* [42]. Likewise, *BNI1*-dependent localization of Kar9p is responsible for mitotic spindle orientation [39,40] and the mitotic spindle orientation function of *BNI1* can be decoupled from RNA localization in *kar9* mutants (DL Beach, P Maddox, K Bloom, unpublished data). Interestingly, the formin containing protein *cappuccino* is also required for localization of the kinesin–β-galactosidase fusion protein and *oskar* mRNA [44,45]. Although this latter result may be indirect, the data nonetheless point toward a key role for the formin protein family in establishing spatial cues for localization of subcellular components.

Finally, this theme of multifunctional scaffolds is also apparent upon inspection of the proteins required for *bicoid* mRNA localization. Mutations in *exu* and *swallow* (*swa*) result in a homogeneous distribution of *bcd* mRNA. The *swa* phenotype is not suppressed in *bcd* mutants, indicating that *swa* has additional functions. *swa* mutants, like *bni1*, display abnormalities in nuclear migration in early embryos [46]. Thus inscuteable, cappuccino, Bni1p, and swallow all have multiple functions in positioning the mitotic spindle as well as localizing protein and mRNA. Studies on the localization of *Bni1* and its interaction with the actin cytoskeleton indicate that it may form a multipurpose cortical scaffold that serves as a docking site for a variety of subcellular components (Figure 3). RNA movement is therefore likely to entail directed active transport systems, as well as passive transport, wherein the final docking event is accomplished by accumulation at asymmetrically positioned anchors.

Conclusions

Dynamic measurements of mRNA movements in yeast provide new insights into the transport and docking processes required for localizing mRNA. Long-range movements are likely to be motor driven, whereas short stochastic movements close to docking sites result in accumulation at specific locales. The docking sites are multifunctional and can be likened to beacons in the cell. Identification of the effectors responsible for particular polarized events represents a new challenge. The mRNA field has not only come of age, it has now opened new avenues of investigation.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Hesketh JE: **Sorting of messenger RNAs in the cytoplasm: mRNA localization and the cytoskeleton.** *Exp Cell Res* 1996, **225**:219-236.

2. •• Bashirullah A, Cooperstock RL, Lipshitz HD: **RNA Localization in development.** *Annu Rev Biochem* 1998, **67**:335-394.
A comprehensive review of current literature detailing the localization of mRNA localized in oocytes and embryos including *Drosophila*, *Xenopus,* and chicks.

3. Lasko P: **RNA sorting in *Drosophila* oocytes and embryos.** *FASEB J* 1999, **13**:421-433.

4. Mowry KL, Cote CA: **RNA sorting in *Xenopus* oocytes and embryos.** *FASEB J* 1999, **13**:435-445.

5. Jansen R-P: **RNA-cytoskeletal associations.** *FASEB J* 1999, **13**:455-466.

6. Etkin LD, Lipshitz HD: **RNA Localization.** *FASEB J* 1999, **13**:419-420.

7. Bassell GJ, Oleynikov Y, Singer RH: **The travels of mRNAs through all cells large and small.** *FASEB J* 1999, **13**:447-454.

8. •• Beach DL, Salmon ED, Bloom K: **Localization and anchoring of mRNA in budding yeast.** *Curr Biol* 1999, **9**:569-578.
A system for visualizing RNA *in vivo* using MS2 coat protein and the RNA-binding site. Using digital enhanced fluorescent microscopy the dynamics of *ASH1* mRNA were observed throughout the cell cycle of wild-type and mutant cells. The *ASH1* mRNA tracks with polarity establishment machinery throughout the cell cycle. Using an analysis strategy to examine stacks of images through time, a role for both *BNI1* and *BUD6* in anchoring the mRNA to the bud tip independent of RNA transport was established.

9. •• Bertrand E, Chartrand P, Schaefer M, Shenoy SM, Singer RH, Long RM: **Localization of *ASH1* mRNA particles in living yeast.** *Mol Cell* 1998, **2**:437-445.
An exogenous RNA-binding protein fused to GFP was used *in vivo* imaging of a reporter RNA containing the target sequence for the protein. *ASH1* mRNA was localized to the bud tip via the 3′UTR. Analysis of *ASH1* localized in *she* mutants demonstrated *MYO4* dependent motility of the *ASH1* transcripts.

10. Macdonald PM, Kerr K: **Mutational analysis of an RNA recognition element that mediates localization of bicoid mRNA.** *Mol Cell Biol* 1998, **18**:3788-3795.

11. • Chartrand P, Meng XH, Singer RH, Long RM: **Structural elements required for localization of *ASH1* mRNA and a GFP reporter particle *in vivo*.** *Curr Biol* 1999, **9**:333-336.
Along with [12•], this paper demonstrates the presence of sequence elements within the *ASH1* coding region and 3′UTR competent to direct mRNA localization.

12. • Gonzalez I, Buonomo SBC, Nasmyth K, von Ahsen U: ***ASH1* mRNA localization in yeast involves multiple secondary structural elements and Ash1 protein translation.** *Curr Biol* 1999, **9**:337-340.
Molecular dissection of elements within the coding region that are required for mRNA localization. In addition, this paper demonstrates a role for translation of *ASH1* mRNA in localization.

13. Rongo C, Gavis ER, Lehmann R: **Localization of *oskar* translation and requires Oskar protein.** *Development* 1995, **121**:2737-2746.

14. Kim-Ha J, Kerr K, Macdonald PM: **Translational regulation of *oskar* mRNA by Bruno, an ovarian RNA-binding protein, is essential.** *Cell* 1995, **81**:403-412.

15. Smibert CA, Wilson JE, Kerr K, Macdonald PM: ***Smaug* protein represses translation of unlocalized *nanos* mRNA in the *Drosophila* embryo.** *Genes Dev* 1996, **10**:2600-2609.

16. • Deshler JO, Highett MI, Schnapp BJ: **A highly conserved RNA binding protein for cytoplasmic mRNA localization in vertebrates.** *Curr Biol* 1998, **8**:489-496.
VG1 mRNA requires Vera (a.k.a. Vg1 RBP) for microtubule-dependent localization in *Xenopus* oocytes. Cloning of the protein reveals strong conservation between functional domains of Vera, the human protein KOC, and the chicken ZBP-1 all of which bind the 3′UTR of localized mRNAs.

17. • Havin L, Git A, Elisha Z, Oberman F, Yaniv K, Schwatrz SP, Standart N, Yisraeli JK: **RNA-binding protein conserved in both microtubule- and microfilament-based RNA localization.** *Genes Dev* 1998, **12**:1593-1598.
Cloning of the Vg1 RBP (a.k.a. Vera) and analysis of the peptide sequence. Vg1-RBP is the same protein as Vera and is homologous to ZBP-1 from chickens and KOC from humans.

18. Ross AF, Oleynikov Y, Kislauskis EH, Taneja KL, Singer RH: **Characterization of a β-actin mRNA zipcode-binding protein.** *Mol Cell Biol* 1997, **17**:2158-2165.

19. Elisha Z, Havin L, Ringle I, Yisraeli JK: **Vg1 RNA binding protein mediates the association of Vg1 RNA with microtubules in Xenopus oocytes.** *EMBO J* 1995, **14**:5109-5114.

20. Yisraeli JK, Sokol S, Melton DA: **A two-step model for the localization of maternal mRNA in *Xenopus* oocytes: involvement of microtubules and microfilaments in the translocation and anchoring of Vg1 mRNA.** *Development* 1990, **108**:289-298.

21. Long RM, Singer RH, Meng X, Gonzalez I, Nasmyth K, Jansen R-P: **Mating type switching in yeast controlled by asymmetric localization of *ASH1* mRNA.** *Science* 1997, **277**:383-387.

22. Takizawa PA, Sil A, Swedlow JR, Herskowitz I, Vale RD: **Actin-dependent localization of an RNA encoding a cell-fate determinant in yeast.** *Nature* 1997, **389**:90-96.

23. Bobola N, Jansen RP, Shin TH, Nasmyth K: **Asymmetric accumulation of Ash1p in postanaphase nuclei depends on a myosin and restricts yeast mating-type switching to mother cells [see comments].** *Cell* 1996, **84**:699-709.

24. Ferrandon D, Elphick L, Nusslein-Volhard C, St Johnson D: **Staufen protein associates with the 3′UTR of *bicoid* mRNA to form particles that move in a microtubule-dependent manner.** *Cell* 1994, **79**:1221-1232.

25. Clark I, Giniger E, Ruohola-Baker H, Jan LY, Jan YN: **Transient posterior localization of a kinesin fusion protein reflects anteroposterior polarity of the *Drosophila* oocyte.** *Curr Biol* 1995, **4**:289-300.

26. Li M, McGrail M, Serr M, Hays TS: **Drosophila cytoplasmic dynein, a microtubule motor that is asymmetrically localized in the oocyte.** *J Cell Biol* 1994, **126**:1475-1494.

27. Carson JH, Worboys K, Ainger K, Barbarese E: **Translocation of myelin basic protein mRNA in oligodendrocytes requires microtubules and kinesin.** *Cell Motil Cytoskeleton* 1997, **38**:318-328.

28. Deshler JO, Highett MI, Schnapp BJ: **Localization of Xenopus Vg1 mRNA by vera protein and the endoplasmic reticulum.** *Science* 1997, **276**:1128-1131.

29. Dabora SL,Sheetz MP: **The microtubule-dependent formation of a tubulovesicular network with characteristics of the ER from cultured cell extracts.** *Cell* 1988, **54**:27-35.

30. Waterman-Storer CM, Gregory J, Parsons SF, Salmon ED: **Membrane/microtubule tip attachment complexes (TACs) allow the assembly dynamics of plus ends to push and pull membranes into tubulovesicular networks in interphase Xenopus egg extracts.** *J Cell Biol* 1995, **130**:1161-1169.

31. Ainger K, Avossa D, Morgan F, Hill SJ, Barry C, Barbarese E, Carson JH: **Transport and localization of exogenous myelin basic protein mRNA microinjected into oligodendrocytes.** *J Cell Biol* 1993, **123**:431-441.

32. Glotzer JB, Saffrich R, Glotzer M, Ephrussi A: **Cytoplasmic flows localize injected oskar RNA in Drosophila oocytes.** *Curr Biol* 1997, **7**:326-337.

33. Wang S, Hazelrigg T: **Implications for *bcd* mRNA localization from spatial distribution of exu protein in *Drosophila* oogenesis.** *Nature* 1994, **369**:400-403.

34. •• Theurkauf WE, Hazelrigg TI: ***In vivo* analysis of cytoplasmic transport and cytoskeletal organization during Drosophila oogenesis: characterization of a multi-step anterior localization pathway.** *Development* 1998, **125**:3655-3666.
Expressing an endogenous RNA-binding protein (EXU) fused to GFP, the authors demonstrate that the bound *bcd* mRNA is localized via microtubule-dependent (within the nurse cells and oocyte) and microtubule-independent (within ring canals) motility within developing *Drosophila* oocytes.

35. Peabody DS: **Translational repression by bacteriophage MS2 coat protein expressed from a plasmid.** *J Biol Chem* 1990, **265**:5684-5689.

36. Shaw SL, Yeh E, Bloom K, Salmon ED: **Imaging green fluorescent protein fusion proteins in *Saccharomyces cerevisiae*.** *Curr Biol* 1997, **7**:701-704.

37. Munchow S, Sauter C, Jansen RP: **Association of the class V myosin Myo4p with a localised messenger RNA in budding yeast depends on She proteins.** *J Cell Sci* 1999, **112**:1511-1518.

38. Erdelyi M, Michon A-M, Guichet A, Glotzer JB, Ephrussi A: **Requirement for *Drosophila* cytoplasmic tropomyosin in *oskar* mRNA localization.** *Nature* 1995, **377**:524-527.

39. Lee L, Klee SK, Evangelista M, Boone C, Pellman D: **Control of mitotic spindle position by the *Saccharomyces cerevisiae* formin Bnilp.** *J Cell Biol* 1999, **144**:947-961.

40. Miller RK, Matheos D, Rose MD: **The cortical localization of the microtubule orientation protein, Kar9p, is dependent upon actin and proteins required for polarization.** *J Cell Biol* 1999, **144**:963-975.

41. Evangelista M, Blundell K, Longtine MS, Chow CJ, Adames N, Pringle JR, Peter M, Boone C: **Bni1p, a yeast formin linking Cdc42p and the Actin cytoskeleton during polarized morphogenesis.** *Science* 1997, **276**:118-122.

42. Li P, Yang XH, Wasser M, Cai Y, Chia W: **Inscuteable and staufen mediate asymmetric localization and segregation of prospero RNA during Drosophila neuroblast cell divisions.** *Cell* 1997, **90**:437-447.

43. Doe CQ: **Spindle orientation and asymmetric localization in Drosophila: both inscuteable?** *Cell* 1996, **86**:695-697.

44. Ephrussi A, Dickinson LK, Lehmann R: ***oskar* organizes the germ plasm and directs localization of the posterior determinant *nanos*.** *Cell* 1991, **66**:37-50.

45. Kim-Ha J, Smith JL, Macdonald PM: ***oskar* mRNA is localized to the posterior pole of the *Drosophila* oocyte.** *Cell* 1991, **66**:23-35.

46. Berleth T, Burri M, Thoma G, Bopp D, Richstein S, Frigerio G, Noll M, Nüsslein-Volhard C: **The role of localization of *bicoid* RNA in organizing the anterior pattern of the *Drosophila* embryo.** *EMBO J* 1988, **7**:1749-1756.