CHAPTER THREE

Polarity and axis formation in the *Drosophila* female germ line

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Contents

Abstract

By the time a *Drosophila* egg is laid, both major body axes have already been defined and it contains all the nutrients needed to develop into a free-living larva in 24 h. By contrast, it takes almost a week to make an egg from a female germline stem cell, during the complex process of oogenesis. This review will discuss key symmetry-breaking steps in *Drosophila* oogenesis that lead to the polarisation of both body axes: the asymmetric divisions of the germline stem cells; the selection of the oocyte from the 16-cell germline cyst; the positioning of the oocyte at the posterior of the cyst; Gurken signalling from the oocyte to polarise the anterior-posterior axis of the somatic follicle cell epithelium around the developing germline cyst; the signalling back from the posterior follicle cells to polarise the anterior-posterior axis of the oocyte; and the migration of the oocyte nucleus that specifies the dorsal-ventral axis. Since each event creates the preconditions for the next, I will focus on the mechanisms that drive these symmetry-breaking steps, how they are linked and the outstanding questions that remain to be answered.

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1. Overview of oogenesis

Drosophila oogenesis takes place in the two ovaries, each containing 16–18 ovarioles that function as production lines of new eggs (Fig. 1A) (Bastock & St Johnston, 2008; Hinnant, Merkle, & Ables, 2020; Spradling, 1993). This process starts at the anterior of the ovariole in a structure called the germarium, which contains the germline stem cells (GSCs). GSCs divide asymmetrically to produce a new stem cell and a cystoblast, which then goes through four incomplete divisions to produce a cyst of 16 germ cells interlinked by ring canals (Fig. 1B). In region 2b of the germarium, one of these cells is selected to become the oocyte and arrests in meiotic prophase, while the remaining 15 cells exit meiosis and endoreplicate to become polyploid nurse cells. At this stage, the germline cyst becomes enveloped in somatic follicle cells to form an egg chamber, which then grows and undergoes morphogenetic changes during the 14 stages of post-germarial development (Fig. 1A). Towards the end of oogenesis, the follicle cells secrete the egg shell and the nurse cells expel their contents into the oocyte to increase its volume, before both cell populations undergo apoptosis, leaving a mature oocyte that is ready to be fertilised.

By the time that the mature oocyte is produced, both body axes of the future embryo are already defined (St Johnston & Nüsslein-Volhard, 1992). The anterior-posterior axis is specified by the localisation of *bicoid* mRNA to the oocyte anterior and the accumulation of *oskar* and *nanos* mRNAs and the other constituents of the pole plasm to the oocyte posterior(Ephrussi, Dickinson, & Lehmann, 1991; Gavis & Lehmann, 1992; Kim-Ha, Smith, & Macdonald, 1991; St Johnston, Driever, Berleth, Richstein, & Nüsslein-Volhard, 1989). By contrast, the dorsal-ventral axis is specified by the expression in the ventral follicle cells of Pipe, which sulphates proteins in the egg shell to provide the ventral cue that activates the Toll signalling pathway, which forms the Dorsal morphogen gradient (Cho, Stevens, Sieverman, Nguyen, & Stein, 2012; Roth, Stein, & Nüsslein-Volhard, 1989; Sen, Goltz, Stevens, & Stein, 1998; Stein, Roth, Vogelsang, & Nüsslein-Volhard, 1991; Zhang, Stevens, & Stein, 2009). Axis formation depends on a series of polarised events during oogenesis that generate the asymmetries that lead to the localisation of these axis determinants. This review will discuss our current understanding of these symmetry-breaking steps that generate polarity and how they are linked, with each step creating the conditions that allow the next step to occur.

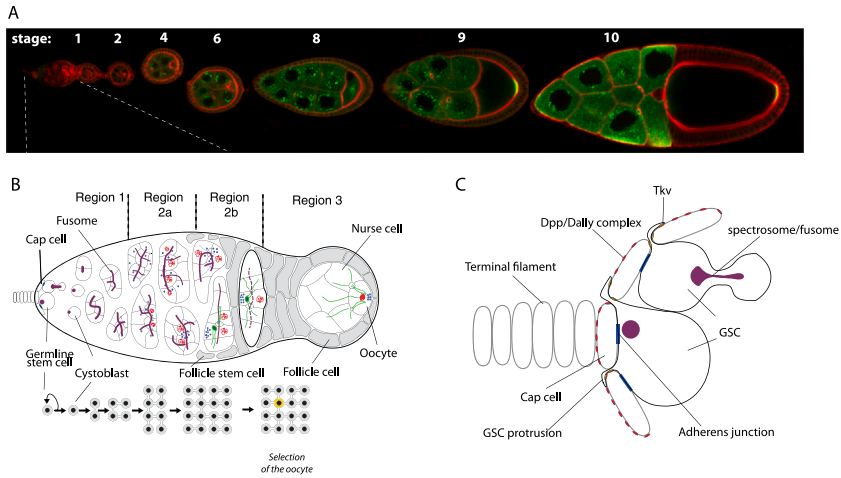

Fig. 1 Overview of *Drosophila* oogenesis. (A) An image of a *Drosophila* ovariole showing egg chambers at increasing stages of development from the anterior (left) to posterior (right). The ovariole expressed GFP-Staufen (green) in the germline cells and was stained for F-actin (red). (B) A diagram of the germarium. Adherens junctions (blue) anchor the germline stem cells (GSCs) to the somatic cap cells at the anterior of the ovariole. The spectrosome (purple) localises to the anterior of the GSC and orients the mitotic spindle so that one daughter cell remains associated with the cap cells, which provide the stem cell niche. During mitosis, new spectrosome material forms in the cleavage furrow and is inherited by the posterior daughter, the cystoblast. The cystoblast then divides four times with incomplete cytokinesis to produce a cyst of 16 germ cells linked by ring canals. New spectrosome material forms in the ring canals at each mitosis to generate a branched structure called the fusome (purple), which anchors one spindle pole at each mitosis to produce an invariant pattern of divisions. In region 2a of the germarium both cells with four ring canals and the two cells with three ring canals start to form the synaptonemal complex (red), which mediates synapsis between homologous chromosomes during meoisis. The follicle cells start to surround each cyst as it enters region 2b. The synaptonemal complex becomes restricted to the two cells with four ring canals (the pro-oocytes) in region 2b, before becoming restricted to one of these cells, the oocyte, in late region 2b. At this stage, the centrosomes (blue dots) and the noncentrosomal microtubule organising centres (green) are localised to the oocyte, where the latter nucleate microtubules that extend through the ring canals into the other germ cells (nurse cells) in the cyst. As the germline cyst moves into region 3, it rounds up with the oocyte coming to lie at the posterior. This coincides with the polarisation of the oocyte, which positions the centrosomes and noncentrosomal microtubule organising centres at the posterior. (C) A diagram of the germline stem cell niche. The cap cells localise the glypican, Dally, on their anterior sides where it sequesters Dpp (red). The GSCs produce protrusions containing the Dpp receptor, Tkv (green), that extend between the cap cells to reach the source of Dpp.

2. Asymmetric stem cell division

The first step of oogenesis is the asymmetric division of a female germline stem cell (GSC) at the anterior tip of the germarium. The GSCs are anchored by adherens junctions and gap junctions to the somatic cap cells that lie at the anterior of the germarium (Gilboa, Forbes, Tazuke, Fuller, & Lehmann, 2003; Song, Zhu, Doan, & Xie, 2002). The cap cells also provide the stem cell niche by producing the bone morphogenetic proteins (BMPs), Dpp and Gbb, which signal to the stem cells to repress Bam transcription and maintain stem cell identity (Chen & McKearin, 2003; Song et al., 2004; Xie & Spradling, 1998, 2000). The asymmetric outcome of the GSC division is extrinsic, as the daughter cell that contacts cap cells receives the BMP signal and remains a stem cell, whereas the daughter cell that does not, expresses Bam and differentiates as a cystoblast. This explains why when the stem cells are lost, other germ cells can replace them by occupying the niche next to the cap cells and de-differentiating into GSCs (Kai & Spradling, 2004). For this system to work, the spread of the Dpp signal from cap cells must be limited to prevent it reaching cells outside the niche. A recent study has revealed an unexpected mechanism to achieve this, in which most Dpp is sequestered by the Heparan-sulphate proteoglycan, Dally on the opposite (anterior) side of the Cap cells from the GSCs (Fig. 1C) (Wilcockson & Ashe, 2019). The GSCs receive this signal by generating dynamic protrusions containing the Dpp receptor, Tkv, that reach between the Cap cells to contact the sequestered Dpp. Thus, most Dpp is anchored where only the GSCs can reach it.

Although the control of daughter cell fate is extrinsic, GSC divisions are also intrinsically asymmetric with respect to the spectrosome, a spherical structure composed of F-actin, endoplasmic reticulum and several actin binding proteins, such as the Adducin, Hu li tai shao (Hts), α and β-spectrin, Tropomodulin and Short stop (Shot) (Cuevas, Lee, & Spradling, 1996; Lighthouse, Buszczak, & Spradling, 2008; Lin, Yue, & Spradling, 1994; Röper & Brown, 2004; Snapp, Iida, Frescas, Lippincott-Schwartz, & Lilly, 2004). The spectrosome localises close to the contact sits with the cap cells and anchors one spindle pole to orient the division so that one daughter remains in contact with the cap cells and becomes a GSC and the other lies posteriorly and differentiates (Deng & Lin, 1997). In this way, the intrinsic polarity of the spectrosome reinforces the extrinsic polarity provided by the niche. During division, the spectrosome migrates from the anterior side of the GSC towards the transient ring canal between the

daughter cells and fuses with new spectrosome material that forms within the ring canal to produce a tubular structure called the fusome (Cuevas & Spradling, 1998). This is then broken at cytokinesis, with two thirds remaining in the GSC and one third in the cystoblast. The fusome behaves similarly in subsequent incomplete divisions, except that the ring canals persist, and the new fusomal material that forms within them fuses with the existing fusome to produce a branched structure that eventually extends into all 16 cells of the cyst. During these mitoses, the fusome recruits dynein to anchor one pole of each spindle, orienting the divisions so that the ring canals always segregate to older cystocyte (McGrail & Hays, 1997). This results in an invariant arrangement of cells in the germline cyst, in which two cells have 4 ring canals, two have 3, four have 2 and eight have 1 ring canal (Fig. 1B).

3. Oocyte determination

A key symmetry-breaking event in oogenesis is the selection of one cell in the 16-cell germline cyst to become the oocyte. This depends on the formation of a microtubule organising centre (MTOC) in the presumptive oocyte that nucleates a microtubule network that extends into all 16 cells of the cyst, allowing the dynein complex and its cargo adaptor proteins Bicaudal-D and Egalitarian to transport oocyte determining factors along this polarised cytoskeleton into one cell (Navarro, Puthalakath, Adams, Strasser, & Lehmann, 2004; Suter & Steward, 1991; Theurkauf, Alberts, Jan, & Jongens, 1993). What has been less clear is how this MTOC is established in the first place. Based on the observation that both cells with four ring canals initially enter meiosis and form synaptonemal complex in region 2 of the germarium, it was proposed that there is a competition between these two cells or "pro-oocytes" to randomly determine which becomes the oocyte (Carpenter, 1975, 1994; Huynh & St Johnston, 2004). An alternative model is that the oocyte is specified by the asymmetric inheritance of some structure during the cyst divisions. For example, one of the cells with four ring canals always ends up with more fusomal material than the other cells because it inherits the original spectrosome from the cystoblast, and this could specify the oocyte (Cuevas & Spradling, 1998; Lin & Spradling, 1995; Lin et al., 1994). A variant of this model proposed that the original centriole inherited by the cystoblast could segregate into one cell to specify the oocyte (Theurkauf, 1994).

This mystery has now been resolved with the discovery that the amount of fusome does indeed determine the future oocyte, but indirectly through a positive feedback loop (Nashchekin, Busby, Jakobs, Squires, & Johnston, 2021). Stable, acetylated microtubules form around the fusome through the activity of the actin/microtubule cross-linker protein, Short stop, which is recruited to the fusome, presumably by binding actin (Fig. 2A) (Grieder, Cuevas, & Spradling, 2000; Röper & Brown, 2004). Shot in turn recruits the microtubule minus end-binding protein, Patronin, and Patronin and the microtubule minus ends are therefore slightly enriched in the cell with most fusome (Fig. 2B). This generates a weakly asymmetric microtubule network, which is then amplified by the dynein-dependent transport of microtubules and Patronin into the cell with most microtubule minus ends (Fig. 2C). This positive feedback loop gradually concentrates Patronin and minus ends in the presumptive oocyte, where Patronin forms non-centrosomal microtubule organising centres (ncMTOCs) (Fig. 2D and E) (Nashchekin et al., 2021). The fusome has disassembled by the time the oocyte is specified and Patronin, dynein and microtubules therefore act as both a memory of the transient fusome asymmetry as well as an amplification mechanism to reliably select one cell from 16 to become the oocyte.

4. Oocyte polarisation

When the oocyte is first selected in region 2b of the germarium, the centrosomes, ncMTOCs and oocyte markers, such as Orb and BicD proteins, accumulate at the cell anterior, but they all translocate to the posterior of the oocyte in region 3 (Huynh, Shulman, Benton, & St Johnston, 2001) (Fig. 2F). This polarisation of the oocyte depends on the classical polarity factors first identified in *C. elegans*: Par-1, Bazooka (*Drosophila* Par-3), Par-6 and atypical protein kinase C (aPKC) (Cox, Lu, Sun, Williams, & Jan, 2001; Cox, Seyfried, Y, & Jan, 2001; Huynh, Petronczki, Knoblich, & St Johnston, 2001; Huynh, Shulman, et al., 2001; Kemphues, Priess, Morton, & Cheng, 1988; Vaccari & Ephrussi, 2002). In mutants in any of these PAR proteins, the centrosomes, ncMTOCs and oocyte fate markers fail to relocate to the posterior and the oocyte exits meiosis and reverts to the nurse cell fate. This early polarisation of the oocyte has not been studied in detail and remains mysterious. Bazooka, aPKC and Par-6 localise to the adherens junctions around the ring canals, whereas Par-1 associates with the fusome during cyst divisions and is weakly enriched on

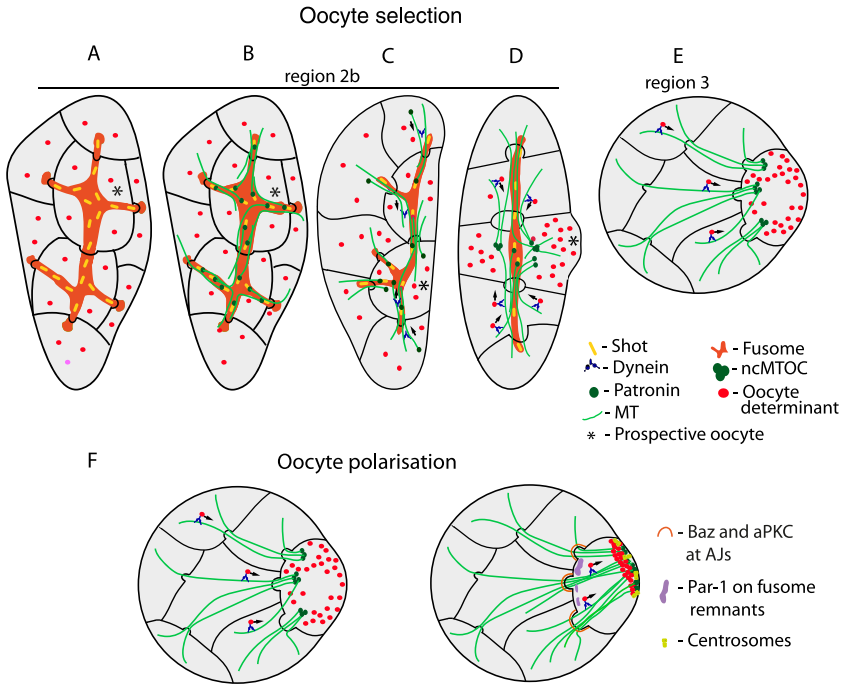

Fig. 2 Oocyte selection and polarisation. (A) Shot protein (yellow) is recruited to the fusome (light brown). The prospective oocyte (asterisk) contains slightly more fusome than the other germ cells, but the germline determinants are uniformly distributed throughout the cyst. (B) Shot recruits the microtubule minus end binding protein, Patronin, to the fusome. Patronin captures and stabilises microtubule minus ends. (C) Dynein (blue) transports microtubules and Patronin (dark green) towards the microtubule minus ends, leading to an enrichment of minus ends in the prospective oocyte, which contains more minus ends because it contains more fusome, Shot and Patronin. (D) The accumulation of microtubule minus ends in the prospective oocyte provides more tracks for dynein-dependent microtubule transport, eventually leading to the concentration of all minus ends and Patronin in this cell, where Patronin forms noncentrosomal microtubule organising centres. Dynein also transports oocyte determinants into this cell, leading to its specification as the oocyte. (E) The oocyte is fully determined before it enters region 3. (F) Oocyte polarisation. The noncentrosomal microtubule organising centres move to the posterior of the oocyte, along with the centrosomes and oocyte specific proteins, such as Orb. This polarisation requires Baz, Par-6 and aPKC, which localise to the adherens junctions around the ring canals and Par-1, which may be enriched on the remnants of the fusome, which breaks down in region 2b.

the fusome remnants at the anterior of the oocyte (Cox, Lu, et al., 2001; Cox, Seyfried, et al., 2001; Huynh, Petronczki, et al., 2001; Huynh, Shulman, et al., 2001). These localisations are not interdependent, as they are in the *C. elegans* zygote, however, and nothing is known about how

they act together to direct the posterior movement of the centrosomes and ncMTOCs. It is also unclear why this early polarity is required to maintain oocyte fate, but the most likely explanation is that it allows the formation of a stable microtubule network that sustains dynein-dependent transport into the oocyte.

5. The posterior positioning of the oocyte

Soon after it has been specified, the oocyte moves to the posterior end of the germline cyst, generating an anterior-posterior asymmetry in the egg chamber that will eventually define the AP axis of the embryo (González-Reyes & St Johnston, 1994). In region 2b of the germarium, the 16 germ cells form a flattened disc that then rounds up to become more spherical as the cyst moves into region 3. Both the oocyte and the posterior follicle cells independently up-regulate the DE-Cadherin adhesion complex in region 2b, causing them to adhere to each other and this adhesion ensures that the oocyte comes to lie posterior to the 15 nurse cells as the germline cyst rounds up (Fig. 3A) (Godt & Tepass, 1998; González-Reyes & St Johnston, 1998b). Thus, oocyte positioning is an in vivo example of a morphogenetic process that is driven by quantitative differences in adhesion, as originally demonstrated in vitro by Steinberg and Takeichi (Steinberg & Takeichi, 1994). If other follicle cells express higher levels of DE-Cadherin than the posterior follicle cells, as in *talin* mutants, these cells outcompete the posterior cells for adhesion to the oocyte, leading to mispositioning of the oocyte and the development of bipolar egg chambers that have an oocyte with two anterior ends (Bécam, Tanentzapf, Lepesant, Brown, & Huynh, 2005). The large class of spindle mutants can also a similar bipolar phenotype, because they delay the specification of the oocyte, which no longer up-regulates DE-cadherin by the time the germline cyst enters region 3 (González-Reyes, Elliott, & St Johnston, 1997). Thus, the correct timing of oocyte selection is essential for normal oocyte positioning. Most spindle loci encode either components of the dsDNA break repair pathway or the piRNA pathway that represses transposons and the delay in oocyte specification results from activation of the DNA damage checkpoint kinase Chk2 by DNA damage (Abdu, Brodsky, & Schüpbach, 2002; Chen, Pane, & Schüpbach, 2007; Ghabrial & Schüpbach, 1999; Ghabrial, Ray, & Schüpbach, 1998; Klattenhoff et al., 2007).

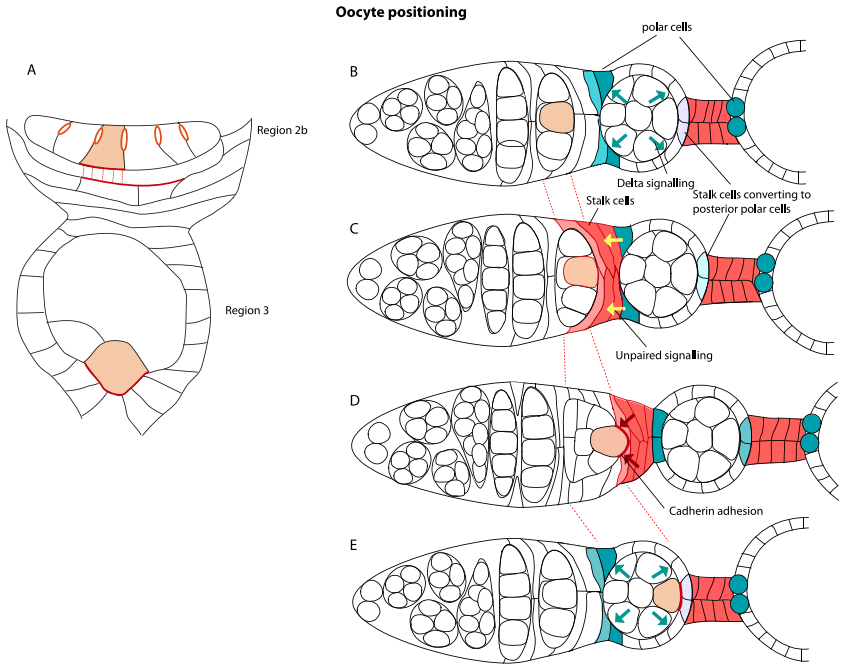

Fig. 3 Oocyte positioning. (A) The components of the E-Cadherin adhesion complex (red) are up-regulated in the oocyte soon after it has been specified in region 2b and adhere to E-cadherin that is highly expressed in the follicle cells posterior to the oocyte. This adhesion ensures that the oocyte moves to the posterior of the cyst as the latter rounds up on entering region 3. (B–E) The relay model of oocyte positioning. (B) On leaving the germarium, the germline cyst expresses Delta (teal) to activate the Notch pathway in the Fringe-expressing polar/stalk precursor cells that contact it, inducing them to differentiate as polar cells (turquoise). Initially 2–4 anterior cells become polar cells before this number is reduced to two later in oogenesis. (C) The polar cells secrete the JAK/STAT pathway ligand, Unpaired (yellow), which induces the anterior polar/stalk cell precursors to differentiate as stalk cells (pale red) and up-regulate the E-cadherin adhesion complex (dark red). (D) The oocyte in the next younger cyst also up-regulates E-cadherin causing it to adhere to the adjacent stalk cells in the stalk between it and the next older cyst, thereby ensuring that it comes to lie posterior to the other germ cells as the cyst rounds up on entering region 3. (E) As the younger cyst exits the germarium, it starts to express Delta to induce the polar/stalk precursors that contact it to become polar cells. The cells on its posterior side have already developed as stalk cells and they are therefore delayed in their differentiation as polar cells.

One question raised by the positioning of the oocyte is why only the posterior follicle cells upregulate DE-cadherin, as the terminal follicle cells at each end of the egg chamber are equivalent until later in oogenesis when Gurken signalling specifies the terminal follicle cells in contact with the

oocyte to adopt a posterior fate, as discussed below (González-Reyes, Elliott, & St Johnston, 1995; Roth, Neuman-Silberberg, Barcelo, & Schüpbach, 1995). This paradox was resolved by the discovery of a relay mechanism in which each egg chamber induces the positioning of the oocyte in the next, younger egg chamber (Fig. 3) (Torres, Lopez-Schier, & St Johnston, 2003). When the follicle cells surround a germline cyst, the cells between adjacent cysts are destined to become either the stalk cells that form the stalk between adjacent cysts, or the polar cells, a pair of round cells at each end of the egg chamber (Tworoger, Larkin, Bryant, & Ruohola-Baker, 1999). These precursor cells express *fringe*, which modifies the Notch receptor to make it responsive to Delta (Grammont & Irvine, 2001). As the egg chamber leaves the germarium, the germline cyst activates Delta signalling, and this specifically induces the stalk/polar precursor cells that contact the germ line at each end of the egg chamber to become polar cells (Fig. 3B) (Lopez-Schier & St Johnston, 2001). However, this does not happen synchronously at each end of the egg chamber and the anterior polar cells differentiate before the posterior polar cells. The polar cells then express Unpaired, the ligand for the JAK/STAT pathway and this induces the adjacent stalk/polar precursors to differentiate to form the stalk between the cyst and the adjacent younger cyst (Fig. 3C) (McGregor, Xi, & Harrison, 2002; Torres et al., 2003). The stalk cells up-regulate E-cadherin at the same time as the oocyte of the next germline cyst, allowing the anterior stalk cells to adhere to the oocyte and induce its posterior positioning (Fig. 3D). These anterior stalk cells will subsequently become the posterior polar cells of the younger cyst once it has exited the germarium and activated Delta signalling (Fig. 3E). Thus, the oocyte is positioned by a transient population of anterior stalk cells before the cyst becomes symmetric again with polar cells at each end. The delay in specifying the posterior polar cells in each egg chamber results from the fact that these cells have already been exposed to Unpaired produced by the adjacent older cyst and have started to differentiate as stalk: as Delta/Notch and JAK/STAT signalling are antagonistic, it takes more Delta signalling to overcome the JAK/STAT signalling and induce polar fate posteriorly (Assa-Kunik, Torres, Schejter, St Johnston, & Shilo, 2007).

6. The polarising signal

At stage 6 of oogenesis, Delta signals from the germ line a second time to induce the follicle cells to exit the endocycle and differentiate

(Deng, Althauser, & Ruohola-Baker, 2001; Lopez-Schier & St Johnston, 2001). At this stage the epithelial follicle cells have already been divided into two distinct populations, the terminal follicle cells at each end of the egg chamber, which are specified by the same Unpaired signal from the polar cells that induces the stalk, and the mainbody follicle cells in the middle (McGregor et al., 2002). This temporal switch, as well as the initial polarisation and positioning of the oocyte set the scene for the establishment of the anterior-posterior axis the during stages 7–9 of oogenesis. As a consequence of oocyte polarisation, *gurken* mRNA is localised to the posterior of the oocyte with the nucleus and centrosomes and it is translated there to produce secreted Gurken protein. Gurken, which is a Transforming Growth Factor-α orthologue, activates the Epidermal Growth Factor receptor (EGFR) in the adjacent terminal follicle cells to induce them to adopt a posterior fate, rather than the default anterior fate, thereby imparting anterior-posterior polarity to the egg chamber (Fig. 4A) (González-Reyes & St Johnston, 1998a; González-Reyes et al., 1995; Roth et al., 1995). The posterior follicle cells then signal back to re-polarise the oocyte to define the anterior-posterior axis of the future embryo (Fig. 4B).

The nature of the polarising signal from the posterior follicle cells is still unknown despite more than 20 years of searching. Posterior follicle cell clones mutant for the catalytic subunit of the myosin phosphatase *flapwing* (PP1β) have increased Myosin activity, which disrupts signalling to polarise the oocyte (Sun, Yan, Denef, & Schupbach, 2011). This is in part due to a defect in Notch signalling, which impairs the differentiation of the posterior follicle cells, but even when this phenotype is rescued by expressing the intracellular domain of Notch, *flapwing* clones still disrupt anterior-posterior axis formation. It is unclear, however, why excessive Myosin activity disrupts signalling, although it is associated with a defect in endocytosis.

More recently, a microarray screen identified 317 genes that are up-regulated in the posterior follicle cells and RNAi knockdown of several of these produced anterior-posterior polarity defects in the oocyte (Wittes & Schupbach, 2019). However, the best candidate for a signal, Semaphorin 1b, did not produce a phenotype when knocked down by CRISPR, even when its paralogue Semaphorin 1a was also mutated. Thus, it is possible that multiple Sema domain containing proteins function redundantly as the polarising signal or that the RNAi polarity phenotypes result from off target effects.

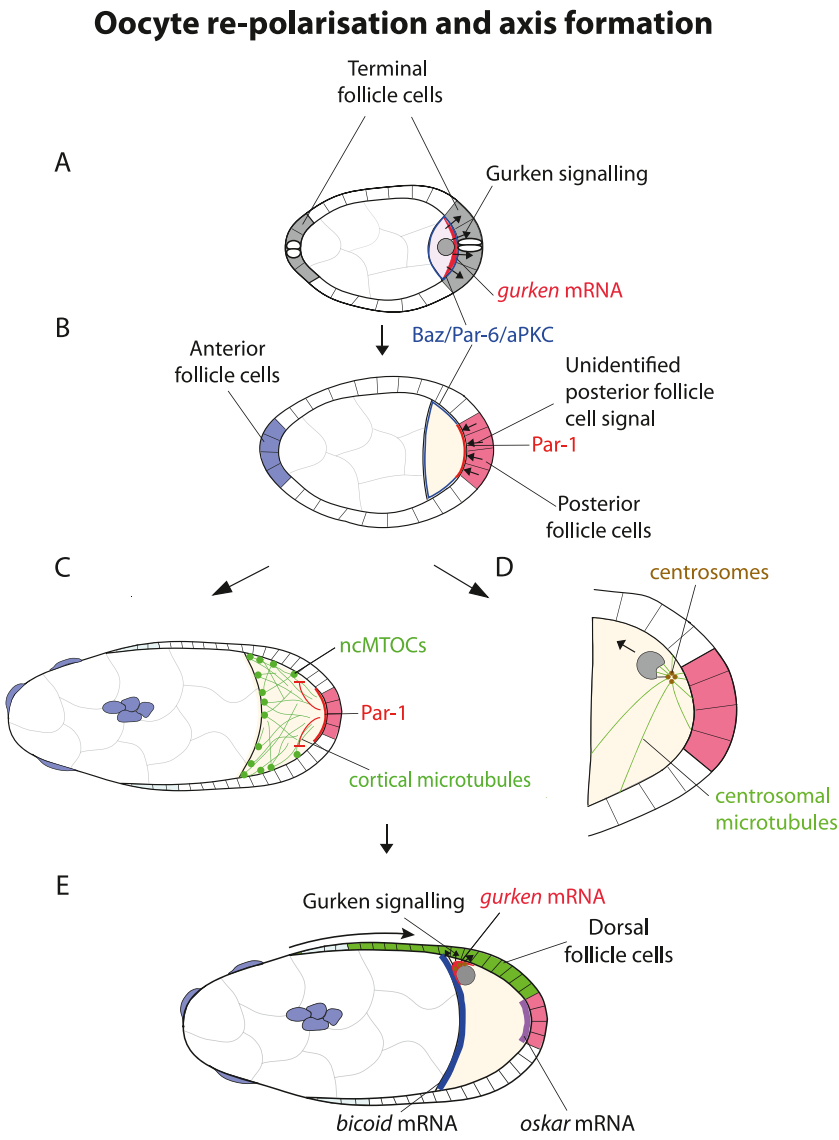

Fig. 4 Oocyte re-polarisation and axis formation. (A) Unpaired signalling from the polar cells at each end of the egg chamber induces the nearby cells to become terminal follicle cells (grey). At stage 6 of oogenesis, *gurken* mRNA (red) is translated at the posterior of the oocyte to produce Gurken protein, which signals to the adjacent follicle cells to induce them to adopt a posterior (pink) rather than an anterior fate (lilac). At this stage, the oocyte appears unpolarised, with Baz, Par-6 and aPKC (blue) localised all around the cortex. (B) The posterior follicle cells send an unknown signal back to polarise the oocyte. This induces the localisation of PAR-1 (red) to the

7. Polarising the anterior-posterior axis

Regardless of its nature, the follicle cell signal induces a re-polarisation of the oocyte that leads to the localisation of the anterior and posterior determinants, *bicoid* and *oskar* mRNAs at opposite ends of the oocyte (González-Reyes et al., 1995; Roth et al., 1995). This new polarity is specified by the localisation of Par-1 to the posterior cortex and Par-6, aPKC and Bazooka (Par-3) to the anterior and lateral cortex (Benton & St Johnston, 2003; Doerflinger et al., 2010; Doerflinger, Benton, Torres, Zwart, & St Johnston, 2006; Shulman, Benton, & St Johnston, 2000; Tomancak et al., 2000). Thus, the anterior-posterior polarity of the oocyte is defined by the same localisation of anterior and posterior PAR proteins as the C. elegans zygote, apart from PAR-2, which is not conserved (Nance & Zallen, 2011; St Johnston & Ahringer, 2010). Furthermore, these complementary cortical PAR domains are maintained by mutual antagonism between Par-1 and the Baz/Par-6/aPKC complex as they are in worms (Figs. 4B and 5C). Par-1 phosphorylates Bazooka to exclude it from the posterior cortex, while aPKC phosphorylates Par-1 to keep it from localising anterior/laterally (Benton & St Johnston, 2003; Doerflinger et al., 2010; Hurov, Watkins, & Piwnica-Worms, 2004; Suzuki et al., 2004). Nonphosphorylatable versions of Par-1 and Bazooka therefore extend all around the oocyte cortex and produce radially symmetric oocytes with opposite phenotypes. *oskar* mRNA localises all around Par-1^{T768A} expressing oocytes, indicating that the entire cortex has posterior identity, whereas the anterior determinant *bicoid* mRNA localises around the whole cortex of Baz^{S151A,S1085A} expressing oocytes (Doerflinger et al., 2010).

posterior cortex of the oocyte and the exclusion of Par-6 and aPKC from this region. (C) Par-1 excludes noncentrosomal microtubule organising centres (green circles) from the posterior cortex, resulting in the formation of a weakly polarised microtubule cytoskeleton with minus ends at the anterior and lateral cortex, with plus ends extending posteriorly. (D) The polarising signal from the posterior follicle cells also induces the release of the nucleus and centrosomes (light brown) from the posterior cortex of the oocyte and the growing microtubules from the centrosomes push the nucleus to the anterior of the oocyte. (E) The nucleus is anchored when reaches the anterior margin of the oocyte, and the centrosomes and *gurken* mRNA move around it to lie between the nucleus and the cortex. Gurken then signals for a second time to induce the main body follicle cells to adopt a dorsal (green) rather than a ventral fate as they migrate to cover the oocyte. The polarised cortical microtubule network defines the anterior posterior axis by directing the kinesin- dependent localisation of *oskar* mRNA (mauve) to the posterior cortex and the dynein-dependent localisation of *bicoid* mRNA (dark blue) to the anterior.

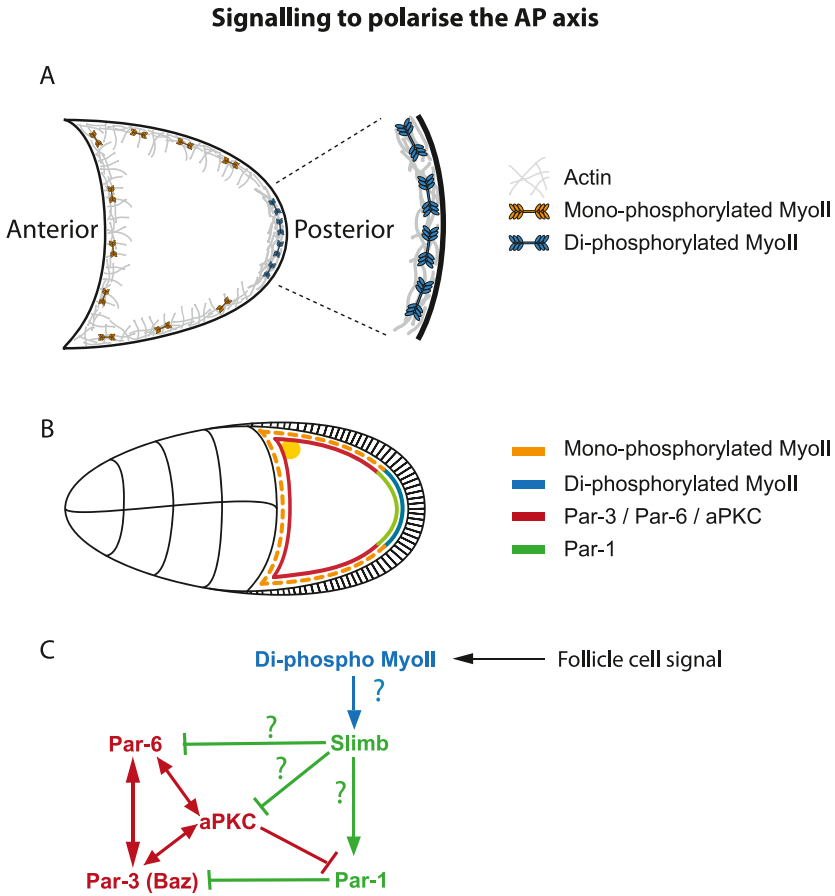

Fig. 5 Signalling to polarise the AP axis. (A) The posterior follicle cell signal induces the di-phosphorylation of the Myosin regulatory light chain (MRLC) at the posterior of the oocyte, resulting in longer pulses of Myosin activity that presumably increase cortical tension at the posterior. Mono-phosphorylated MRLC is found around the rest of the cortex, which has shorter Myosin pulses and lower tension. (B) Posterior MRLC di-phosphorylation induces the exclusion of PAR-6 and aPKC from the posterior cortex and the recruitment of PAR-1. (C) Models of the possible signalling pathways that lead to the posterior localisation of Par-1.

Interestingly, oocytes expressing both nonphosphorylatable constructs show the same phenotype as Par-1^{T768A} alone, indicating that Par-1 acts downstream of the anterior Par proteins in specifying oocyte polarity.

The localisation of *bicoid* and *oskar* mRNAs depends on the formation of a weakly polarised microtubule cytoskeleton, in which microtubules grow from microtubule organising centres (MTOCs) along the anterior and

lateral cortex of the oocyte, with more plus ends extending posteriorly (Clark, Giniger, Ruohola-Baker, Jan, & Jan, 1994; Clark, Jan, & Jan, 1997; Parton et al., 2011; Theurkauf, Smiley, Wong, & Alberts, 1992). This organisation provides the tracks for kinesin-1 to transport *oskar* mRNA to the posterior and for dynein to transport *bicoid* mRNA anteriorly (Brendza, Serbus, Duffy, & Saxton, 2000; Cha, Koppetsch, & Theurkauf, 2001; Duncan & Warrior, 2002; Januschke et al., 2002; Trovisco et al., 2016; Zimyanin et al., 2008). Par-1 plays a key role in organising the microtubules by excluding the spectraplakin Short stop (Shot) from the posterior cortex (Fig. 4C) (Nashchekin, Fernandes, & St Johnston, 2016). Shot therefore localises along the anterior and lateral cortex through its N-terminal actin binding domain and recruits the microtubule minus end-binding protein Patronin to form noncentrosomal MTOCs that nucleate most microtubules in the oocyte. Indeed, simply forming a gradient of ncMTOCs along the anterior and lateral cortex is sufficient to account for the observed microtubule organisation and *bicoid* and *oskar* mRNA localisations in wildtype and polarity mutants (Trong, Doerflinger, Dunkel, St Johnston, & Goldstein, 2015).

Much less is known about how the cortical asymmetry of the Par proteins is first established in response to the posterior follicle cell signal. Prior to the signal, Par-6, aPKC and Bazooka are uniformly distributed around the oocyte cortex, whereas Par-1 is largely cytoplasmic (Doerflinger et al., 2006; Doerflinger et al., 2010). This symmetry is broken by the recruitment of Par-1 to the posterior cortex at stage 7 and the concomitant disappearance of Par-6 and aPKC from this region, in a process that depends on the polarising follicle cell signal and cortical F-actin (Doerflinger et al., 2006; Doerflinger et al., 2010). Bazooka persists at the posterior until stage 9, indicating that the loss of Par-6 and aPKC from this region is not driven by the phosphorylation and exclusion of Baz by Par-1. The clearance of Par-6 and aPKC from the posterior cortex and the recruitment of Par-1 require Slimb, the substrate recognition subunit of the SCF Ubiquitin ligase (Morais-de-Sá, Mukherjee, Lowe, & St Johnston, 2014). Furthermore, the levels of aPKC and Par-6 are increased in *slimb* mutants, whereas Par-1 and Baz are unaffected. This suggests a simple model, in which the polarising signal induces the ubiquitination of Par-6, aPKC or an unidentified component of the complex to trigger its degradation and abolish aPKC activity at the posterior. This in turn would allow Par-1 to localise posteriorly, where it can phosphorylate and exclude Bazooka, thereby reinforcing polarity by removing the anchor for Par-6 and aPKC.

It has recently been discovered that activation of nonmuscle myosin (Myosin II) also plays a key role in polarising the oocyte (Doerflinger, Zimyanin, & St Johnston, 2021). Myosin is activated by the phosphorylation of Serine 21 in the myosin regulatory light chain (MRLC; Sqh in Drosophila), but the MRLC can also phosphorylated on Threonine 20, although the effects of this second phosphorylation are unclear (Heissler & Sellers, 2014; Vasquez, Heissler, Billington, Sellers, & Martin, 2016). Both Serine 21 and Threonine 20 of the MRLC are phosphorylated specifically at the posterior of the oocyte in response to the polarising follicle cell signal (Fig. 5A). This induces larger foci of Myosin that persist for longer, suggesting that Myosin generates more force and hence cortical tension at the posterior (Doerflinger et al., 2021). Furthermore, reducing the force generated by Myosin or reducing the amount of MRLC-2P by overexpressing a form of MRLC in which the Threonine is changed to a non-phosphorylatable Alanine impairs the posterior recruitment of Par-1. Thus, the second phosphorylation of MRLC on Threonine 20 increases the lifetime of Myosin foci and is required for oocyte polarisation. MRLC phosphorylation is not affected by *slimb* mutants, indicating that Myosin activation acts upstream of Slimb to re-polarise the oocyte. How the unknown follicle cell signal leads to Myosin activation and how this is transduced via Slimb to remove aPKC activity and recruit Par-1 at the posterior cortex are currently unknown (Fig. 5B and C).

8. Polarising the dorsal ventral-axis

While the anterior-posterior axis is ultimately determined by the architecture of the ovariole and the positioning of the oocyte at the posterior of the germline cyst, the dorsal-ventral axis is a true symmetry-breaking event. At stage 6 of oogenesis, *gurken* mRNA is localised at the posterior of the oocyte with the nucleus, but the polarising follicle cell signal triggers the movement of the nucleus and *gurken* mRNA to a point on the anterior margin of the oocyte, where Gurken signals a second time to induce the adjacent mainbody follicle cells to adopt a dorsal rather than a ventral fate (Fig. 4E) (González-Reyes et al., 1995; Roth et al., 1995). This specifies the dorsal-ventral axis of the embryo because the ventral follicle cells express Pipe, which acts as the cue that initiates Toll signalling to induce the formation of the nuclear concentration gradient of the Dorsal morphogen (Roth et al., 1989; Sen et al., 1998; St Johnston & Nüsslein-

Volhard, 1992). Since the main-body follicle cells are all equivalent before Gurken signalling, and there are no markers that predict where the nucleus will migrate to on the anterior margin of the oocyte, it is thought that the movement is random. Further support for this view comes from the observation that in oocytes with two nuclei, the nuclei migrate to random positions with respect to each other (Roth, Jordan, & Karess, 1999).

The nucleus is pushed towards the anterior by the force exerted on it by growing microtubules that emanate from the centrosomes, which are positioned at the posterior cortex during the initial polarisation of the oocyte (Fig. 4D) (Tissot et al., 2017; Zhao, Graham, Raposo, & St Johnston, 2012). Although the centrosomes are largely inactive early in oogenesis, they nucleate growing microtubules that create an indentation on the posterior side of the nucleus both before and after the nucleus starts to move anteriorly (Zhao et al., 2012). Both the nucleus and centrosomes must therefore be tethered in some way to the posterior cortex before migration, with the polarising signal acting to break this tether. Since the centrosomes move around the nucleus once it reaches the anterior and come to lie between the nucleus and the dorsal cortex, the nucleus must also be anchored at the anterior to counteract the pushing force exerted by the centrosomal microtubules. This anchoring depends on several dynein-associated factors, suggesting that the microtubule minus end-directed motor, dynein exerts pulling forces to counteract the pushing forces from the growing microtubules (Duncan & Warrior, 2002; Januschke et al., 2002; Lei & Warrior, 2000; Swan & Suter, 1996; Swan, Nguyen, & Suter, 1999). If the anchoring of the nucleus at the posterior and dorsal/anterior cortex occur by the same mechanism, one attractive possibility is that the release and migration of the nucleus is induced simply by inactivating dynein in response to the polarising signal and then reactivating it once the nucleus has reached the anterior margin of the oocyte.

9. Concluding remarks

The development of the egg chamber is intricate and highly-coordinated process, in which each polarised event depends on the successful and timely completion of all the steps that precede it (Fig. 6). The asymmetric division of the germline stem cell not only creates the cystoblast that gives rise to a new germline cyst, but also generates the asymmetry in the segregation of the spectrosome that ensures that one cell in the 16-cell cyst

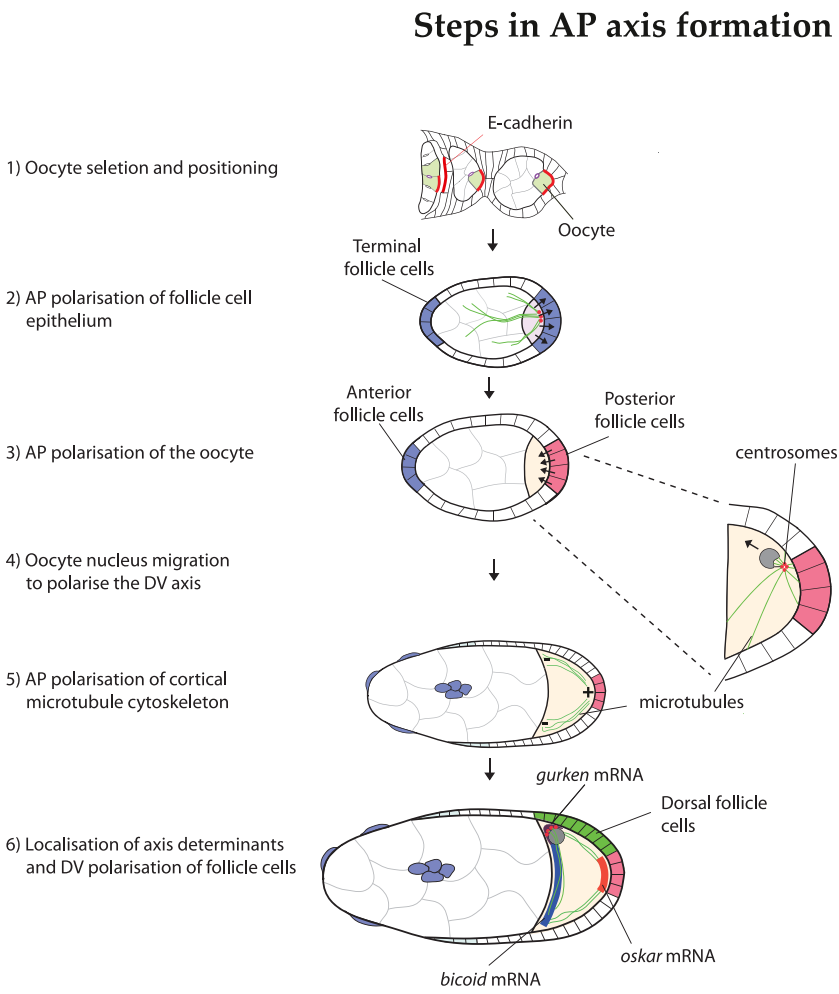

Fig. 6 A summary of the symmetry-breaking steps in *Drosophila* axis formation.

will have more fusome material than the others, initiating the positive feedback loop that specifies the oocyte. Oocyte polarisation and selection are in turn essential preconditions for the positioning of the oocyte at the posterior of the cyst, a process that also depends on the signalling to pattern the polar and stalk cells in the adjacent older cyst in the ovariole. Both the polarisation of the oocyte in the germarium and its positioning at the posterior of the cyst ensure that *gurken* mRNA is localised at the oocyte cortex facing the adjacent terminal cells, so that Gurken is in the correct place to induce them to adopt a posterior fate. Finally, the initial oocyte

polarity positions the centrosomes behind the nucleus at the posterior of the oocyte, so that they are in the correct position nucleate the microtubules that push the nucleus towards the anterior in response to the polarising signal from the posterior follicle cells.

Three of the steps in this process correspond to classical cell polarisation. The germline stem cell is polarised by its adhesion to the cap cells and by its exposure to Dpp and Gbb on its anterior processes, although little is known about how these cues position of the spectrosome to orient the mitotic spindle (Yadlapalli & Yamashita, 2012). The initial polarisation of the oocyte in the germarium is also mysterious: it involves the canonical polarity factors of the Baz/Par-6/aPKC complex and Par-1, but they do not appear to regulate each other as in other polarised cells, and nothing is known about how they mediate the posterior movement of the ncMTOCs and centrosomes. By contrast, anterior-posterior axis formation in the oocyte in response to the signal from the posterior follicle cells shares several common features with anterior-posterior axis formation in the *C. elegans* zygote. In both cases, Par-3, Par-6 and aPKC localise to the anterior cortex and Par-1 the posterior; both involve the mutual antagonism between the anterior and posterior PAR proteins; and Myosin acts upstream of the PAR proteins in each system to establish polarity (Lang & Munro, 2017; Motegi & Seydoux, 2013).

Nevertheless, there are several crucial differences between polarity in the *Drosophila* oocyte and the *C. elegans* zygote. Firstly, Myosin is inhibited at the posterior of the worm zygote in response to sperm entry, triggering a cortical contraction towards the anterior that polarises the AP axis by dragging the anterior PAR proteins away from the posterior through advection (Cheeks et al., 2004; Munro, Nance, & Priess, 2004). By contrast, Myosin is activated at the posterior of the fly oocyte without inducing any observable cortical movement, where it acts to recruit Par-1 rather than the anterior PARs. Secondly, the relative size of the anterior and posterior domains in the worm zygote is determined by the relative amounts of anterior and posterior PARs and the mutual antagonism between them (Goehring et al., 2011; Lang & Munro, 2017; Sailer, Anneken, Li, Lee, & Munro, 2015). The boundary between the anterior and posterior PAR domains is not established this way in the fly oocyte, however, and is determined instead by the size of the contact region between the oocyte and the posterior follicle cells. Furthermore, mutual antagonism between PAR proteins is not sufficient to maintain the complementary anterior and posterior domains in

the oocyte, since acute inhibition of MRLC phosphorylation causes a rapid and reversible loss of the posterior Par-1 domain after it has formed (Doerflinger et al., 2021). Thus, the posterior pole of the oocyte seems to be defined by continuous signalling from the posterior follicle cells, rather than having distinct establishment and maintenance phases. These differences most likely reflect the different states of the fly oocyte and worm zygote. The former is arrested in meiotic prophase and has many hours to polarise and localise the determinants in response to a stable positional cue, whereas the latter must respond to the transient cue provided by sperm entry and establish polarity before the first cell division. This demonstrates the versatility of the PAR system in responding to different signals to polarise cells in different ways depending on the developmental context.

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