Received: 13 April 2020 | Revised: 13 May 2020 | Accepted: 17 May 2020

DOI: 10.1002/wrna.1610

ADVANCED REVIEW

WIREs RNA WILEY

Patterning the *Drosophila* embryo: A paradigm for RNA-based developmental genetic regulation

Paul Lasko[1,2]

[1]Department of Biology, McGill University, Montréal, Québec, Canada

[2]Department of Human Genetics, Radboudumc, Nijmegen, Netherlands

Correspondence
Paul Lasko, Department of Biology, McGill University, Montréal, QC, Canada.
Email: paul.lasko@mcgill.ca

Funding information
Natural Sciences and Engineering Research Council of Canada, Grant/Award Number: RGPIN-2014-06340; Radboud Universiteit, Grant/Award Number: Visiting Professorship

Abstract

Embryonic anterior–posterior patterning is established in *Drosophila melanogaster* by maternally expressed genes. The mRNAs of several of these genes accumulate at either the anterior or posterior pole of the oocyte via a number of mechanisms. Many of these mRNAs are also under elaborate translational regulation. Asymmetric RNA localization coupled with spatially restricted translation ensures that their proteins are restricted to the position necessary for the developmental process that they drive. Bicoid (Bcd), the anterior determinant, and Oskar (Osk), the determinant for primordial germ cells and posterior patterning, have been studied particularly closely. In early embryos an anterior–posterior gradient of Bcd is established, activating transcription of different sets of zygotic genes depending on local Bcd concentration. At the posterior pole, Osk seeds formation of polar granules, ribonucleoprotein complexes that accumulate further mRNAs and proteins involved in posterior patterning and germ cell specification. After fertilization, polar granules associate with posterior nuclei and mature into nuclear germ granules. Osk accumulates in these granules, and either by itself or as part of the granules, stimulates germ cell division.

This article is categorized under:

RNA Export and Localization > RNA Localization

Translation > Translation Regulation

RNA in Disease and Development > RNA in Development

KEYWORDS

embryonic patterning, ribonucleoprotein complexes, translational control

1 | INTRODUCTION

Establishment of the anterior–posterior axis of the *Drosophila* embryo is among the most intensively studied developmental processes. The insight of Christiane Nüsslein-Volhard, Eric Wieschaus and their colleagues who carried out comprehensive forward genetic screens to find mutations affecting this process, and their generosity in sharing data and making their mutants available to other researchers enabled the molecular identification by the early 1990s of many of the key genes involved and basic elucidation of the relevant pathways. Since that time a wealth of new

information has been obtained, leading not only to a deep understanding of this developmental process, but also providing important insights into RNA-level mechanisms of gene regulation that are widely employed in biology. Now, 30 years later, remarkable new discoveries still emerge from studying this system, providing new knowledge about how physical processes underlie regulation of genes responsible for cellular and developmental switches. Nevertheless, important questions also still remain to be answered.

The central events of anterior–posterior patterning can be summarized as follows. *bcd* and *oskar* (*osk*) mRNAs are transcribed in the nurse cells and transported through cytoplasmic bridges called ring canals to the presumptive oocyte, where, during mid-oogenesis, they later accumulate at the anterior and posterior poles respectively (Berleth et al., 1988; Ephrussi, Dickinson, & Lehmann, 1991; Kim-Ha, Smith, & Macdonald, 1991). Upon egg deposition, *bcd* is translated to produce an anterior-to-posterior gradient of Bcd protein, a homeodomain-containing transcription factor that regulates expression of zygotic patterning genes in a concentration-dependent manner (Driever & Nüsslein-Volhard, 1988a, 1988b). Osk is the key maternal determinant for germ cell specification and for establishing the posterior somatic pole of the embryo (Ephrussi et al., 1991; Kim-Ha et al., 1991; Lehmann & Nüsslein-Volhard, 1986). *osk* RNA localizes to the posterior pole of the oocyte, where it is translated, via three sequential mechanisms described in more detail below. Osk protein nucleates the assembly of germ plasm in the late oocyte and formation of polar granules in the early embryo (Ephrussi et al., 1991; Ephrussi & Lehmann, 1992; Smith, Wilson, & Macdonald, 1992). Polar granules are considered to be determinants of primordial germ cells (PGCs), as cytoplasm containing polar granules induces pole cell formation at an ectopic site (Illmensee & Mahowald, 1974). Vasa (Vas), a DEAD-box RNA helicase, Tudor (Tud), and Aubergine (Aub), a Piwi-family protein that interacts with piwi-interacting RNAs (piRNAs), are key functional components of polar granules that are recruited downstream of Osk during later stages of oogenesis (Bardsley, McDonald, & Boswell, 1993; Breitwieser, Markussen, Horstmann, & Ephrussi, 1996; Hay, Jan, & Jan, 1990; Lasko & Ashburner, 1990; Wilson, Connell, & Macdonald, 1996). Many other specific mRNAs are also recruited to polar granules, including *nanos* (*nos*), *germ cell-less* (*gcl*), and *polar granule component* (*pgc*) (Jongens, Hay, Jan, & Jan, 1992; Nakamura, Amikura, Mukai, Kobayashi, & Lasko, 1996; Wang & Lehmann, 1991; Wharton & Struhl, 1991). *nos* encodes the main determinant for posterior pattern, while *gcl* and *pgc* are required for development of PGCs (Gavis & Lehmann, 1992; Nakamura et al., 1996). While *nos* is not required for PGC specification, it is essential for their subsequent development (Asaoka-Taguchi, Yamada, Nakamura, Hanyu, & Kobayashi, 1999; Forbes & Lehmann, 1998). After fertilization and the migration of zygotic nuclei to the posterior pole, Osk also nucleates the formation of nuclear germ granules, ribonucleoprotein particles (RNPs) that are related to polar granules but are compositionally distinct (Kistler et al., 2018). Through an unknown mechanism, nuclear germ granules, or perhaps Osk specifically, promote pole cell division.

Space constraints do not permit a comprehensive review of all relevant literature, so here I will focus on recent advances toward understanding deployment and regulation of Bicoid (Bcd), the central determinant for anterior fate, and on the assembly of RNP) complexes called polar granules, which specify posterior fate and PGCs (often called pole cells in *Drosophila*).

2 | ESTABLISHMENT OF THE BCD GRADIENT

2.1 | *bcd* RNA movement is microtubule-dependent but randomly directed

bcd mRNA is transcribed in nurse cells and transferred through the ring canals into the oocyte in later stages of oogenesis. Through several intermediate steps (Figure 1) it becomes tightly localized at the anterior pole of the oocyte, and its localization depends upon the activities of three other genes, *staufen* (*stau*), *swallow* (*swa*), and *exuperantia* (*exu*), and the ESCRT-II endosomal sorting complex (Irion & St Johnston, 2007; St Johnston, Driever, Berleth, Richstein, & Nüsslein-Volhard, 1989). Depolymerization of microtubules with drugs blocks all steps of *bcd* localization (Pokrywka & Stephenson, 1991). During late oogenesis, anterior localization of *bcd* is achieved through continuous microtubule-dependent active transport (Weil, Forrest, & Gavis, 2006). However, beginning at the end of oogenesis *bcd* is anchored at the anterior in an actin-dependent process (Weil, Parton, Davis, & Gavis, 2008).

A model for anterior *bcd* localization by which the motor protein dynein transports *bcd* toward the minus-ends of microtubules emerged based primarily on observations that disruption of dynein function by overexpressing dynamitin results in dispersion of *bcd* RNA (Duncan & Warrior, 2002; Januschke et al., 2002). This model gained widespread acceptance, and was supported by experiments showing that injected *bcd* mRNA into nurse cells recruits dynein-associated proteins required for mRNA transport (Bullock & Ish-Horowicz, 2001; Clark, Meignin, & Davis, 2007).

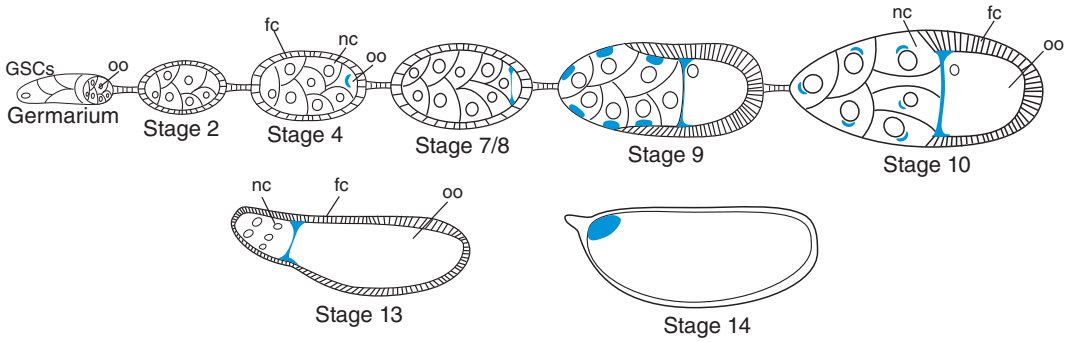

FIGURE 1 Cartoon illustrating various stages of *Drosophila* oogenesis, with sites of *bcd* RNA accumulation shown in blue. Germline stem cells (GSCs) at the anterior end of the germarium undergo four transit-amplifying divisions and become enveloped by follicle cells (fc) to form egg chambers. The four mitotic divisions that germ cells undergo have incomplete cytokinesis, so the 16 germline cells remain interconnected by cytoplasmic bridges called ring canals. One of the germline cells that is connected to four other germline cells by ring canals becomes specified as the oocyte (oo) and is always located at the posterior end of the egg chamber. The other germline cells become nurse cells (nc). Nurse cells synthesize RNAs and proteins that can be transferred into the oocyte through ring canals. After stage 10, the cytoplasm of nurse cells is rapidly transferred into the oocyte. The nurse cells subsequently die and the mature stage 14 egg remains

However, a more recent study using improved imaging techniques to visualize *bcd* RNA in living oocytes confirms that *bcd* localization requires dynein, but paints a more complex picture than simple minus-end directed transport (Trovisco et al., 2016). This study used a construct, *bcdMS2*, that expresses the *bcd* 3′ UTR, which is sufficient for all stages of localization (Macdonald & Struhl, 1988) fused to 11 copies of a stem-loop element called MS2, which can be bound with very high affinity by the MS2 coat protein (MCP). Live imaging of *bcd* is then accomplished by coexpressing MCP conjugated to the fluorescent protein GFP (Bertrand et al., 1998; Weil et al., 2006). Using this approach, Trovisco and colleagues imaged small *bcdMS2* containing particles that moved at speeds up to 2.2 μm/s. The mean velocity of these particles slowed in a hypomorphic dynein mutant background, increased in a kinesin mutant background, and the particles stopped moving altogether upon treatment with colcemid, a drug that depolymerizes microtubules. Consistent with earlier results, these experiments indicated that *bcd* RNA localization is dependent on dynein and microtubules and antagonized by kinesin, a plus-end directed motor. However, assessment of the directionality of *bcdMS2* particle movement showed almost no directional bias toward the anterior over the posterior, and an equal dependence on dynein for movement in either direction. These observations rule out a major contribution of directed transport to *bcd* RNA localization. Rather, anterior accumulation of *bcd* depends upon essentially random movement followed by actin-dependent anchoring. This conclusion was supported by fluorescence recovery after photobleaching (FRAP) experiments that revealed a slow-recovering population of *bcd* RNA at the anterior pole. This work also showed that the RNA-binding protein Exu (Lazzaretti et al., 2016) is necessary to assemble *bcdMS2* particles. How anterior *bcd* anchoring is maintained after egg activation remains to be fully determined.

2.2 | *bcd* is translationally repressed until egg activation

bcd mRNA is translationally repressed throughout oogenesis and until egg activation (Sallés, Lieberfarb, Wreden, Gergen, & Strickland, 1994), but exactly how this is accomplished remains unclear. While early evidence suggested that translational regulation of *bcd* involved alterations in poly(A) tail length (Sallés et al., 1994), as occurs for many maternal mRNAs (Lim, Lee, Son, Chang, & Kim, 2016; Rouget et al., 2010), recent work using poly(A)-tail length profiling by sequencing (PAL-seq) (Subtelny, Eichhorn, Chen, Sive, & Bartel, 2014) showed no change in *bcd* poly(A) tail length upon its translational activation in early embryos (Eichhorn et al., 2016). In oocytes the average *bcd* poly(A) tail length was similar to those of several well-translated mRNAs, suggesting that a repressive mechanism independent of the poly (A) tail is in operation. This mechanism remains unknown.

2.3 | Bcd gradient formation occurs mostly at the protein level

Precisely how the Bcd gradient is established has been the topic of intense study. The initial model was that Bcd is translated from localized mRNA at the anterior pole, and forms a gradient by diffusion toward the posterior (Driever &

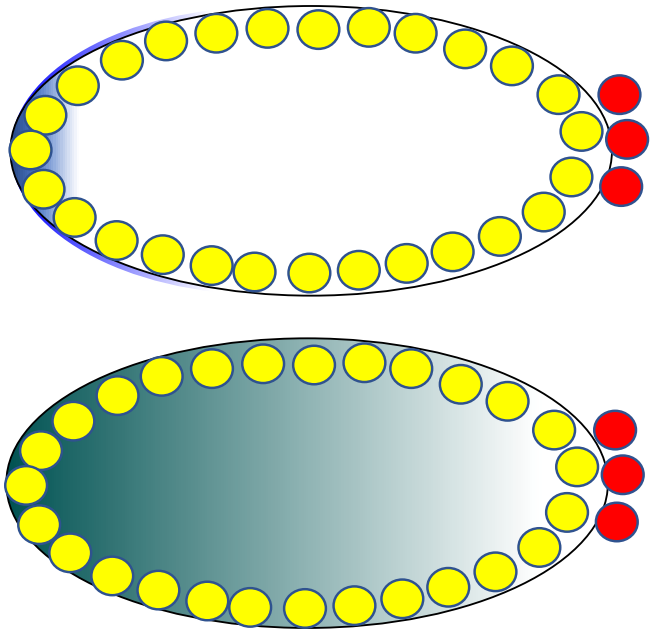

FIGURE 2 Early *Drosophila* embryos are syncytial, containing many nuclei that share a common cytoplasm. From a tight anterior cap of *bcd* RNA (blue, top panel) a gradient of Bcd protein (green, lower panel) is produced, so that nuclei are exposed to different levels of Bcd depending upon their position along the anterior–posterior gradient. Somatic nuclei are depicted in yellow while primordial germ cells (pole cells) are in red

Nüsslein-Volhard, 1988a; Gregor, Wieschaus, McGregor, Bialek, & Tank, 2007; Figure 2). Localized synthesis at the anterior and a short protein half-life ensures that a uniform Bcd concentration from the anterior to posterior is never reached. Several alternative models have also been proposed. One involves establishment of a *bcd* RNA gradient that extends throughout a substantial fraction of the embryo that presages the protein gradient (Spirov et al., 2009). Another postulates that nuclear trapping, rather than degradation, is important to establish the Bcd gradient (Coppey, Berezhkovskii, Kim, Boettiger, & Shvartsman, 2007). While translational control determines the time at which Bcd protein first appears, it does not seem to be a major factor in establishing its graded distribution. An early report that *bcd* is translationally repressed by Nanos and Pumilio in early embryos (Gamberi, Peterson, He, & Gottlieb, 2002) has recently been called into question (Wharton, Nomie, & Wharton, 2018).

The various models proposed make different predictions about the dynamics of synthesis, diffusion, and degradation of *bcd* RNA and protein along the anterior–posterior axis in early embryos. Because measuring these parameters in living embryos is technically very challenging, there have been inconsistent results among the different groups that investigate this question. However, a recent paper that used a tandem fluorescent protein timer to measure the age of Bcd protein at different positions along the anterior–posterior axis of the embryo improved upon earlier technologies and supports the original protein diffusion based model (Durrieu et al., 2018). To create such a timer, Bcd was tagged with two different fluorophores, a green one that matures quickly, and a red one that matures slowly. Newly synthesized protein is therefore mostly green, while both tags fluoresce in older protein, which consequently appears yellow. This construct was expressed in embryos and the tagged protein was quantitatively imaged with confocal multi-view light-sheet microscopy, with the important result that Bcd protein is older toward the posterior end of the embryo than at the anterior pole. This argues in favor of the original model of Bcd gradient formation involving protein diffusion from a localized anterior source and against the existence of an mRNA prepattern, as with an mRNA gradient newly synthesized protein would be present distant from the anterior pole.

3 | LOCALIZATION AND TRANSLATIONAL REGULATION OF *OSK*

3.1 | *osk* is the primary determinant for germ cell specification and posterior patterning

Several lines of evidence support the primary role of *osk* in germ cell specification and posterior patterning. Ectopic expression of *osk* at the anterior can induce a second set of pole cells and a bicaudal embryonic segmentation pattern with mirror-image posterior segments (Ephrussi & Lehmann, 1992; Smith et al., 1992). Several maternal-effect mutants produce bicaudal embryos, in which posterior segments are duplicated at the anterior end, sometimes in a mirror-image

symmetry. A second focus of *osk* mRNA at the anterior pole is observed in bicaudal embryos produced by *Bicaudal-D* or *ik2* females (Chang et al., 2011; Ephrussi et al., 1991; Shapiro & Anderson, 2006). In contrast, embryos from hypomorphic *osk* females lack posterior segmentation and pole cells (Lehmann & Nüsslein-Volhard, 1986). *osk* is one of a set of maternally expressed genes, called posterior-group genes, that can mutate to give this phenotype (Nüsslein-Volhard, Frohnhöfer, & Lehmann, 1987). The products of many of these other genes exert their effects through *osk*. For instance, the posterior-group genes *cappuccino*, *chickadee*, *spire*, and *stau* are required to establish or maintain *osk* at the posterior pole (Manseau, Calley, & Phan, 1996; Micklem, Adams, Grünert, & St Johnston, 2000). The mRNAs and/or proteins from other posterior-group genes, such as *vas*, *tud*, *nos*, and *aub*, accumulate in pole plasm dependent upon *osk* function and operate downstream (Hay, Ackerman, Barbel, Jan, & Jan, 1988; Lasko & Ashburner, 1990; Wang & Lehmann, 1991; Wilson et al., 1996).

3.2 | Multiple mechanisms achieve posterior *osk* RNA localization

Step 1: *Localization of osk to the early oocyte via dynein-directed transport along microtubules*

During oogenesis, *osk* mRNA accumulates at the posterior pole of the oocyte through three different sequential mechanisms (Figure 3). The first of these begins in the germarium, where *osk* accumulates into the presumptive oocyte, where it is readily detectable from stage 2 of oogenesis. In these early stages, the microtubule cytoskeleton is organized such that a single microtubule organizing center (MTOC) forms in the oocyte, and microtubules emanate from the MTOC through ring canals into the nurse cells (Theurkauf, Alberts, Jan, & Jongens, 1993). Thus microtubules are polarized so that their minus-ends are concentrated in the oocyte and their plus-ends are in the nurse cells. As will be seen, this organization is not retained in later stages of oogenesis.

Several reports demonstrate that *osk* mRNA localization into the early oocyte depends upon minus-end directed transport by the motor protein dynein. Dynein, as assessed by immunostaining for the dynein heavy chain Dhc64C, accumulates into the early oocyte in a similar manner to *osk* mRNA (Li, McGrail, Serr, & Hays, 1994). In early egg chambers dynein forms part of a multiprotein complex that also contains Egalitarian (Egl), Bicaudal-D (Bic-D), and Lissencephaly-1 (Lis-1), and mutations affecting any dynein complex gene block oocyte differentiation (Gepner

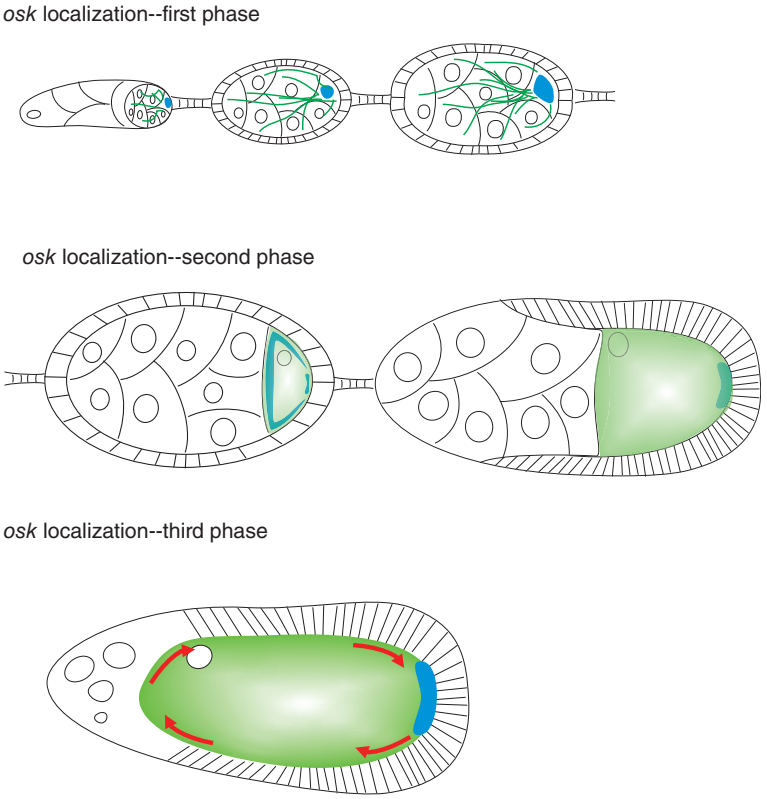

FIGURE 3 *osk* mRNA localization proceeds through three distinct mechanisms. In early oogenesis (top panel), a microtubule organizing center (MTOC) forms in the oocyte, and microtubules (green lines) emanate from there through the ring canals into the nurse cells. The motor protein dynein transports *osk* (blue) along these microtubules into the oocyte. During stages 8–9 of oogenesis (middle panel), microtubules (shaded green) reorganize so that their minus-ends accumulate all around the oocyte cortex except at the posterior pole. The plus-end directed motor kinesin drives *osk* (blue) away from the cortex and it accumulates at the posterior thanks to a weak plus-end microtubule bias. In late oogenesis (lower panel) rapid cytoplasmic streaming (red arrows) takes place, which transports macromolecules throughout the oocyte cytoplasm. *osk* mRNA is carried along by these movements and is anchored at the posterior, where there is a relative absence of microtubules, by myosin V

et al., 1996; Liu, Xie, & Steward, 1999; Mach & Lehmann, 1997; McGrail & Hays, 1997; Navarro, Puthalakath, Adams, Strasser, & Lehmann, 2004; Swan, Nguyen, & Suter, 1999). RNA injection experiments discussed in the preceding section examined both *bcd* and *osk* RNAs and suggested that this early stage of *osk* localization involves dynein-directed transport (Clark et al., 2007).

Further insights into the mechanism of *osk* mRNA accumulation into the oocyte came from mapping of *cis*-acting sequences that are required for this stage of transport. As measured by immunostaining for the proxy protein Stau, that associates with *osk* RNA throughout oogenesis, the *osk* 3′ UTR alone can localize to the oocyte (Jenny et al., 2006). The *osk* 3′ UTR can also rescue the oogenesis arrest observed in *osk*-null alleles, indicating that it has a noncoding function. The necessary element within the 3′ UTR for oocyte entry was mapped to a 67-nucleotide stem-loop called the oocyte entry signal (OES) that is separable from another element, to be discussed below, that is required for posterior localization in subsequent stages of oogenesis (Jambor, Mueller, Bullock, & Ephrussi, 2014). An injection assay was established to measure dynein-directed transport of RNAs to the apical surface of syncytial *Drosophila* embryos (Bullock & Ish-Horowicz, 2001; Wilkie & Davis, 2001). A segment of the *osk* 3′ UTR containing the OES localizes in this assay, but localization is strongly impaired when the OES is absent (Jambor et al., 2014). However, the OES is only active in the context of larger regions of the 3′ UTR, and other partially redundant elements operate in concert with it, leading to the proposal that the presence of multiple weak 3′ UTR signals driving oocyte entry facilitates transfer of *osk* mRNA to the posterior localization pathway (Ryu, Kenny, Gim, Snee, & Macdonald, 2017).

As mentioned above, mutations in genes encoding dynein complex components block oocyte differentiation. While this phenotype supports a role for dynein-mediated RNA transport in oogenesis, for many years it prevented direct analysis of the role of the dynein complex in *osk* localization. In the absence of an oocyte *osk* does not accumulate in a single cell, so this confounds cause and effect. One component of the dynein complex, Egl, is an RNA binding protein that links RNAs carrying defined localization elements to Bic-D and dynein (Dienstbier, Boehl, Li, & Bullock, 2009). Driving short-hairpin RNA (shRNA) targeting Egl using a maternal α-tubulin driver that is active only after oocyte specification permits the isolation of oocytes that are depleted for it (Sanghavi, Liu, Veeranan-Karmegam, Navarro, & Gonsalvez, 2016). In egg chambers thus depleted for Egl, *osk* mRNA is completely delocalized, and is present throughout the oocyte and in the cytoplasm of nurse cells. *osk* localization could be fully rescued by coexpression of an Egl transgene made resistant to the shRNA by introducing several point mutations into the recognition sequence. Further analysis using *egl* missense mutations abrogating either dynein or BicD binding supports a model for stepwise assembly of a transport complex. The first step is that Egl dimerizes through an interaction with dynein light chain, then dimerized Egl binds RNA and Bic-D, relieving autoinhibition of Bic-D and association with the dynein motor (Goldman, Neiswender, Veeranan-Karmegam, & Gonsalvez, 2019). This is consistent with results from reconstitution studies using purified components (McClintock et al., 2018; Sladewski et al., 2018).

Step 2: *Localization of osk to the oocyte posterior involves kinesin and microtubules*

During stages 7 and 8 of oogenesis the organization of the microtubule cytoskeleton changes. Microtubules form an anterior–posterior density gradient, with a higher concentration at the anterior (Clark, Jan, & Jan, 1997). Their minus-ends become anchored all around the cortex, a process that requires the minus-end microtubule binding protein Patronin and Short stop (Shot), a protein that crosslinks microtubules and filamentous actin (Nashchekin, Fernandes, & St Johnston, 2016). Shot is excluded from the posterior cortex so fewer microtubules are anchored there, resulting in slightly more microtubule plus-ends being oriented toward the posterior pole (Khoc Trong, Doerflinger, Dunkel, St Johnston, & Goldstein, 2015; Nashchekin et al., 2016; Parton et al., 2011; Zimyanin et al., 2008).

This raises the possibility that kinesin, a plus-end directed motor protein, might conduct directed transport of *osk* RNA to the posterior pole of the oocyte. In fact, in *kinesin heavy chain* (*khc*) mutant oocytes posterior localization of *osk* fails and it accumulates around the anterior and lateral cortex (Brendza, Serbus, Duffy, & Saxton, 2000; Cha, Serbus, Koppetsch, & Theurkauf, 2002). As kinesin is however required for several cellular processes within the oocyte including cytoplasmic streaming, distinguishing direct from indirect effects remained a problem for some time. Ultimately, live imaging analysis of labeled *osk* in wild-type and *khc* mutant oocytes produced convincing evidence that kinesin-dependent transport toward the plus ends of microtubules results in exclusion of *osk* from the oocyte cortex and concentration, thanks to the weak posterior bias of plus-ends, at the posterior pole (Parton et al., 2011; Zimyanin et al., 2008). Kinesin-1 associates with *osk*-containing ribonucleoprotein particles upon nuclear export, a process requiring the atypical RNA-binding tropomyosin Tm1-I/C (Gáspár, Sysoev, Komissarov, & Ephrussi, 2016).

A peculiar aspect of this second stage of *osk* localization is that it requires components of the exon-junction complex (EJC) and splicing of the first intron of *osk* (Hachet & Ephrussi, 2001; Hachet & Ephrussi, 2004; Mohr, Dillon, & Boswell, 2001; Newmark & Boswell, 1994; Palacios, Gatfield, St Johnston, & Izaurralde, 2004). Splicing is linked to *osk* localization because it creates a localization element (the spliced *oskar* localization element, or SOLE), from the final 18 nucleotides of exon 1 of *osk* and the first 10 nucleotides of exon 2 (Ghosh, Marchand, Gáspár, & Ephrussi, 2012). Both the SOLE and EJC components are required for *osk* localization, because when SOLE is present in an RNA without splicing being required for its formation, the RNA remains unlocalized, and RNAs loaded with EJC but lacking SOLE also fail to localize (Ghosh et al., 2012). It is believed that SOLE recruits EJC, either directly or through an unknown third component (Ghosh, Obrdlik, Marchand, & Ephrussi, 2014; Simon, Masiewicz, Ephrussi, & Carlomagno, 2015). The SOLE, EJC, and 3′ UTR together are necessary for posterior transport, as interfering with any of these components results in *osk* mislocalization.

Step 3: *Posterior osk accumulation through cytoplasmic streaming and anchorage*

After stage 8 the oocyte undergoes a period of rapid growth, and during stages 10–12 the cytoplasm of the nurse cells is also rapidly transferred through the ring canals into the oocyte. Fast rotary cytoplasmic streaming occurs within the oocyte during this time, which is dependent on kinesin and the microtubule cytoskeleton (Lu, Winding, Lakonishok, Wildonger, & Gelfand, 2016; Palacios & St Johnston, 2002; Serbus, Cha, Theurkauf, & Saxton, 2005). This enables cytoplasmic content to cycle around the oocyte from the anterior to the posterior and vice versa, so in the absence of anchoring mechanisms, localized molecules would become homogeneously distributed. Conversely, cytoplasmic streaming can provide a mechanism for localizing molecules, provided that a coupled trapping mechanism also exists.

An early study showed that injected fluorescently tagged *osk* RNA could localize via such a mechanism (Glotzer, Saffrich, Glotzer, & Ephrussi, 1997). For some time, the importance of cytoplasmic streaming and anchoring for localization of endogenous *osk* remained unclear, although this mechanism became well established for RNAs that localize to the pole plasm only in late oogenesis such as *nos* (Forrest & Gavis, 2003). Ironically, analysis of two *trans*-acting factors required for *nos* localization, Rumpelstiltskin (Rump) and Lost, revealed that cytoplasmic streaming and anchoring also contribute to endogenous *osk* localization (Jain & Gavis, 2008; Sinsimer, Jain, Chatterjee, & Gavis, 2011). Oocytes doubly mutant for *lost* and *rump* support *osk* localization through stage 10 of oogenesis, but the resulting embryos have clearly reduced posterior *osk* localization and form fewer pole cells. A defect in *osk* localization in late oogenesis in *lost rump* mutants was also directly shown by live imaging of GFP-tagged *osk* RNA.

Kinesin-1 is required both for cytoplasmic streaming (Lu et al., 2016; Palacios & St Johnston, 2002; Serbus et al., 2005) and for the initial posterior localization of *osk*. This complicated the unraveling of the relative importance of the two mechanisms. To resolve this, a mutant was produced that blocked microtubule sliding required for cytoplasmic streaming (Khc^{mutA}, carrying four missense mutations in the C-terminal microtubule binding site) (Lu et al., 2018). Simultaneous RNA interference targeting *klc* and *btz* was used to specifically block directed transport. Using these strains it was found that cytoplasmic streaming can partially rescue posterior localization of *osk* and fluorescently labeled Stau when the initial microtubule-dependent transport phase is blocked. Supporting redundancy between these two localization mechanisms, over 80% of embryos produced by Khc^{mutA} or *klc btz* double knockdown females produced pole cells (Lu et al., 2018).

How is *osk* anchored at the posterior pole during cytoplasmic streaming? Several lines of evidence suggest that anchoring involves an actomyosin-based mechanism. From a forward-genetic screen aimed at identifying new maternal-effect lethal loci, several alleles of *didum*, which encodes myosin V, were obtained (Krauss, López de Quinto, Nüsslein-Volhard, & Ephrussi, 2009; Luschnig et al., 2004). These mutants presented a posterior-group phenotype related to that of *osk* mutants, and *didum* oocytes showed defects in *osk* mRNA localization with some *osk* being retained in the central region of the oocyte. This work led to the suggestion that transport or entrapment of *osk* through a process dependent on F-actin and myosin V is a necessary final step in its localization. However, as myosin V is present throughout all the oocyte cortex, not just the posterior pole (Krauss et al., 2009) it remained unclear how its activity becomes spatially restricted.

A very recent elegant study resolves this issue, showing an antagonistic relationship between cortical myosin V and microtubules with respect to *osk* accumulation (Lu et al., 2020). Consistent with previous work (Clark et al., 1997; Nashchekin et al., 2016; Zimyanin et al., 2008), an anterior-to-posterior gradient of microtubule density with a relative absence at the posterior pole was demonstrated. Depolymerization of microtubules by germline overexpression of Klp10A, a depolymerizing kinesin, resulted in uniform accumulation of the *osk* proxy Stau around the entire oocyte cortex, while overexpression of Patronin, which prevents depolymerization and increases microtubule density, resulted

in accumulation of Stau in the center of the oocyte cytoplasm (Lu et al., 2020). Next, optogenetic tools were used to recruit Klp10A to particular spatial locations on the oocyte cortex, depleting microtubules at the site of recruitment. When this was done, Stau accumulated in the same positions, and this was reversible when the light stimulus leading to Klp10A recruitment was removed. These experiments confirm a model whereby *osk*/Stau localization and anchoring critically depends upon local microtubule density, with low density favoring cortical trapping of *osk*/Stau by myosin V, and high density favoring cortical exclusion of *osk*/Stau by kinesin-1.

3.3 | *osk* is under complex translational control

osk mRNA generates two protein isoforms from different translational start sites (Markussen, Michon, Breitwieser, & Ephrussi, 1995). The long isoform (Long Osk) anchors mRNAs and mitochondria in the pole plasm (Hurd et al., 2016; Tanaka, Kato, Matsuda, Hanyu-Nakamura, & Nakamura, 2011; Tanaka & Nakamura, 2008; Vanzo & Ephrussi, 2002; Vanzo, Oprins, Xanthakis, Ephrussi, & Rabouille, 2007). Short Osk, meanwhile, supports all Osk functions in assembly of polar granules and in posterior patterning and germ cell specification (Markussen et al., 1995). It is unknown how initiation from these two start sites, which are in the same open reading frame, is controlled. Complex temporal and spatial regulation of translation ensures that Osk accumulates abundantly only at the posterior pole, not while the mRNA is in transit, and not before stage 9 of oogenesis (Figure 4). Failure of these mechanisms results in lethality. Bruno1 (Bru1) represses *osk* translation in early oogenesis and outside the pole plasm, through interactions with binding sites in the *osk* 3′ UTR called Bru1 response elements (BREs). Deleting or mutating the BREs generally results in ectopic Osk expression (Kim-Ha, Kerr, & Macdonald, 1995; Reveal et al., 2010; Webster, Liang, Berg, Lasko, & Macdonald, 1997). Bru1 represses *osk* through two distinct, but coupled mechanisms. The first such mechanism to be revealed involves Bru1-mediated recruitment of Cup to *osk* mRNA. Cup is a competitive inhibitor of eIF4G for binding to eIF4E, therefore it inhibits assembly of an active cap-binding complex (Nakamura, Sato, & Hanyu-Nakamura, 2004) (see Box for further details). *osk* translation is also inhibited by oligomerization of *osk* mRNA into large RNPs that are not readily accessed by ribosomes and the rest of the translational machinery (Besse, López de Quinto, Marchand, Trucco, & Ephrussi, 2009; Chekulaeva, Hentze, & Ephrussi, 2006; Kim et al., 2015). The two mechanisms are linked. Concentrating *osk* mRNA molecules into RNPs brings them into close juxtaposition and facilitates regulation in *trans*; Bru1 bound to the 3′ end of one *osk* mRNA molecule can recruit Cup to eIF4E bound to the 5′ cap structure of an adjacent *osk* mRNA molecule (Macdonald, Kanke, & Kenny, 2016; Reveal et al., 2010). *osk* translation is tightly linked to maintenance of posterior localization of *osk* mRNA localization. In nonsense *osk* alleles or when translational activation elements are mutated, *osk* mRNA localizes only transiently to the pole plasm (Ephrussi et al., 1991; Kim-Ha et al., 1991; Munro, Kwon, Schnapp, & St Johnston, 2006).

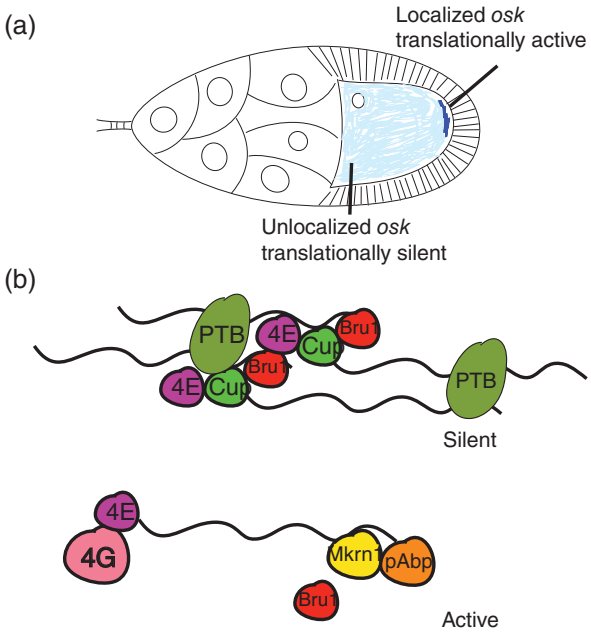

FIGURE 4 *osk* is under complex translational control. (a). Unlocalized *osk* is translationally repressed by oligomerization and by the translational repressor Bru1. *osk* is oligomerized by PTB, which can bind to multiple sites on the *osk* 3' UTR. Bru1 binds to specific sites in the *osk* 3' UTR and recruits Cup, a competitive inhibitor for eukaryotic initiation factor (eIF) 4E (4E) binding to eIF4G. (b). In the pole plasm *osk* translation is activated. Makorin1 (Mkrn1) displaces Bru1 and recruits poly(A) binding protein (pAbp), which stimulates translation

BOX 1 REGULATION OF CAP-DEPENDENT TRANSLATION

The step of translation initiation that is regulated by some of the proteins discussed here is the assembly of the 48S initiation complex. This complex includes the mRNA, the initiator tRNA, the 40S (small) ribosomal subunit, and various translation initiation factors (eIFs) (Eliseev et al., 2018). eIF4F plays a central role in assembling this complex. It is composed of three subunits: eIF4E, which binds the cap structure present at the 5′ end of mRNA; eIF4A, an RNA helicase, and eIF4G, which binds both eIF4E and eIF3. eIF4G binding to eIF4E is necessary to assemble eIF4F at the cap, and eIF4G binding to eIF3 enables recruitment of the small ribosomal subunit, which is also bound by eIF3. Translational repressors discussed here disrupt eIF4F formation in different ways. Cup competes with eIF4G for binding to eIF4E, thus prohibiting eIF4F assembly on mRNAs to which it is bound. Conversely, 4EHP binds the cap structure but does not bind eIF4G, sequestering mRNA to which it is bound away from eIF4F and ribosomes.

Less is known about how *osk* translation is activated in the pole plasm, although several proteins have been implicated in this process (Chang, Tan, & Schedl, 1999; Micklem et al., 2000; Wilson et al., 1996). One aspect of *osk* translational activation is inhibition of Bru1 (Kim et al., 2015), and one BRE-containing region in the distal part of the *osk* 3′ UTR (BRE-C) functions in activation as well as in repression (Reveal et al., 2010). A recent study implicated the RNA binding protein Makorin-1 (Mkrn1) as a key activator of *osk* translation (Dold et al., 2020). Mkrn1 binds the *osk* 3′ UTR at an A-rich region immediately adjacent to BRE-C, where it antagonizes Bru1 binding and promotes recruitment of poly(A) binding protein.

3.4 | Osk associates with Vas and binds RNA

Despite extensive analysis of the developmental role of Osk, its precise molecular function has long remained unclear. Structural analysis of two domains of Osk, its N-terminal LOTUS domain (Osk-N), and its C-terminal hydrolase-like domain (Osk-C), indicates that it can directly associate with RNA (Yang et al., 2015), which could explain, at least in part, its role in polar granule assembly. The LOTUS domain is an established RNA binding motif (Anantharaman, Zhang, & Aravind, 2010; Callebaut & Mornon, 2010), but in Osk-N it homodimerizes in a manner that masks the nucleic acid binding surface. Osk-C forms a structure resembling a lipase-like fold, but with nonconservative changes in several key amino acids that would rule out any catalytic activity. However, Osk-C was found to avidly bind some RNAs, including the *osk* and *nos* 3′ UTRs, in EMSA assays. Thus sequence similarity analysis in this case is misleading, and Osk-C, rather than the predicted Osk-N, possesses a direct RNA binding activity. Point mutation analysis implicated three specific Arg residues within Osk-C in RNA binding, which all reside on a positively charged ridge that can accommodate RNA. Several other residues present in this ridge and/or spatially proximate to these Arg residues have been implicated by mutation in Osk-dependent developmental functions (Ephrussi et al., 1991; Kim-Ha et al., 1991; Lehmann & Nüsslein-Volhard, 1986; Rongo, 1996; Yang et al., 2015).

The DEAD-box helicase Vas appears to be a cofactor for many functions of Osk, and as described above the two proteins are often present in close association within RNPs. Osk interacts directly with Vas through an interaction between the LOTUS domain and an alpha-helical region of Vas, with two phenylalanine residues of Vas (F504 and F508) essential for Osk binding (Jeske et al., 2015; Jeske, Müller, & Ephrussi, 2017). Mutation of one of these residues (F504E) blocks recruitment of Vas to the pole plasm. Vas also functions in piRNA biogenesis and localizes to the perinuclear nuage in nurse cells, the structure where piRNA processing takes place (ElMaghraby et al., 2019; Zhang et al., 2012). Two other LOTUS domain proteins, Tejas and Tapas, also interact with Vas and play roles in piRNA-mediated processes (Jeske et al., 2017; Patil, Anand, Chakrabarti, & Kai, 2014; Patil & Kai, 2010).

4 | POLAR GRANULE FORMATION INVOLVES SPECIFIC MOLECULAR ASSOCIATIONS AND NONSPECIFIC PHYSICAL PROCESSES

Key proteins and mRNAs involved in germ cell specification associate in polar granules, RNA-protein complexes that are linked to germ cell development throughout the animal kingdom. Polar granules are one class of related particles that are collectively called germ granules. Polar granules in early *Drosophila melanogaster* embryos are nonmembrane

bound, electron-dense, approximately round particles with diameters ranging from 200 to 500 nm (Mahowald, 1962). They are first seeded by localization of single RNA molecules, which subsequently recruit additional transcripts from the same gene through homotypic interactions (Niepielko, Eagle, & Gavis, 2018). They later become associated with the embryonic nuclei that are destined to become PGCs (Lerit & Gavis, 2011), and further grow in size and number through the cellular blastoderm stage of development. During this period they are referred to as nuclear germ granules (Kistler et al., 2018). Beginning in the 1980s, molecular genetic studies revealed numerous protein and RNA components of polar granules, many of which have been described above. However, the mechanisms by which these particles assemble only began to come to light in recent years, and important questions about this remain to be answered.

A critical insight was provided by work on P granules, structures analogous to polar granules in the nematode *Caenorhabditis elegans*. This work showed that P granules exhibit physical properties similar to those of a liquid with a viscosity comparable to that of glycerol (Brangwynne et al., 2009). Furthermore, it provided compelling evidence that accumulation of P granules in the germ plasm is not dependent on active transport or on cytoplasmic flows. Rather, accumulation comes about through establishment, by polarity proteins including MEX-5 and PAR-1, of a greater likelihood of condensation versus dissolution of P granule material into droplets at the posterior pole where the germ plasm forms.

4.1 | Homotypic RNA–RNA interactions underlie germ granule assembly

In contrast, evidence began to emerge in *Drosophila* that higher-order supramolecular assemblies arise in the pole plasm through specific RNA–RNA and RNA–protein interactions. Much earlier work had already shown that Stau, which is required for a late stage of *bcd* localization, binds best to RNA when a base-paired structure involving two *bcd* RNA molecules is present (Ferrandon, Koch, Westhof, & Nüsslein-Volhard, 1997). In a study of *osk* RNA in mid-oogenesis, Ephrussi and colleagues showed that polypyrimidine tract binding (PTB) protein can bind to several sites in its *osk* 3′ UTR, contributing to its translational repression prior to its posterior localization (Besse et al., 2009). PTB has four RNA recognition motifs (RRMs), and the third and fourth of these domains are oriented in such a way that they can bind to PTB binding sites on different RNA molecules, thus serving as a bridge and potentially serving as a basis for assembly of higher-order structures. Interestingly, *osk*-containing RNPs in stage-9 oocytes that are precursors to polar granules are smaller and more numerous in *hephasteus* mutants (*hephasteus* encodes PTB).

Subsequent advances in microscopy have enabled more detailed study of the molecular topography of polar granules and related RNPs, providing evidence for internal organization built around homotypic clustering of RNA molecules. Super-resolution, single-molecule level imaging of polar granules in early embryos showed that while Osk, Vas, Tud, and Aub proteins all occupy the same space within the granules, different RNA components of polar granules have different distributions, suggesting that polar granules have internal structure (Trcek et al., 2015; Figure 5). *cycB* and *nos* RNAs largely colocalized with the four proteins, while *pgc* and *gcl* RNA occupy more peripheral regions of the polar granule. Despite its importance in seeding pole plasm assembly, *osk* RNA is ultimately excluded from polar granules. However, it is present in other particles, one type including only Vas and another type including only *pgc* among the molecules examined. Exclusion of *osk* from embryonic polar granules was also reported in other studies (Herpers, Xanthakis, & Rabouille, 2010; Little et al., 2015).

4.2 | Pole plasm RNPs fall into several classes that assemble by different mechanisms

Such a highly organized internal structure argues against a model of *Drosophila* polar granule formation proceeding simply by liquid droplet condensation, although concentration-dependent biophysical processes surely have an important role. Importantly, internal structure has also been reported in *C. elegans* P granules using a similar approach (Wang et al., 2014).

Another study used high-resolution fluorescent in situ hybridization (FISH) to follow the assembly of germ plasm RNPs during oogenesis and early embryogenesis (Little et al., 2015). From this work it was concluded that germ granules share a common set of proteins including Osk, Vas, and Tud, but are heterogeneous in terms of their RNA composition. Germ granules with different RNAs assemble by different mechanisms. RNPs containing single copies of *nos* RNA assemble into granules containing an average of 10–20 *nos* RNA molecules at the posterior oocyte cortex. Conversely, *osk* RNA already resides within multi-copy RNPs during its transport, and assembles into large granules often containing >100 RNA molecules that are compositionally distinct from *nos* particles. This work was further extended

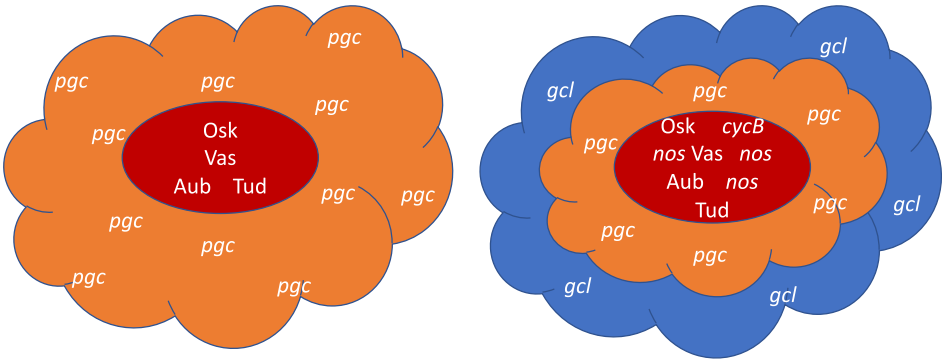

FIGURE 5 Cartoon illustrating the structured and heterogeneous nature of polar granules. Osk, Vas, Aub, and Tud proteins accumulate in the core region of polar granules along with *cycB* and *nos* mRNAs. *pgc* and *gcl* mRNAs accumulate in more peripheral regions (Trcek et al., 2015). Some polar granules contain only one species of mRNA while others accumulate several (Little, Sinsimer, Lee, Wieschaus, & Gavis, 2015)

(Niepielko et al., 2018) to show that single-copy *nos* and *pgc* RNPs can first populate the same granule, and then granules grow by self-recruitment of additional RNA molecules into homotypic clusters, a process that requires simultaneous acquisition of Osk protein. Polar granule targeting and assembly into homotypic clusters appears to involve different 3′ UTR elements, at least for *pgc* RNA (Eagle, Yeboah-Kordieh, Niepielko, & Gavis, 2018). A more recent study indicates that two polar granule proteins, Aub and Tud, also form distinct clusters within the granules (Vo et al., 2019).

4.3 | Many RNAs associate with germ granules by base-pairing with Aub-associated piRNAs

All of the studies discussed above looked at a few canonical polar granule RNAs (*osk*, *nos*, *pgc*, *cycB*) but well over 100 other mRNAs accumulate in pole plasm (Frise, Hammonds, & Celniker, 2010; Jambor et al., 2015; Lécuyer et al., 2007; Rangan et al., 2009). These do not include extra-mitochondrial *mtlrRNA*, which was reported based on electron microscopy evidence to be present in the pole plasm (Akiyama & Okada, 1992; Amikura, Kashikawa, Nakamura, & Kobayashi, 2001; Iida & Kobayashi, 1998; Kobayashi, Amikura, & Okada, 1993). Colocalization analysis using quantitative single-molecule RNA FISH showed essentially complete overlap between mtlrRNA and EYFP-labeled mitochondria in the pole plasm (Hurd et al., 2016). Localization of other pole plasm RNAs is abrogated in *osk* mutants, suggesting a mechanism in common with their better characterized counterparts. How then are all these different RNAs targeted? Vourekas, Alexiou, Vrettos, Maragkakis, and Mourelatos (2016) demonstrated a central role for Aub and piwi-interacting RNAs (piRNAs) in this process. Aub interacts with piRNAs, many of which have complementary sequences to transposon-encoded mRNAs, to form piRNPs, which interact with target mRNAs through base pairing and silence their expression (Siomi, Sato, Pezic, & Aravin, 2011). However, Aub-loaded piRNAs are highly diverse and more than half are derived from mRNA, not transposon, sequences (Barckmann et al., 2015; Vourekas et al., 2016). Consistent with the high concentration of Aub in polar granules, Aub-associated mRNAs from wild-type embryos, but not from embryos produced by *tud* mutant females, are highly enriched for those that are localized to the posterior of the embryo and/or to germ cells (Barckmann et al., 2015; Vourekas et al., 2016). This suggests that Aub-associated piRNAs recognize and play a role in posterior accumulation of germ plasm mRNAs. Transcriptome-wide prediction of targets for Aub-associated piRNAs shows that sites with modest complementarity are present essentially at random. However, posterior-localized mRNAs are generally longer than average, therefore they tend to contain more such sites, and thus a random process becomes biased toward specific mRNAs (Vourekas et al., 2016). Stabilization of posterior-localized mRNAs is further accomplished through Aub-mediated recruitment of the poly(A) polymerase Wispy (Dufourt et al., 2017).

4.4 | Short Osk nucleates germ granules in pole cell nuclei, which promote their proliferation

After fertilization, particles containing Vas and *nos* are recruited by dynein-dependent transport along centrosome-associated astral microtubules to become associated with posterior nuclei (Lerit & Gavis, 2011). These particles are similar

in size and shape to polar granules. However, as soon as pole cells form, nuclear Osk- and Vas-containing particles increase in size and number, becoming prominent in germ cells by the cellular blastoderm stage of development (Kistler et al., 2018). Nuclear germ granules behave as phase-transitioned condensates, with both liquid-like and hydrogel-like properties (Kistler et al., 2018). They are seeded by Short Osk and, like polar granules, are nonmembrane bound RNPs. While they contain Vas, they do not accumulate several other polar granule components including Aub and Tud proteins nor *nos*, *pgc*, or *gcl* RNAs. Nuclear germ granules are not completely liquid-like and labile, because they do not fuse with one another; however, they are dynamic in that they can exchange Osk and Vas from particle to particle. When formation of nuclear germ granules is prevented by deleting the Osk nuclear localization signal, the number of pole cells, but not of pole buds, is reduced, indicating a role for nuclear Osk and potentially these granules in promoting germ cell division (Kistler et al., 2018). Each pole bud normally forms two pole cells (Pae, Cinalli, Marzio, Pagano, & Lehmann, 2017) but in OskΔNLS embryos the division that accomplishes this often fails to occur, and subsequent divisions occur even less frequently (Kistler et al., 2018).

5 | CONCLUSION

Despite all that has been learned important questions about the structure and function of polar granules remain unresolved. Some of these are specific, for instance more needs to be learned about how *bcd* RNA anchoring is maintained at the anterior cortex, and about how *bcd* translation is repressed until egg activation. We also lack understanding about how translation from the two initiation codons that produce the long and short isoforms of Osk is properly balanced.

Other open questions are wider in scope. For instance, is there a functional importance of the restricted structural domains within polar granules? Since the position of an RNA within a granule does not specify the onset of its translation, and granules do not reorganize as some RNAs become translationally active (Trcek et al., 2015), perhaps there is none. Related to this, it is unclear how the translational machinery can gain access to RNAs embedded within polar granules, as association into large RNPs like polar granules more typically masks RNAs from translation. Finally, it has long been known that polar granules in different Drosophilid species have different characteristic shapes and sizes (Counce, 1963; Mahowald, 1968). In some manner Osk controls these species-specific differences, as *D. immigrans* Osk expressed in *D. melanogaster* oocytes lacking endogenous *osk* produces *D. immigrans*-type polar granules, which perhaps surprisingly are functional, rescuing the *osk* mutant phenotype (Jones & Macdonald, 2007). How any of the mechanisms described above can influence higher-order structure at the scale of nearly a micron remains to be determined.

ACKNOWLEDGMENTS

I am very grateful to Sanjay Ghosh for critical reading of the manuscript and helpful comments. I am also grateful to NSERC for support from Discovery Grant RGPIN-2014-06340 and from a Visiting Professorship from the Radboud University Excellence Initiative.

CONFLICT OF INTEREST

The author has declared no conflicts of interest for this article.

ORCID

Paul Lasko https://orcid.org/0000-0002-4037-3501

RELATED WIREs ARTICLE

Posttranscriptional regulation in Drosophila oocytes and early embryos

FURTHER READING

Others have recently reviewed this general topic, each with their own different emphasis. The interested reader should also consult the following references (Goldman & Gonsalvez, 2017; Lehmann, 2016; Ma, He, Xie, & Deng, 2016; Quinlan, 2016; Rojas-Rios & Simonelig, 2018; Ryder & Lerit, 2018; Suter, 2018; Trcek & Lehmann, 2019; Wieschaus, 2016).

Briscoe, J., & Small, S. (2015). Morphogen rules: Design principles of gradient-mediated embryo patterning. Development, 142(23), 3996–4009. http://doi.org/10.1242/dev.129452

Cho, P. F., Poulin, F., Cho-Park, Y. A., Cho-Park, I. B., Chicoine, J. D., Lasko, P., & Sonenberg, N. (2005). A new paradigm for translational control: Inhibition via 5′-3′ mRNA tethering by Bicoid and the eIF4E cognate 4EHP. Cell, 121(3), 411–423. http://doi.org/10.1016/j.cell.2005.02.024

Rivera-Pomar, R., Niessing, D., Schmitt-Ott, U., Gehring, W. J., & Jäckle, H. (1996). RNA binding and translational suppression by Bicoid. Nature, 379(6567), 746–749. http://doi.org/10.1038/379746a0

Rödel, C. J., Gilles, A. F., & Averof, M. (2013). MicroRNAs act as cofactors in Bicoid-mediated translational repression. Current Biology, 23(16), 1579–1584. http://doi.org/10.1016/j.cub.2013.06.041

Sinsimer, K. S., Lee, J. J., Thiberge, S. Y., & Gavis, E. R. (2013). Germ plasm anchoring is a dynamic state that requires persistent trafficking. Cell Reports, 12(5), 1169–1177. http://doi.org/10.1016/jcelrep.2013.10.045

Winding, M., Kelliher, M. T., Lu, W., Wildonger, J., & Gelfand, V. I. (2016). Role of kinesin-1-based microtubule sliding in *Drosophila* nervous system development. Proceedings of the National Academy of Sciences of the United States of America, 113(34), E4985–E4994. http://doi.org/10.1073/pnas.1522416113

REFERENCES

Akiyama, T., & Okada, M. (1992). Spatial and developmental changes in the respiratory activity of mitochondria in early *Drosophila* embryos. *Development*, *115*(4), 1175–1182.

Amikura, R., Kashikawa, M., Nakamura, A., & Kobayashi, S. (2001). Presence of mitochondria-type ribosomes outside mitochondria in germ plasm of *Drosophila* embryos. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(16), 9133–9138. http://doi.org/10.1073/pnas.171286998

Anantharaman, V., Zhang, D., & Aravind, L. (2010). OST-HTH: A novel predicted RNA binding domain. *Biology Direct*, *5*, 13. http://doi.org/10.1186/1745-6150-5-13

Asaoka-Taguchi, M., Yamada, M., Nakamura, A., Hanyu, K., & Kobayashi, S. (1999). Maternal Pumilio acts together with Nanos in germline development in *Drosophila* embryos. *Nature Cell Biology*, *1*(7), 431–437. http://doi.org/10.1038/15666

Barckmann, B., Pierson, S., Dufourt, J., Papin, C., Armenise, C., Port, F., … Simonelig, M. (2015). Aubergine iCLIP reveals piRNA-dependent decay of mRNAs involved in germ cell development in the early embryo. *Cell Reports*, *12*(7), 1205–1216. http://doi.org/10.1016/j.celrep.2015.07.030

Bardsley, A., McDonald, K., & Boswell, R. E. (1993). Distribution of Tudor protein in the *Drosophila* embryo suggests separation of functions based on site of localization. *Development*, *119*(1), 207–219.

Berleth, T., Burri, M., Thoma, G., Bopp, D., Richstein, S., Frigerio, G., … Nüsslein-Volhard, C. (1988). The role of localization of *bicoid* RNA in organizing the anterior pattern of the *Drosophila* embryo. *The EMBO Journal*, *7*(6), 1749–1756.

Bertrand, E., Chartrand, P., Schaefer, M., Shenoy, S. M., Singer, R. H., & Long, R. M. (1998). Localization of *ASH1* mRNA particles in living yeast. *Molecular Cell*, *2*(4), 437–445. http://doi.org/10.1016/S1097-2765(00)80143-4

Besse, F., López de Quinto, S., Marchand, V., Trucco, A., & Ephrussi, A. (2009). *Drosophila* PTB promotes formation of high-order RNP particles and represses *oskar* translation. *Genes & Development*, *23*(2), 195–207. http://doi.org/10.1101/gad.505709

Brangwynne, C. P., Eckmann, C. R., Courson, D. S., Rybarska, A., Hoege, C., Gharakhani, J., … Hyman, A. A. (2009). Germline P granules are liquid droplets that localize by controlled dissolution/condensation. *Science*, *324*(5935), 1729–1732. http://doi.org/10.1126/science.1172046

Breitwieser, W., Markussen, F.-H., Horstmann, H., & Ephrussi, A. (1996). Oskar protein interaction with Vasa represents an essential step in polar granule assembly. *Genes & Development*, *10*(17), 2179–2188. http://doi.org/10.1101/gad.10.17.2179

Brendza, R. P., Serbus, L. R., Duffy, J. B., & Saxton, W. M. (2000). A function for kinesin I in the posterior transport of *oskar* mRNA and Staufen protein. *Science*, *289*(5487), 2120–2122. http://doi.org/10.1126/science.289.5487.2120

Bullock, S. L., & Ish-Horowicz, D. (2001). Conserved signals and machinery for RNA transport in *Drosophila* oogenesis and embryogenesis. *Nature*, *414*(6864), 611–616. http://doi.org/10.1038/414611a

Callebaut, I., & Mornon, J. P. (2010). LOTUS, a new domain associated with small RNAs in the germline. *Bioinformatics*, *26*(9), 1140–1144. http://doi.org/10.1093/bioinformatics/btq122

Cha, B. J., Serbus, L. R., Koppetsch, B. S., & Theurkauf, W. E. (2002). Kinesin I-dependent cortical exclusion restricts pole plasm to the oocyte posterior. *Nature Cell Biology*, *4*(8), 592–598. http://doi.org/10.1038/ncb832

Chang, C. W., Nashchekin, D., Wheatley, L., Irion, U., Dahlgaard, K., Montague, T. G., … St Johnston, D. (2011). Anterior-posterior axis specification in *Drosophila* oocytes: Identification of novel *bicoid* and *oskar* mRNA localization factors. *Genetics*, *188*(4), 883–896. http://doi.org/10.1534/genetics.111.129312

Chang, J. S., Tan, L., & Schedl, P. (1999). The *Drosophila* CPEB homolog, Orb, is required for Oskar protein expression in oocytes. *Developmental Biology*, *215*(1), 91–106. http://doi.org/10.1006/dbio.1999.9444

Chekulaeva, M., Hentze, M. W., & Ephrussi, A. (2006). Bruno acts as a dual repressor of *oskar* translation, promoting mRNA oligomerization and formation of silencing particles. *Cell*, *124*(3), 521–533. http://doi.org/10.1016/j.cell.2006.01.031

Clark, A., Meignin, C., & Davis, I. (2007). A dynein-dependent shortcut rapidly delivers axis determination transcripts into the *Drosophila* oocyte. *Development*, *134*(10), 1955–1965. http://doi.org/10.1242/dev.02832

Clark, I., Jan, L. Y., & Jan, Y. N. (1997). Reciprocal localization of Nod and kinesin fusion proteins indicates microtubule polarity in the *Drosophila* oocyte, epithelium, neuron, and muscle. *Development*, *124*(2), 461–470.

Coppey, M., Berezhkovskii, A. M., Kim, Y., Boettiger, A. N., & Shvartsman, S. Y. (2007). Modeling the Bicoid gradient: diffusion and reversible nuclear trapping of a stable protein. *Developmental Biology*, *312*(2), 623–630. http://doi.org/10.1016/j.ydbio.2007.09.058.

Counce, S. J. (1963). Developmental morphology of polar granules in *Drosophila* including observations on pole cell behavior and distribution during embryogenesis. *Journal of Morphology*, *112*, 129–145.

Dienstbier, M., Boehl, F., Li, X., & Bullock, S. L. (2009). Egalitarian is a selective RNA-binding protein linking mRNA localization signals to the dynein motor. *Genes & Development*, *23*(13), 1546–1558. https://doi.org/10.1101/gad.531009

Dold, A., Han, H., Liu, N., Hildebrandt, A., Brüggemann, M., Rücklé, C., … Lasko, P. (2020). Makorin 1 controls embryonic patterning by alleviating Bruno1-mediated repression of *oskar* translation. *PLoS Genetics*, *16*(1), e1008581. http://doi.org/10.1371/journal.pgen.1008581

Driever, W., & Nüsslein-Volhard, C. (1988a). A gradient of Bicoid protein in *Drosophila* embryos. *Cell*, *54*(1), 83–93. http://doi.org/10.1016/0092-8674(88)90182-1

Driever, W., & Nüsslein-Volhard, C. (1988b). The Bicoid protein determines position in the *Drosophila* embryo in a concentration-dependent manner. *Cell*, *54*(1), 95–104. http://doi.org/10.1016/0092-8674(88)90183-3

Dufourt, J., Bontonou, G., Chartier, A., Jahan, C., Meunier, A.-C., Pierson, S., … Simonelig, M. (2017). piRNAs and Aubergine cooperate with Wispy poly(A) polymerase to stabilize mRNAs in the germ plasm. *Nature Communications*, *8*, 1305. http://doi.org/10.1038/s41467-017-01431-5

Duncan, J. E., & Warrior, R. (2002). The cytoplasmic dynein and kinesin motors have interdependent roles in patterning the *Drosophila* oocyte. *Current Biology*, *12*(23), 1982–1991. http://doi.org/10.1016/S0960-9822(02)01303-9

Durrieu, L., Kirrmaier, D., Schniedt, T., Kats, I., Raghavan, S., Hufnagel, L., … Knop, M. (2018). Bicoid gradient formation mechanism and dynamics revealed by protein lifetime analysis. *Molecular Systems Biology*, *14*(9), e8355. http://doi.org/10.15252/msb.20188355

Eagle, W. V. I., Yeboah-Kordieh, D. K., Niepielko, M. G., & Gavis, E. R. (2018). Distinct cis-acting elements mediate targeting and clustering of *Drosophila* polar granule RNAs. *Development*, *145*(22), dev164657. http://doi.org/10.1242/dev164657

Eichhorn, S. W., Subtelny, A. O., Kronja, I., Kwasnieski, J. C., Orr-Weaver, T. L., & Bartel, D. P. (2016). mRNA poly(A)-tail changes specified by deadenylation broadly reshape translation in *Drosophila* oocytes and embryos. *eLife*, *5*, e16955. http://doi.org/10.7554/eLife.16955

Eliseev, B., Yeramala, L., Leitner, A., Karuppasamy, M., Raimondeau, E., Huard, K., … Schaffitzel, C. (2018). Structure of a human cap-dependent 48S translation pre-initiation complex. *Nucleic Acids Research*, *46*(5), 2678–2689. https://doi.org/10.1093/nar/gky054

ElMaghraby, M. F., Andersen, P. R., Pühringer, F., Hohmann, U., Meixner, K., Lendl, T., … Brennecke, J. (2019). A heterochromatin-specific RNA export pathway facilitates piRNA production. *Cell*, *178*(4), 964–979. http://doi.org/10.1016/j.cell.2019.07.007

Ephrussi, A., Dickinson, L. K., & Lehmann, R. (1991). Oskar organizes the germ plasm and directs localization of the posterior determinant nanos. *Cell*, *66*(1), 37–50. http://doi.org/10.1016/0092-8674(91)90137-n

Ephrussi, A., & Lehmann, R. (1992). Induction of germ cell formation by *oskar*. *Nature*, *358*(6385), 387–392. http://doi.org/10.1038/358387a0

Ferrandon, D., Koch, I., Westhof, E., & Nüsslein-Volhard, C. (1997). RNA-RNA interaction is required for the formation of specific *bicoid* mRNA 3' UTR-STAUFEN ribonucleoprotein particles. *The EMBO Journal*, *16*(7), 1751–1758. http://doi.org/10.1093/emboj/16.7.1751

Forbes, A., & Lehmann, R. (1998). Nanos and Pumilio have critical roles in the development and function of *Drosophila* germline stem cells. *Development*, *125*(4), 679–690.

Forrest, K. M., & Gavis, E. R. (2003). Live imaging of endogenous RNA reveals a diffusion and entrapment mechanism for *nanos* mRNA in *Drosophila*. *Current Biology*, *13*(14), 1159–1168. http://doi.org/10.1016/s0960-9822(03)00451-2

Frise, E., Hammonds, A. S., & Celniker, S. E. (2010). Systematic image-driven analysis of the spatial *Drosophila* embryonic expression landscape. *Molecular Systems Biology*, *6*, 345. http://doi.org/10.1038/msb.2009.102

Gamberi, C., Peterson, D. S., He, L., & Gottlieb, E. (2002). An anterior function for the *Drosophila* posterior determinant Pumilio. *Development*, *129*(11), 2699–2710.

Gáspár, I., Sysoev, V., Komissarov, A., & Ephrussi, A. (2016). An RNA-binding atypical tropomyosin recruits kinesin-1 dynamically to *oskar* mRNPs. *The EMBO Journal*, *36*(3), 319–333. http://doi.org/10.15252/embj.201696038

Gavis, E. R., & Lehmann, R. (1992). Localization of *nanos* RNA controls embryonic polarity. *Cell*, *71*(2), 301–313. http://doi.org/10.1016/0092-8674(92)90358-j

Gepner, J., Li, M., Ludmann, S., Kortas, C., Boylan, K., Iyadurai, S. J., … Hays, T. S. (1996). Cytoplasmic dynein function is essential in *Drosophila melanogaster*. *Genetics*, *142*(3), 865–878.

Ghosh, S., Marchand, V., Gáspár, I., & Ephrussi, A. (2012). Control of RNP motility and localization by a splicing-dependent structure in *oskar* mRNA. *Nature Structural & Molecular Biology*, *19*(4), 441–449. http://doi.org/10.1038/nsmb.2257

Ghosh, S., Obrdlik, A., Marchand, V., & Ephrussi, A. (2014). The EJC binding and dissociating activity of PYM is regulated in *Drosophila*. *PLoS Genetics*, *10*(6), e1004455. http://doi.org/10.1371/journal.pgen.1004455

Glotzer, J. B., Saffrich, R., Glotzer, M., & Ephrussi, A. (1997). Cytoplasmic flows localize injected *oskar* RNA in *Drosophila* oocytes. *Current Biology*, *7*(5), 326–337. http://doi.org/10.1016/s0960-9822(06)00156-4

Goldman, C. H., & Gonsalvez, G. B. (2017). The role of microtubule motors in mRNA localization and patterning within the *Drosophila* oocyte. *Results and Problems in Cell Differentiation*, *63*, 149–168. http://doi.org/10.1007/978-3-319-60855-6_7

Goldman, C. H., Neiswender, H., Veeranan-Karmegam, R., & Gonsalvez, G. B. (2019). The Egalitarian binding partners Dynein light chain and Bicaudal-D act sequentially to link mRNA to the dynein motor. *Development*, *146*, dev176529. http://doi.org/10.1242/dev176529

Gregor, T., Wieschaus, E. F., McGregor, A. P., Bialek, W., & Tank, D. W. (2007). Stability and nuclear dynamics of the Bicoid morphogen gradient. *Cell*, *130*(1), 141–152. http://doi.org/10.1016/j.cell.2007.05.026.

Hachet, O., & Ephrussi, A. (2001). *Drosophila* Y14 shuttles to the posterior of the oocyte and is required for *oskar* mRNA transport. *Current Biology*, *11*(21), 1666–1674. http://doi.org/10.1016/s0960-9822(01)00508-5

Hachet, O., & Ephrussi, A. (2004). Splicing of *oskar* RNA in the nucleus is coupled to its cytoplasmic localization. *Nature*, *428*(6986), 959–963. http://doi.org/10.1038/nature02521

Hay, B., Ackerman, L., Barbel, S., Jan, L. Y., & Jan, Y. N. (1988). Identification of a component of *Drosophila* polar granules. *Development*, *103*(4), 625–640.

Hay, B., Jan, L. Y., & Jan, Y. N. (1990). Localization of Vasa, a component of *Drosophila* polar granules, in maternal-effect mutants that alter embryonic anteroposterior polarity. *Development*, *109*(2), 425–433.

Herpers, B., Xanthakis, D., & Rabouille, C. (2010). ISH-IEM: A sensitive method to detect endogenous mRNAs at the ultrastructural level. *Nature Protocols*, *5*(4), 678–687. http://doi.org/10.1038/nprot.2010.12

Hurd, T. R., Herrmann, B., Sauerwald, J., Sanny, J., Grosch, M., & Lehmann, R. (2016). Long Oskar controls mitochondrial inheritance in *Drosophila melanogaster*. *Developmental Cell*, *39*(5), 560–571. https://doi.org/10.1016/j.devcel.2016/11.004

Iida, T., & Kobayashi, S. (1998). Essential role of mitochondrially encoded large rRNA for germ-line formation in *Drosophila* embryos. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(19), 11274–11278. http://doi.org/10.1073/pnas.95.19.11274

Illmensee, K., & Mahowald, A. (1974). Transplantation of posterior polar plasm in *Drosophila*. Induction of germ cells at the anterior pole of the egg. *Proceedings of the National Academy of Sciences of the United States of America*, *71*(4), 1016–1020. http://doi.org/10.1073/pnas.71.4.1016

Irion, U., & St Johnston, D. (2007). *bicoid* RNA localization requires specific binding of an endosomal sorting complex. *Nature*, *445*(7127), 554–558. http://doi.org/10.1038/nature05503

Jain, R. A., & Gavis, E. R. (2008). The *Drosophila* hnRNP M homolog Rumpelstiltskin regulates *nanos* mRNA localization. *Development*, *135*(5), 973–982. http://doi.org/10.1242/dev.015438

Jambor, H., Mueller, S., Bullock, S. L., & Ephrussi, A. (2014). A stem-loop structure directs *oskar* mRNA to microtubule minus ends. *RNA*, *20*(4), 429–439. http://doi.org/10.1281/rna.041566.113

Jambor, H., Surendranath, V., Kalinka, A. T., Mejstrik, P., Saalfeld, S., & Tomancak, P. (2015). Systematic imaging reveals features and changing localization of mRNAs in *Drosophila* development. *eLife*, *4*, e05003. http://doi.org/10.7554/eLife.05003

Januschke, J., Gervais, L., Dass, S., Kaltschmidt, J. A., Lopez-Schier, A., St Johnston, D., ... Guichet, A. (2002). Polar transport in the *Drosophila* oocyte requires Dynein and kinesin I cooperation. *Current Biology*, *12*(23), 1971–1981. http://doi.org/10.1016/S0960-9822(02)01302-7

Jenny, A., Hachet, O., Závorszky, P., Cyrklaff, A., Weston, M. D. J., St Johnston, D., ... Ephrussi, A. (2006). A translation-independent role of *oskar* RNA in early *Drosophila* oogenesis. *Development*, *133*(15), 2827–2833. http://doi.org/10.1242/dev02456

Jeske, M., Bordi, M., Glatt, S., Müller, S., Rybin, V., Müller, C. W., & Ephrussi, A. (2015). The crystal structure of the *Drosophila* germline inducer Oskar identifies two domains with distinct Vasa helicase- and RNA-binding activities. *Cell Reports*, *12*(4), 587–598. http://doi.org/10.1016/jcelrep.2015.06.055

Jeske, M., Müller, C. W., & Ephrussi, A. (2017). The LOTUS domain is a conserved DEAD-box RNA helicase regulator essential for the recruitment of Vasa to the germ plasm and nuage. *Genes & Development*, *31*(9), 939–952. http://doi.org/10.1101/gad.297051.117

Jones, J. R., & Macdonald, P. M. (2007). Oskar controls morphology of polar granules and nuclear bodies in *Drosophila*. *Development*, *134*(2), 233–236. http://doi.org/10.1242/dev02729

Jongens, T. A., Hay, B., Jan, L. Y., & Jan, Y. N. (1992). The *germ cell-less* gene product: A posteriorly localized component necessary for germ cell development in *Drosophila*. *Cell*, *70*(4), 569–584. http://doi.org/10.1016/0092-8674(92)90427-e

Khoc Trong, P., Doerflinger, H., Dunkel, J., St Johnston, D., & Goldstein, R. E. (2015). Cortical microtubule nucleation can organise the cytoskeleton of *Drosophila* oocytes to define the anteroposterior axis. *eLife*, *4*, e06088. http://doi.org/10.7554/eLife.06088

Kim, G., Pai, C. I., Sato, K., Person, M. D., Nakamura, A., & Macdonald, P. M. (2015). Region-specific activation of *oskar* mRNA translation by inhibition of Bruno-mediated repression. *PLoS Genetics*, *11*(2), e1004992. http://doi.org/10.1371/journal.pgen.1004992

Kim-Ha, J., Kerr, K., & Macdonald, P. M. (1995). Translational regulation of *oskar* mRNA by Bruno, an ovarian RNA-binding protein, is essential. *Cell*, *81*(3), 403–412. http://doi.org/10.1016/0092-8674(95)90393-3

Kim-Ha, J., Smith, J. L., & Macdonald, P. M. (1991). *oskar* mRNA is localized to the posterior pole of the *Drosophila* oocyte. *Cell*, *66*(1), 23–35. http://doi.org/10.1016/0092-8674(91)90136-m

Kistler, K. E., Trcek, T., Hurd, T. R., Chen, R., Liang, F. X., Sall, J., ... Lehmann, R. (2018). Phase-transitioned nuclear Oskar promotes cell division of *Drosophila* primordial germ cells. *eLife*, *7*, e37949. http://doi.org/10.7554/eLife.37949

Kobayashi, S., Amikura, R., & Okada, M. (1993). Presence of mitochondrial large ribosomal RNA outside mitochondria in germ plasm of *Drosophila melanogaster*. *Science*, *260*(5113), 1521–1524. http://doi.org/10.1126/science.7684857

Krauss, J., López de Quinto, S., Nüsslein-Volhard, C., & Ephrussi, A. (2009). Myosin-V regulates *oskar* mRNA localization in the *Drosophila* oocyte. *Current Biology*, *19*(23), 1058–1063. http://doi.org/10.1016/j.cub.2009.04.062

Lasko, P. F., & Ashburner, M. (1990). Posterior localization of Vasa protein correlates with, but is not sufficient for, pole cell formation. *Genes & Development*, *4*(6), 905–921. http://doi.org/10.1101/gad.4.6.905

Lazzaretti, D., Veith, K., Kramer, K., Basquin, C., Urlaub, H., Irion, U., & Bono, F. (2016). The *bicoid* mRNA localization factor Exuperantia is an RNA-binding pseudonuclease. *Nature Structural and Molecular Biology*, *23*(8), 705–713. http://doi.org/10.1038/nsmb.3254

Lécuyer, E., Yoshida, H., Parthasarathy, N., Alm, C., Babak, T., Cerovina, T., ... Krause, H. M. (2007). Global analysis of mRNA localization reveals a prominent role in organizing cellular architecture and function. *Cell*, *131*(1), 174–187. https://doi.org/10.1016/j.cell.2007.08.003

Lehmann, R. (2016). Germ plasm biogenesis—An Oskar-centric perspective. *Current Topics in Developmental Biology*, *116*, 679–707. http://doi.org/10.1016/bs.ctdb.2015.11.024

Lehmann, R., & Nüsslein-Volhard, C. (1986). Abdominal segmentation, pole cell formation, and embryonic polarity require the localized activity of *oskar*, a maternal gene in *Drosophila*. *Cell*, *47*(1), 141–152. http://doi.org/10.1016/0092-8674(86)90375-2

Lerit, D. A., & Gavis, E. R. (2011). Transport of germ plasm on astral microtubules directs germ cell development in *Drosophila*. *Current Biology*, *21*(6), 439–448. http://doi.org/10.1016/j.cub.2011.01.073

Li, M. G., McGrail, M., Serr, M., & Hays, T. S. (1994). *Drosophila* cytoplasmic dynein, a microtubule motor that is asymmetrically localized in the oocyte. *The Journal of Cell Biology*, *126*(6), 1475–1494. http://doi.org/10.1083/jcb.126.6.1475

Lim, J., Lee, M., Son, A., Chang, H., & Kim, V. N. (2016). mTAIL-seq reveals dynamic poly(A) tail regulation in oocyte-to-embryo development. *Genes & Development*, *30*(14), 1671–1682. https://doi.org/10.1101/gad.284802.116

Little, S. C., Sinsimer, K. S., Lee, J. J., Wieschaus, E., & Gavis, E. R. (2015). Independent and coordinate trafficking of single *Drosophila* germ plasm RNAs. *Nature Cell Biology*, *17*(5), 558–568. http://doi.org/10.1038/ncb3143

Liu, Z., Xie, T., & Steward, R. (1999). Lis1, the *Drosophila* homolog of a human lissencephaly gene, is required for germline cell division and oocyte differentiation. *Development*, *126*, 4477–4488.

Lu, W., Lakonishok, M., Liu, R., Billington, N., Rich, A., Glotzer, M., … Gelfand, V. I. (2020). Competition between kinesin-1 and myosin-V defines *Drosophila* posterior determination. *eLife*, *14*(9), e54216. http://doi.org/10.7554/eLife.54216

Lu, W., Lakonishok, M., Serpinskaya, A. S., Kirchenbüchler, D., Ling, S. C., & Gelfand, V. I. (2018). Ooplasmic flow cooperates with transport and anchorage in *Drosophila* oocyte posterior determination. *The Journal of Cell Biology*, *217*(10), 3497–3511. http://doi.org/10.1083/jcb/201709174

Lu, W., Winding, M., Lakonishok, M., Wildonger, J., & Gelfand, V. I. (2016). Microtubule-microtubule sliding by kinesin-1 is essential for normal cytoplasmic streaming in *Drosophila* oocytes. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(34), E4995–E5004. http://doi.org/10/1073/pnas.1522424113

Luschnig, S., Moussian, B., Krauss, J., Desjeux, I., Perkovic, C., & Nüsslein-Volhard, C. (2004). An F1 genetic screen for maternal-effect mutations affecting embryonic pattern formation in *Drosophila melanogaster*. *Genetics*, *167*(1), 325–342. http://doi.org/10.1534/genetics.167.1.325

Ma, J., He, F., Xie, G., & Deng, W. M. (2016). Maternal AP determinants in the *Drosophila* oocyte and embryo. *Wiley Interdisciplinary Reviews: Developmental Biology*, *5*(5), 562–581. http://doi.org/10.1002/wdev.235

Macdonald, P. M., Kanke, M., & Kenny, A. (2016). Community effects in regulation of translation. *eLife*, *5*, e10965. http://doi.org/10.7554/eLife.10965

Macdonald, P. M., & Struhl, G. (1988). *cis*-acting sequences responsible for anterior localization of *bicoid* mRNA in *Drosophila* embryos. *Nature*, *336*(6199), 595–598. http://doi.org/10.1038/336595a0

Mach, J. M., & Lehmann, R. (1997). An Egalitarian-Bicaudal-D complex is essential for oocyte specification and axis determination in *Drosophila*. *Genes & Development*, *11*(4), 423–435. http://doi.org/10.1101/gad.11.4.423

Mahowald, A. P. (1962). Fine structure of pole cells and polar granules in *Drosophila melanogaster*. *The Journal of Experimental Zoology*, *151*(3), 201–215. https://doi.org/10.1002/jez.1401510302

Mahowald, A. P. (1968). Polar granules in *Drosophila*. II. Ultrastructural changes during early embryogenesis. *The Journal of Experimental Zoology*, *167*(2), 237–262. https://doi.org/10.1002/jez.1401670211

Manseau, L., Calley, J., & Phan, H. (1996). Profilin is required for posterior patterning of the *Drosophila* oocyte. *Development*, *122*(7), 2109–2116.

Markussen, F.-H., Michon, A. M., Breitwieser, W., & Ephrussi, A. (1995). Translational control of *oskar* generates short OSK, the isoform that induces pole plasma assembly. *Development*, *121*(11), 3723–3732.

McClintock, M. A., Dix, C. I., Johnson, C. M., McLaughlin, S. H., Maizels, R. J., Hoang, H. T., & Bullock, S. L. (2018). RNA-directed activation of cytoplasmic dynein-1 in reconstituted transport RNPs. *eLife*, *7*, e36312. https://doi.org/10.7554/eLife.36312

McGrail, M., & Hays, T. S. (1997). The microtubule motor cytoplasmic dynein is required for spindle orientation during germline cell divisions and oocyte differentiation in *Drosophila*. *Development*, *124*(12), 2409–2419.

Micklem, D. R., Adams, J., Grünert, S., & St Johnston, D. (2000). Distinct roles of two conserved Staufen domains in *oskar* mRNA localization and translation. *The EMBO Journal*, *19*(6), 1366–1377. https://doi.org/10.1093/emboj/19.6.1366

Mohr, S., Dillon, S., & Boswell, R. (2001). The RNA-binding protein Tsunagi interacts with Mago Nashi to establish polarity and localize *oskar* mRNA during *Drosophila* oogenesis. *Genes & Development*, *15*(21), 2886–2899. http://doi.org/10.1101/gad.927001

Munro, T. P., Kwon, S., Schnapp, B. J., & St Johnston, D. (2006). A repeated IMP-binding motif controls *oskar* mRNA translation and anchoring independently of *Drosophila melanogaster* IMP. *The Journal of Cell Biology*, *172*(4), 577–588. http://doi.org/10.1083/jcb.200510044

Nakamura, A., Amikura, R., Mukai, M., Kobayashi, S., & Lasko, P. F. (1996). Requirement for a noncoding RNA in *Drosophila* polar granules for germ cell establishment. *Science*, *274*(5295), 2075–2079. https://doi.org/10.1126/science.274.5295.2075

Nakamura, A., Sato, K., & Hanyu-Nakamura, K. (2004). *Drosophila* Cup is an eIF4E binding protein that associates with Bruno and regulates *oskar* mRNA translation in oogenesis. *Developmental Cell*, *6*(1), 69–78. https://doi.org/10.1016/s1534-5807(03)00400-3

Nashchekin, D., Fernandes, A. R., & St Johnston, D. (2016). Patronin/Shot cortical foci assemble the noncentrosomal microtubule array that specifies the *Drosophila* anterior-posterior axis. *Developmental Cell*, *38*(1), 61–72. http://doi.org/10.1016/j.devcel.2016.06.010

Navarro, C., Puthalakath, H., Adams, J. M., Strasser, A., & Lehmann, R. (2004). Egalitarian binds dynein light chain to establish oocyte polarity and maintain oocyte fate. *Nature Cell Biology*, *6*(5), 427–435. http://doi.org/10.1038/ncb1122

Newmark, P., & Boswell, R. (1994). The *mago nashi* locus encodes an essential product required for germ plasm assembly in *Drosophila*. *Development*, *120*(5), 1303–1313.

Niepielko, M. G., Eagle, W. V. I., & Gavis, E. R. (2018). Stochastic seeding coupled with mRNA self-recruitment generates heterogeneous *Drosophila* germ granules. *Current Biology*, *28*(12), 1872–1881. https://doi.org/10.1016/j.cub.2018.04.037

Nüsslein-Volhard, C., Frohnhöfer, H.-G., & Lehmann, R. (1987). Determination of anteroposterior polarity in *Drosophila*. *Science*, *238*(4834), 1675–1681. http://doi.org/10.1126/science.3686007

Pae, J., Cinalli, R. M., Marzio, A., Pagano, M., & Lehmann, R. (2017). GCL and CUL3 control the switch between cell lineages by mediating localized degradation of an RTK. *Developmental Cell*, *42*(2), 130–142. http://doi.org/10.1016/j.devcel.2017.06.022

Palacios, I., Gatfield, D., St Johnston, D., & Izaurralde, E. (2004). An eIF4AIII-containing complex required for mRNA localization and nonsense-mediated mRNA decay. *Nature*, *427*(6976), 753–757. http://doi.org/10.1038/nature02351

Palacios, I. M., & St Johnston, D. (2002). Kinesin light chain-independent function of the Kinesin heavy chain in cytoplasmic streaming and posterior localization in the *Drosophila* oocyte. *Development*, *129*(23), 5473–5485. http://doi.org/10.1242/dev.00119

Parton, R. M., Hamilton, R. S., Ball, G., Yang, L., Cullen, C. F., Lu, W., ... Davis, I. (2011). A PAR-1-dependent orientation gradient of dynamic microtubules directs posterior cargo transport in the *Drosophila* oocyte. *The Journal of Cell Biology*, *194*(1), 121–135. http://doi.org/10.1083/jcb.201103160

Patil, V. S., Anand, A., Chakrabarti, A., & Kai, T. (2014). The Tudor domain protein Tapas, a homolog of the vertebrate Tdrd7, functions in piRNA pathway to regulate retrotransposons in germline of *Drosophila melanogaster*. *BMC Biology*, *12*, 61. http://doi.org/10.1186/s12915-014-0061-9

Patil, V. S., & Kai, T. (2010). Repression of retroelements in *Drosophila* germline via piRNA pathway by the Tudor domain protein Tejas. *Current Biology*, *20*(8), 724–730. https://doi.org/10.1016/j.cub.2010.02.046

Pokrywka, N. J., & Stephenson, E. C. (1991). Microtubules mediate the localization of *bicoid* RNA during *Drosophila* oogenesis. *Development*, *113*(1), 55–66.

Quinlan, M. E. (2016). Cytoplasmic streaming in the *Drosophila* oocyte. *Annual Review of Cell and Developmental Biology*, *32*, 173–195. http://doi.org/10.1146/annurev-cellbio-111315-125416

Rangan, P., DeGennaro, M., Jaime-Bustamente, K., Coux, R.-X., Martinho, R. G., & Lehmann, R. (2009). Temporal and spatial control of germ-plasm RNAs. *Current Biology*, *19*(1), 72–77. https://doi.org/10.1016/j.cub.2008.11.066

Reveal, B., Yan, N., Snee, M. J., Pai, C. I., Gim, Y., & Macdonald, P. M. (2010). BREs mediate both repression and activation of *oskar* mRNA translation and act in *trans*. *Developmental Cell*, *18*(3), 496–502. http://doi.org/10.1016/j.devcel.2009.12.021

Rojas-Rios, P., & Simonelig, M. (2018). piRNAs and PIWI proteins: Regulators of gene expression in development and stem cells. *Development*, *145*(17), dev161786. http://doi.org/10.1242/dev.161786

Rongo, C. G. (1996). *The role of RNA localization and translational regulation in Drosophila germ cell determination*. (PhD thesis). Massachusetts Institute of Technology. Available at http://hdl.handle.net/1721.1/10562

Rouget, C., Papin, C., Boureux, A., Meunier, A. C., Franco, B., Robine, N., ... Simonelig, M. (2010). Maternal mRNA deadenylation and decay by the piRNA pathway in the early *Drosophila* embryo. *Nature*, *467*(7319), 1128–1132. http://doi.org/10.1038/nature09465

Ryder, P. V., & Lerit, D. A. (2018). RNA localization regulates diverse and dynamic cellular processes. *Traffic*, *19*(7), 496–502. http://doi.org/10.1111/tra.12571

Ryu, Y. H., Kenny, A., Gim, Y., Snee, M., & Macdonald, P. M. (2017). Multiple *cis*-acting signals, some weak by necessity, collectively direct robust transport of *oskar* mRNA to the oocyte. *Journal of Cell Science*, *130*(18), 3060–3071. http://doi.org/10.1242/jcs.202069

Sallés, F. J., Lieberfarb, M. E., Wreden, C., Gergen, J. P., & Strickland, S. (1994). Coordinate initiation of *Drosophila* development by regulated polyadenylation of maternal messenger RNAs. *Science*, *266*(5193), 1996–1999. http://doi.org/10.1126/science.7801127

Sanghavi, P., Liu, G., Veeranan-Karmegam, R., Navarro, C., & Gonsalvez, G. B. (2016). Multiple roles for Egalitarian in polarization of the *Drosophila* egg chamber. *Genetics*, *203*(1), 415–432. http://doi.org/10.1534/genetics.115.184622

Serbus, L. R., Cha, B. J., Theurkauf, W. E., & Saxton, W. M. (2005). Dynein and the actin cytoskeleton control kinesin-driven cytoplasmic streaming in *Drosophila* oocytes. *Development*, *132*(16), 3743–3752. http://doi.org/10.1242/dev.01956

Shapiro, R. S., & Anderson, K. V. (2006). *Drosophila* Ik2, a member of the I kappa B kinase family, is required for mRNA localization during oogenesis. *Development*, *133*(8), 1467–1475. http://doi.org/10.1242/dev.02318

Simon, B., Masiewicz, P., Ephrussi, A., & Carlomagno, T. (2015). The structure of the SOLE element of *oskar* mRNA. *RNA*, *21*(8), 1444–1453. http://doi.org/10.1261/rna.049601.115

Sinsimer, K. S., Jain, R. A., Chatterjee, S., & Gavis, E. R. (2011). A late phase of germ plasm accumulation during *Drosophila* oogenesis requires *lost* and *rumpelstiltskin*. *Development*, *138*(16), 3431–3440. http://doi.org/10.1242/dev.065029

Siomi, M. C., Sato, K., Pezic, D., & Aravin, A. A. (2011). PIWI-interacting small RNAs: The vanguard of genome defense. *Nature Reviews Molecular Cell Biology*, *12*(4), 246–258. http://doi.org/10.1038/nrm3089

Sladewski, T. E., Billington, N., Ali, M. Y., Bookwalter, C. S., Lu, H., Krementsova, E. B., ... Trybus, K. M. (2018). Recruitment of two dyneins to an mRNA-dependent Bicaudal D transport complex. *eLife*, *7*, e36306. http://doi.org/10.7554/eLife.36306

Smith, J. L., Wilson, J. E., & Macdonald, P. M. (1992). Overexpression of *oskar* directs ectopic activation of *nanos* and presumptive pole cell formation in *Drosophila* embryos. *Cell*, *70*(5), 849–859. http://doi.org/10.1016/0092-8674(92)90318-7

Spirov, A., Fahmy, K., Schneider, M., Frei, E., Noll, M., & Baumgartner, S. (2009). Formation of the Bicoid morphogen gradient: an mRNA gradient dictates the protein gradient. *Development*, *136*(4), 605–614. http://doi.org/10.1242/dev.031195.

St Johnston, D., Driever, W., Berleth, T., Richstein, S., & Nüsslein-Volhard, C. (1989). Multiple steps in the localization of *bicoid* RNA to the anterior pole of the *Drosophila* oocyte. *Development*, *107*(Suppl.), 13–19.

Subtelny, A. O., Eichhorn, S. W., Chen, G. R., Sive, H., & Bartel, D. P. (2014). Poly(A)-tail profiling reveals an embryonic switch in translational control. *Nature*, *508*(7494), 66–71. http://doi.org/10.1038/nature13007

Suter, B. (2018). RNA localization and transport. *Biochimica et Biophysica Acta – Gene Regulatory Mechanisms*, *1861*, 938–951. https://doi.org/10.1016/j.bbagrm.2018.08.004

Swan, A., Nguyen, T., & Suter, B. (1999). Drosophila Lissencephaly-1 functions with Bic-D and Dynein in oocyte determination and nuclear positioning. *Nature Cell Biology*, *1*(7), 444–449. http://doi.org/10.1038/15680

Tanaka, T., Kato, Y., Matsuda, K., Hanyu-Nakamura, K., & Nakamura, A. (2011). *Drosophila* Mon2 couples Oskar-induced endocytosis with actin remodeling for cortical anchorage of the germ plasm. *Development*, *138*(12), 2523–2532. http://doi.org/10.1242/dev.062208

Tanaka, T., & Nakamura, A. (2008). The endocytic pathway acts downstream of Oskar in *Drosophila* germ plasm assembly. *Development*, *135*(6), 1107–1117. http://doi.org/10.1242/dev.017293

Theurkauf, W. E., Alberts, B., Jan, Y. N., & Jongens, T. A. (1993). A central role for microtubules in the differentiation of *Drosophila* oocytes. *Development*, *118*(4), 1169–1180.

Trcek, T., Grosch, M., York, A., Shroff, H., Lionnet, T. L., & Lehmann, R. (2015). *Drosophila* germ granules are structured and contain homotypic RNA clusters. *Nature Communications*, *6*, 7962. http://doi.org/10.1038/ncomms8962

Trcek, T., & Lehmann, R. (2019). Germ granules in *Drosophila*. *Traffic*, *20*(9), 650–660. http://doi.org/10.1111/tra.12674

Trovisco, V., Belaya, K., Nashchekin, D., Irion, U., Sirinakis, G., Butler, R., … St Johnston, D. (2016). *bicoid* mRNA localises to the *Drosophila* oocyte anterior by random Dynein-mediated transport and anchoring. *eLife*, *5*, e17537. http://doi.org/10.7554/eLife.17537

Vanzo, N., Oprins, A., Xanthakis, D., Ephrussi, A., & Rabouille, C. (2007). Stimulation of endocytosis and actin dynamics by Oskar polarizes the *Drosophila* oocyte. *Developmental Cell*, *12*(4), 543–555. http://doi.org/10.1016/j.devcel.2007.03.002

Vanzo, N. F., & Ephrussi, A. (2002). Oskar anchoring restricts pole plasm formation to the posterior of the *Drosophila* oocyte. *Development*, *129*(15), 3705–3714.

Vo, H. D. L., Wahlduzzaman, Tindell, S. J., Zheng, J., Gao, M., & Arkov, A. (2019). Protein components of ribonucleoprotein granules from *Drosophila* germ cells oligomerize and show distinct spatial organization during germline development. *Scientific Reports*, *9*(1), 19190. https://doi.org/10.1038/s41598-019-55747-x

Vourekas, A., Alexiou, P., Vrettos, N., Maragkakis, M., & Mourelatos, Z. (2016). Sequence-dependent but not sequence-specific piRNA adhesion traps mRNAs to the germ plasm. *Nature*, *531*(7594), 390–394. https://doi.org/10.1038/nature17150

Wang, C., & Lehmann, R. (1991). Nanos is the localized posterior determinant in *Drosophila*. *Cell*, *66*(4), 637–647. http://doi.org/10.1016/0092-8674(91)90110-k

Wang, J. T., Smith, J., Chen, B. C., Schmidt, H., Rasoloson, D., Paix, A., … Seydoux, G. (2014). Regulation of RNA granule dynamics by phosphorylation of serine-rich intrinsically disordered proteins in *C. elegans*. *eLife*, *3*, e04591. http://doi.org/10.7554/eLife.04591

Webster, P. J., Liang, L., Berg, C. A., Lasko, P., & Macdonald, P. M. (1997). Translational repressor Bruno plays multiple roles in development and is widely conserved. *Genes & Development*, *11*(19), 2510–2521. http://doi.org/10.1101/gad.11.19.2510

Weil, T. T., Forrest, K. M., & Gavis, E. R. (2006). Localization of *bicoid* mRNA in late oocytes is maintained by continual active transport. *Developmental Cell*, *11*(2), 251–262. http://doi.org/10.1016/j.devcel.2006.06.006

Weil, T. T., Parton, R., Davis, I., & Gavis, E. R. (2008). Changes in *bicoid* mRNA anchoring highlight conserved mechanisms during the oocyte-to-embryo transition. *Current Biology*, *18*(14), P1055–P1061. https://doi.org/10.1016/j.cub.2008.06.046

Wharton, R. P., & Struhl, G. (1991). RNA regulatory elements mediate control of *Drosophila* body pattern by the posterior morphogen *nanos*. *Cell*, *67*(5), 955–967. http://doi.org/10.1016/0092-8674(91)90368-9

Wharton, T. H., Nomie, K. J., & Wharton, R. P. (2018). No significant regulation of *bicoid* mRNA by Pumilio or Nanos in the early *Drosophila* embryo. *PLoS One*, *13*(3), e0194865. https://doi.org/10.1371/journal.pone.0194865

Wieschaus, E. (2016). Positional information and cell fate determination in the early *Drosophila* embryo. *Current Topics in Developmental Biology*, *117*, 567–579. http://doi.org/10.1016/bs.ctdb.2015.11.020

Wilkie, G. S., & Davis, I. (2001). *Drosophila wingless* and pair-rule transcripts localize apically by dynein-mediated transport of RNA particles. *Cell*, *105*(2), 209–219. http://doi.org/10.1016/s0092-8674(01)00312-9

Wilson, J. E., Connell, J. E., & Macdonald, P. M. (1996). *aubergine* enhances *oskar* translation in the *Drosophila* ovary. *Development*, *122*(5), 1631–1639.

Yang, N., Yu, Z., Hu, M., Wang, M., Lehmann, R., & Xu, R.-M. (2015). Structure of *Drosophila* Oskar reveals a novel RNA binding protein. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(37), 11541–11546. http://doi.org/10.1073/pnas.1515568112

Zhang, F., Wang, J., Xu, J., Zhang, Z., Koppetsch, B. S., Schultz, N., … Theurkauf, W. E. (2012). UAP56 couples piRNA clusters to the perinuclear transposon silencing machinery. *Cell*, *151*(4), 871–884. http://doi.org/10.1016/j.cell.2012.09.040

Zimyanin, V. L., Belaya, K., Pecreaux, J., Gilchrist, M. J., Clark, A., Davis, I., … St Johnston, D. (2008). In vivo imaging of *oskar* mRNA transport reveals the mechanism of posterior localization. *Cell*, *134*(5), 843–853. http://doi.org/10.1016/j.cell.2008.06.053

How to cite this article: Lasko P. Patterning the *Drosophila* embryo: A paradigm for RNA-based developmental genetic regulation. *WIREs RNA*. 2020;11:e1610. https://doi.org/10.1002/wrna.1610