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The orthogonal axes of *Drosophila* are established during oogenesis through a hierarchical series of symmetry-breaking steps, most of which can be traced back to asymmetries inherent in the architecture of the ovary. Oogenesis begins with the formation of a germline cyst of 16 cells connected by ring canals. Two of these 16 cells have four ring canals, whereas the others have fewer. The first symmetry-breaking step is the selection of one of these two cells to become the oocyte. Subsequently, the germline cyst becomes surrounded by somatic follicle cells to generate individual egg chambers. The second symmetry-breaking step is the posterior positioning of the oocyte within the egg chamber, a process mediated by adhesive interactions with a special group of somatic cells. Posterior oocyte positioning is accompanied by a *par* gene-dependent repolarization of the microtubule network, which establishes the posterior cortex of the oocyte. The next two steps of symmetry breaking occur during midoogenesis after the volume of the oocyte has increased about 10-fold. First, a signal from the oocyte specifies posterior follicle cells, polarizing a symmetric prepattern present within the follicular epithelium. Second, the posterior follicle cells send a signal back to the oocyte, which leads to a second repolarization of the oocyte microtubule network and the asymmetric migration of the oocyte nucleus. This process again requires the *par* genes. The repolarization of the microtubule network results in the transport of *bicoid* and *oskar* mRNAs, the anterior and posterior determinants, respectively, of the embryonic axis, to opposite poles of the oocyte. The asymmetric positioning of the oocyte nucleus defines a cortical region of the oocyte where *gurken* mRNA is localized, thus breaking the dorsal–ventral symmetry of the egg and embryo.

In *Drosophila*, the symmetry-breaking events that establish both the anterior–posterior (AP) and dorsal–ventral (DV) axes are completed at midstage of oogenesis, long before fertilization and egg deposition (Roth 2003). Ovarian development in *Drosophila* is tractable to extensive genetic screening and genetic mosaic analysis. All stages of ovarian development starting with the divisions of germline and somatic stem cells are accessible to in-depth microscopic analyses and some of them are amenable to live imaging. Furthermore,

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oogenesis in *Drosophila* is a slow process (10 d) in comparison to embryogenesis (1 d), allowing one to follow single steps of polarity establishment with great spatiotemporal resolution (Spradling 1993).

The term symmetry breaking as it will be used in the following requires some specification. All the events that are described in this article, with one possible exception (oocyte nucleus migration, see the following discussion), can be traced back to prior asymmetries. Thus, strictly speaking, they do not represent symmetry-breaking processes. However, the egg chambers or eggs that are produced in absence of any one of these processes are indeed symmetric or lack at least one major axis of polarity (Fig. 1). Thus, when using the term "symmetry breaking," we refer to processes that when absent result in symmetry, rather than to processes that establish the initial anatomical asymmetries that are present throughout oogenesis. The described events fall into two categories. Either they enhance weak existing asymmetries or they represent inductive processes transferring asymmetries established within one group of cells to another group of cells. In any case, if the process does not take place, existing spatial information is lost and the system collapses into symmetry.

OUTLINE OF *DROSOPHILA* OOGENESIS

In *Drosophila*, each of the two ovaries is composed of 16–20 independent strings of egg chambers called ovarioles (Spradling 1993). The ovarioles are the functional units of egg production (Fig. 2). At a given time, each ovariole typically contains six to seven sequentially more mature egg chambers (also called follicles) connected by somatic stalk cells. Egg chamber development has been divided into 14 stages; we will refer to stages 1–6 as early, stages 7–10 as mid, and stages 11–14 as late oogenesis. The ovariole is essentially a tubelike structure with a long axis formed by the string of connected egg chambers and perpendicular to it, a short axis (defining the plane of a cross section through an egg chamber). These two axes of the ovariole correspond to the future AP (long) axis and DV (short) axis of the egg and embryo.

The anterior tip of each ovariole is composed of the germarium, a structure that harbors the germline and somatic stem cells (Fig. 2). The egg chambers are assembled at the posterior end of the germarium. A single egg chamber contains a cluster of 16 germ cells, which are connected by cytoplasmic bridges, called ring canals. Only one of the 16 germ cells develops as an oocyte, whereas the remaining 15 become nurse cells. Oocyte growth

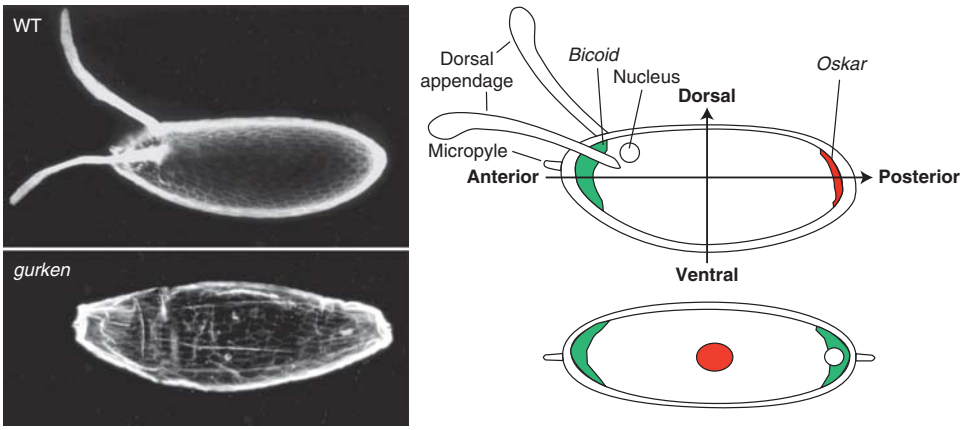

Figure 1. Loss of polarity along both main-body axes in *gurken* mutants. *Left*: egg shell preparation. *Right*: schematic drawing of eggs showing the orientation of the main body axes, the localization of *bicoid* and *oskar* mRNA and the oocyte nucleus.

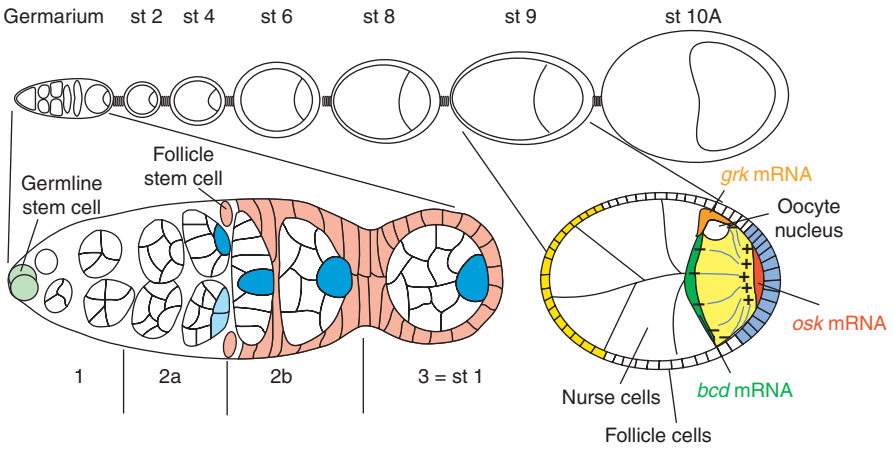

Figure 2. The ovariole. *Top*: schematic drawing of ovariole with the germarium at the anterior tip and egg chambers of increasing age. *Bottom*: Magnified view of germarium and stage 9 egg chamber.

depends on the nurse cells, which become polyploid and transport mRNAs, proteins, and endomembrane structures (e.g., ER and Golgi) via the ring canals into the oocyte. The nurse cell–oocyte cluster is surrounded by a monolayer epithelium of somatic follicle cells, which play a key role in axis determination.

During midstages of *Drosophila* oogenesis, the future embryonic axes are specified by the localization of three messenger RNAs within the oocyte (van Eeden and St Johnston 1999; Riechmann and Ephrussi 2001) (Fig. 2). *bicoid* mRNA is localized to the anterior pole of the oocyte facing the nurse cells, whereas *oskar* mRNA is localized to the opposite, posterior pole. The polar localization of these two mRNAs is maintained throughout the rest of oogenesis and well into early embryogenesis in which *bicoid* specifies anterior (head and thorax) and *oskar* posterior (abdomen) regions of the embryonic AP axis (St Johnston and Nusslein-Volhard 1992). Concomitantly with the localization of *bicoid* and *oskar*, a third mRNA, encoded by *gurken*, accumulates in an anterior–dorsal position of the oocyte and thereby defines the DV axis of the egg. *gurken* mRNA localization, however, is only transient and acts indirectly through a signaling process involving the follicle cells to determine the embryonic DV axis (Roth 2003; Moussian and Roth 2005). These three types of RNA localization are the key events that lead to embryonic axis formation. Because they normally take place in a stereotypic manner with regard to the ovariole architecture, one may ask which processes sense the ovariole architecture causing the reproducible localization of theses mRNAs. It turns out that, by far, the most complex series of events is required to establish AP polarity, whereas DV polarity appears to be a necessary consequence of the final step of AP polarization.

PRELUDE: CYST FORMATION AND THE FUSOME: GENERATING A GRADIENT OF POLARITY BY ORIENTED ASYMMETRIC DIVISIONS

The germline stem cells (GSC) at the anterior tip of the germarium undergo asymmetric divisions, producing one self-renewing GSC and one cell, the cystoblast, which starts differentiating (Fuller and Spradling 2007). It undergoes four incomplete mitotic divisions, which produce a cyst of 16 cells (called cystocytes) interconnected by ring canals. The divisions are oriented, leading to a stereotypic pattern of cell–cell connections: Two cells of the cyst have four, two have three, four have two, and eight have one ring canal (Spradling 1993). One of the two cells with four ring canals, also referred to as pro-oocytes, will assume the

oocyte fate (Huynh and St Johnston 2004). Judging from the pattern of cell connections, the cyst is apparently symmetric with both pro-oocytes, having an equal chance to assume the oocyte fate. Moreover, all cystocytes enter prophase of meiosis I, but a synaptonemal complex forms only in both cells with four ring canals and to a lesser degree in the two cells with three ring canals (Carpenter 1975). Nevertheless, only the future oocyte will complete meiosis, whereas the other cystocytes leave the meiotic program and eventually start the endocycles characteristic for the nurse cells. These observations, together with mutant analyses (Theurkauf et al. 1993; Huynh and St Johnston 2004), suggest that a dynamic competition process takes place in which only one of the two pro-oocytes normally wins. Thus, the selection of one of the two cells with four ring canals looks like a typical example for a symmetry-breaking event.

A closer look at cyst formation, however, has revealed that the future oocyte appears to be selected already at the first incomplete division (the cystoblast division) (de Cuevas and Spradling 1998). Moreover, cyst formation is accompanied by repeated cell rearrangements, which establish intrinsic AP polarity within the germline cluster and even within the future oocyte (de Cuevas and Spradling 1998).

All of these features depend on the formation of the fusome, a membranous branched structure that runs through the cytoplasmic bridges and connects all cystocytes (Lin and Spradling 1995). In *Drosophila* oogenesis, the fusome is derived from a spherical structure present within the GSC, called the spectrosome, which is composed of membranous vesicles and components of the submembranous cytoskeleton, like α- and β-spectrin, ankyrin, and the adducin-like protein Hu-li tai shao (Hts) (Yue and Spradling 1992; Lin et al. 1994; Snapp et al. 2004; Roper 2007; Lighthouse et al. 2008). During GSC division, one third of the spectrosome is inherited by the cystoblast and gives rise to the fusome.

The fusome orients the cystoblast and cystocyte divisions, because during metaphase, one pole of the mitotic spindle is attached to the fusome (Deng and Lin 1997; McGrail and Hays 1997; Grieder et al. 2000). This leads to an asymmetric division as the old fusome always resides in one of the daughter cells, whereas the other daughter cell initially lacks fusome material (Fig. 3A). During interphase, however, new fusome material forms within the cytoplasmic bridge, connecting the two daughter cells. The old and the newly formed fusome of the cytoplasmic bridge are subsequently brought into close contact and eventually fuse, resulting in the directional growth and branching of the fusome (in Fig. 3A, this is depicted for the second cystocyte division).

The juxtaposition of old and newly synthesized fusome material is brought about by a movement of the cytoplasmic bridges toward each other, which also moves the cystocytes closer to each other, causing the clustering of the later formed cystocytes at one side of the cyst. Thus, the resulting cyst structure is polarized (rosette shape) with the cells with fewer ring canals at one and the two cells with four ring canals at the opposite site of the cyst (de Cuevas and Spradling 1998; Grieder et al. 2000). Moreover, the two cells with four ring canals are intrinsically polarized because their ring canals cluster at one side. Because this arrangement is stabilized by adherens junctions and maintained throughout oogenesis, it is possible to predict that the part of the cell with the clustered ring canals will become the anterior pole (Godt and Tepass 1998; Gonzalez-Reyes and St Johnston 1998a). One cell with four ring canals has the largest piece of fusome material going back to the fusome initially present in the cystoblast (de Cuevas and Spradling 1998; Grieder et al. 2000). This cell apparently assumes the oocyte fate. Because the fusome is a transient structure, which disassembles before obvious signs of oocyte differentiation (the stable accumulation of oocyte determinants, restriction of meiosis), this conclusion is not easy to draw. Currently, it is based on the preferential accumulation of some RNAs and the behavior of the centrosomes (see the following section).

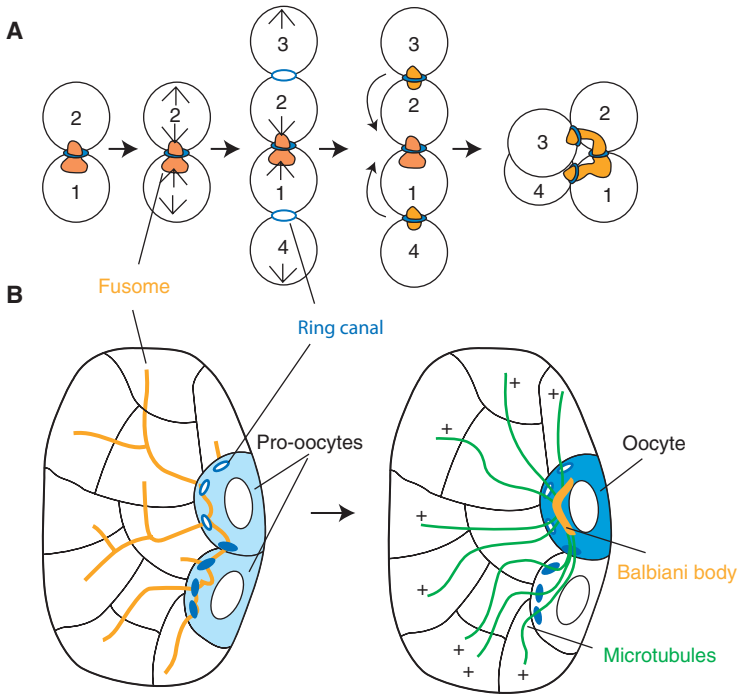

Figure 3. Cyst formation and oocyte determination. (*A*) Second cystocyte division producing a four-cell cyst. At metaphase, one spindle pole attaches to the old fusome. Cystokinesis is incomplete, leaving the cystocytes connected by ring canals. Within the ring canals, new fusome material forms. The ring canals move toward each other, leading to clustering of the cystocytes. (*B*) Oocyte determination. In region 2a of the germarium, the cystocytes are connected by the branched fusome. Both cells with four ring canals (pro-oocytes) show signs of oocyte specification. Later, the fusome is replaced by a polarized microtubule (MT) network emanating from one microtubule organizing center (MTOC) that resides in one of the two pro-oocytes. It is positioned in the region of the Balbiani body, anterior to the oocyte nucleus.

OOCYTE DETERMINATION: MAKING A DISCRETE DECISION THROUGH DIRECTED TRANSPORT

The process of oocyte determination cannot be separated from that of oocyte differentiation. A disruption of oocyte differentiation, even at late stages at which the oocyte is clearly discernable, leads to a reversion to the nurse cell fate and the formation of egg chambers with 16 nurse cells (Huynh et al. 2001b). During oocyte determination, two characteristics of the later oocyte are established in parallel. First, the oocyte needs to become the target of nurse cell–oocyte transport, which is largely microtubule-based during early stages of oogenesis (Fig. 3B). Thus, the MT network of the cyst has to be polarized such that the major routes of transport are directed toward the oocyte. However, the MT-dependent accumulation of certain proteins and mRNAs itself provides also the major means of oocyte determination (Theurkauf et al. 1993). Second, the meiotic program has to be restricted to the oocyte and the oocyte has to successfully go through the prophase of meiosis I and condense its DNA to form the karyosome. Because this review focuses on spatial patterning, the nuclear differentiation of the oocyte will not be pursued in more detail (for review, see Huynh and St Johnston 2004). With regard to spatial asymmetries, the key question is how fusome polarity gives rise to a stable polarization of the MT cytoskeleton.

The MT polarization of the cyst emerges gradually after the last cystocyte division. The minus ends of MTs become enriched within the two pro-oocytes and eventually are all concentrated within one of them (Fig. 3B). At this stage, MTs can be visualized to run from the pro-oocytes through the ring canals into the neighboring pronurse cells (Grieder et al. 2000). Although the MT network spans the entire cyst, only the centrosomes within the pro-oocytes are active, whereas those in the pronurse cells are inactivated. Remarkably, the inactive centrosomes migrate through the ring canals and collect within the oocyte (Mahowald and Strassheim 1970; Grieder et al. 2000; Bolivar et al. 2001).

Although the centrosome migration has no apparent function (Stevens et al. 2007), it provided an important clue for understanding the MT organization of the cyst. Colchicine treatment, which depolymerizes only dynamic MTs, prevents the accumulation of specific proteins and mRNAs within the oocyte, but does not block the migration of the centrosomes or restriction of meiosis to one cell (Huynh and St Johnston 2000; Bolivar et al. 2001). Centrosome migration, however, has been shown to be Dynein-dependent, implying the existence of colchicine-resistant MTs. Thus, the polarized MT network of early cysts is apparently composed of two types of MTs: those that are unstable and dynamic, and those that are stable.

Indeed, the *Drosophila* Spectraplakin homolog Short Stop (Shot) has been identified as a fusome component that is required for centrosome migration and appears to stabilize MTs (Roper and Brown 2004). Shot is a huge multidomain protein with a carboxy-terminal MT binding domain and accumulates along the fusome in a polar fashion. In absence of Shot, not only the dynamic, but also stable, acetylated MTs are lost from the fusome. Thus, Shot is likely to be a key component that stabilizes MTs and links them to the fusome. Potentially, this occurs in polar fashion, allowing the dynein-dependent transport of the centrosomes and providing the seed for the formation of a dynamic MT network responsible for the majority of all other transport processes.

Dynein is likely to play a crucial role for sensing the weak initial asymmetries of the stable MT network and for the formation of the dynamic MT network. This hypothesis is based on the observation that two dynein regulators, Bicaudal-D (BicD) and Egalitarian (Egl), are specifically required for oocyte determination without affecting the fusome structure or the movement of the centrosomes (Suter et al. 1989; Swan and Suter 1996; Mach and Lehmann 1997; Bolivar et al. 2001; Navarro et al. 2004). In the absence of either of these factors, oocyte-specific proteins like Orb and mRNAs like *osk* do not accumulate in the oocyte and, most significantly, the polarized dynamic MT network does not form.

What role do BicD and Egl play in these processes? Besides interacting with each other, each protein has been shown to bind proteins involved in the function or regulation of the Dynein complex: Egl to dynein light chain, and BicD to dynamitin, a subunit of the dynactin complex, which regulates dynein activity (Mach and Lehmann 1997; Navarro et al. 2004). In addition, BicD and Egl have been shown to recruit the dynein motor complex to mRNAs and initiate minus end-directed mRNA transport in other developmental contexts (Bullock and Ish-Horowicz 2001). Thus, BicD and Egl likely play a critical role in dynein-dependent transport of mRNAs and proteins critical for oocyte specification to the future oocyte.

Interestingly, *BicD* mRNA, BicD protein, Egl protein, and dynein themselves concentrate in the oocyte in a *BicD*- and *egl*-dependent fashion. This suggests a positive feedback mechanism generating a gradient of minus end-directed motor activity, which is more and more focused to the future oocyte. Some features of *egl* null mutants support a MT independent pathway of oocyte selection (Huynh and St Johnston 2000; Navarro et al. 2004). However, the enhancement of dynein-dependent transport toward the future oocyte seems to be the main mechanism that finally enforces a sharp cell fate decision

between one cell and its neighbor. How is this possible? In the cyst dynein, transport is self-amplifying because it moves some of its positive regulators from the prenurse cells into the oocyte and, in an unknown fashion, polarizes the MT network, which further focuses the transport toward the oocyte. If the dynein cargo included proteins stabilizing MT minus ends or determinants of the oocyte fate, such proteins would be depleted from pronurse cells and more concentrated in the future oocyte, leading finally to sharp fate decision between the two pro-oocytes. This process has formal similarity to the mechanism of substrate depletion known from pattern formation theory (Koch and Meinhardt 1994).

INTERLUDE: GERMARIUM STRUCTURE AND THE ENCAPSULATION OF THE CYST

So far, we have looked at the germline cyst as an isolated object but have not considered its positioning within the ovariole. The early events of the formation of the fusome and the selection of the oocyte can be understood without invoking interactions of the cyst with its environment as they are based primarily on intrinsic processes within cystocytes, or interactions among the cystocytes. For all subsequent steps in axis formation, interactions between the germline cyst and specialized somatic cells will be crucial. The contact between germline cysts and somatic cells first arises within the germarium (Fig. 2).

The germarium has been subdivided into four regions (Spradling 1993). Region 1 includes the anterior tip of the germarium with the stem cell niche for the GSCs and the adjacent posterior zone where the cystoblasts and the dividing cysts reside (also see Yamashita et al. 2009). Region 2a begins when the cystocytes have completed their final division. Oocyte selection takes place and meiosis of the selected oocyte proceeds to the pachytene stage. The follicle cell stem cells (FSC) are positioned at the border between region 2a and region 2b (Nystul and Spradling 2007). Exactly two FSCs are positioned in fixed locations on opposite lateral edges of the germarium. The FSCs produce the somatic cells that start to migrate between and establish close contacts with the cysts. At the same time, the cysts flatten to become one-cell thick discs spanning the whole width of the germarium. In region 3, the cysts have rounded up and are largely covered by follicle cells. The cells separating region 2b from region 3 cysts start to differentiate into stalk cells and polar cells (Fig. 4). In region 3, the oocyte is usually positioned posterior to the nurse cells, close to the stalk cells of the next egg chamber. With the beginning of stalk formation and the positioning of the oocyte, the geometry of the ovariole is fixed. The stalk cells define the long axis of the ovariole and thus the orientation of the AP axis of the cyst, including the future AP axis of the posteriorly positioned oocyte. The future DV axis has to be established within a plane perpendicular to this axis. How are oocyte positioning and stalk cell formation coordinated?

THE FORMATION OF A POLARIZED EGG CHAMBER: ALIGNING GERMLINE AND SOMA

One of the important prerequisites for the formation of polarized egg chambers is the establishment of two separate populations of somatic cells with distinct morphogenetic programs (the precursors of stalk/polar cells and of the epithelial follicle cells) (Tworoger et al. 1999). The genetic mechanisms that lead to this crucial distinction are not known, neither is it known how the different behavior of these cells is regulated. However, we have some knowledge about the interaction of these cells with the germline cyst that establishes egg chamber polarity.

Interestingly, AP asymmetry is established through a relay mechanism, which propagates polarity from older to younger cysts (Torres et al. 2003; Assa-Kunik et al. 2007). In essence, this mechanism translates a temporal sequence into spatial order. For this relay mechanism, the stalk/polar cell precursors are the crucial players (Fig. 4). They are involved in a series of inductive interactions. First, they receive a

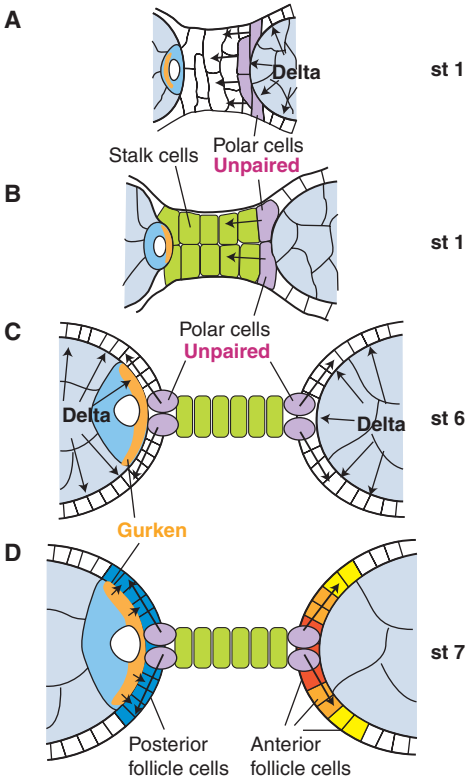

Figure 4. The role of polar and cell stalk for axis formation. The region connecting two egg chambers is schematically depicted with anterior and the younger egg chamber pointing to the left side. (*A*) In region 3 of the germarium, Delta signals from the germline to specify the anterior polar cells. These in turn express the JAK/STAT signaling ligand Unpaired and signal toward the anterior prestalk cells to induce the stalk cell fate. (*B*) A two-cell-wide stalk forms. Adhesive interactions between the oocyte of the younger cyst and the stalk cells tightly position the oocyte at the posterior pole. The first round of oocyte repolarization takes place indicated by the shift of mRNAs (orange crescent) from the anterior to the posterior pole of the oocyte. (*C*) At stage 6, a second round of Delta signaling from the germline induces the differentiation of the epithelial follicle cells, which acquire competency to react to a gradient of Unpaired emanating from the polar cells and to Gurken signaling emanating from the oocyte. (*D*) Unpaired induces terminal cell fates: In the absence of Gurken signaling, the three types of anterior follicle cells form (red, orange, and yellow); in the presence of Gurken signaling, the posterior follicle cells form (blue).

signal from the older germline cyst (region 3), which is transmitted backwards toward the younger cyst, resulting in a soma–germline interaction with the younger cyst (region 2b). The signal propagation is largely mediated by the Notch and the JAK/STAT pathways (Grammont and Irvine 2001; Roth 2001; McGregor et al. 2002). When propagation begins, region 2b and region 3 cysts are separated by a pool of uncommitted stalk/polar cell precursors (Fig. 4A). In region 3 cysts, the Notch ligand Delta begins to signal to the surrounding somatic cells. This leads to high Notch activation in stalk/polar cell precursors, which are in direct contact with the germline cyst and causes them to differentiate into polar cells. In turn, the polar cells start to express the JAK/STAT ligand Unpaired. Unpaired is able to activate JAK/STAT signaling only in cells that lack high levels of Notch signaling (Assa-Kunik et al. 2007). Thus, Unpaired cannot act on the follicle cells surrounding the germline cyst, nor on the polar cells themselves, which all receive high levels of Notch activation from the germline. Therefore, Unpaired produced by the polar cells can only signal in a vectorial fashion to more anterior stalk/polar precursors. Activation of JAK/STAT signaling in these anterior cells induces them to differentiate into stalk cells. Stalk cells intercalate with each other and converge toward the middle of the ovariole, forming a two-cell-wide stalk (Fig. 4B). This stalk directly contacts the younger cyst. At this stage, there are no intervening polar cells, as the posterior polar cells of each egg chamber are generated much later. The stalk cells in direct contact with the young cyst up-regulate the cell adhesion molecule DE-cadherin (Godt and Tepass 1998; Gonzalez-Reyes and St Johnston 1998a; Becam et al. 2005), which is also up-regulated in the oocyte in comparison to the nurse cells. Thus, the oocyte preferentially adheres to the posterior stalk cells. This positions the oocyte to the

posterior pole of the egg chamber and brings it in line with the axis defined by the stalk.

Why is oocyte positioning a symmetry-breaking event? The pro-oocytes are already in an asymmetric position with regard to the pronurse cells in stage 2a cysts. However, this is only a weak asymmetry and most importantly, it is not oriented with regard to the somatic cells encapsulating and separating the cyst. Indeed, it turns out that the fixed arrangements of the oocyte–nurse cell cluster with regard to follicle and polar/stalk cells is one of the most important steps in axis formation. If the oocyte does not establish contact with the anterior stalk cells, egg chambers develop in which the oocyte resides in a middle position between the nurse cells. Oocyte and nurse cell differentiation and growth proceed normally, but the oocyte never contacts the polar cells or the neighboring epithelial follicle cells. These egg chambers give rise to eggs lacking both AP and DV polarity. Mutants in the spindle genes cause such phenotypes (Gonzalez-Reyes and St Johnston 1994; Gonzalez-Reyes et al. 1997).

In *dicephalic* mutants, the oocyte is occasionally positioned anteriorly, rather than posteriorly, to the nurse cells (Gonzalez-Reyes and St Johnston 1998b; Ligoxygakis et al. 2001). Strikingly, under these conditions, egg chamber development proceeds in a normal fashion, just with a reverted AP axis of the egg. The eggs are even deposited and support normal embryonic development (Ligoxygakis et al. 2001). Thus, it is not essential that the oocyte is localized posteriorly, but rather that it assumes an asymmetric position with regard to the nurse cells and along the axis defined by the stalk cells.

Why is it so important that the oocyte is aligned with the stalk/polar cells, either anterior or posterior to the nurse cells? The answer has to do with a signal from the oocyte to a specific group of follicle cells that themselves are specified via polar cell signaling. Before we continue with the processes of follicle cell patterning and ooycte-to-follicle cell signaling, we have to turn our attention to the internal structure of the oocyte, which changes around the time of posterior oocyte positioning.

THE FIRST ROUND OF OOCYTE POLARIZATION: THE ESTABLISHMENT OF THE OOCYTE POSTERIOR POLE

As described previously, the cyst structure already imposes internal polarity on the presumptive oocyte: The four ring canals are clustered at one pole. When the oocyte becomes positioned to one side of the nurse cells by external forces (stalk cell interaction), this internal polarity becomes even more pronounced. The anterior of the oocyte is not only marked by the ring canals (Fig. 3B). The MTs that grow from the presumptive oocyte through the ring canals into the pronurse cells emerge from an MTOC that resides at the anterior of the oocyte between the ring canals and the oocyte nucleus (Grieder et al. 2000; Huynh et al. 2001b). Several components that are transported in a MT- and dynein-dependent fashion to the oocyte remain close to this anterior MTOC. These include the centrosomes, BicD, Egl, Orb, and Cup protein, *osk* and *orb* mRNAs, and the mitochondria (Huynh and St Johnston 2000; Huynh et al. 2001b; Vaccari and Ephrussi 2002). An anterior mitochondrial cloud forms, which is enriched in Golgi vesicles and overlaps with the MTOC, the localized proteins and mRNAs. Because of its resemblance to similar structures found in oocytes of many species, including *Xenopus* and humans, it has been termed the Balbiani body (Fig. 3B, Fig. 4A).

In region 3, when oocyte positioning is completed, a reorganization of the MT network of the oocyte takes place, which shifts the anterior MTOC to the posterior pole (Huynh et al. 2001b; Vaccari and Ephrussi 2002). Whether the MTOC disassembles anteriorly and is newly formed posteriorly or whether it is maintained and moves around the oocyte nucleus is not known and can only be resolved by live imaging. When the MTOC shifts position, the Balbiani body disassembles and the anterior localized centrosomes, mRNAs, and proteins translocate to the posterior pole (Fig. 4B). The mitochondria of the Balbiani body disperse in the oocyte and supposedly provide all the mitochondria for subsequent oocyte growth

because in later stages the nurse cell-to-oocyte transport of mitochondria seems to be blocked (Cox and Spradling 2003). A small subpopulation of the Balbiani body mitochondria becomes localized to the posterior cortex where later the germplasm will form. (Cox and Spradling 2003).

The posterior translocation of Balbiani body components leads to the first establishment of a cytoplasmic region within the oocyte, which continues to be present until embryogenesis. It also defines the posterior oocyte cortex, which later will be crucial for the exchange of signals with the follicular epithelium. Mutations that prevent this first internal step of oocyte polarization reveal that it also represents an important check point for ovarian development (Huynh and St Johnston 2004). If it does not take place, the oocyte reverts to the nurse cell fate and further egg chamber development and growth is essentially blocked.

Despite its importance, the mechanisms involved in early oocyte polarization are not well understood. The process depends on an intact MT network and requires BicD as well as dynein (Vaccari and Ephrussi 2002). Strikingly, it also depends on the *par* genes, a group of genes first discovered in *Caenorhabditis elegans*, which has a conserved role in cell polarization throughout animal evolution (also see McCaffrey and Macara 2009). It has been shown that *Drosophila* homologs of all conserved *par* genes (*par-1*, *par-3*, *par-4*, *par-5*, and *par-6*) and of aPKC are required for early oocyte polarization (Cox et al. 2001a; Cox et al. 2001b; Huynh et al. 2001a; Huynh et al. 2001b; Benton et al. 2002; Vaccari and Ephrussi 2002; Martin and St Johnston 2003).

In many polarized cells, like in the *C. elegans* zygote, the Par proteins are involved in the formation and stabilization of complementary cell cortex domains (Munro 2006; Goldstein and Macara 2007). A conserved ternary complex of Par-3, Par-6, and aPKC localizes to one pole of the cell, whereas Par-1 is found at the opposite pole. The Par-3/Par-6/aPKC complex and Par-1 are involved in inhibitory interactions, which lead to mutually exclusive membrane localization.

Interestingly, in *Drosophila*, Par-1 is localized to the fusome and, like other oocyte-specific proteins, becomes restricted to the oocyte in a MT-dependent manner (Cox et al. 2001a; Huynh et al. 2001b; Vaccari and Ephrussi 2002). First, it localizes to the anterior pole and later relocalizes with the MTOC to the posterior pole. However, the relocalization process requires *par-1* itself and *bazooka* (*baz*), the *par-3* homolog of *Drosophila*. Par-3 protein is localized at the anterior pole, where it remains when Par-1 translocates to the posterior pole (Huynh et al. 2001a; Vaccari and Ephrussi 2002). In *par-1* mutants, Par-3 does not remain restricted to the anterior pole. Thus, like in *C. elegans* zygotes and other polarized cells, the first internal polarization step of the *Drosophila* oocyte is accompanied by the establishment of complementary mutually exclusive domains of Par proteins. However, the changes in MT polarity and the Par protein localization seem to be interdependent, which indicates a more complex mechanism so far unknown from other systems. In addition, it is not clear how the polarization process is initiated. Polarization coincides with the adhesive interactions between oocyte and stalk cells. Thus, it is conceivable that a signal derived from somatic cells induces repolarization. This view is supported by the finding that the extracellular matrix receptor dystroglycan is required in the germline for early oocyte repolarization (Deng et al. 2003). Oocytes lacking dystroglycan also show a defect in F-actin accumulation at the posterior pole. This might hint to a similarity with other systems in which it is well established that changes in the actomyosin network initiate the formation of Par protein asymmetries (Murno 2006).

FOLLICLE CELL PATTERNING AND THE EMERGENCE OF AP POLARITY WITHIN THE FOLLICLE CELL LAYER

After the egg chamber leaves the germarium, it is fully surrounded by a monolayer of epithelial follicle cells, and the posterior pair of polar cells has formed so that each egg chamber has an

identical pair of polar cells at its termini and thus, the somatic cells surrounding and connecting the egg chambers are symmetric (McGregor et al. 2002). After mitotic divisions regulated by mechanical stress of the growing oocyte and Hh signaling, the epithelial follicle cells stop dividing and begin to differentiate in response to a second round of Delta signaling from the germline beginning at stage 6/7 (Fig. 4C) (Wang and Riechmann 2007; Zhang and Kalderon 2000; Lopez-Schier and St Johnston 2001). How Notch controls the cell cycle switch in the follicle cells has been well investigated (Sun and Deng 2007). However, for the purposes of this review, it is only important that the epithelial follicle cells during stage 6/7 become competent to respond to signals emanating from the polar cells and from the oocyte.

At stage 6/7, the egg chamber essentially forms an ellipsoidal structure with the polar cells defining the endpoints of the long axis (Fig. 2, Fig. 4C,D). Now, the polar cells act as organizers of surrounding epithelial follicle cells (Grammont and Irvine 2002). Unpaired secreted by polar cells leads to a gradient of JAK/STAT signaling, which specifies about 200 epithelial follicle cells at each end of the egg chamber to assume the terminal follicle cell fate (Xi et al. 2003). The prepattern that is established within the follicular epithelium by JAK/STAT is mirror-image symmetric (Fig. 4C). This is apparent in the absence of a further modulation of JAK/STAT signaling from the germline, when the terminal cells at both ends of the egg chamber differentiate into different types of anterior follicle cells (Gonzalez-Reyes and St Johnston 1998b). JAK/STAT signaling not only specifies the terminal fate as such, but apparently also accounts for terminal cell type diversity. Unpaired seems to function as a morphogen in specifying three anterior terminal cell types as a function of distance from the polar cells (Xi et al. 2003) (Fig. 4D).

At the time Notch signaling provides competency to differentiate and JAK/STAT signaling provides spatial patterning information, the epithelial follicle cells surround a highly polarized germline cyst. The oocyte, positioned posterior to the nurse cells, has grown in size so that it just abuts the terminal follicle cells (Fig. 4C,D). MT- and dynein-dependent transport from the nurse cells has led to the accumulation of several mRNAs within the oocyte. In the next step of axis formation, this inner polarity of the germline cyst is transferred to the follicular epithelium (Gonzalez-Reyes and St Johnston 1994; Gonzalez-Reyes et al. 1995; Roth et al. 1995). Among the mRNAs that have begun to accumulate within the oocyte already in the germarium is that of the *gurken* (*grk*) gene. *grk* codes for a transforming growth factor α (TGFα) homolog, a member of the epidermal growth factor receptor (EGFR) ligand family (Neuman-Silberberg and Schuepbach 1993). At stage 6/7, Grk protein is secreted by the oocyte and activates the EGFR in the adjacent terminal follicle cells (Peri et al. 1999; Queenan et al. 1999; Chang et al. 2008). Like other mRNAs that accumulate in the early oocyte *grk* mRNA becomes concentrated at the posterior cortex already at stage 1 (Fig. 4B), and it has been suggested that efficient secretion of Grk toward the adjacent follicle cells requires the tight localization of *grk* mRNA to the posterior cortex of the oocyte (Clegg et al. 1997). Thus, posterior *grk* mRNA localization might provide a link between the early *par*-dependent polarization of the oocyte and the later EGF-dependent polarization of the egg chamber (Huynh and St Johnston 2004). EGFR activation in the terminal follicle cells leads to the transcription of several immediate target genes of EGF signaling (Morimoto et al. 1996; Ghiglione et al. 1999; Reich et al. 1999) only in cells that have received prior Notch activation and JAK/STAT signaling (Xi et al. 2003). The final outcome of this Grk signal is the induction of the posterior follicle cell (PFC) fate.

A combination of JAK/STAT signaling and EGF activation can induce the PFC fate also in ectopic positions of the follicular epithelium (Xi et al. 2003). However, almost nothing is known about the genes downstream of the two pathways that specify the PFC fate. In contrast to the three types of anterior terminal

follicle cells (AFCs) that go through a complex program of morphological changes, the PFCs are apparently not composed of different subpopulations and do not show obvious morphological differences compared with adjacent main body follicle cells. Only genetic mosaic analysis reveals a sharp border between the two cell types (Gonzalez-Reyes and St Johnston 1998b).

Interestingly, recent work shows that besides JAK/STAT and EGF signaling, there are two additional pathways required for normal cell behavior of the PFCs, but not the main body follicle cells (Riechmann 2007). First, both the upstream and the core components of the Hippo tumor-suppressor pathway are involved in controlling differentiation and cell division of the PFCs (Meignin et al. 2007; Polesello and Tapon 2007). The Hippo pathway promotes Notch signaling and prevents multilayering of the PFCs (Yu et al. 2008). Second, multilayering of the PFC epithelium is also observed when integrin function is lost (Fernandez-Minan et al. 2007). It is not known why the PFCs are particularly sensitive to a loss of these two pathways. However, it has been suggested that this might be linked to mechanical constraints because of greater tissue curvature at the termini as compared with the more lateral regions (Riechmann 2007).

BACK SIGNALING AND THE SECOND ROUND OF OOCYTE POLARIZATION

At stages 7–9, the oocyte nucleus changes its position from posterior to anterior. This is accompanied by a restructuring of the MT cytoskeleton (Theurkauf et al. 1992; Steinhauer and Kalderon 2006). The posterior MTOC disappears and MTs begin to emanate from the lateral and anterior cortex, projecting the plus ends into the center of the oocyte. Second, MT-dependent transport leads to mRNA localization to the opposite poles of the oocyte (Steinhauer and Kalderon 2006). So far, only the posterior cortex of the oocyte was defined as a distinct region for mRNA localization. Now the anterior cortex of the oocyte is established as a separate destination for mRNA targeting. *osk* mRNA is transported in a Kinesin-dependent way toward the plus ends of MTs and becomes localized to the posterior cortex (Brendza et al. 2000; Januschke et al. 2002). *bcd* mRNA is transported in a dynein-dependent way toward the minus ends of MTs and becomes localized to the anterior cortex (Januschke et al. 2002).

When PFCs are lacking, or do not correctly differentiate, the oocyte nucleus does not migrate normally and mRNA localization is aberrant (Gonzalez-Reyes and St Johnston 1994; Gonzalez-Reyes et al. 1995; Roth et al. 1995; Meignin et al. 2007). These observations have led to the conclusion that the PFCs produce a signal that is received by the oocyte and triggers changes in the MT cytoskeleton (Gonzalez-Reyes and St Johnston 1994; Poulton and Deng 2007). Because the PFCs have just been established through a germline signal at stage 6/7, this second signaling process from the soma back to the germline has been termed back signaling.

The molecular nature of the signal emanating from the PFCs is unknown. However, there is good evidence that the signal requires direct contact between PFCs and the posterior surface of the oocyte (Poulton and Deng 2007). First, mutants preferentially disrupting the contact between PFCs and the oocyte cause polarity defects in the oocyte (Martin et al. 2003). Second, if cell clones mutant for EGF or JAK/STAT signaling components do not encompass all PFCs, such that some wild-type PFCs remain, *osk* mislocalization is restricted to the region of the oocyte cortex that is in direct contact with the mutant cells, whereas *osk* is normally localized in regions abutting wild-type PFCs (Frydman and Spradling 2001; Xi et al. 2003; Poulton and Deng 2006). Thus, the polarizing signal can be transmitted to the oocyte in a very local fashion.

Within the follicle cells, only three components have been found so far to be required for back signaling without affecting the PFC fate. All three highlight the importance of the ECM for the production of the polarizing signal. PFC clones mutant for laminin A

(Lan) or the transmembrane tyrosine phosphatase Dlar, a possible receptor for Lan, prevent proper oocyte polarization (Deng and Ruohola-Baker 2000; Frydman and Spradling 2001). It also has been shown that downregulation of the Lan receptor, dystroglycan (DG), in PFCs is required for proper back signaling (Poulton and Deng 2006). In all three cases, loss of function or overexpression clones in PFCs show locally restricted effects on the oocyte cortex as described for EGF and JAK/STAT signaling components. Despite this, Lan, Dlar, and DG are likely to play an indirect role in signal production (Poulton and Deng 2006).

At present, not much is known about the nature of the signal produced by the PFCs or the way this signal is perceived by the oocyte. Several components are required within the oocyte to establish correct MT polarity, but not for the PFC fate. Such components might be a part of the intracellular pathway that transduces the signal. However, some of them might also act in parallel to the signaling process and not be involved in providing spatial information. Candidates for back signaling factors fall into four categories: (1) Protein kinase A (Lane and Kalderon 1994; Steinhauer and Kalderon 2005), (2) Components of the Exon junction complex (EJC) (Micklem et al. 1997; Newmark et al. 1997; Hachet and Ephrussi 2001; Mohr et al. 2001) and heterogeneous nuclear ribonucleoproteins (hnRNPs) (Yano et al. 2004; Steinhauer and Kalderon 2005), (3) Proteins involved in vesicle transport (Coutelis and Ephrussi 2007; Januschke et al. 2007; Tanaka and Nakamura 2008), and (4) Par proteins and the tumor suppressor protein Lethal giant larvae (Lgl). We will focus our discussion on the *par* genes and *lgl* because only for these has a connection between cytoskeletal reorganization and back signaling been established.

The early requirement of the *par* genes makes it difficult to investigate their function during later stages of oogenesis. However, by using weak alleles of *par-1*, it was possible to identify a specific requirement of *par* genes for axis formation during midoogenesis (Shulman et al. 2000). At this stage, reduced Par-1 levels lead to a lack of posterior *osk* localization, which instead concentrates in the center of the oocyte; *bcd* mRNA localization is only weakly affected (Benton et al. 2002). The phenotype correlates with changes in the MT network: The posterior MTOC does not disassemble and MTs emanate from all cortical regions of the oocyte projecting the plus ends to the center. Polarity defects have recently also been reported for *lgl* mutants (Tian and Deng 2008). LGL is an evolutionary conserved WD40 domain containing protein, which acts together with Par-1 to establish polarity in a number of cell types (Munro 2006; see also McCaffrey and Macara 2009; Prehoda 2009). Loss of *lgl* in the oocyte leads to partially penetrant mislocalization of *osk* and *bcd*, as well as to defects in oocyte nucleus migration. Both Par-1 (the N1 isoforms) and Lgl are localized to the posterior cortex of stage 7 oocytes and thus belong to the earliest known markers responding to the polarizing signal (Doerflinger et al. 2006; Tian and Deng 2008).

It has been shown that Par-1N1 weakly accumulates at the posterior cortex even before the oocyte nucleus migrates and the MT network repolarizes. The posterior recruitment of Par-1N1 itself is not MT-dependent, but it requires F-actin. Par-1 and Lgl form protein complexes and Lgl can be inactivated through phosphorylation by aPKC (Tian and Deng 2008). However, the involvement of aPKC in axis formation during midoogenesis is still controversial.

The Par-1 kinase is able to destabilize MTs. Overexpression of Par-1 prevents the formation of MTs around the entire oocyte cortex (Tian and Deng 2009). Thus, posterior localized Par-1 might initiate the first asymmetry of the MT network by preventing MT growth from the posterior cortex. The mechanism by which Par-1 controls MT stability is still controversial. In contrast to earlier reports (Doerflinger et al. 2003), Tian and Deng (2009) suggest that the MT binding protein Tau is a target for phosphorylation by Par-1. Phosphorylated Tau can no longer bind to and stabilize MTs. The *tau* mutant phenotypes, however, are fairly weak,

suggesting that Par-1 has additional targets. Taken together, Par-1 seems to be the perfect link between PFC derived signals and oocyte polarity, given its spatial and temporal pattern of localization and its suggested protein function.

However, the Par-1 mutant phenotype as well as that of mutants in any of the other components mentioned above, with the possible exception of the EJC protein Mago nashi, do not lead to a complete lack of oocyte polarity. Thus, it is also possible that the crucial components responsible for transmitting the PFC signal have not been found so far, or that several signaling processes act in parallel. Indeed, the changes of the MT network at stage 7 might be decomposed into independent elements. The stabilization of minus ends at the lateral and anterior cortex of the oocyte might occur independent from the destabilization of MTs at the posterior pole induced by the PFCs. In addition, the migration of the oocyte nucleus might require an independent trigger. The oocyte nucleus itself has MTOC characteristics. Thus, its position within the oocyte has a profound influence on the overall structure of the oocyte MT network (Guichet et al. 2001; Januschke et al. 2002; Gervais et al. 2008). Januschke et al. 2006 have even proposed that the movement of the oocyte nucleus is the actual polarizing event. The posterior MTOC is not disassembled, but rather moves with the nucleus to the anterior pole where preformed MTs are released from the nucleus and anchored to the anterior and lateral cortex. In this scenario, nuclear migration does not depend on a repolarized MT cytoskeleton, but is driven by the growth of MTs emanating from the nucleus. The main function of the polarizing signal would be the initiation of nuclear migration.

Irrespective of the steps initiating MT repolarization at stage 7, MT polarity is further stabilized by feedback mechanisms occurring at two levels during stages 8 and 9. First, the *par* genes establish mutually exclusive membrane domains, which are maintained until stage 10A (Benton and St Johnston 2003). Par-1 kinase phosphorylates Par-3 and thereby restricts its localization to lateral and anterior membranes. Second, an Osk-dependent positive feedback loop is initiated, which re-enforces MT polarity (Zimyanin et al. 2007). Initially, only a weak MT gradient has been established on posterior Par-1N1 localization. This gradient is sufficient to initiate *osk* mRNA transport, first to the center of the oocyte and from there to the posterior pole, where it can be translated. Osk protein at the posterior cortex recruits more Par-1 protein, which in turn re-enforces the MT gradient. Par-1 in turn stabilizes Osk protein through direct phosphorylation, generating a second positive feedback loop (Riechmann et al. 2002). Thus, symmetry breaking of the MT network results from multiple dynamic interactions, including a spatially highly restricted Osk-dependent positive feedback loop.

So far, we have only provided a crude description of the of structure MT network at stage 9 when the steps of mRNA localization take place, which are crucial for axis formation of the later embryo. This description is largely based on the behavior of β-Gal fusion proteins containing the motor domains of the minus end-directed motor Nod, which localizes anteriorly, and the plus end-directed motor kinesin, which moves to the posterior (Clark et al. 1994; Clark et al. 1997). From these observations, the idea emerged that the MT network of the oocyte is highly polarized, with minus ends pointing toward the anterior and plus ends toward posterior pole. Direct staining for MTs, however, did not provide a clear picture of how the MTs are organized and often lead to conflicting results depending on fixation and staining procedures (Theurkauf et al. 1992; Theurkauf et al. 1993; Cha et al. 2002; Januschke et al. 2006; Wang and Riechmann 2008). The problem has not been resolved, but recent in vivo imaging approaches show that the overall MT network is much less polarized than expected (MacDougall et al. 2003; Zimyanin et al. 2008). There seems to be only a 20% excess of MTs with their plus end pointing posteriorly even after completion of MT polarity re-enforcement during stages 7 and 8, suggesting that the initial overall MT polarity

induced by the polarizing signal from PFCs was even weaker (Zimyanin et al. 2008).

THE EMERGENCE OF DV POLARITY

The posterior-to-anterior movement of the oocyte nucleus accompanies the repolarization of the MT network at stage 7 and we have already mentioned that it is currently impossible to specify the causal relationship between both events (Januschke et al. 2002; Januschke et al. 2006). In contrast, the role of the oocyte nucleus in establishing DV asymmetry is well supported (Roth 2003). The oocyte nucleus moves from a central posterior position toward the anterior side, the nurse cell–oocyte interface (Fig. 5). When the nucleus arrives at the anterior pole, it occupies a particular position at the circular circumference of the oocyte. This position corresponds to the future dorsal side of the egg chamber and the later embryo. *grk* mRNA is targeted to the nucleus and a second round of EGF signaling takes place, which leads to the DV patterning of the follicular epithelium (Neuman-Silberberg and Schuepbach 1993; Gonzalez-Reyes et al. 1995; Roth et al. 1995).

Several observations have shown that nuclear movement is not just correlated with, but is required to establish DV polarity. *grk* mRNA localization to the dorsal side requires correct nuclear positioning. If the nucleus is wrongly positioned, it is still targeted by *grk* mRNA (Roth et al. 1995). This explains why laser ablation of the nucleus causes a loss of DV polarity of the egg chamber (Montell et al. 1991). If nuclear movement fails to occur, as in *mago* mutants, or when the Grk signal is delayed, eggs develop that have AP polarity in the follicular epithelium, but lack DV polarity orthogonal to the AP axis (Micklem et al. 1997; Newmark et al. 1997; Peri and Roth 2000) (Fig. 5). Together, such observations show that nuclear movement is necessary for

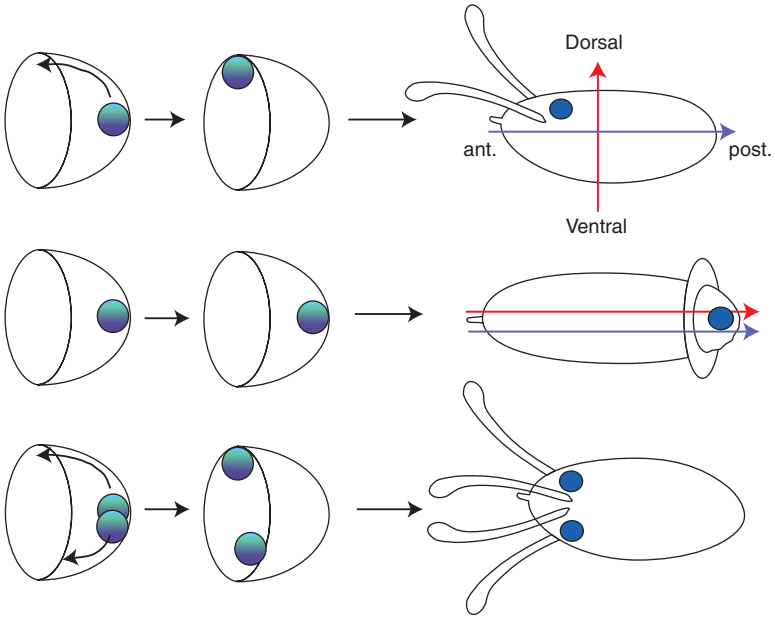

Figure 5. Oocyte nucleus migration and dorsoventral axis formation. Schematic drawings of stage 7 oocytes (*left*) and mature eggs. *Top*: Wild type. The posterior-to-anterior movement of the oocyte nucleus forces the nucleus to acquire an asymmetrical position, which determines the dorsal side of the egg and establishes orthogonality between the AP and the DV axes. *Middle*: If nuclear movement does not occur, AP and DV axes are parallel to each other. *Bottom*: In binuclear oocytes, both nuclei move to the anterior pole to adopt random position at the anterior cortex. Both nuclei induce dorsal egg shell structures.

the orthogonal orientation of the AP and DV axes. However, they do not prove that the nucleus determines the dorsal side of the egg chamber. It is conceivable that other processes specify this side before nuclear migration and the nucleus moves to this prespecified position. This scenario is unlikely, however. First, the DV axes of different egg chambers within an ovariole are randomly oriented with regard to each other and with regard to the DV axis of the female. Second, in binuclear oocytes, both nuclei move to the anterior cortex and induce dorsal chorion fates (Roth et al. 1999). A statistical analysis shows that they choose their position randomly with regard to each other. This behavior is most compatible with the assumption that the egg chamber completely lacks DV asymmetry before nuclear movement. Thus, among all the symmetry-breaking steps described so far, nuclear movement might provide the only case in which no bias exists that orients the process.

CONCLUDING REMARKS

The process of axis formation that we have described looks overwhelmingly complex and seems to take several detours. At several instances, polarity is established that later is lost. Thus, early AP polarity (rosette shape) of the germline cluster in region 2a of the germarium seems to be a by-product of the internal polarization of the oocyte (clustering of the ring canals). The rosette shape apparently disappears when the cyst flattens in region 2b. Likewise, the AP asymmetry of polar cell differentiation, required for oocyte positioning, is later lost, giving rise to a symmetric arrangement of polar and stalk cells. This symmetric arrangement is required for PFC induction by Grk. The first MT polarization of the oocyte provides another example. This process establishes the posterior pole of the oocyte and it has been suggested that mitochondria localized there at stage 1 are later incorporated into the pole plasm (Cox and Spradling 2003). However, this posterior pole can only be maintained and the pole plasm can only be assembled when the second round of MT repolarization takes place, which reverses the early MT polarity. Early MT polarity, however, was a prerequisite for posterior *grk* mRNA localization, which might be important for PFC induction by Grk signaling. In all of these instances, polarities arise transiently either as a secondary consequence or as a means of another step of symmetry breaking.

Another apparent detour in axis formation is represented by the reciprocal signaling between germline and soma. Why is polarity first established in the germline, then transferred to the follicular epithelium and from there back to the germline? The enormous increase in size of the oocyte might provide an explanation. The volume of the oocyte increases by four orders of magnitude from the time of oocyte determination to egg deposition. During these growth processes, axis information has to be accurately maintained. The follicular epithelium in close contact with the oocyte provides a means to store spatial information in a stable way and reuse it at a later stage after which the oocyte might have grown considerably. This is definitively an issue for DV axis formation, which we have not described in detail, but it might also be relevant to back signaling required for AP polarity. Using the follicular epithelium to store spatial information has another advantage. The border between oocyte and follicle cells provides a sharp discontinuity. Thus, back signaling from the follicle cells can be spatially highly confined.

Finally, most steps of axis formation during *Drosophila* oogenesis do not represent true symmetry-breaking events, but rather sense and enhance asymmetries of the ovariole architecture. Therefore, the question arises of how ovariole architecture is established in the first place. The embryonic and larval ovary of *Drosophila* consists of a growing spherical mass of intermingled mesenchymal somatic and primordial germ cells (King 1970; Gilboa and Lehmann 2006). During late larval and early pupal stages, somatic cells rearrange to form stacks of disc-shaped cells (Godt and Laski 1995). The first stacks give rise to the terminal filaments, which provide the anterior anchoring point of each ovariole.

Subsequently, cell stacks form, which give rise to the first interfollicular stalks and to the basal stalks. The linear arrangement of these cell stacks defines the long axis of the ovariole. The type of cell rearrangement and cell recruitment, which initiated stack formation, is known as convergent extension (Godt and Laski 1995; Stern 2004). A similar process is required for AP axis formation during gastrulation in most animals (also see Vladar et al. 2009). The convergent extension processes that establish the ovariole organization continue to play a role in the adult ovary when the prestalk cells converge to form the two-cell wide (Fig. 4A,B) and later the one-cell wide interfollicular stalks (Fig. 4C,D), which connect and align the individual egg chambers.

Taken together, axis formation during *Drosophila* oogenesis combines examples of most every process known so far to be involved in the generation spatial asymmetries, including cell shape changes and cell migration (stalk formation), differential cell adhesion (egg chamber orientation), inductive signaling (stalk cell and follicle cell specification, back-signaling), pattern formation in epithelial sheets (JAK/STAT signaling), asymmetric cell divisions (cyst formation), cell polarization (first and second round of par-dependent oocyte polarization), and intracellular patterning (oocyte migration, formation of cortical domains for RNA localization).

To what degree is this highly intricate process conserved in evolution? The fixed linear arrangement of developing egg chambers as represented in the ovariole structure seems to be an insect invention: Within insects, it represents a very stable character already present in the most primitive forms, whereas such structures are generally not found in other animal ovaries (Gutzeit and Sander 1985; Büning 1994). The connection of each ovariole to its own group of germline and somatic stem cells, the ordered sequence of maturation, and the intimate relation between the oocyte and the follicle cells producing the protective eggshell layers allowed for high fecundity and huge variety of eggshell structures, which was likely important in allowing insects to exploit a wide variety of environmental niches. In addition, the polar organization of the ovariole made it possible to shift aspects of axis formation, which typically take place during embryogenesis, to earlier and earlier stages of oogenesis. Posterior cytoplasmic determinants specifying the AP axis and the asymmetric positioning of the oocyte nucleus correlating with dorsal side of the egg are found already in ancestral insect orders (Sander 1976). However, there is also considerable variation in ovariole structure mainly connected to the presence or absence and the position of nurse cells (Büning 1994). Moreover, the molecular details of axis formation are highly variable even within the more advanced holometabolous insects (Fonseca et al. 2009). It will be interesting to see what aspects of *Drosophila* ovarian symmetry-breaking processes are conserved in other insect species, and whether the unique properties of the ovariole structure are exploited in different ways.

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