Published in final edited form as:

Semin Cell Dev Biol. 2007 April ; 18(2): 163–170. doi:10.1016/j.semcdb.2007.01.008.

How does an mRNA find its way? Intracellular localisation of transcripts

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Abstract

The localisation of transcripts to specific regions of the cell probably occurs in all cell types and has many distinct functions that go from the control of body axis formation to learning and memory. mRNAs can be localised by a variety of mechanisms including local protection from degradation, diffusion to a localised anchor, and active transport by motor proteins along the cytoskeleton. In this review, I consider the evidence for each of these mechanisms using a limited, but illustrative, number of examples of localised mRNAs.

Keywords

RNA localisation; Mechanisms; Anchoring

1. Introduction

In eukaryotic cells, gene expression is regulated at several levels from chromatin structure to protein stability. One essential step in this regulation is the localisation of transcripts to a specific region of the cell and the translation activation of the localised RNA. This results in an asymmetric distribution of RNAs and proteins that provides localised activities in polarised cells and developing embryos. The list of RNAs known to localise is over 100, with at least 25 transcripts in yeast alone. This number is probably a small representation of the total number of localised RNAs, since an estimated 71% of the total RNAs show some kind of subcellular localisation in the embryo of *Drosophila melanogaster* (H. Krause, personal communication) and around 300 are protected from degradation at the posterior of the egg (H. Lipshitz, personal communication). Thus, finding out how transcripts are sorted to their correct intracellular localisation is a key question in the understanding of how gene expression is regulated and how cells achieve polarity.

In this review, I am presenting the recent progress in understanding the way that RNAs get localised, which involves at least one of these five mechanisms: diffusion and localised entrapment, localised degradation, localised synthesis, active transport, and polarised nuclear export. In addition, once localised the RNAs need to be anchored. Since space is limiting and other chapters of this issue cover in detail specific aspects of RNA localisation, I only present cases of RNAs that are either well understood or convey important concepts.

2. Mechanisms of mRNA localization

2.1. Diffusion and localised entrapment

Diffusion allows the movement of RNAs, not only within the nucleus [1], but also once the RNA is exported and needs to be localised. For diffusion to provide a means of localisation, the RNA needs to be trapped by previously localised anchors. Although not much is known about the nature of these anchors, this seems to be the case for *nanos* (*nos*) mRNA. *nos* represents a class of RNAs whose localisation to the posterior pole of the *Drosophila* egg does not occur until late oogenesis – when the cytoskeleton shows no anterior–posterior (AP) polarity – and depends on the prior formation of the pole plasm [2]. These observations led to the proposal of diffusion and entrapment as the mechanism of *nos* localisation, which has since been confirmed by the analysis of fluorescent *nos* RNA *in vivo* [3]. Treatment with microtubule (MT)-depolymerising drugs does not abolish, but reduces the posterior localisation of *nos*, which further suggests diffusion, and not active transport as the mechanism responsible for localisation. However, the observed reduction shows that *nos* RNA localisation also requires a MT-dependent step, which might be the enhancement of its diffusion by cytoplasmic flows. Localised RNAs are then organised in particles that are trapped by an actin-dependent association with the germ plasm, which lies below the cortex at the posterior pole.

Diffusion and entrapment allows for the localisation of both *Xcat2* mRNA, which codes for a Nos related protein, and *Xdazl* mRNA to the vegetal pole during early stages of oogenesis in *Xenopus* [4]. At stage I, the RNAs localise to a structure called the mitochondrial cloud (MC), which lies next to the nucleus. Then, the MC moves towards the vegetal cortex, bringing the RNAs with it. This early RNA localisation seems not to involve active transport, since MT depolymerisation had no effect on mRNA localisation. In fact, recent experiments suggest that *Xcat2* and *Xdazl* transcripts localise to the MC through diffusion and association with the ER within the cloud [5]. Upon injection into previtellogenic oocytes, fluorescent *Xcat2* RNA forms particles that accumulate at the MC in a linear manner. FRAP experiments either in the cytoplasm, at the edge of, and within the MC show that the particles are diffusing and get trapped at the MC, where they are immobilised. The cytoplasmic levels of the RNA remain constant, which supports a progressive entrapment and not a selective degradation as the mechanism for localisation.

2.2. Localised degradation

In *Drosophila*, several RNAs, including *hsp83* mRNA, localise to the pole cells, the precursors of the germ cells, when they are formed at the posterior of the embryo. *hsp83* mRNA is uniformly distributed earlier in the fertilised egg, but as the nuclear divisions advance the levels of total RNA reduce dramatically, except in the pole plasm where the RNA is protected [6]. The nuclei that reach this region will bud off the embryo forming the pole cells and taking the components of the pole plasm, which include the localised transcript. Although not much is known about the stabilising machinery, *hsp83* RNA is degraded everywhere in mutants that lack the pole plasm. Conversely, in *smaug* mutants, the degradation of *hsp83* RNA is not triggered, and consequently, localisation does not occur. Smaug seems to be acting by recruiting the CCR4/POP2/NOT deadenylase complex, which induces deadenylation and destabilisation [7]. This model is based on the observations that Smaug associates with both the *hsp83* RNA and the deadenylase complex. Furthermore, in *smaug* mutant embryos the deadenylation and degradation of *hsp83* mRNA is abolished.

2.3. Localised synthesis

Local transcription as a mechanism for local accumulation of RNAs has been described in either a syncytial, when several nuclei share a cytoplasm, or when RNAs are exported from

localised mitochondria [8]. For example, in multinucleate myofibers, the mRNAs encoding the δ - and ϵ -subunit of the acetylcholine receptor (AChR) concentrate beneath the neuromuscular junction (NMJ) by the spatially restricted activation of their expression in the nuclei close to the synapse. This local transcription allows the accumulation of the receptors at the synapses and is regulated by locally acting factors from the nerve and by muscle activity. In the case of the AChR ϵ gene, it seems that neural agrin induces the focal binding of muscle-derived neuregulins at the NMJ, which in turn serves to activate transcription of the AChR ϵ gene by Egr-1 and a neuregulin response element ([9,10] and references therein).

2.4. Polarised export

Although an attractive model for RNA localisation, it is not clear whether the vectorial export of RNAs from the nucleus is linked to their asymmetric distribution in the cytoplasm. However, a study on the nuclear architecture of the biflagellated single-cell algae *Chlamydomonas reinhardtii* reveals that this may be the case [11]. The analysis of the nuclear pore complexes (NPC) showed that they are preferentially at one side of the nucleus, opposite to the flagella at the anterior of the cell. This nuclear asymmetry is especially clear during the upregulation of genes encoding flagellar proteins following deflagellation. At this point, the newly synthesised $\beta 2$ tubulin transcript localises to the posterior polysome-rich cytoplasmic region adjacent to the nuclear area with the highest NPC density. Although the causal relationship between the NPCs asymmetric distribution and the cytoplasmic localisation of the $\beta 2$ tubulin mRNA is not determined, the NPCs might redistribute to allow the localisation of mRNAs to the most active translation site.

2.5. Active transport along the cytoskeleton

As discussed by Simon Bullock in chapter xxx [12], molecular motors move directionally along the cytoskeleton. They are widely used to traffic organelles and other cargoes, including RNAs, within the cytoplasm. Localised transcripts need to be selected from the general population of RNAs, and although any RNA might inefficiently move along the cytoskeleton, the presence of specific localisation sequences increases the probability of specific transport [13]. Basically, active transport involves that specific elements in the RNA (*cis*-acting elements) are recognised by *trans*-acting factors that specifically recruit motor proteins that move along the cytoskeleton towards the final destination. Although active transport is the most commonly used mechanism for RNA localisation, it is also hard to prove, requiring either *in vivo* visualisation of the RNA moving in a motor-dependent manner, or the functional and biochemical characterisation of the RNA–motor interaction.

2.5.1. ASH1 mRNA—In budding yeast, *ASH1* mRNA is localised to the bud of the daughter cell by its association with the myosin Myo4 and actin. For *ASH1* mRNA to localise and for Myo4 to co-localise and co-precipitate with the transcript, the proteins She2 and She3, and several *cis*-acting elements are necessary [14]. She2 seems to bind these elements in the nucleus. This interaction enhances the affinity of She2 for She3, which itself binds Myo4p. The artificial tethering of She3 to *ASH1* mRNA allows the transcript to localise even in the absence of She2 [15]. This suggests that the She3/Myo4 complex is modulated by the *cis*-acting elements and the sole function of She2 is to recruit the complex to the RNA [16]. However, the artificial tethering of She2 to *ASH1* mRNA through high affinity binding affects the localisation of the transcript by disrupting its anchoring [17]. So, although the function of She2 during transport might be to associate *ASH1* RNA to Myo4/She3, the natural interaction of She2 with the RNA is required for anchoring, presumably by allowing the remodelling of the complex and the switching to a She3/Myo4-dependent, She2-independent anchoring.

2.5.2. Pair-rule and wingless mRNAs—The combination of *in vivo* visualisation and genetics has been very powerful towards the understanding of the mechanism of localisation of RNAs in *Drosophila* embryos. The best characterised examples are *wingless* and the pair-rule transcripts which localise to the apical cytoplasm above the nuclei by the MT minus-end-directed motor dynein [18–20]. Injected fluorescent pair-rule and *wingless* RNAs assemble into particles that move in a MT-dependent manner towards the apical cytoplasm at approximately 0.5 $\mu\text{m/s}$ [19,20]. Since MTs are nucleated from an apical MTOC, it was suggested that a minus-end-directed motor directs the transport of the transcripts. Indeed, co-injection of the RNAs with antibodies against dynein or injection into mutants with reduced dynein function inhibits apical localisation or reduces the movement of the particles, respectively. However, how the RNA interacts with dynein and how dynein activity is regulated is not yet fully understood, but the localisation signals and a complex formed by the proteins Egalitarian (Egl) and Bicaudal-D (BicD) seem to have an important role in these processes ([21] and references therein). Although it has not been possible to detect dynein with the injected RNA, a complex that contains both can be isolated biochemically in a BicD-dependent manner [21], and Egl binds directly to the dynein light chain [22].

2.5.3. Gurken, bicoid and oskar mRNAs—At early stages of oogenesis, when the oocyte is acquiring its fate, the dynein-BicD-Egl complex is required for the transport of molecules, including mRNAs, from the nurse cells (NCs) into the oocyte [23]. This complex is essential for the establishment of the oocyte fate and if mutated oogenesis stops [22,24]. Later in oogenesis, three of these early localised RNAs, *bicoid* (*bcd*), *gurken* (*grk*) and *oskar* (*osk*) undergo a new localisation within the oocyte that seems to be mediated by dynein (in the case of *bcd* and *grk* mRNAs) and kinesin (in the case of *osk* mRNA).

2.5.3.1. Gurken mRNA: There is compelling evidence to suggest that the transport of *grk* mRNA to the dorsal-anterior (DA) corner of the oocyte is mediated by dynein. First, over-expression of dynamin, a regulator of dynein processivity, disrupts *grk* mRNA localisation. Second, injected fluorescent *grk* mRNA forms particles that preferentially move first to the anterior pole and then dorsally at an average speed of 0.25 $\mu\text{m/s}$, which is consistent with active transport ([25] and references therein). These two steps of movement are dynein-dependent, since they are abolished if the RNA is co-injected with antidynein antibodies. Furthermore, the injected RNA localises to the DA corner in 85% of wild type oocytes, but only in 21% of the oocytes with reduced dynein function.

What might mediate the interaction of *grk* mRNA with dynein? Since mutations in *squid* and *hrb27C* block the *grk* localisation to the DA corner, and Squid and Hrb27C interact with each other and bind *grk* mRNA, it is likely that they are components of the localisation complex [26,27]. However, if any of these factors link the RNA to dynein, it would only do so in the second step, since endogenous and injected *grk* mRNA localise to the anterior in *squid* and *hrb27C* mutants. Alternatively, Squid and Hrb27C might modulate the choice of the MT network by dynein. It has been suggested that the two steps represent the transport of *grk* RNA on two distinct populations of MTs, with the minus ends either at the anterior or at the DA corner (for detail see [12]). In *squid* and *hrb27C* mutants, dynein might only be able to recognise the first population of MTs. However, since Squid and Hrb27C also function in other polarity and RNA processing events [28], their role on *grk* RNA localisation could be an indirect one.

2.5.3.2. Bicoid mRNA: The transport of *bcd* mRNA to the anterior pole of the oocyte occurs during different stages of oogenesis and involves various *cis*- and *trans*-acting factors. The first phase of *bcd* localisation might depend on dynein and involves anteriorly nucleated MTs and the formation of a complex that contains both *bcd* RNA and Exuperantia

in the NCs [29]. However, how Exu contributes to the RNA-dynein interaction and how the complex recognises a specific set of MTs is not known. A second phase is the movement of *bcd* RNA from an anterior ring to cover the anterior cortex. This step requires γ -tubulin37C and Grip75, than when mutated affect the anterior population of MTs [30], and the protein Swallow, that co-localises with the RNA and interacts with the dynein light chain, suggesting dynein to be involved [31].

A final step, which localises the majority of *bcd* RNA [32], requires the dynein-dependent movement of a *bcd*-Staufen (Stau) complex on MTs. Fluorescently labeled *bcd* RNA accumulates, as the endogenous transcript, at the anterior during late oogenesis. This step requires MTs, the actin-dependent NC-to-oocyte dumping of material and Stau. Furthermore, *bcd* RNA and Stau form particles that are either transported and/or anchored in a MT- and actin-dependent manner, since disruption of the two cytoskeletons results in the mislocalisation of the particles. To distinguish whether the cytoskeleton functions in anchoring or transport, FRAP and FLIP experiments were performed in which either the localised RNA or a region posterior to the localisation domain was photobleached, respectively. In the first case, there is a recovery of fluorescence to 50% the initial value. In the second case, the signal of localised RNA decreases by 24%. These results suggest that the association of *bcd* RNA with the anterior cortex is dynamic and requires active transport. This is further supported by the fact that the anterior accumulation of *bcd* is decreased or absent in oocytes that either have a reduced dynein function, over-express the dynamitin subunit, or have been treated with an ATPase inhibitor.

In addition to *bcd*, Stau is also required for the kinesin-dependent transport of *osk* mRNA to the posterior of the oocyte. How does Stau function in the differential localisation of two RNAs via two motor proteins, dynein and kinesin, in the same cell? One possibility is that the specificity in this case is determined by the expression of Stau interactors at different stages of oogenesis. In fact, the interaction of Stau with *bcd* RNA is modulated by the action of a complex formed by two proteins, Vps36 and Vps22 (U. Irion and D. St Johnston, personal communication). Mutations in Vps22 and Vps36 abolish the final Stau-dependent step in *bcd* localisation, but have no effect on the Stau-dependent transport of *osk* mRNA. Vps36 binds directly and specifically to *bcd* 3' UTR, localises to the anterior cortex in a *bcd* RNA-dependent manner, and is required for the recruitment of Stau to the *bcd* complex.

2.5.3.3. Oskar mRNA: In contrast to *bcd* and *grk* mRNAs, which are likely transported by dynein towards the anterior and DA cortex, respectively, *osk* mRNA localisation to the posterior pole requires the heavy chain of kinesin (KHC), a plus-end-directed motor protein [33,34]. However, whether kinesin directly transports *osk* mRNA or it mediates other processes that contribute to its posterior localisation is not yet clear. For example, it was observed that if *osk* RNA is injected in the proximity of the posterior, it localises in a MT-independent manner, suggesting that diffusion and entrapment contribute to *osk* localisation [35]. Since kinesin is essential for all cytoplasmic flows in the oocyte [34], it could play a role in *osk* transport by facilitating its diffusion. However, recent analysis of *kinesin* alleles that partially affect motor function show that *osk* RNA localises efficiently to the posterior even when the ooplasmic flows are strongly reduced, questioning their contribution [36].

In addition to the localisation of *osk* mRNA to the posterior and cytoplasmic movements in the oocyte, KHC is also required for the exclusion of *osk* RNA from the oocyte cortex [37]. This and the lack of a good visualisation of MTs at the posterior led to a model in which *osk* localisation is a consequence of kinesin transporting it away from the minus-ends at all regions of the cortex, except the posterior, where MTs would not exist. This model is consistent with the observation that at early stages of its transport, *osk* RNA is detected in the middle of the oocyte, where presumably the plus-ends would be. However, it does not

explain how plus-end binding proteins, such as Nod [38], and kinesin itself [39] localise to the posterior at the time when *osk* RNA is found there. It also does not explain that in oocytes with a reduced kinesin activity, the localisation of *osk* RNA at the center of the oocyte persists through later stages, suggesting that the shift to the posterior is slowed [36]. To obtain clear answers to these questions, advances in the analysis of the polarity of the cytoskeleton, the interaction of *osk* with the motor, and the imaging of *osk* RNA movements are needed.

One of the reasons for a delay in the understanding of the movement of *osk* RNA is that splicing is required to form a localising complex including Mago, Y14 and eIF4AIII [40]. These proteins are components of the exon-exon junction complex (EJC). When mutated the posterior localisation of *osk* RNA is prevented, and the transcript is instead found at the anterior [41,42]. In addition to the EJC, mutations in *barentsz*, *hrp48* and *stau* also abolish the transport of *osk* to the posterior [39]. All these proteins co-localise with *osk* RNA to the posterior in a transcript-dependent manner, which further suggests that they are components of the localisation complex. How this complex is assembled to achieve the final localisation of *osk* is not yet understood, but it seems to take place in a stepwise manner, as suggested by the distribution of the proteins and their function in other organisms (Fig. 1E). Furthermore, how these factors link the RNA to kinesin, and whether they contribute to the activity of the motor still needs to be determined.

2.5.4. *Vg1* mRNA—This stepwise assembly of an RNA localisation complex is also the case for *Vg1* mRNA, a transcript that localises to the vegetal pole of *Xenopus* oocytes. An initial step is the interaction of hnRNP I [43] and Vg1RBP/Vera [44] with the transcript. Both proteins localise to the vegetal cortex and mutations in the RNA that impair protein binding also affect RNA localisation. 40LoVe ([45,46] and references therein) can then join this complex by both protein-protein and protein-RNA interactions. Although these associations are likely to happen in the nucleus, *Vg1* transcripts that are microinjected into the cytoplasm move to the vegetal cortex, which suggest that the complex can also form outside of the nucleus. Once the complex is exported to the cytoplasm, it is remodeled and additional transport factors, such as Stau, are recruited, suggesting their function in *Vg1* RNA transport [47] (Fig1F). In fact, the injection of a dominant negative form of Stau strongly reduces the localisation of *Vg1* RNA [48]. At the stage when *Vg1* mRNA is localised, the MTs are arranged with their minus ends to the cortex and their plus ends extending towards the center of the oocyte [49], suggesting that a minus-end directed motor transports *Vg1* RNA. However, two plus-end motors, kinesin I and II, have been involved in *Vg1* localisation: kinesin I was shown to co-immunoprecipitate with Stau and *Vg1* mRNA [48], while injection of antibodies against kinesin II and a dominant negative form of the motor inhibit *Vg1* RNA localisation [50]. It is still unclear whether either or both kinesins directly transport *Vg1* RNA along the MTs or they might play a more indirect role by, for example recycling a minus-end-directed motor.

2.5.5. CaMKII α and A2RE mRNAs—CaMKII α mRNA in mammals is found at synapses where its synthesis is regulated by neural activity. CaMKII α mRNA forms particles that, upon depolarisation, preferentially move in an anterograde manner and that require cytoplasmic polyadenylation elements (CPE) and the binding of CPEB. Furthermore, CPEB forms granules that occasionally contain a CPE-containing RNA, and in addition, CPEB co-immunoprecipitates and partially co-localises with kinesin and dynein. Thus, it seems that the transport of CaMKII α mRNA is mediated by association of CPEB via the CPE to form granules that associate with motor proteins ([56] and references therein). CaMKII α mRNA is also found in particles that contained several RNA-binding proteins (RBPs) and interact with the KHC KIF5 [54]. The movement of these particles is enhanced by over-expression of KIF5, and suppressed by a dominant negative KIF5 or

RNAi against the RBPs. Furthermore, kinesin co-localises with *CaMKII α* mRNA in *Drosophila*, suggesting that both mammals and insects transport CaMKII α mRNA to the synapses by kinesin [55,56].

Another transcript that seems to localise in a kinesin-dependent manner is the Myelin Basic Protein (MBP) mRNA in cultured oligodendrocytes. This RNA targeting depends on the *cis*-acting sequence A2RE and its recognition by hnRNP A2. Similarly, in hippocampal neurons a microinjected A2RE-containing RNA is recruited into granules that are transported along neurites in an hnRNP A2-dependent manner. Several transcripts including HIV viral RNAs and dendritically localised RNAs contain A2RE-like sequences, suggesting that interaction of this motif with hnRNP A2 – and presumably their transport along the MTs by kinesin – is a general pathway to localise RNAs ([57] and references therein).

2.6. Anchoring

After being transported, localised RNAs must be retained at their destinations. Although an essential step in RNA targeting, little is known about how RNA anchoring is achieved. Actin and actin-binding proteins play an important role in the anchoring of several transcripts that are transported in an actin- or in a MT-dependent manner, such as *ASH1* and β -actin mRNAs or *osk* and *Vg1* mRNAs, respectively ([58] and references therein). In some cases, this actin requirement might be a structural one, as for *Vg1* RNA, where actin keeps the integrity of the cytokeratin filaments, or as for *bcd*, where actin anchors a population of MTs that is required for the transport of the transcript [32]. A surprising example of an anchoring protein is dynein itself [58]. Using an RNA anchoring assay, it was shown that the apical anchoring of pair-rule mRNAs depends on MTs, and involves dynein as a static anchor after having transported the transcripts there. It would be very interesting to analyse whether this is a general principle that could also apply to other dynein cargoes and other molecular motors.

3. Concluding remarks

Although considerable progress has been made in identifying the *cis*- and *trans*-acting factors required for the localisation of RNAs, and their interactions with the localisation machinery, a lot of questions remain unanswered. For instance, what traps and protects RNAs at the posterior of the *Drosophila* egg? How are the localisation complexes formed and remodeled? What is the molecular function of individual *trans*-acting factors? How do RNAs interact with the motor complex?

In addition, other aspects of RNA localisation need to be kept in mind. For example, although it is likely that most RNAs use one major mechanism of localisation, several RNAs use multiple mechanisms. *osk* RNA is localised by dynein in early oocytes, but by kinesin in late oocytes. Also in the oocyte, local synthesis in the nucleus might contribute to *grk* mRNA localisation [59], although the transcript produced in the NC nuclei is sufficient for axis determination [60]. For transcripts that are already enriched in a cytoplasmic region, localised degradation contributes to their final localisation and ensures that any unlocalised RNA is not translated. For example, an estimated 4% of *nos* mRNA is localised by diffusion/entrapment, with the degradation of the unlocalised transcript contributing largely to its distribution [61].

Finally, how is RNA localisation coordinated with other RNA processing events, such as degradation? Although just an emerging field, it has recently become clear that the localisation and degradation machinery are somehow related and that RNA degradation factors such as Me31B and Dcp1 are required for *osk* RNA localisation ([62,63] and references therein). Also, is there a direct link between ER and RNA trafficking, and if so,

how is it coordinated? For example, in budding yeast, a coordinated movement of the ER and *ASH1* mRNA has been observed [64]. *ASH1* RNA and the tubular ER co-localise and co-migrate to the bud in a Myo4/She3/She2-dependent manner. Thus, it seems likely that a coordinated localisation of the ER and RNAs is a common mechanism to achieve local translation and asymmetrical localisation of proteins.

Acknowledgments

I am grateful to U. Irion, D. St Johnston, H. Krause and H. Lipshitz for sharing unpublished data and to Philippe Loiseau, Ines Alvarez-Garcia and Simon Bullock for discussions and/or comments. I apologise to all whose work could not be cited due to space constraints. Isabel M Palacios is funded by the Royal Society.

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Fig. 1.

(A–D) Mechanisms of mRNA localization. (A) Diffusion and entrapment: diffusion (facilitated by cytoplasmic flows (blue spiral)) through the cytoplasm and entrapment by a specific anchor (red). (B) Localised degradation and protection: although nothing is known about the nature of the protecting machinery (green), the degradation of *hsp83* mRNA is mediated by Smaug and the CCR4/POP2/NOT deadenylase complex (blue). (C) Local synthesis: the local transcription of AChR mRNAs at the neuromuscular junction (NMJ) – regulated by locally acting factors from the nerve and the muscle (yellow arrows) – allows the local accumulation of the receptors. (D) Active transport by motor proteins: *bcd* and *grk* mRNAs seem to be transported by dynein to the anterior pole of the oocyte along an anteriorly nucleated population of MTs (blue). Once at the anterior, *grk* RNA shifts to another population of MTs (red) that mediates its dynein-dependent localisation to the anterior-dorsal corner. In the same cell, *osk* RNA accumulates at the posterior pole in a KHC-dependent manner, likely by the active transport of the transcript by the motor from the minus ends (at the anterior and cortex, green MTs) to the plus-ends. (E) The nuclear history of a transcript is important for its cytoplasmic localisation. *osk* mRNA is transcribed in and exported from the nurse cell (NC) nuclei, transported from the NC into the oocyte and finally localised to the posterior. The splicing of *osk* mRNA and its interaction in the nucleus with several factors, is essential for its localisation. The assembly probably starts by the co-transcriptional binding of Hrp48, and upon splicing, the EJC. Barentsz recognises the complex as it is exported from the nucleus by interaction with eIF4AIII, and finally, Stau joins in the cytoplasm of the NCs. The *osk* particle moves then from the NCs into the oocyte where presumably by interacting with the KHC, it localises to the posterior pole. (F) Remodeling of the localising complex is probably an essential step in RNA localisation. As shown in the localisation of *Vg1* RNA in the *Xenopus* oocyte, hnRNP I and Vg1RBP/Vera bind directly to each other and to the transcript in the nucleus. Upon export, the complex is remodeled such that these two proteins no longer interact and additional factors become part

of the localisation complex, such as Stau and Prp. The transport of *Vg1* mRNA to the vegetal pole is then somehow mediated by a mechanism that seems to require kinesin I and II. NPC: nuclear pore complex; RC: ring canal.