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The Origin of Asymmetry: Early Polarisation of the *Drosophila* Germline Cyst and Oocyte

Review

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The anterior–posterior axis of *Drosophila* is established before fertilisation when the oocyte becomes polarised to direct the localisation of *bicoid* and *oskar* mRNAs to opposite poles of the egg. Here we review recent results that reveal that the oocyte acquires polarity much earlier than previously thought, at the time when it acquires its fate. The oocyte arises from a 16-cell germline cyst, and its selection and the initial cue for its polarisation are controlled by the asymmetric segregation of a germline specific organelle called the fusome. Several different downstream pathways then interpret this asymmetry to restrict distinct aspects of oocyte identity to this cell. Mutations in any of the six conserved Par proteins disrupt the early polarisation of the oocyte and lead to a failure to maintain its identity. Surprisingly, mutations affecting the control of the mitotic or meiotic cell cycle also lead to a failure to maintain the oocyte fate, indicating crosstalk between the nuclear and cytoplasmic events of oocyte differentiation. The early polarity of the oocyte initiates a series of reciprocal signaling events between the oocyte and the somatic follicle cells that leads to a reversal of oocyte polarity later in oogenesis, which defines the anterior–posterior axis of the embryo.

Introduction

In many invertebrates and vertebrates, one or more of the body axes are already set up in the egg [1]. For instance, the anterior–posterior and dorsal–ventral axes in *Drosophila* are established during oogenesis by the asymmetric localisation of *bicoid*, *oskar,* and *gurken* mRNAs within the oocyte (the future egg) [2,3]. The localisation of these transcripts depends on the polarised organization of the oocyte cytoskeleton, and, therefore, on the polarity of the oocyte itself. Recent data show that the first asymmetry that leads to this polarisation of the oocyte can be traced back to earlier and earlier stages of oogenesis, ultimately leading to the very first steps at which the oocyte is determined.

The determination of the *Drosophila* oocyte has puzzled biologists for more than a century, because it arises from a syncytium that is formed by four rounds of incomplete division of a single germ cell, producing a germline cyst of 16 sister cells that share the same cytoplasm [4,5]. Only one cell will become the oocyte, however, while the other 15 cells differentiate as nurse cells, which provide the oocyte with nutrients and cytoplasmic components. This means that the selection of the oocyte can be viewed as the polarisation of the cyst cytoplasm and membrane [6]. In addition, the oocyte may inherit some of its polarity from this cyst asymmetry. The determination and polarisation of the oocyte are, therefore, two intimately linked issues, which will be the main focus of this review.

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Early Oogenesis in *Drosophila*

The *Drosophila* ovary is composed of 16–20 ovarioles, each of which contains a chain of progressively more and more mature egg chambers [7]. New egg chambers are generated at the anterior of the ovariole in a region called the germarium, which has been divided into four regions according to the developmental stage of the cyst (Figure 1). Oogenesis begins in region 1, when a germline stem cell divides asymmetrically to produce a posterior cystoblast, and a new germline stem cell, which remains attached to the neighboring somatic cells at the anterior (for reviews see [8,9]). The cystoblast then undergoes precisely four rounds of mitosis with incomplete cytokinesis to form a cyst of 16 germline cells, which are interconnected by stable cytoplasmic bridges called 'ring canals'. During these divisions, a cytoplasmic structure called the fusome anchors one pole of each mitotic spindle (see movie 1 and 2 in supplemental data) and, therefore, ensures that cells follow an invariant pattern of division [10]. This leads to the formation of a symmetric cyst comprising two cells with four ring canals, two with three ring canals, four with two and eight with one. This invariant pattern is important, as the oocyte always differentiates from one of the two cells with four ring canals, which are, therefore, called the pro-oocytes [11].

Once the 16 cell cyst has formed, it enters region 2a of the germarium. At this stage, all the cells of one cyst look the same, but by the time it reaches region 2b, one cell will have differentiated as an oocyte. This differentiation can be followed with several types of marker (Figure 1): First, oocyte-specific proteins, such as BicD, Orb, Btz and Cup and mRNAs, such as *osk*, *BicD* and *orb* first concentrate in the two pro-oocytes, and come to lie on either side of the largest ring canal that connects them. By the end of region 2a, they accumulate just in the oocyte [12–17]. Live imaging of GFP-labeled mitochondria reveals that they show a similar pattern of localisation to one cell [18]. Second, microtubules are initially diffusely distributed throughout the cyst in association with the fusome, but their minus ends gradually become restricted to the future oocyte [19,20]. Third, the centrioles appear to be inactivated after the last mitotic division, and they migrate along the fusome into

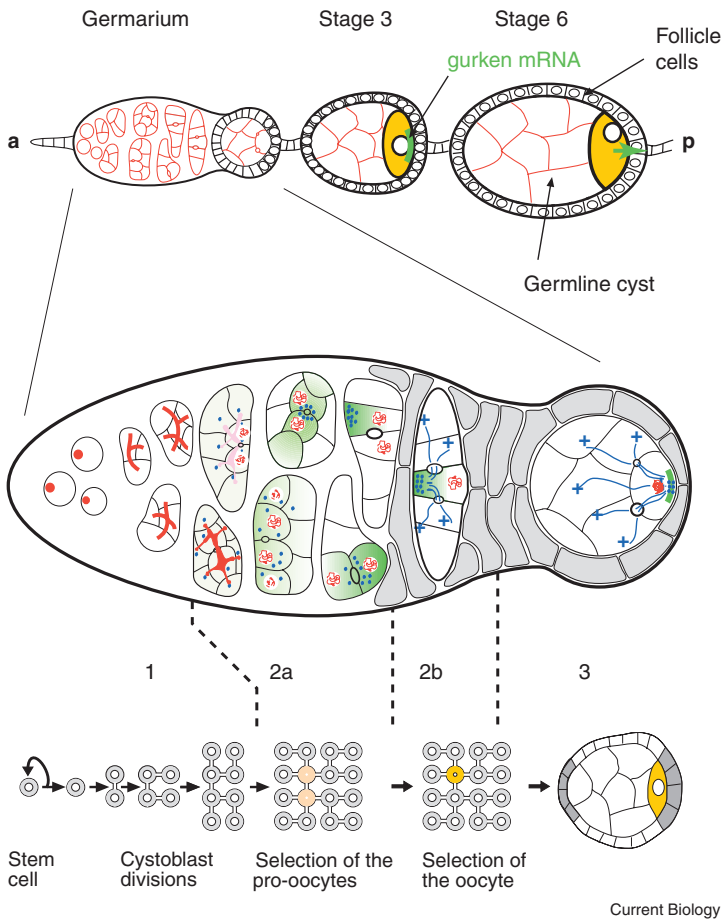

Figure 1. *Drosophila* early oogenesis.
Each ovariole is made of a chain of progressively more mature egg chambers toward the posterior (p). An egg chamber comprises 16 germline cells surrounded by a monolayer of follicle cells. The egg chambers are produced in a specialized structure, called the germarium, at the anterior (a) of the ovariole. The germarium is divided into four morphological regions along the anterior–posterior axis. The germline stem cells reside at the anterior tip of the germarium (left) and divide to produce cystoblasts, which divide four more times in region 1 to produce 16 cell germline cysts that are connected by ring canals. The stem cells and cystoblasts contain a spectrosome (red circles), which develops into a branched structure called the fusome, which orients each division of the cyst. In early region 2a, the synaptonemal complex (red lines) forms along the chromosomes of the two cells with four ring canals (pro-oocytes, yellow) as they enter meiosis. The synaptonemal complex then appears transiently in the two cells with three ring canals, before becoming restricted to the pro-oocytes in late region 2a. By region 2b, the oocyte has been selected, and is the only cell to remain in meiosis. In region 2a, cytoplasmic proteins, mRNAs and mitochondria (green), and the centrosomes (blue circles) progressively accumulate at the anterior of the oocyte. In region 2b, the minus-ends of the microtubules are focused in the oocyte, and the plus-ends extend through the ring canals into the nurse cells. The follicle cells (gray) also start to migrate and surround the germline cells. As the cyst moves down to region 3, the oocyte adheres strongly to the posterior follicle cells and repolarises along its anterior–posterior axis, with the microtubule minus-ends and specific cytoplasmic components now localized at the posterior cortex.

the pro-oocytes, and then into the oocyte [18–20]. Fourth, although the oocyte is the only cell to go through meiosis, electron microscopic studies show that the other pro-oocyte also enters meiotic prophase, and reaches the pachytene stage before becoming a nurse cell, while the two cells with 3 ring canals reach the zygotene stage [21]. This can be followed with antibodies against components of the synaptonemal complex, a proteinaceous structure that forms between paired homologous chromosomes [22–24].

In region 2b, the cyst changes shape and becomes a one cell-thick disc that spans the whole width of the germarium. Oocyte-specific factors now become concentrated in the oocyte and a microtubule organising centre (MTOC) is clearly detectable. At the same time, somatic follicle cells start to migrate and surround the cyst. As the cyst moves down to region 3 (also called stage 1), the mitochondria, centrosomes, Golgi vesicles, proteins and mRNAs organize at the anterior of the oocyte to form a typical Balbiani body [18–20]. At the same time, the cyst rounds up to form a sphere with the oocyte always lying at the posterior pole. The cyst then leaves the germarium and enters the vitellarium. The oocyte becomes polarised, as proteins, mRNAs, centrosomes and a subset of the mitochondria move to the posterior cortex, its DNA becomes highly condensed to form a structure called the karyosome, whereas the nurse cells start to polyploidize [25,26].

Formation of a Polarised Cyst

The Fusome and Determination of the Oocyte

Two main models have been proposed to explain how the oocyte is selected. One model is based on the symmetrical behavior of the two pro-oocytes until mid-late region 2a, and proposes that there is a competition between the two pro-oocytes to become the oocyte [21,27]. The ‘winning’ cell would become the oocyte, while the ‘losing’ cell would revert to the nurse cell fate. A second model suggests that the choice of the oocyte is biased by the establishment of some asymmetry as early as the first cystoblast division, which is maintained until the overt differentiation of the oocyte [6,28]. The role played by the fusome and the analysis of its formation provide the stongest evidence in support of this model [28–32].

The fusome arises from a spherical structure called the spectrosome in the germline stem cell, which is made of small membranous vesicles kept together by components of the sub-membraneous cytoskeleton, such as α-spectrin, β-spectrin, and Hts (an adducin-like

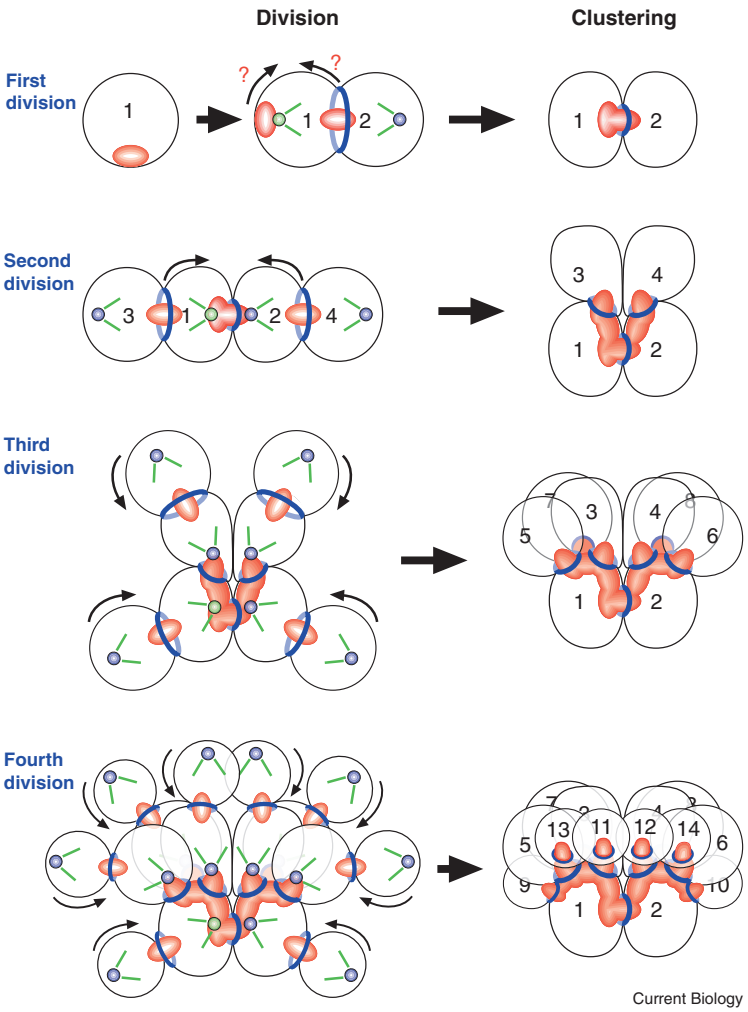

Figure 2. Formation of the fusome.
During the first incomplete division, the spectrosome (red) of the cystoblast interacts with one of the centrosomes (green and blue spheres) to anchor one pole of the mitotic spindle (green lines). A fusome plug (red) forms in the arrested furrow or ring canal (blue). The spectrosome (or 'original' fusome) and the fusome plug come together to fuse. The direction of these movements is not known. The same mechanism is repeated for the second, third and fourth division: first, one pole of each mitotic spindle is anchored by the fusome and a new fusome plug forms in each ring canal. Then the ring canals move centripetally for the fusome plugs to fuse with the central fusome (black curved arrows). This behavior has several crucial consequences: cystocyte 1 has more fusome than the other cystocytes; the same centrosome (green sphere) could be inherited by cystocyte 1 from the first division through the fourth division; and the fusome always marks the anterior of cystocyte 1, after the clustering of the ring canals.

protein) [10,33]. The cystoblast inherits one third of the fusome from the GSC [30]. When the cystoblast divides, one pole of the spindle is anchored by the inherited fusome (the 'original' fusome), while a new fusome plug forms in the arrested ring canal, at the opposite pole of the cell [29,32]. The two fusomes then come together to fuse, so that one cell contains the 'original' fusome plus half of the plug, whereas the other cell only retains the other half of the fusome plug (Figure 2). This asymmetric behaviour of the fusome is then repeated during the next three divisions [32]. The oldest cell, therefore, retains the 'original' fusome and accumulates three more fusome plugs. Thus, this cell has more fusome than all of the other cells and can be identified throughout the divisions.

Unfortunately, most of the fusome has already degenerated by the time oocyte-specific proteins such as BicD or Orb accumulate in a single cell in late region 2a. However, the preferential accumulation of the centrosomes as well as of *osk* and *orb* mRNAs in one cell can be detected earlier in region 2a, and this is always the cell with the most fusome [18,19]. This is particularly obvious in *egl* and *BicD* mutants, in which the fusome perdures longer, and the centrosomes clearly accumulate in the cell with the largest piece of fusome remnant [20]. These data strongly suggest that the 'original' fusome marks the future oocyte, thus supporting the second model. It does not rule out the possibility that both pro-oocytes can become the oocyte, but shows that if there is a competition, it is strongly biased.

What is the link between the asymmetric inheritance of the fusome and the specification of the oocyte? The simplest model is that an oocyte-determining factor is asymmetrically distributed at each division with the 'original' fusome into the future oocyte. It has been proposed that one of the cystoblast centrioles could stay in contact with the fusome during each division, and, because of the semi-conservative replication of the centrosome, could be inherited by the oocyte [6]. Consequently, oocyte determinants could co-segregate with this centriole. Indeed, it has recently been shown that such a mechanism mediates the segregation of *dpp* and *eve* mRNAs into specific cells during the asymmetric divisions of the early embryo of the snail *Ilyanassa obsoleta* [34]. Alternatively, the oocyte could inherit more of some protein or activity associated with the fusome, and this early bias could initiate a feedback loop that induces the transport of oocyte determinants toward this cell.

The Fusome and the Polarisation of the Oocyte

It is not known what drives the movement of the fusome plugs, but several lines of evidence suggest that it is microtubule-dependent. Mutations in the microtubule minus-end directed motor, Dynein, or in its associated regulator, Lis1, affect the formation of the fusome, which appears less branched, and mutant cysts contain fewer than 16 cells [20,31,35]. It is not yet clear, however, if these mutations affect the movement and formation of the plugs, or the anchoring of the mitotic spindles to the fusome, or both. Because of the centripetal gathering of the ring canals, the cyst adopts a 'rosette' shape [4]. In this configuration, the distance between the ring canals is minimized [32]. This shape is later on stabilized by the formation of adherens junctions around the ring canals, which can be visualized in EM sections and by the localisation of Armadillo, E-Cadherin and Bazooka/Par-3 proteins ([36] and A.P. Mahowald, personal communication).

It is reasonable to assume that as early as the two-cell stage, the central fusome occupies a fixed position, while the plugs move towards it. The fusome, therefore, marks the 'inner' part of each cell. In particular in region 2b, the fusome remnant is always located at the anterior of the oocyte, where all the cytoplasmic components accumulate to form a Balbiani body [18]. The fusome thus marks the future anterior side of the oocyte, suggesting that the anterior–posterior polarity of the oocyte is inherited from the polarised cyst divisions.

In conclusion, the fusome marks the future oocyte and also the future anterior side of the oocyte, strongly suggesting that it plays a direct role in the specification and polarisation of the oocyte. This has been difficult to prove, however, because of the earlier functions of the fusome. For example, *hts* and α-*spectrin* mutants lack a fusome and often fail to specify an oocyte, but the divisions also become asynchronous and randomly oriented, resulting in cysts with a variable number of cells, and this latter defect could be the primary cause of the failure in oocyte determination [10,33,37].

The Read-Out of Fusome Polarity: Nurse Cell Versus Oocyte Differentiation

Although the oocyte appears to be selected early in region 1, its identity only becomes obvious two days later, in late region 2a. The differentiation of the cyst in region 2a is gradual and twofold. In the cytoplasm, the oocyte accumulates specific components and organelles, and in the nucleus, it enters meiosis and arrests in prophase I. Recent results have revealed that there are at least three pathways to restrict different aspects of oocyte identity to one cell.

Cytoplasmic Differentiation

One of the main features of cyst differentiation is the formation of a microtubule array that is polarised toward the oocyte and extends through the ring canals into the other cells of the cyst [38]. As the microtubules form along the fusome, this polarisation has been suggested to be a direct readout of fusome polarity [19]. Indeed, microtubules are essential for the determination of the oocyte, as treatment with the microtubule depolymerising drug, colchicine, results in egg chambers with 16 nurse cells and no oocyte [39]. Furthermore, none of the oocyte-specific proteins or mRNAs is asymmetrically localized within these cysts [38]. Thus, the transport of these proteins and mRNAs is microtubule-dependent, suggesting that the formation of the polarised microtubule cytoskeleton precedes and predicts the specification of the oocyte.

Although colchicine disrupts the localisation of proteins and mRNAs to the oocyte, it does not disrupt the migration of the centrioles or the restriction of meiosis to one cell [20,22]. Because microtubule-destabilizing drugs like colchicine only affect dynamic microtubules, one possibility is that the centrioles migrate along stable microtubules that are not affected by these treatments. In support of this view, it has recently been found that antibodies against acetylated tubulin, a marker for stable microtubules, label a population of microtubules associated with the fusome [40]. Furthermore, mutations in the *Drosophila* spectroplakin Shot disrupt these stable microtubules without affecting the fusome itself, and this blocks the migration of the centrioles as well as of mRNAs and proteins into one cell. Shot is a component of the fusome, and contains a GAS-domain, which has been shown to bind and stabilize microtubules. This suggests a model in which Shot assembles stable microtubules on the fusome, along which the centrioles migrate. The situation is less clear for the restriction of meiosis, as it is only partially disrupted in *shot* mutant germline clones. This raises the possibility that a third microtubule-independent pathway directly reads the fusome polarity to control entry into meiosis (Figure 3).

Independent evidence for three distinct pathways that restrict different aspects of oocyte identity comes from the analysis of the phenotypes of *Bicaudal-D* (*BicD*) and *egalitarian* (*egl*) mutants. Egl and BicD proteins co-purify with each other, and null mutations in either gene give rise to egg chambers with 16 nurse cells and no oocyte, without affecting the asymmetric distribution of the fusome [20,32,41–43]. Oocyte-specific proteins and mRNAs remain evenly distributed within mutant cysts, and this defect correlates with a failure to polarize the microtubule cytoskeleton, as observed with GFP-tubulin and several microtubule minus-end markers. However, the migration of the centrioles is normal in *BicD* and *egl* null mutants, indicating that these genes act downstream of the initial asymmetry that selects the oocyte [20]. This provides further evidence that the movement of the centrioles occurs by a mechanism that is distinct from the transport of mRNAs and proteins.

Although *egl* and *BicD* cause identical defects on mRNA and protein localisation and MT organization, they have opposite effects on meiosis. In *egl* mutants, all 16 cells of the cyst enter meiosis and reach the full pachytene stage, before they revert to the nurse cell fate [22,27]. In contrast, none of the cells enters meiosis in *BicD* mutants, and they all become nurse cells directly [22]. Thus, an upstream asymmetry must regulate the activity of the BicD/Egl complex to promote meiosis in the oocyte and inhibit it in the nurse cells.

In addition to its role in oocyte determination, the BicD/Egl complex is required for the localisation of

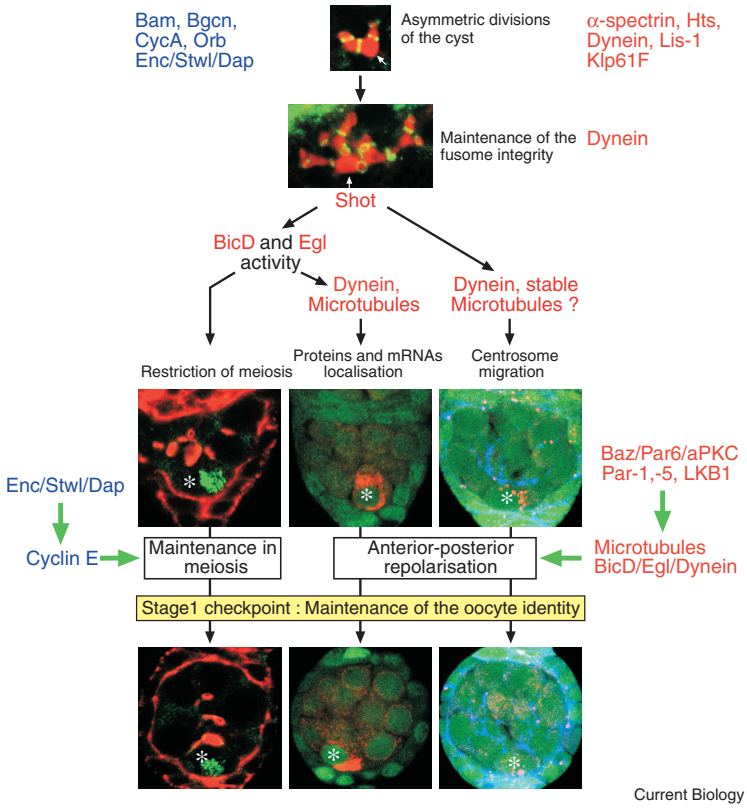

Figure 3. The early steps in the determination and polarisation of the oocyte.
The early differentiation of the oocyte is a multistep process. Genes involved at each step are indicated though the list is not exhaustive. Regulators of cytoplasmic differentiation are shown in red, whereas regulators of the cell cycle are shown in blue. The top panel shows a 4-cell cyst (α-spectrin, in red, marks the fusome and anilin, in green, marks the ring canals). One cell has more fusome than the other cells (white arrow). The panel below shows a 16-cell cyst after the last division with one cell having more fusome (white arrow). There are three different pathways to restrict oocyte identity to one cell (asterisk). The left panels show the actin in red and the synaptonemal complex in green. In the middle panels, nuclear GFP is shown in green, and Orb (an oocyte-specific cytoplasmic protein) is labelled in red. On the right panels, γ-tubulin marks the centrosomes in red, α-spectrin marks the fusome in blue, and nuclear GFP is shown in green. Orb and the centrosomes are clearly seen migrating from the anterior of the oocyte to the posterior, revealing the repolarisation of the oocyte.

oskar, K10 and *gurken* mRNAs in the stage 9–10 oocyte, and for the apical transport of *wingless* and pair rule transcripts in the syncytial blastoderm embryo [43–45]. Injection experiments in the embryo have demonstrated that BicD and Egl are required to recruit the dynein motor complex to mRNAs, so that it can transport them to the apical cytoplasm [45,46]. Consistent with this, a conserved amino-terminal domain of the mammalian homologs of BicD has been shown to bind the dynamitin subunit of the dynein/dynactin complex and to activate dynein motility, while a carboxy-terminal region binds to GTP-Rab6, and acts as an adaptor for the dynein-dependent transport of Rab6 positive Golgi membranes [47–49].

Very recent results indicate that Egl also plays a role in coupling cargoes to the dynein motor complex, as a newly identified carboxy-terminal domain of the protein has been shown to bind the dynein light chain [50]. Furthermore, *egl* mutations that specifically disrupt this binding site abolish mRNA and protein localisation to the oocyte, as do mutants in the *dynein light chain* (*dlc*) itself. These results suggest that Egl is an essential adaptor that links the dynein motor complex to the mRNAs and proteins that are transported into the oocyte. Surprisingly, in contrast to the *egl* null mutations, neither the *egl* alleles that disrupt the interaction with the Dlc nor *dlc* mutants affect the restriction of meiosis to one cell. These data strongly support the idea that Egl has two separable functions in oocyte selection, an early dynein-independent function that controls meiosis, and a later function in the dynein-dependent transport of mRNAs and proteins into the oocyte.

Germline clones mutant for specific alleles of the dynein–dynactin complex, such as *Dhc64C*$^{4\text{-}19}$, *Lis1*^{E415}, and *dynamitin*^{K16109} have similar phenotypes to *BicD* and *egl,* as they give rise to egg chambers with 16 nurse cells and no oocyte [20,31,35,51,52]. However, the requirements for the BicD–Egl and dynein–dynactin complexes are not identical during early oogenesis. Firstly, the Dynein heavy chain (Dhc) and Lis1 are required during the cyst divisions in region 1, whereas BicD and Egl are not. Secondly, unlike BicD and Egl, Dhc is required for the migration of the centrioles into the oocyte, as well as the localisation of meiosis and oocyte-specific mRNAs and proteins [20]. This observation suggests that dynein may transport the centrioles into the oocyte along stable microtubules, in addition to moving mRNAs and proteins along colchicine-sensitive microtubules. These results need to be interpreted with caution, however, as *Dhc* mutants also disrupt the fusome, which starts to fragment when the cyst enters region 2a. This may be the primary cause of the total lack of polarisation in *dynein* germline clones [20].

In summary, although it was originally thought that the oocyte was specified by the transport of determinants along a single polarised microtubule cytoskeleton, recent results have uncovered a more complex reality, in which multiple processes function in parallel to restrict different aspects of oocyte identity to one cell. However, all of these processes probably depend on the initial polarity of the fusome, which may act in three distinct ways to select the oocyte: First, the fusome organizes a polarised network of dynamic microtubules that direct the localisation of

oocyte-specific proteins and mRNAs into one cell, presumably through the Dynein-dependent transport of cargoes that are linked to the motor through BicD, Egl and the Dynein light chain. Second, the fusome also nucleates stable microtubules that are associated with Shot, and the centrioles may migrate along these in a process that could also involve Dynein. Finally, the fusome appears to regulate a Dynein light chain and microtubule-independent activity of the BicD/Egl complex that controls entry into meiosis. Although the molecular mechanisms are only emerging, these results also suggest an exciting link between oocyte differentiation, vesicular trafficking and mRNA transport.

Nuclear Differentiation

The differentiation of the oocyte is marked by several cell cycle changes and as the future female gamete, the oocyte is the only cell to complete meiosis. In contrast, the nurse cells go through several rounds of endoreplication without mitosis to become highly polyploid. Interestingly, recent results show that cell cycle regulators also control the identity of the oocyte.

Cell-Cycle Regulators and Maintenance of the Oocyte Fate

The first link between the control of the cell cycle and the identity of the oocyte came from the analysis of mutations in *encore*, which encodes a novel protein with a putative RNA binding domain [53,54]. At 25°C, one group of alleles induces precisely one more division in the germline, producing cysts with 32 cells and one oocyte. At 18°C, a second group of alleles produce cysts with 16 polyploid nurse cells and no oocyte. However, in these cysts *oskar* mRNA initially accumulates into only one cell, but then diffuses away [53]. This demonstrates that the initial selection of the oocyte is not affected by *encore* mutations, and defines a novel step in the determination of the oocyte, which is the maintenance of its fate. Encore interacts with a splicing factor called Half-pint in yeast two-hybrid assays, and mutations in *half-pint* produce cysts that go through one less division and contain only 8 cells, which sometimes all become nurse cells [55]. Interestingly, Half-pint regulates the splicing of *ovarian tumor* (*otu*) mRNA, and mutations in *otu* also affect the number of cyst divisions, and the determination of the oocyte [56,57]. Although these genes are not direct regulators of the cell cycle, they reveal a link between the regulation of the number of cyst divisions and the determination of the oocyte. The lack of an oocyte does not seem to be a consequence of the abnormal number of divisions, however, as the two phenotypes are produced by different alleles or under different conditions. The same is true for mutations in the transcription factor *stonewall* (*stwl*). A high percentage of *stwl* mutant cysts contain 16 polyploid cells, whereas a lower percentage go through one extra division to produce 32 cells cysts [58]. Interestingly, *stwl* also affects the maintenance of the oocyte fate rather than its selection, as *orb* mRNA initially accumulates normally into one cell.

Direct regulators of the cell cycle also participate in the determination of the oocyte. Overexpression of *string/cdc25* inhibits the last cyst division to produce cysts with 8 cells, 50% of which lack an oocyte [59]. A very similar phenotype is produced by loss-of-function mutations in *tribbles*, which is a negative regulator of *string* [59]. In contrast, overexpression of *tribbles* induces a fifth division to produce cysts with 32 cells and 2 oocytes. Surprisingly, these effects are the opposite of those observed in other tissues, where overexpression of string and mutants in *tribbles* induce extra mitoses, suggesting that there is something different about the regulation of mitosis in the female germ line.

Mutants in cyclin E, which regulates entry into S-phase, can also affect the number of cyst divisions: a subset of mutant egg chambers contain only 8 cells (7 nurse cells and one oocyte), and another subset have 16 cells, with two or three cells having oocyte-like nuclear features, such as low ploidy and condensed chromatin [60]. This phenotype is dominantly suppressed by mutations in the cyclin-dependent kinase inhibitor, *dacapo* (*dap*) [24]. Furthermore, egg chambers homozygous mutant for *dap* contain 16 polyploid nurse cells. Interestingly, these egg chambers initially restrict SC, Orb and BicD into one cell, but proteins and mRNAs diffuse away from the oocyte at stage 1, instead of moving to the posterior pole. Thus, *dap* is also required for the maintenance of the oocyte fate, rather than its specification [24]. One possible explanation for this phenotype is that the elevated levels of cyclin E in *dap* mutants cause the oocyte to undergo endocycles and become polyploid, and that the oocyte loses its cytoplasmic differentiation as a consequence. Thus, an abnormal cell cycle state blocks the nuclear and cytoplasmic differentiation of the oocyte, and induces it to revert to the nurse cell fate, much like the losing pro-oocyte does normally.

The Spindle *Genes: The Meiotic Checkpoint and RNA Silencing*

Mutants in the spindle group genes affect the formation of the anterior–posterior and dorsal–ventral axes, and delay the specification of the oocyte [61–64]. This delay is characterised by an arrest in the formation of the karyosome, a mispositioning of the oocyte, a failure to localize the cytoplasmic components of the oocyte to the posterior pole, and the differentiation of the losing pro-oocyte as a second oocyte. These defects are transient, however, and there is usually only one oocyte at later stages, indicating that the selection of the oocyte is not abolished but merely slowed down [22,64]. Despite the puzzling pleiotropy of phenotypes, the molecular characterisation of some of the genes has revealed a common cause. The spindle-class comprises the 5 *spindle* genes, *spn-A*, *-B*, *-C*, *-D*, *-E* and *okra*, *aubergine*, *vasa*, and *maelstrom* [41,61,65]. *spn-A, -B, -D* and *okra* encode homologs of yeast RAD51 and RAD54, which are involved in repairing dsDNA breaks [66–68]. Furthermore, the patterning defects of *spn-A, -B, -C* and *-D* and *okra* mutants can be suppressed by mutations in *meiW68*, which block the formation of the dsDNA breaks that initiate recombination, and also by mutations in *meiP41* or *chk2*, which are components of the DNA damage checkpoint pathway [68,69]. Thus, the primary defect in these *spindle* mutations appears to be a failure to repair the dsDNA breaks

formed during recombination, and the patterning defects and most probably the delay in the oocyte differentiation are a consequence of the activation of a meiotic checkpoint pathway (reviewed in [70,71]).

Not all of the spindle genes are involved in the repair of dsDNA breaks, and it has recently emerged that a second class, comprising *spindle-E*, *maelstrom*, *aubergine*, and the newly identified gene *armitage* are all required for translational silencing by micro-RNAs, and RNA interference [72–76]. Mutants in these genes produce a similar spectrum of phenotypes to the other spindle mutants, including a delay in the selection and polarisation of the oocyte, while *spindle-E* and *maelstrom* mutants have also been found to cause premature cytoplasmic streaming in the oocyte at stage 9 [77–80]. Interestingly, all of these mutants also cause the precocious translation of *oskar* mRNA in the nurse cells, strongly suggesting that *oskar* is regulated by micro-RNAs [76]. These results indicate that a number of key processes in development of the germline cysts is regulated by post-transcriptional gene silencing by microRNAs, although the relevant mRNA targets in early oogenesis have not yet been identified.

Early Polarisation of the Oocyte

When a germline cyst reaches region 2b, the oocyte specific proteins and mRNAs, as well as the centrosomes and mitochondria have been transported along the fusome into the presumptive oocyte. These components remain associated with the fusome remnants and, therefore, accumulate at the anterior of the oocyte to form a Balbiani body [18]. When the oocyte moves through region 3, all of the components of the Balbiani body disassociate and move around the oocyte nucleus to form a tight crescent at the posterior cortex. This movement is the first sign of anterior–posterior polarity in the oocyte, and is a crucial step in the maintenance of its identity [26,81].

The importance of this polarisation step was first demonstrated by the analysis of a null mutation in the *par-1* gene, which specifically disrupts this process [26]. PAR-1 is the *Drosophila* homolog of *Caenorhabditis elegans* PAR-1, a serine/threonine kinase which is required for the polarisation of the *C. elegans* zygote [82]. In *Drosophila*, *par-1* mutant egg chambers contain 16 nurse cells and lack an oocyte [26,83]. The oocyte appears to be selected normally, as the centrosomes, the SC, and Orb accumulate in one cell in region 2b/3 [26]. However, these components do not translocate to the posterior of the oocyte in region 3 and the oocyte de-differentiates as a nurse cell, by exiting meiosis and becoming polyploid.

Anterior–posterior polarity in *C. elegans* also requires a number of other PAR proteins: the PDZ-domain proteins PAR-3, and PAR-6, which form a complex with atypical protein kinase C at the anterior of the zygote; the RING finger protein, PAR-2, which is required to recruit PAR-1 to the posterior cortex, as well as the 14-3-3 homolog, PAR-5, and the homolog of the mammalian kinase LKB1, PAR-4, both of which are not localized [84–89]. It has subsequently been found that the *Drosophila* homologs of all of these proteins, except for PAR-2, which is not conserved, are also required for the polarisation of the *Drosophila* oocyte and the maintenance of its fate [36,83,90,91]. Thus, there is a striking homology between the earliest polarisation of the AP axis in worms and flies.

The fact that mutations in the *par* genes, which are widely involved in cell polarisation, and in cell cycle regulators such as *dacapo* (see above) produce strikingly similar phenotypes demonstrates that the nuclear and the cytoplasmic development of the oocyte are intimately linked, and that stage 1 (region 3) of oogenesis is a crucial developmental checkpoint (Figure 3). If the oocyte is not in the appropriate cell cycle state by this stage or if it fails to polarize along its anterior–posterior axis, it will revert to the nurse cell fate.

The Par proteins define and maintain complementary anterior and posterior cortical domains in the *C. elegans* embryo, with the PAR-3/PAR-6/aPKC complex localising to the anterior cortex, and PAR-1 and PAR-2 to the posterior (reviewed in [92]). The spatial relationship between the Par proteins in the *Drosophila* oocyte appears more complex, as most antibodies and GFP fusions place the PAR-3 homologue Bazooka (Baz) and aPKC at the adherens junctions that form around the ring canals, and PAR-1 on the fusome [36,93]. However, isoform specific antibodies detect Baz at the anterior of the oocyte and PAR-1 at the posterior pole [94]. Furthermore, the localisation of these proteins appears to be interdependent, as Baz extends to the posterior in *par-1* null cysts, and PAR-1 remains anterior in *baz* null clones. Recent data have begun to elucidate the molecular mechanisms that may underlie these mutually exclusive localisations. PAR-1 has been found to phosphorylate two sites in Baz to recruit 14-3-3 (PAR-5), which then disrupts the Baz/PAR-6/aPKC complex by blocking oligomerization of Baz and its interaction with aPKC [95,96]. Furthermore, mammalian aPKC can phosphorylate PAR-1 to inhibit its activity and localisation to the plasma membrane [97]. Thus, the mutual antagonism between the Baz/PAR-6/aPKC complex and PAR-1/14-3-3 may provide a general mechanism to establish complementary cortical domains in polarised cells, including the early *Drosophila* oocyte.

One likely target of the PAR proteins in the *Drosophila* oocyte is the microtubule cytoskeleton [98]. In oocytes of *par* null mutants, the microtubule minus-ends fail to switch from the anterior of the nucleus to the posterior cortex in region 3 [26,90,91,93,94]. Furthermore, the polarity defects of the *par* mutants can be phenocopied by mild colchicine treatments [94]. In addition, several of the par proteins appear to control microtubule organization later in oogenesis, because stage 8–9 oocytes mutant for *par-1, lkb1* and *14-3-3*ε have a disorganised microtubule cytoskeleton, in which the minus-ends lie all around the oocyte cortex, and the plus-ends point toward the center of the oocyte, instead of the posterior pole [90,91,98]. How direct the regulation of microtubules by the Par-cassette is remains unknown. Indeed, BicD, Egl or Dynein could also be targets as they give very similar phenotypes to *par* mutations. In oocytes mutant for hypomorphic alleles of *BicD* or *Dhc*, proteins accumulate into one cell, but fail to translocate to the posterior

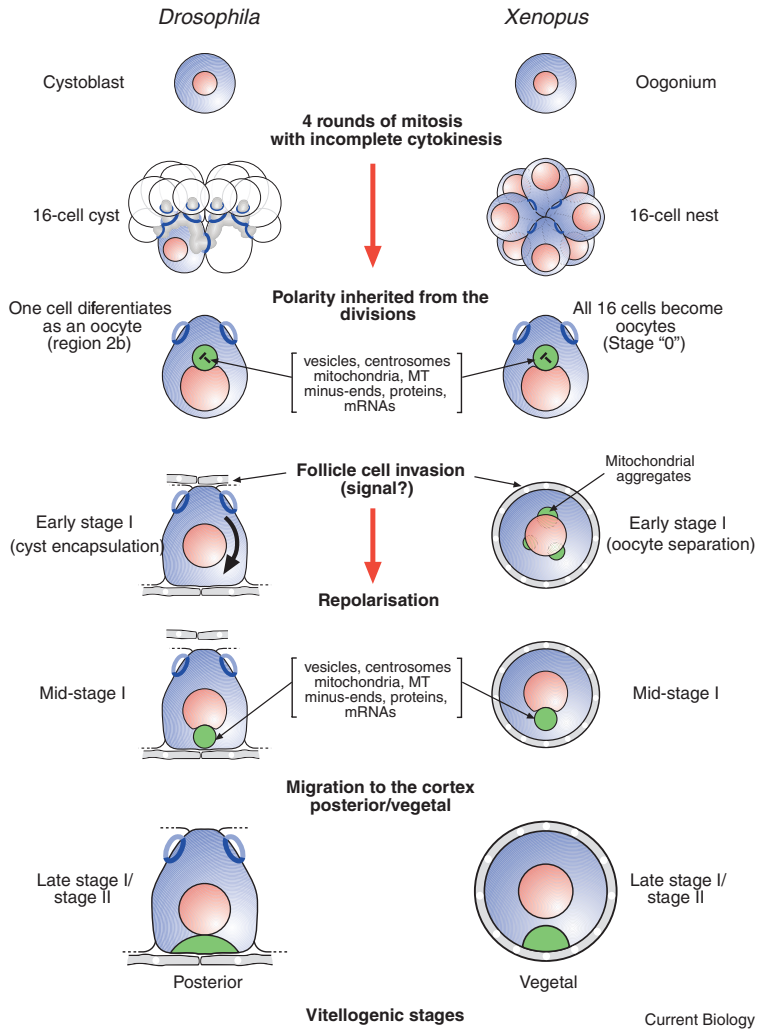

Figure 4. Similarities between the early steps of *Drosophila* and *Xenopus* oogenesis.
In both *Drosophila* and *Xenopus*, the oocyte inherits an anterior–posterior axis of symmetry from the cyst divisions (for a review on *Xenopus* early oogenesis, see [111–115]). Specific cytoplasmic components (green) accumulate in a depression above the nucleus (red) [18,81,111, 113,116]. The oocyte then polarises along this axis when the somatic follicle cells surround it. We suggest that a signal coming from the follicle cells may trigger this polarisation. This polarisation is clearly seen in *Drosophila* with the translocation of specific cytoplasmic proteins, mRNAs and the centrosomes to the posterior of the oocyte (black arrow). The situation is less clear in *Xenopus*, as the cell rounds up and seems to lose any polarity [113,115,117]. However, we propose that the same components that were located above the nucleus after the cyst division, are now part of the Balbiani body on the vegetal side (green sphere). At the following stage, these components migrate to the posterior/vegetal cortex of the oocyte. This has been clearly demonstrated in *Xenopus*, and is here hypothesized for *Drosophila* [117–119]. Both oocytes then enter the vitellogenic stages.

[22,94]. Similarly, in *BicD* and *egl* null mutant oocytes, the centrosomes remain at the anterior of a pseudo-oocyte [20]. It will, therefore, be very informative to work out the relationships between the Par-cassette, the BicD/Egl/Dynein complex and the microtubules.

Upstream regulators remain more elusive. In *C. elegans*, the sperm entry provides an extrinsic cue that triggers the Par-dependent polarisation of the embryo [92]. This cannot be the case in the germarium, as fertilisation only occurs at the end of oogenesis, about a week later. In other cell-types, such as epithelial cells, the localisation of the PAR3/PAR6/aPKC complex is regulated by an apical complex of Crumbs and Stardust proteins, as well as by the lateral proteins, Discs Large, Scribble, and Lethal Giant Larvae [99,100]. Oocyte polarity appears to be regulated differently, however, as this early polarisation step is unaffected in germline clones of null alleles of all these genes (J.-R. H. and Uwe Irion, unpublished data). These results suggest that the polarisation of the oocyte may rely not only on intrinsic mechanisms, but may also require an extrinsic signal. It is interesting to note that oocyte re-polarisation occurs exactly when the follicle cells first surround and contact the oocyte. A signal from the follicle cells might, therefore, induce the reorganisation of oocyte polarity in region 3. In support of this hypothesis, *Dystroglycan*, which encodes a receptor for multiple extracellular matrix molecules, has been shown to be required in the germline for the repolarisation of the oocyte at this precise stage [101].

In conclusion, the fusome establishes an axis of polarity as early as in region 1, but this polarity is reorganised in a Par-dependent manner in region 3. However, this is not the final polarity of the oocyte, as it repolarises at stage 7 to define the anterior–posterior axis of the embryo, raising the question of how these two polarisation events are related.

The Positioning of the Oocyte Links the Early and Late Polarisation of the Egg Chamber

The oocyte acquires its final anterior–posterior polarity in a two step process. First, *gurken* mRNA is translated at the posterior of the oocyte and the resulting protein signals to the adjacent terminal follicle cells to induce them to adopt a posterior rather than an anterior fate [102,103]. These posterior follicle cells then send an unknown signal back to the oocyte at stage 7, which induces a repolarisation of the microtubule cytoskeleton,

which directs the transport of *bicoid* mRNA to the anterior of the oocyte and of *oskar* mRNA to the posterior. This chain of polarisation depends on at least two events taking place in the germarium.

Firstly, *gurken* mRNA must be localized at the posterior of the oocyte for Gurken protein to signal to the adjacent follicle cells (Figure 1). As *gurken* mRNA is part of the Balbiani body, its posterior localisation is a consequence of the Par-dependent repolarisation of the oocyte in region 3. The posterior localisation of *gurken* mRNA, therefore, provides a crucial link between the early polarisation events taking place in the germarium and the final anterior–posterior polarity acquired at stage 7. This is best demonstrated by the phenotype of *maelstrom* mutants, in which oocyte identity is maintained, but *gurken* mRNA fails to switch from the anterior to the posterior of the oocyte [78,79]. As a consequence, all the subsequent polarisation steps are disrupted.

Secondly, the oocyte needs to be localized at the posterior of the egg chamber, in contact with the terminal follicle cells that are competent to receive the Gurken signal [104]. Indeed, mutants that disrupt the movement of the oocyte to the posterior of the cyst abolish all late anterior–posterior asymmetry, and give rise to symmetric egg chambers with two sets of anterior follicle cells, and oocytes that localize *bicoid* mRNA to both poles and *oskar* mRNA to the center. This invariant localisation of the oocyte is due to an up-regulation of DE-Cadherin in the oocyte and in somatic cells that contact the posterior of the oocyte [105,106]. The oocyte, therefore, out-competes the nurse cells for adhesion to these posterior follicle cells, and is pulled to the posterior when the cyst changes shape on entering region 3. Thus, the AP axis originates from the adhesiveness of the posterior follicle cells, raising the question of how these cells are specified and positioned.

The role of the posterior follicle cells in oocyte positioning raises a paradox. On the one hand, the anterior and posterior follicle cells are thought to be equivalent until Gurken signaling breaks the symmetry. On the other hand, these cells must already be different in the germarium to up-regulate Cadherin and thus to position the oocyte. This paradox has recently been resolved with the discovery that the early difference between the anterior and posterior follicle cells is only temporary, and arises from a series of inductive signals that are transmitted from the anterior follicle cells of the adjacent older egg chamber to the posterior follicle cells of the younger, anterior egg chamber [107]. Each cyst signals through Delta to induce the differentiation of the anterior and posterior pairs of polar follicle cells at each end of the egg chamber, but the anterior polar cells differentiate much earlier than the posterior ones. These cells turn on Unpaired, the ligand for the JAK/STAT pathway, which induces the adjacent anterior polar stalk cell precursors to become stalk cells, and it is thought that these cells then up-regulate Cadherin to adhere to the oocyte of the adjacent younger egg chamber. Therefore, the anterior–posterior axis is established by a relay mechanism in which each cyst induces the positioning of the oocyte of the next cyst.

These last results emphasize the importance of the communication between the germline and the somatic cells to coordinate the formation of an egg chamber. Signals from the germline to the somatic cells control the migration and differentiation of the follicle cells, whereas signals from the somatic cells to the germline are required to position and polarize the oocyte.

Conclusion and Perspectives

Although we have focused on *Drosophila* oogenesis in this review, the formation of a germline cyst seems widely conserved throughout the animal kingdom [108]. In particular, the first steps of *Drosophila* oogenesis show a striking similarity to the early steps of *Xenopus* oogenesis (Figure 4) [109]. Furthermore, PAR-6b has recently been found to localize to the animal pole of unfertilised mouse eggs, suggesting that PAR proteins may play a conserved role in oocyte polarity in mammals [110]. Thus, the eggs of many species, including vertebrates, may be polarised much earlier than previously thought, and the *Drosophila* egg chamber will provide a useful paradigm for understanding this process.

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Supplemental data

Supplemental data are available at http://www.current-biology.com/cgi/content/full/14/11/R438/DC1/

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