REVIEWS

MOVING MESSAGES: THE INTRACELLULAR LOCALIZATION OF mRNAs

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Abstract | mRNA localization is a common mechanism for targeting proteins to regions of the cell where they are required. It has an essential role in localizing cytoplasmic determinants, controlling the direction of protein secretion and allowing the local control of protein synthesis in neurons. New methods for *in vivo* labelling have revealed that several mRNAs are transported by motor proteins, but how most mRNAs are coupled to these proteins remains obscure.

DEVELOPMENTAL CELL BIOLOGY

Polarized cell behaviours depend on the asymmetric distribution of proteins in the cells, and this is often done by localizing the corresponding mRNAs. For example, 10% of randomly selected mRNAs localize to the anterior of the *Drosophila melanogaster* oocyte[1], whereas an estimated 400 mRNAs are targeted to the dendrites of mammalian neurons[2,3]. In some cases, the proteins that are encoded by the localized mRNAs also contain their own targeting signals, and the localization of the transcript contributes to efficient protein sorting, without being essential for this process. For example, most of the mRNAs that are transported to the bud tip in *Saccharomyces cerevisiae* encode proteins that still localize to the bud tip in mutants in which mRNA localization is abolished[4]. Similarly, the localization of *inscuteable* (*insc*) and *prospero* mRNAs in *D. melanogaster* neuroblasts shows a partial redundancy with the targeting of the proteins they encode, although both are important for the asymmetric divisions of these cells under certain circumstances[5–8].

However, many localized mRNAs encode proteins that lack their own sorting signals, and the subcellular distribution of the protein is therefore entirely determined by the localization of its transcript. These transcripts have many functions, including localizing cytoplasmic determinants, targeting protein secretion to specific membrane domains, contributing to the polarization of the cytoskeleton, and allowing the local control of gene expression through translational regulation (see Supplementary information S1 (TABLE)).

There are two important reasons why proteins are localized through their mRNAs rather than directly. First, mRNA localization not only targets the protein to the correct region of the cell, but also prevents its expression elsewhere. This is important for localized cytoplasmic determinants, which will alter the pattern of the embryo if present in the wrong regions. For example, the mislocalization of *oskar* or *nanos* mRNAs in the fly egg induces the development of a second abdomen in the place of the head and thorax[9,10]. For other localized mRNAs, it might not be possible to localize the encoded proteins to the correct compartments, because they bind to other factors wherever they are made. This is the case for Tau and MAP2, which will bind to any microtubules, and must therefore be localized as mRNAs to axons or dendrites, respectively[11].

The second reason for localizing a protein through its mRNA is that this devolves the control of protein expression to individual regions of the cytoplasm. This allows a cell to respond rapidly to a local requirement for the protein, and makes it possible to regulate gene expression independently in different parts of the cell. These factors are particularly important in large, highly polarized cells, such as neurons, where the translation of localized mRNAs in growth cones might be important for axon guidance, and the local control of protein synthesis in dendrites is thought to contribute to synaptic plasticity[12].

Whatever the function of a localized mRNA, it must be targeted to the appropriate region of the cell by one

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of four basic processes: local synthesis, local protection from degradation, diffusion and local trapping, or active transport along the cytoskeleton. Here, I review recent progress in elucidating the mechanisms of mRNA localization, and discuss how RNA-binding proteins recognize *cis*-acting RNA localization elements to assemble functional RNA transport complexes.

Mechanisms of mRNA localization

Local synthesis. The simplest way by which an mRNA can be localized to a particular region of the cell is by local synthesis, although this mechanism is rare. For example, the mRNAs for the δ- and ε-subunits of the acetylcholine receptor are transcribed in the nuclei that underlie the neuromuscular junctions of mammalian myofibres, but not in the other nuclei of these large SYNCYTIAL cells, thereby concentrating the mRNAs near the synapses where the receptors are required[13,14]. This mechanism is also thought to contribute to the localization of *gurken* mRNA on one side of the *D. melanogaster* oocyte nucleus[15]. However, this cannot be the only way that *gurken* mRNA is localized, as some mRNA is transcribed in NURSE CELLS and imported into the oocyte, and injected mRNA localizes efficiently above the nucleus, even though it is obviously not transcribed there[16,17].

Local protection from degradation. mRNAs can also be localized by degrading all the transcripts that are not in the correct place. This mechanism has been shown to restrict *hsp83* mRNA to the pole plasm at the posterior of the *D. melanogaster* egg[18]. This posterior enrichment requires two distinct *cis*-acting elements in the 3′ untranslated region (UTR) of the RNA: a degradation element that targets the mRNA for destruction in all regions of the egg or embryo, and a protection element that stabilizes the mRNA at the posterior[19]. Both *nanos* and *pgc* (*polar granule component*) RNAs become restricted to the posterior pole plasm by a similar mechanism — for example, only ~4% of *nanos* mRNA is localized in the freshly laid egg, but this mRNA is stable, whereas the mRNA that is not in the pole plasm is rapidly degraded[19,20]. This pathway for germline localization seems to be evolutionarily conserved, as a remarkably similar process restricts *vasa* and *nanos* mRNAs to the primordial germ cells of zebrafish[21–23] (FIG. 1).

Diffusion and anchoring. mRNAs can also become localized by passively diffusing through the cytoplasm until they are trapped by a localized anchor. Several transcripts, such as *nanos*, *gcl* (*germ cell-less*) and *Cyclin B* mRNAs, become enriched in the *D. melanogaster* pole plasm in this way. These mRNAs accumulate at the posterior of the oocyte late in oogenesis, when the cytoskeleton is no longer polarized along the anterior–posterior axis, and their localization depends on the earlier translation of *oskar* mRNA at the posterior and the subsequent assembly of polar granules[24–28]. The mRNAs therefore seem to move randomly through the oocyte cytoplasm until they are trapped wherever the pole plasm assembles. This model is supported by experiments in which *nanos* mRNA was fluorescently labelled *in vivo* (BOX 1), showing that its localization is enhanced by microtubule-dependent cytoplasmic streaming in the oocyte, which presumably facilitates its diffusion towards the posterior anchor[29].

Active transport. Most of the best-characterized examples of mRNA localization are thought to occur by active transport along the cytoskeleton. This is difficult to show, however, and has only been convincingly demonstrated in a few cases. One effective approach is to follow the behaviour of the mRNA in living cells (BOX 1). If the mRNA moves directionally at speeds that are too fast to be explained by cytoplasmic flow, it is presumably being transported by a motor. This does not mean that the mRNA is necessarily the direct cargo of the motor, however, and it might be hitchhiking on some other structure or organelle.

The second way to show that mRNA transport occurs by an active transport mechanism is to show that the mRNA is linked to a motor, and that this motor is required for the localization of the mRNA and co-localizes with it. These approaches have revealed that mRNAs can be transported along actin or microtubules, and by members of all three main families of motor proteins — the myosins, dyneins and kinesins.

Myosin-mediated mRNA transport

ASH1 *mRNA.* The best understood example of active transport of mRNA is the localization of *ASH1* mRNA to the bud tip in *S. cerevisiae*, which results in the repression of MATING TYPE SWITCHING in the daughter cell (FIG. 1a,b). This localization is actin dependent and requires the type-V myosin Myo4 (also known as She1), which co-localizes and co-precipitates with *ASH1* mRNA[30–34]. These observations indicate that Myo4 transports the mRNA along actin cables to the bud tip, and this has been confirmed by labelling *ASH1* mRNA *in vivo*[34–36]. The labelled mRNA moves at speeds of 200–440 nM sec^{-1} into the bud tip, which is consistent with myosin-based motility, but moves very little in *myo4* mutants.

The interaction of Myo4 with *ASH1* mRNA depends on two other proteins, She2 and She3, and four *cis*-acting localization elements in the mRNA[33,34,37,38]. She2 is a novel RNA-binding protein that binds as a dimer to each of the four localization elements in *ASH1* mRNA[34,39–41]. Binding to RNA increases the affinity of She2 for the C terminus of She3, which binds Myo4 through its N terminus. So, Myo4 is directly coupled through these two adaptor proteins to its RNA cargo.

Whereas Myo4 is required for the localization of She3–She2–RNA complexes, the reverse is also true[42,43]. It seems that the association of the motor with the mRNA is important, as the binding of RNA directly to She3 bypasses the requirement for She2 (REF. 40). Although the nature of myosin regulation by its RNA cargo is unclear, one possibility is that the RNA increases the localization of the motor by stimulating its activity or by making it more processive. Alternatively, the myosin and the other components of the localization complex might be anchored in the bud by the translation of the mRNA once it is localized[38,43,44].

SYNCYTIAL
Describes cells that contain multiple nuclei in a common cytoplasm.

NURSE CELL
An auxiliary cell that supplies the *Drosophila melanogaster* oocyte with synthesized mRNAs and proteins during insect oogenesis through large cytoplasmic bridges, known as ring canals.

MATING TYPE SWITCHING
The process by which the active mating type locus of a haploid yeast cell is replaced by one of the opposite mating type from a silent cassette elsewhere in the genome. Only mother cells switch mating type, because the transcription of the HO endonuclease, which initiates mating type switching, is repressed in daughter cells by ASH1.

Box 1 | Methods for visualizing mRNA localization *in vivo*

Injection of fluorescent mRNAs
The injection of fluorescently labelled *in vitro* transcripts provides a quick and easy way to study mRNA localization in living cells, and can be used to map localization elements[61,62,65,78,91,102]. However, injection can damage cells, and mRNAs whose localization depends on nuclear events, such as pre-mRNA splicing, will not localize unless injected into the nucleus.

Injection of molecular beacons
Molecular beacons provide an elegant way to label endogenous mRNAs directly, and can be used to follow mRNAs whose localization requires nuclear events, such as pre-mRNA splicing[186]. One potential drawback of this technique is its low sensitivity, as the beacon introduces a single fluorophore into the mRNA.

GFP tagging of RNA-binding proteins
The simplest non-invasive technique for labelling endogenous mRNA localization complexes *in vivo* is to generate transgenic lines that express a green fluorescent protein (GFP)-tagged version of an RNA-binding protein that specifically associates with the mRNA[79,87,109]. A disadvantage of this approach is that most RNA-binding proteins associate with more than one mRNA species, and it is therefore necessary to show that any moving particles that are observed contain the RNA of interest.

MS2–GFP tagging
An ingenious solution to the potential problems that are caused by the promiscuity of endogenous RNA-binding proteins is to insert multiple copies of the recognition site for a heterologous RNA-binding protein into the mRNA of interest, and to co-express this protein as a GFP fusion protein. For example, Bertrand *et al.*[35] used a GFP-tagged version of the MS2 phage coat protein, and an *ASH1* mRNA construct containing multiple copies of the 19-nucleotide MS2-binding site. By inserting a nuclear localization signal into the MS2–GFP fusion protein, they could distinguish free MS2–GFP from that bound to the mRNA, which is exported with the latter into the cytoplasm. This approach has now been used successfully to visualize the localization of several mRNAs[29,34–36,52,53,104].

ACTIN STRESS FIBRE
Long, stable F-actin bundle that forms from focal adhesions in cells that are under mechanical tension.

MOLECULAR BEACON
An oligonucleotide complementary to an mRNA of interest, with a fluorophore at one end and a quencher at the other. The ends of the beacon base pair in the free probe to bring the quencher next to the fluorophore, thereby preventing fluorescence, but this structure unwinds on hybridizing to the target mRNA and the fluorophore becomes active.

PAIR-RULE GENE
A class of segmentation gene that divides the anterior–posterior axis of the fly embryo into segments. Each pair-rule gene is expressed in a stripe in every second segment (seven stripes in total) under the control of the Gap genes.

β-Actin *and prolamine mRNAs.* Myosin can also transport *β-actin* mRNA along actin filaments to the lamellae at the leading edge of motile fibroblasts. This localization is actin dependent, and is enhanced by treatments that increase the formation of ACTIN STRESS FIBRES[45–47]. In addition, the proportion of cells that show *β-actin* mRNA localization is reduced by treatment with the myosin inhibitor butanedione monoxime and in mouse embryonic fibroblasts that are mutant for myosin IIB[46]. Although the inhibition of myosin might disrupt localization indirectly, these results are consistent with a model in which myosin IIB transports *β-actin* mRNA along stress fibres to the lamellae. This is further supported by studies on the RNA-binding protein ZBP1 (zip-code-binding protein 1) (BOX 2). ZBP1 binds to two repeats in the *β-actin* localization element (the zip code) *in vitro* and co-localizes with *β-actin* mRNA *in vivo*[48–50]. Green fluorescent protein (GFP)-tagged ZBP1 forms particles that move towards newly forming protrusions at up to 0.6 μm sec^{-1}, which is the speed that is expected for active transport by a myosin[51]. These particles probably represent *β-actin* mRNA transport particles, as direct labelling of the mRNA with MOLECULAR BEACONS (BOX 1) shows a similar rapid flow of RNA from old to new lamellae[52].

The same mechanism might localize prolamine mRNA to specific regions in rice endosperm[53]. Fluorescently tagged mRNA forms particles that move unidirectionally at speeds of ~0.3–0.4 μm sec^{-1} towards specific regions of the cortical endoplasmic reticulum (ER). These movements are abolished by treatment with actin-destabilizing drugs, which indicates that they involve active transport along actin.

***prospero* mRNA.** The localization of *prospero* mRNA to the basal cortex of *D. melanogaster* neuroblasts is also actin and myosin dependent[6]. *prospero* mRNA localization requires the double-stranded (ds)RNA-binding protein Staufen, which couples the mRNA to Miranda protein, which, in turn, co-purifies with both myosin II (Zipper) and the myosin VI (Jaguar)[5,6,54–57]. Jaguar protein is transiently enriched basally, and mutants that reduce Jaguar activity impair the basal localization of Miranda, indicating that this myosin might anchor or transport Miranda–Staufen–*prospero* mRNA complexes to the basal cortex[57]. Zipper is also required for this localization, as formation of the basal Miranda crescent is abolished by mutations in the myosin regulatory light chain and by Rho kinase inhibitors, which block Zipper activation[58,59].

Unlike Jaguar, Zipper shows a reciprocal localization to Miranda, as it is recruited to the apical cortex by the apical cell polarity complex PAR3–PAR6–aPKC, which inhibits the myosin repressor lethal giant larvae (LGL)[58,59]. In the absence of LGL, Zipper localizes to the entire cortex and Miranda remains in the cytoplasm. Miranda protein and *prospero* mRNA therefore seem to be localized by cortical exclusion by Zipper on the apical side, and by cortical anchoring by Jaguar basally.

mRNA transport towards microtubule minus ends

Pair-rule transcripts. A combination of *D. melanogaster* genetics and the ability to observe mRNA localization in real time have provided compelling evidence that the minus-end-directed motor protein dynein actively transports mRNAs along microtubules. The best-characterized example is the PAIR-RULE mRNAs, which localize to the apical cytoplasm above the nuclei of *D. melanogaster* syncytial blastoderm embryos[60] (FIG. 1c). When fluorescently labelled pair-rule mRNAs are injected into the basal cytoplasm, they localize apically. This localization depends on the microtubules, which are nucleated from an apical MICROTUBULE ORGANIZING CENTRE (MTOC) and extend around the nuclei and into the cytoplasm[61].

Furthermore, high-resolution imaging has shown that these mRNAs form particles that move apically at speeds of 0.5 μm sec^{-1} (REF. 62). More importantly, the movement of these particles is abolished by co-injection of monoclonal antibodies against the dynein heavy chain protein (DHC), whereas the speed of transport is reduced in a *Dhc* HYPOMORPHIC mutant combination, which demonstrates that these mRNAs are transported by dynein. Interestingly, the activity of dynein seems to be regulated by its RNA cargo in a similar way to Myo4 in yeast, as weak mutations in a pair-rule localization element decrease both the speed and frequency of particle movement. This indicates that the velocity and the processivity of dynein is reduced[63].

MICROTUBULE ORGANIZING CENTRE
(MTOC). A large organelle that organizes most of the microtubules in the cell through the activity of the γ-tubulin ring complex, which nucleates new microtubules from their minus ends. In most somatic cells, the MTOC is the centrosome, which contains the paired centrioles, but the centrosomes disappear in female germ cells, which contain more diffuse MTOCs.

HYPOMORPHIC ALLELE
An allele that reduces the level or activity of a gene product, without eliminating it entirely, often causing a less extreme phenotype than a loss-of-function (or null) allele.

The dynein–BicD–EGL pathway. The same dynein-dependent pathway localizes mRNAs at other stages of fly development. A number of mRNAs, including *K10*, *bicoid* (*bcd*) and *gurken*, are synthesized in nurse cells during early fly oogenesis and are then transported through cytoplasmic bridges, known as ring canals, into the oocyte[64]. This localization depends on microtubules that are nucleated from an MTOC in the oocyte and extend through ring canals into nurse cells, and also requires the function of Bicaudal-D (BicD), egalitarian (EGL) and DHC.

Remarkably, all the mRNAs that localize to the oocyte are transported apically when injected into the syncytial blastoderm embryo, whereas pair-rule transcripts are efficiently transported into the oocyte when ectopically expressed in nurse cells[65]. Furthermore, the apical localization of the pair-rule mRNAs requires BicD and EGL, and both proteins accumulate apically with injected mRNAs. BicD and EGL are also required for the localization of *insc* mRNA in embryonic neuroblasts, and co-localize with the mRNA at the apical cortex[8]. So, dynein, BicD and EGL function together to transport mRNAs to the minus ends of microtubules in various *D. melanogaster* cell types.

It is unclear how dynein is coupled to its mRNA cargoes, as no RNA-binding proteins have been implicated in this transport, but BicD and EGL are good candidates to have a role in this process[66]. The two proteins are found in the same complex and are required for dynein-dependent mRNA transport, but not for several other functions of the motor. Furthermore, the C terminus of EGL binds directly to the dynein light chain (DLC), and mutations in this binding site or in *Dlc* strongly impair dynein-mediated transport into the oocyte[67]. As the N terminus of EGL is required for its association with BicD, this indicates that it might function as an adaptor between dynein and a BicD–cargo complex. This is unlikely to be the whole story, however, as a mammalian homologue of BicD has been shown to bind to the dynamitin subunit of the dynactin–dynein complex to stimulate dynein motor activity[68–70]. BicD and EGL might therefore associate with dynein independently, and these proteins could have a number of possible functions, such as the regulation of dynein motility or dynein interaction with its cargoes.

gurken *mRNA.* Dynein has also been implicated in *gurken* mRNA localization in the oocyte. The cytoskeleton is polarized in an anterior-to-posterior gradient during stages 7–10 of oogenesis, with the microtubule minus ends at the anterior and lateral cortex, and the plus ends extending towards the posterior pole[71,72]. All mRNA localization in the oocyte is microtubule dependent, which indicates that mRNAs that localize anteriorly, such as *bicoid* and *gurken*, are transported by minus-end-directed motors such as dynein. This has been most clearly shown for *gurken* mRNA by analysing the movements of injected fluorescent mRNAs[17]. When *gurken* mRNA is injected at the posterior of the oocyte, it localizes rapidly above the dorsal–anterior nucleus. This localization is abolished by the co-injection of anti-dynein antibodies, and occurs more slowly in females that carry a viable combination of hypomorphic dynein alleles. Furthermore, the localization of endogenous *gurken* mRNA is inhibited by the overexpression of dynamitin, which disrupts dynein function[73,74].

As the minus ends of the microtubules are localized across the anterior and lateral cortex, these experiments raise the question of how the mRNA is targeted specifically to the dorsal–anterior corner. In tracking experiments, *gurken* mRNA particles move first to the anterior of the oocyte, and then change direction to move dorsally[17]. In addition, live imaging with the microtubule-binding protein Tau fused to GFP indicates that the oocyte nucleus nucleates a specific population of microtubules that extend around the anterior cortex. So, dynein seems to transport *gurken* mRNA particles sequentially along two distinct populations of microtubules, first to the anterior of the oocyte and then towards the dorsal nucleus (FIG. 2a,b).

The first step in *gurken* mRNA localization is a general step, because all the mRNAs that are transported into the oocyte by the dynein–BicD–EGL pathway also localize to the anterior cortex. The second step is specific to *gurken* mRNA, and has distinct requirements from the first, as it is specifically disrupted by *K10* and *squid* mutants[75]. *gurken* mRNA therefore seems to contain signals that modify the behaviour of the dynein motor complex so that it can move dorsally on the microtubules nucleated from the nucleus, unlike other anteriorly localized mRNAs.

bicoid *mRNA.* Injection experiments indicate that *bicoid* mRNA transport complexes have a similar ability to discriminate between different populations of oocyte microtubules (FIG. 2a,c). During stages 7–9 of oogenesis, *bicoid* mRNA is transported from nurse cells into the oocyte, where it localizes to the anterior cortex[76]. This localization is microtubule dependent, and might also be mediated by dynein[73,74,77]. Unlike *gurken*, however, *bicoid* mRNA does not localize specifically to the anterior when it is injected directly into the oocyte, and it instead moves to the nearest region of the anterior or lateral cortex[78]. As these regions correspond to the areas that nucleate the minus ends of microtubules, *bicoid* mRNA is presumably transported along these microtubules by dynein. Surprisingly, if the mRNA is first injected into nurse cells, withdrawn and then re-injected into the oocyte, it now localizes specifically to the anterior cortex. Factors in nurse cell cytoplasm must therefore confer on *bicoid* mRNA the ability to distinguish between the microtubules that are nucleated from the anterior cortex and those that are nucleated laterally, so that it is only transported along the former (FIG. 2c).

These early stages of *bicoid* mRNA localization depend on exuperantia (EXU) protein, and EXU–GFP localizes to the anterior cortex[79]. Interestingly, EXU is required in nurse cells to render *bicoid* competent to localize to the anterior, as mRNAs that are exposed

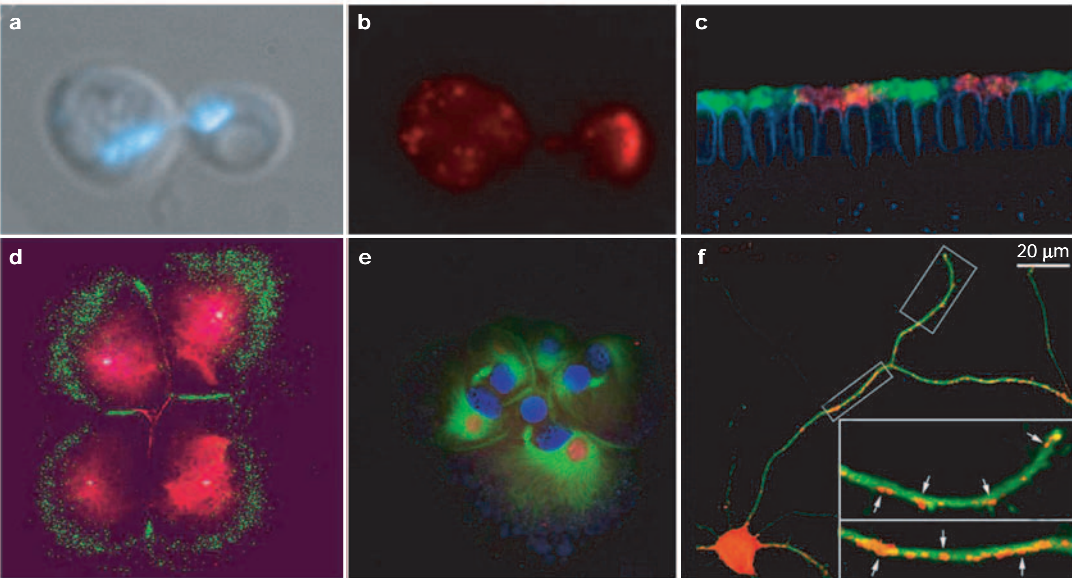


Figure 1 | **Examples of localized mRNAs. a** | A phase-contrast image of *Saccharomyces cerevisiae* in mitosis shows the budding of the daughter cell (right) from the mother cell (left). The chromosomes have been labelled with DAPI (4,6-diamidino-2-phenylindole). **b** | *ASH1* mRNA (red) localizes to the bud tip in the cell shown in (**a**) and is therefore inherited by only the daughter cell, where Ash1 represses mating type switching. Image courtesy of Florian Boehl, Gurdon Institute, University of Cambridge. **c** | The apical localization of *hairy* (green) and *even-skipped* (red) mRNAs in the *Drosophila melanogaster* syncytial blastoderm embryo. Image courtesy of Simon Bullock, MRC Laboratory of Molecular Biology, Cambridge. **d** | The localization of *vasa* mRNA (green) to the cleavage planes of a four-cell zebrafish embryo. This leads to the segregation of the mRNA into the primordial germ cells. Red shows β-catenin. Image courtesy of Holger Knaut, Skirball Institute, New York University School of Medicine. **e** | *dpp* mRNA (red) localization to the centrosome in the MACROMERES of an eight-cell *Ilyanassa obsoleta* embryo (microtubules, green; DNA, blue). The mRNA moves from the centrosome to the adjacent cortex during prophase, and is asymmetrically segregated into the second quartet of MICROMERES[192]. Image courtesy of Lisa Nagy, Department of Molecular and Cellular Biology, University of Arizona. **f** | *β-Actin* mRNA granules (red) in the developing neurites of a cultured hippocampal neuron. The axonal marker tau is shown in green. Arrows indicate *β-actin* mRNA particles. Image courtesy of Gary Bassell, Department of Neuroscience, Albert Einstein College of Medicine.

to *exu* mutant nurse cells and then injected into wild-type oocytes move to both the anterior and lateral cortex[78]. EXU is also required for transport to the lateral cortex, however, as *bicoid* mRNA fails to localize when injected into *exu*-mutant oocytes. EXU therefore seems to be necessary in the oocyte for coupling *bicoid* mRNA to the motor, and in nurse cells for determining the ability to discriminate between anterior and lateral microtubules. However, the molecular function of EXU in *bicoid* mRNA localization is still unclear, as it co-immunoprecipitates with *oskar* mRNA, but not *bicoid*, and purifies in a large protein complex that represses translation in nurse cells[80,81].

Unlike most other mRNAs that localize to the anterior of the oocyte, and which stay there only transiently, *bicoid* mRNA remains at the anterior throughout the rest of oogenesis, until it is released into cytoplasm at egg activation. From stage 10b onwards, the retention of bicoid at the anterior requires Swallow protein, which becomes enriched at the anterior cortex[76,82–84]. Furthermore, Swallow binds to DLC through a COILED-COILED DOMAIN[83]. As the localization of *swallow* and *bicoid* mRNA are microtubule dependent, this indicates that dynein either anchors or transports *bicoid* mRNA to the minus ends of microtubules at the anterior cortex at this stage.

bicoid mRNA moves from an anterior ring at stage 10a to cover the entire anterior cortex of the oocyte at stage 10b. In addition to Swallow, this localization requires γ-tubulin 37C and Grip75, which are components of the γ-tubulin ring complex that nucleates microtubules[85]. Mutants in these proteins do not disrupt all microtubules in the oocyte — the cortical microtubules that drive cytoplasmic streaming seem normal. However, they do abolish the localization of the putative minus-end marker NOD–β-galactosidase (β-gal) to the anterior cortex. These results have led to the model that the γ-tubulin ring complex nucleates a specific population of anterior microtubules that retain *bicoid* mRNA at stages 10b–11.

The final stage of *bicoid* mRNA localization requires Staufen to maintain the mRNA at the anterior of the oocyte after stage 11 (REFS 76,86) (BOX 2). Staufen co-localizes with *bicoid* from stage 10b onwards, and as mutants in a conserved dsRNA-binding domain (dsRBD) disrupt *bicoid* anchoring[87,88], Staufen presumably binds directly to the RNA to anchor it at the anterior cortex. This step is also likely to be microtubule dependent, as injected *bicoid* mRNA recruits Staufen into large particles that localize to the poles of the mitotic spindles of the early embryo[86].

MACROMERES
The larger cells that are produced when early blastomeres undergo unequal divisions in invertebrate embryos.

MICROMERES
The smaller cells that are produced when early blastomeres undergo unequal divisions in invertebrate embryos.

COILED-COIL DOMAIN
A protein structural domain that mediates subunit oligomerization. Coiled coils contain between two and five α-helices that twist around each other to form a supercoil.

Transport towards the plus ends of microtubules

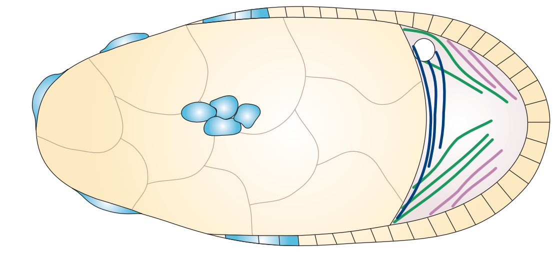
oskar *mRNA.* Whereas *bicoid* and *gurken* mRNAs are presumably transported by dynein, the localization of *oskar* mRNA to the posterior of the oocyte requires the plus-end-directed microtubule motor protein, kinesin[89]. Furthermore, at least a proportion of the microtubule plus ends extend to the posterior pole, as a constitutively active fusion protein that contains the motor domain of the kinesin heavy chain (KHC) and β-gal localizes to the posterior cortex at the same time as *oskar* mRNA[71,90] (FIG. 2a,d).

Although the results above indicate that kinesin transports *oskar* mRNA to the plus ends of microtubules at the posterior pole, it remains to be shown that *oskar* mRNA associates with kinesin or that it moves at speeds that are compatible with active transport. Two alternative mechanisms of *oskar* mRNA localization have been proposed. Glotzer *et al.* observed that when fluorescently labelled *oskar* mRNA was injected in the vicinity of the posterior pole, some of it could still localize after microtubule depolymerization. This led them to suggest that *oskar* mRNA might be passively trapped at the posterior by a pre-localized anchor[91]. According to this view, kinesin might be required to generate cytoplasmic flows that circulate the mRNA in the oocyte so that it is efficiently delivered to the posterior pole. In support of this, the oocyte shows significant cytoplasmic flows at stage 9 that are kinesin dependent[92].

Although this might account for the localization of some *oskar* mRNA, two arguments suggest that it is unlikely to explain its initial localization to the posterior at stage 9. First, the localization of the injected mRNA requires Oskar protein, whereas the endogenous mRNA still localizes to the posterior in *oskar* nonsense mutations but is not anchored there[9,93]. This indicates that the localized anchor is Oskar protein itself, and as *oskar* mRNA is not translated until it reaches the posterior, the first mRNA must localize by a different mechanism[94,95]. Second, the localization of endogenous *oskar* mRNA requires pre-mRNA splicing, whereas the injected mRNAs were transcribed from a cDNA clone without introns, and they therefore lack a key signal required for the localization of the endogenous mRNA[96].

A different model has been put forward by Cha *et al.*, who propose that kinesin transports *oskar* mRNA away from all regions of the oocyte cortex, except the posterior pole[72]. This model is based on the observation that *oskar* mRNA localizes to the centre of the wild-type

a Microtubule populations in the *Drosophila melanogaster* oocyte



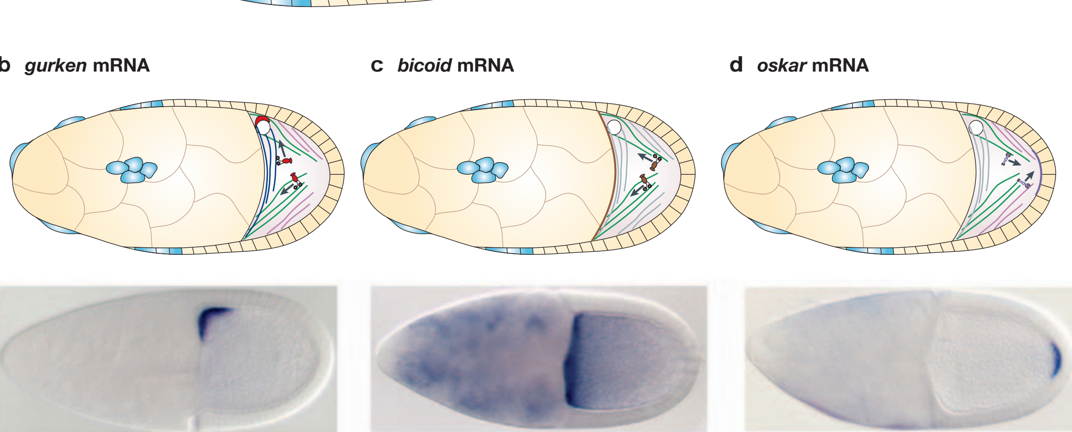


Figure 2 | **Localization of *gurken*, *bicoid* and *oskar* mRNAs along different populations of microtubules in the *Drosophila melanogaster* oocyte. a** | Three different populations of microtubules are thought to exist in the oocyte. Most microtubules are nucleated from the anterior cortex and extend towards the posterior (green). Some microtubules are also nucleated from the lateral cortex, but not from the posterior (pink). The oocyte nucleus nucleates microtubules that extend around the anterior cortex (dark blue). **b** | *gurken* mRNA (red) has been proposed to localize to the dorsal–anterior corner of the oocyte in a two-step process. Dynein first transports the mRNA to the anterior along the microtubules nucleated from the anterior cortex (green), and then transports it dorsally along the microtubules nucleated by the nucleus (dark blue)[17]. **c** | A model for the localization of *bicoid* mRNA (brown)[78]. *bicoid* mRNA is transported towards the minus ends of the microtubules that are nucleated from the anterior cortex (green), but does not move on the microtubules that are nucleated from the lateral cortex or the oocyte nucleus (grey). **d** | *oskar* mRNA (purple) might localize to the posterior by kinesin-dependent transport towards the plus ends of the microtubules nucleated from the anterior (green) and lateral cortex (pink)[92]. The images in **b–d** show *in situ* hybridizations to *gurken*, *bicoid* and *oskar* mRNAs (courtesy of Trent Munro, Gurdon Institute, University of Cambridge).

oocyte before it reaches the posterior pole, whereas it localizes all around the cortex in *Khc*-mutants. One problem with this view is that it cannot easily explain why the putative plus-end marker, KHC–β-gal, localizes to the posterior of wild-type oocytes at stage 9. Furthermore, endogenous kinesin also accumulates posteriorly at stage 9, which strongly indicates that it transports its cargoes there[92].

If *oskar* mRNA is transported by kinesin, it is certainly not the only cargo in the oocyte; the dynein–dynactin complex also localizes to the posterior in a kinesin-dependent manner, and this localization is not affected by mutations that abolish the localization of *oskar* mRNA[73,74,92,97,98]. So, a further possibility is that *oskar* mRNA hitchhikes to the posterior on some other organelle. Moreover, neither the posterior localization of dynein nor *oskar* mRNA requires the kinesin light chain (KLC), which normally couples the KHC to its cargoes. Kinesin must therefore be linked to the complexes it localizes in the oocyte by a novel mechanism.

Unlike *bicoid* mRNA, the anchoring of *oskar* mRNA at the posterior does not require microtubules, and instead depends on the cortical actin cytoskeleton, which is anchored to the plasma membrane by the *D. melanogaster* moesin-like protein MOE[99]. It is not known how *oskar* mRNA is anchored, but this requires the redundant function of two actin-associated proteins, Homer and Bifocal, as well as Oskar itself[100,101].

MBP *and* CaMKIIα *mRNAs.* Kinesin has also been implicated in the localization of myelin basic protein mRNA (*MBP*) and calcium/calmodulin-dependent kinase-2α mRNA (*CaMKIIα*) to the myelinating processes of oligodendrocytes and dendrites of hippocampal neurons, respectively. *MBP* mRNA was the first transcript to be visualized in living cells by the injection of fluorescent RNAs and was found to form particles that move from the oligodendrocyte cell body to myelinating processes[102]. Although most granules are immobile, some show oscillatory movements and others move unidirectionally towards the periphery, at up to 0.2 μm sec^{-1}. This anterograde movement probably depends on kinesin, as antisense oligonucleotides directed against KHC block the peripheral localization of injected mRNA[103].

Fluorescently labelled *CaMKIIα* mRNA particles show a similar pattern of movements in the dendrites of cultured neurons[104]. Most particles are stationary or oscillate, but a small percentage move at speeds of up to 0.2 μm sec^{-1} in either an anterograde or retrograde direction. This movement might depend in part on the binding of cytoplasmic polyadenylation element-binding protein (CPEB) to cytoplasmic polyadenylation elements (CPEs) in the CaMKIIα 3′ UTR, as these elements are sufficient for the dendritic enrichment of the mRNA, and overexpression of CPEB enhances this localization, whereas it is reduced in a CPEB mutant[105]. Furthermore, CPEB co-immunoprecipitates with both dynein and kinesin, which could account for the bidirectional motility of the *CaMKIIα* mRNA particles. *CaMKIIα* mRNA also co-localizes with several RNA-binding proteins that associate with the tail of the KIF5 kinesins, providing further evidence of a link between the motor and this mRNA[106]. Moreover, a dominant-negative KIF5 mutant reduces the distance that *CaMKIIα* mRNA can be found from the cell body, whereas RNA interference (RNAi)-mediated knockdown of four of these RNA-binding proteins (PURα, PSF, Staufen or hnRNPU) restricts the distribution of labelled *CaMKIIα* mRNA. Taken together, these results indicate that kinesin transports *CaMKIIα* mRNA along dendrites in large particles that contain multiple RNA-binding proteins.

The localization of other dendritic mRNAs probably follows the same pathway. The dsRNA-binding protein Staufen co-fractionates with the non-coding RNA *BC1* as well as *CaMKIIα* mRNA, and co-localizes with these transcripts to particles in dendrites[107]. When these particles are labelled with Staufen–GFP, they show comparable movements to the labelled *CaMKIIα* mRNA particles, which indicates that they might represent the same population of RNA transport complexes[108]. GFP tagging of the *β-actin*-mRNA-binding protein ZBP1 labels particles that show similar motility in dendrites[109]. Furthermore, imaging of single *β-actin* mRNA molecules in COS cells reveals stochastic movements in both directions along microtubules[110]. Although reporter mRNAs lacking localization signals show similar stochastic motility, the inclusion of the *β-actin* zip code increases both the frequency and distance of mRNA movement.

Kinesin family motors have also been implicated in the localization of *Vg1* mRNA to the vegetal pole of the *Xenopus laevis* oocyte. Yoon and Mowry found that the *X. laevis* Staufen protein localizes to the vegetal pole of the oocyte with *Vg1* mRNA, and co-immunoprecipitates with a protein that is recognized by a pan-kinesin antibody, which migrates with the expected size for kinesin-1 (REF. 111). Using a different approach, Betley *et al.* observed that kinesin-2 is somewhat enriched at the vegetal cortex during the stages when *Vg1* mRNA is localized, and antibodies against kinesin-2 and a dominant-negative kinesin-2 protein inhibit *Vg1* mRNA localization[112]. However, it is still unclear whether either kinesin transports *Vg1* mRNA, and it is hard to reconcile such a role for these plus-end motors with the microtubule organization of the stage IV oocyte, in which the most microtubules have their minus ends at the cortex[113].

mRNA localization signals

Whichever mechanism is used to localize an mRNA, it must contain *cis*-acting localization elements that are recognized by RNA-binding proteins that couple it to the localization machinery. These elements usually reside in 3′ UTRs, where they are least likely to interfere with translation, but they are occasionally found elsewhere[37,38,96,114–118].

In principle, mRNA localization could be directed by a simple sequence that is recognized by a single *trans*-acting factor. The only clear example of this is *MBP* mRNA, which contains an 11-nucleotide so-called A2RE element that is necessary and sufficient to direct

Box 2 | **Proteins with a conserved role in mRNA localization**

Although it is not known how RNA-binding proteins link mRNAs to the localization machinery, several such proteins have been implicated in the localization of multiple mRNAs in different organisms.

Staufen

Staufen was first identified because of its role in *Drosophila melanogaster* axis formation, where it is required for the microtubule-dependent localization of *oskar* mRNA to the posterior of the oocyte[9,93,159] and *bicoid* mRNA to the anterior[76,86], and for the actin-dependent localization of *prospero* mRNA to the basal cortex of embryonic neuroblasts[5,6,143]. Staufen contains five conserved double-stranded RNA-binding domains (dsRBDs), and dsRBD5 binds to Miranda to mediate *prospero* mRNA localization[54–56]. However, this domain is not necessary for *oskar* mRNA localization, which depends on a conserved insertion in dsRBD2. So, different regions of the protein couple Staufen–RNA complexes to actin- and microtubule-dependent transport pathways[142]. Vertebrate Staufen homologues have also been implicated in mRNA transport. *Xenopus laevis* Staufen is required for the microtubule-dependent localization of *Vg1* mRNA[111], and mammalian STAU1 forms particles that move along microtubules in mammalian dendrites and has been implicated in the localization of *CaMKIIα* mRNA[106–108].

ZBP1

The RNA RECOGNITION MOTIF (RRM)- and KH-DOMAIN-containing protein ZBP1 binds specifically to the *β-actin* localization element (the zip code) that directs the actin-dependent localization of chicken *β-actin* mRNA in fibroblasts. It has also been implicated in the microtubule-dependent localization of *β-actin* mRNA in neurons[48,109,187]. The *X. laevis* ZBP1 homologue VERA/Vg1RBP recognizes the *Vg1* and *VegT* mRNA localization signals, which are necessary for microtubule-mediated transport to the vegetal pole of the oocyte[130,132,135,136]. Finally, the mammalian ZBP1 homologue IMP1 associates with the axonal localization signal in *tau* mRNA[188].

hnRNPA/B proteins

Mammalian hnRNPA2 binds specifically to the 11-nucleotide A2RE element that is both necessary and sufficient to target *MBP* mRNA to oligodendrocyte processes[119,120]. The *D. melanogaster* hnRNPA/B homologue Squid is required for the localization of *gurken* mRNA to the dorsal anterior corner of the oocyte[75,189]. Another *D. melanogaster* hnRNPA/B homologue, HRP48, binds to *oskar* mRNA to mediate its posterior localization and to regulate its translation[161,162]. HRP48 interacts with Squid, and might also have a role in *gurken* mRNA localization[166].

RNA RECOGNITION MOTIF
This motif defines a domain found in many proteins that recognize single-stranded RNA sequences. The RNA-binding site is formed by a four-stranded β-sheet on one face of the domain that contains the highly conserved RNP1 and RNP2 motifs.

KH DOMAIN
An evolutionary conserved RNA-binding domain, which was originally identified in the human hnRNPK protein, and that recognizes single-stranded RNA sequences. Many RNA-binding proteins contain multiple copies of the KH domain.

its transport towards oligodendrocyte processes[119–121]. This short element is recognized by the hnRNPA2 protein, and mutants in the RNA that abolish this specific binding, as well as depletion of hnRNPA2, disrupt transport to the periphery (BOX 2).

Multistep localization and secondary structure. The A2RE element is not sufficient for all steps in *MBP* mRNA localization, however, and its movement from the processes into the myelinating compartment depends on a second nucleotide sequence, RLR[121]. This highlights a common feature of mRNA localization, which is often a multistep process, with different elements directing each step. For example, different regions of *oskar* mRNA are required for its transport from nurse cells of the fly egg chamber into the oocyte and its subsequent localization to the posterior pole[122], whereas *Xcat2* mRNA contains two distinct localization elements that target it to the MITOCHONDRIAL CLOUD and the germinal granules in the *X. laevis* oocyte[123].

All other well-characterized RNA localization elements are more complex than the A2RE element, and one reason for this is the capacity of RNA to fold into higher-order secondary structures. For example, short stem-loop structures direct the apical localization of *hairy* mRNA in the fly embryo and nurse cell to oocyte transport of *K10* and *orb* mRNAs. Mutational analysis indicates that both the sequence and the structure of the double-stranded stems are important[63,124]. The recognition of the four localization elements in *ASH1* mRNA also seems to depend on their structure, because they share no obvious sequence homologies, but are predicted to fold into similar secondary structures, and are all bound specifically by She2 (REFS 37–39,125).

Localization signals often contain multiple elements that are partially redundant. For example, each of the four elements in *ASH1* mRNA directs some localization on its own, but robust accumulation requires the presence of multiple elements[114]. A similar form of redundancy is observed with *D. melanogaster nanos* RNA, which contains four partially redundant regions that direct localization to the posterior pole[20,126–128], and with chicken *β-actin* and *X. laevis fatvg* mRNAs, which each contain two partially redundant localization elements[49,129].

***Vegetal localization signals in* X. laevis.** A more complex form of redundancy is found in *X. laevis Vg1* and *VegT* mRNAs, which require multiple copies of two repeated elements for their localization to the vegetal pole of the oocyte at stage III–IV: an E2 element (UUCAC), which binds VERA/Vg1RBP (BOX 2), and the VM1 motif (YYUCU), which binds Vg1RBP60/hnRNPI (REFS 130–137). Many mRNAs that localize to the vegetal pole in *X. laevis* contain multiple copies of various motifs that all contain the core sequence CAC[138]. Although some of these correspond to E2 elements, distinct CAC motifs have been proposed to target mRNAs to the vegetal pole earlier in oogenesis, in association with the mitochondrial cloud[118,138].

Although these results highlight the importance of multiple copies of short sequences in mRNA localization to the vegetal pole of the *X. laevis* oocyte, this is unlikely to be the whole story. First, FBP2/KSRP, PrrP and hnRNPD also bind specifically to the *Vg1* localization element (VLE), although their precise binding sites remain to be defined[139–141]. Second, the *X. laevis* Staufen homologue also has a role in this process, as it co-localizes and co-purifies with *Vg1* mRNA, and overexpression of a truncated form of Staufen in *X. laevis* disrupts *Vg1* mRNA localization[111]. As the only conserved regions of Staufen are the dsRBDs, this indicates that the VLE must fold into a secondary structure that contains double-stranded regions[142].

The complex case of bicoid localization. The complexity of RNA localization signals reaches its apogee with the *bicoid* localization element, which spans > 600 nucleotides and folds into a secondary structure containing 5 large stem-loops[143–145]. No small region of the element is absolutely essential for localization, but stem-loops IV and V are sufficient to target RNA to the anterior of the oocyte during stages 6–10 (REFS 146–148). Furthermore, point mutations in the BLE1 element in the distal portion of stem V block the localization of stem IV/V, but do not

disrupt the localization of the whole 3′ UTR. So, there seem to be two redundant elements that direct *bicoid* localization during early and mid-oogenesis[148].

Interestingly, the non-redundant stem IV/V localization element forms a high-molecular-weight RIBONUCLEOPROTEIN (RNP) COMPLEX in ovarian extracts, which contains several proteins, including Swallow and the RNA-binding proteins Modulo, polyA-binding protein and the hnRNPI homologue, Smooth[149]. However, none of these proteins binds specifically to the stem IV/V element, indicating that this RNP is the result of the relatively nonspecific and low-affinity binding of proteins that combine to form a specific complex.

Although no specific RNA-binding proteins have been implicated in the early stages of *bicoid* mRNA localization, Staufen is specifically recruited to *bicoid* mRNA in the late oocyte and early embryo. This interaction requires stems III, IV and V of the *bicoid* localization element, which strongly indicates that Staufen binds directly to these double-stranded regions[86]. Staufen recruitment also requires base pairing between the distal loop of stem III in one *bicoid* molecule and an internal bulge in stem III of another molecule[150–152]. The association of Staufen therefore requires the formation of *bicoid* dimers, and so depends on the quaternary structure of the RNA.

The ability of *bicoid* mRNA to dimerize might also explain the redundancy in the early localization signals. These signals were mapped by expressing mutant forms of the *bicoid* localization element in the presence of a wild-type *bicoid* gene[146]. RNAs that are mutant for the BLE1 element in stem loops IV and V, but have a normal stem-loop III, could therefore form dimers with the endogenous RNA, and hitchhike with it to the anterior pole.

Combinatorial* oskar *localization. The *oskar* mRNA localization signal was originally mapped to its 3′ UTR[122]. However, it has recently emerged that an essential localization element is not present in the mature mRNA, as the splicing of the first intron is required for posterior localization[96]. The function of splicing seems to be to recruit the EXON JUNCTION COMPLEX (EJC), which is deposited on the mRNA 20–24 nucleotides upstream of where the intron has been removed[153]. Mutants in two core EJC components, Mago nashi and Y14 (Tsunagi), abolish the posterior localization of *oskar* mRNA, and a third EJC component, the RNA helicase eIF4AIII, has also been implicated in posterior transport[154–157]. eIF4AIII binds to the cytoplasmic protein Barentsz (BTZ), which is also necessary for *oskar* mRNA localization, indicating that the EJC recruits BTZ to the mRNA when it is exported into the cytoplasm[157,158]. The formation of the *oskar* mRNA localization complex therefore depends on its nuclear history (BOX 3).

oskar mRNA localization also requires Staufen, which is thought to bind to stem-loop regions in the *oskar* 3′ UTR[9,93,159,160]. Unlike the EJC components, which only localize transiently to the posterior, Staufen remains associated with the mRNA throughout oogenesis, and is also necessary for the translation and anchoring of *oskar* mRNA at the posterior[95,100,142,159].

In addition to Staufen and the EJC, *oskar* mRNA localization requires the hnRNPA/B family member HRP48 (REF. 161). HRP48 binds to three sites in the *oskar* 3′ UTR, as well as to the 5′ end of the mRNA, and also regulates its translation[94,162]. Several missense alleles of *Hrp48* specifically disrupt *oskar* mRNA localization, without affecting translation, and two of these alleles alter residues in the C-terminal glycine-rich domain that are thought to mediate dimerization[161]. These *Hrp48* mutants lead to the uniform distribution of the mRNA and the disappearance of Staufen–*oskar* mRNA particles. The dimerization of HRP48 might therefore be necessary for the assembly of higher-order *oskar* mRNA complexes that are the substrate for transport.

Although more *trans*-acting factors have been identified for *oskar* mRNA than for any other localized transcript, it is still unclear how it is linked to the motor (probably kinesin) that transports it to the posterior. The EJC, HRP48 and Staufen seem to associate with the RNA independently, and none of these factors is specific for *oskar* mRNA[86,163–166]. So, it is presumably the combination of these RNA-binding proteins, and perhaps others, that single out *oskar* mRNA for transport to the posterior.

mRNA particles. Several lines of evidence indicate that the assembly of functional localization complexes often involves the formation of higher-order RNP structures. Co-injection of two labelled mRNAs into the same oligodendrocyte has revealed that transcripts are localized in large particles that contain ~30 mRNA molecules[167]. Moreover, both *oskar* and *bicoid* mRNAs must assemble into transport particles with multiple mRNAs, as the former can hitchhike on other *oskar* mRNA molecules, while the latter dimerizes[96,150]. Indeed, most observations of mRNA localization in living cells rely on the formation of large RNP particles that can be visualized under the light microscope. Although some of these might be caused by the expression or injection of non-physiological concentrations of RNA, similar particles can be detected in neurons stained with RNA dyes[168].

The idea that localized mRNAs assemble into large particles has received further support from biochemical studies. Sedimentation experiments on neuronal extracts have led to the identification of dense particles of up to 1 μm in diameter that contain large numbers of ribosomes and are enriched for several localized transcripts[169]. Furthermore, large RNP particles (up to 1000S) can be purified from mouse brain extracts on affinity columns containing the C-terminal domain of kinesin KIF5, and these contain several dendritically localized mRNAs[106]. So, a crucial step in the localization of some mRNAs is the assembly of transport particles that contain multiple mRNA molecules and both nuclear and cytoplasmic RNA-binding proteins.

Translational control

mRNA localization can only restrict a protein to a particular region of a cell if the mRNA is not translated until it reaches its destination, and many localized mRNAs are therefore subject to translational control. Although a detailed discussion of this topic is beyond the scope of

MITOCHONDRIAL CLOUD
Also known as the Balbiani body. An aggregate of mitochondria surrounded by electron-dense material that forms next to the nucleus of pre-vitellogenic amphibian oocytes. It subsequently moves to the vegetal pole of the oocyte, where it is thought to have a central role in the assembly of germ plasm.

RIBONUCLEOPROTEIN (RNP) COMPLEX
A complex of protein and RNA.

EXON JUNCTION COMPLEX
(EJC). A protein complex that is deposited as a consequence of pre-mRNA splicing 20–24 nucleotides upstream of splicing-generated exon–exon junctions of newly synthesized mRNA. The EJC is required for efficient nuclear export, nonsense-mediated mRNA decay in mammals and the posterior localization of *oskar* mRNA.

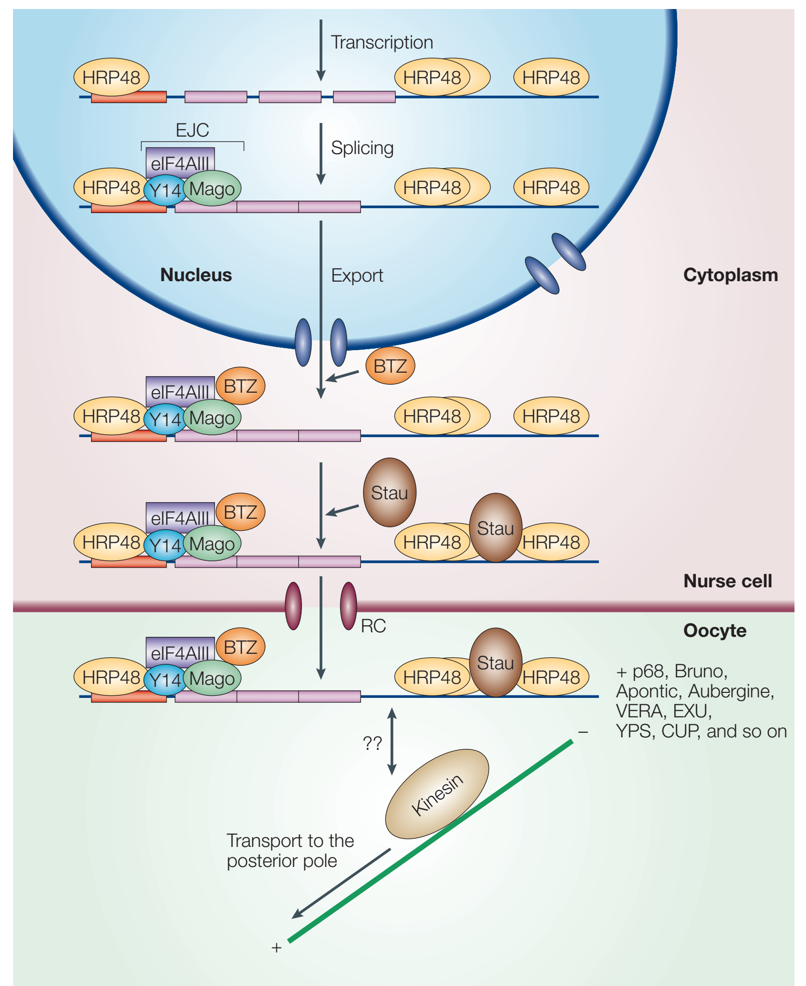
hnRNP
(Heterogeneous nuclear ribonucleoprotein). A group of >20 proteins that associate with high-molecular-weight nuclear RNA. Some hnRNP proteins, such as members of the hnRNPA/B family, shuttle in and out of the nucleus, whereas others are strictly nuclear.

Box 3 | **The role of nuclear history in cytoplasmic mRNA localization**

The analysis of the *trans*-acting factors that associate with *oskar* mRNA highlights an emerging theme in mRNA localization, in that the 'nuclear history' of the mRNA has an important role in determining its fate in the cytoplasm. For example, HRP48 probably binds to *oskar* mRNA co-transcriptionally, whereas the exon junction complex (EJC) binds during splicing, upstream of where each intron has been removed (see figure). Barentsz (BTZ) protein is then recruited by the EJC component eIF4AIII, as the mRNA is exported from the nucleus. Finally, Staufen is thought to associate with *oskar* mRNA in the nurse cell cytoplasm. The mRNA is then transported from nurse cells into the oocyte, where these factors direct its localization to the posterior pole. The *oskar* mRNA localization complex therefore seems to assemble in a stepwise manner, in which factors that associate with the mRNA in the nucleus are required for the recruitment of other essential localization factors in the cytoplasm. Several additional proteins associate with *oskar* mRNA in the cytoplasm to regulate its translation, which indicates the complexity of the RNA transport complex.

The cytoplasmic localization of several other mRNAs also depends on the binding of proteins in the nucleus. For example, Vg1RBP60/hnRNPI and VERA/Vg1RBP have been shown to associate with *Vg1* mRNA in the nucleus, whereas Prrp and Staufen are only recruited in the cytoplasm[190]. Furthermore, both proteins that bind specifically to the *β-actin* mRNA localization element seem to associate with the mRNA in the nucleus: ZBP1 (zip-code-binding protein 1) accumulates at sites of *β-actin* mRNA transcription, whereas ZBP2 is a shuttling heterogeneous nuclear ribonucleoprotein (hnRNP) that is predominantly nuclear, but co-localizes with *β-actin* mRNA in cytoplasmic particles[51,191]. Finally, the strictly nuclear dsRNA-binding protein Loc1 is required for the efficient localization of *ASH1* mRNA to the yeast bud tip[125]. She2 has been shown to shuttle in and out of the nucleus, and probably binds to *ASH1* mRNA in this compartment[43], and Loc1 might therefore function to facilitate She2 binding to *ASH1* mRNA in the nucleus.

EXU, exuperantia; Mago, Mago nashi: RC, ring canals: Stau, Staufen.



this review, in some cases, translational control is also essential for the localization of the mRNA. For example, *ASH1* mRNA needs to be translationally repressed to be efficiently localized to the yeast bud tip, and this requires two proteins, Khd1 and Puf6, which bind directly to the RNA[44,170]. Binding of She2 to the three localization elements in the coding region also seems to contribute to repression, as Ash1 is overexpressed if these are moved to the 3′ UTR[114]. The anchoring of the mRNA once localized requires its translation, but how the switch from repression to activation occurs is not known.

Translational control has a similar role in *oskar* mRNA localization. The translation of unlocalized *oskar* mRNA is repressed by the binding of the Bruno protein and HRP48 to three sites in its 3′ UTR[94,95,162]. It has recently been shown that Bruno recruits the CUP protein, which, by binding to the translation initiation factor eIF4E, inhibits translation[171,172]. Mutants in *cup* therefore cause the premature translation of *oskar* mRNA, and this partially disrupts its localization, presumably because the passage of ribosomes along the mRNA displaces the EJC. Mutants in components of the RNA silencing pathway, such as *spindle-E* and *armitage*, also disrupt *oskar* mRNA localization by causing premature translation, implicating microRNAs in *oskar* translational control[173–175].

Whereas translational silencing of unlocalized *oskar* mRNA is necessary for its localization, anchoring requires the translational activation of the mRNA once it is localized, which depends on the region between two alternative start codons and the *trans*-acting factors Staufen and Aubergine[94,100,142,176]. Unlike *ASH1* mRNA, where translation *per se* is important, Oskar protein has a direct role in its own anchoring, as the long isoform of Oskar maintains its own mRNA at the posterior cortex[177].

In many other cases, the translational control of the localized mRNA is not directly linked to its localization, and is instead temporally regulated. For example, *bicoid* mRNA only becomes poly-adenylated and translated when the egg is activated, and this requires a short element in its 3′ UTR that is distinct from the localization signal[178]. A different mechanism is employed by *Vg1* mRNA, which is repressed by a translation control element (VTE) downstream of the VLE[179]. During late oogenesis, Vg1 RBP71 protein binds to the VLE to catalyse the cleavage of the mRNA at an internal poly-adenylation site, and this removes the 3′ VTE to activate translation[180]. Finally, *nanos* mRNA translation is both spatially and temporally regulated. The translation of unlocalized *nanos* mRNA is repressed by a translational control element (TCE) in its 3′ UTR that contains two stem-loops[127]. One of these stem-loops represses translation during oogenesis[181], whereas the other binds the CUP-binding protein Smaug to mediate repression in the embryo[182–185]. The TCE can also direct the posterior localization of the mRNA, leading to a model in which the binding of localization factors to the TCE prevents the interaction with the translational repressors, thereby allowing the activation of the localized mRNA[20].

Concluding remarks

The recent developments in the *in vivo* imaging of mRNA localization have begun to reveal the mechanisms by which motor proteins transport mRNAs around the cell, and have provided a powerful approach for investigating how the motors themselves are regulated by their cargoes. Despite these advances, we still do not know how most localized transcripts are linked to the motors that move them. In some cases, this requires the stepwise assembly of RNA transport particles containing multiple RNA-binding proteins that function in combination, to control both the localization and translation of the RNA. An important goal in the future will therefore be to determine both the structure and composition of these RNPs to define the substrate for mRNA transport.

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The author declares no competing financial interests.

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