Asymmetric Divisions of Germline Cells

re Fichelson and Jean-René Huynh

Abstract

In most vertebrates and invertebrates, germ cells produce female and male gametes after one or several rounds of asymmetric cell division. Germline-specific features are used for the asymmetric segregation of fates, chromosomes and size during gametogenesis. In *Drosophila* females, for example, a germline-specific organelle called the fusome is used repeatedly to polarize the divisions of germline stem cells for their self-renewal, and during the divisions of cyst cells for the specification of the oocyte among a group of sister cells sharing a common cytoplasm. Later during oogenesis of most species, meiotic divisions produce a striking size asymmetry between a large oocyte and small polar bodies. The strategy used to create this asymmetry may involve the microtubules or the actin microfilaments or both, depending on the considered species. Despite this diversity and species-particularities, recent molecular data suggest that the PAR proteins, which control asymmetric cell division in a wide range of organisms and somatic cell types, could also play an important role at different steps of gametogenesis in many species. Here, we review the asymmetric features of germline cell division, from mitosis of germline stem cells to the extrusion of polar bodies after meiotic divisions.

1 Introduction

In several species, the formation of reproductive cells, called gametes, rely on one or several rounds of asymmetric cell division (Deng and Lin 2001; Huynh and St Johnston 2004; Wong et al. 2005). Asymmetric cell division is a process in which one cell divides into two cells with different developmental potentials. This is a fundamental way to generate cell diversity. In the germline, asymmetric division is often used to allow the simultaneous production of a differentiating cell and of a self-renewing stem cell (Deng and Lin 2001; Wong et al. 2005). Depending on the considered specie and gender, asymmetric germline stem cell division occurs during a defined time window or throughout the entire life of the organism. The differentiating cells produced after stem cell mitosis undergo several rounds of divisions,

Institut Jacques Monod, CNRS, Universités Paris 6 et 7, 2, place Jussieu, F-75251 Paris. Cedex 05, France. E-mail: huynh@ijm.jussieu.fr

Progress in Molecular and Subcellular Biology
Alvaro Macieira-Coelho (Ed.)
Asymmetric Cell Division

leading to an increase in germline cell number. These divisions are symmetric in males and ultimately lead to the production of spermatozoids. In contrast, in females of some insects, germline cell divisions are asymmetric and give rise to one oocyte together with several non-reproductive cells (de Cuevas et al. 1997; Huynh and St Johnston 2004). Finally, a widespread phenomenon occurring during late oogenesis is the asymmetric meiotic divisions which lead to the formation of small polar bodies and of a large oocyte, maintaining maternal stores required for embryogenesis (Maro and Verlhac 2002).

Asymmetric cell divisions may involve extrinsic and/or intrinsic factors (Jan and Jan 1998; Knoblich 2001). With extrinsic factors, daughter cells are initially equivalent but adopt different fates as the result of the interactions of the daughter cells with each other or with their environment. With intrinsic factors, unequal amounts of cell-fate determinants are segregated into the two daughter cells which therefore adopt distinct identities. Intrinsic mechanisms can also control the formation of daughter cells with different sizes by acting on spindle displacement, positioning and/or asymmetry. The processes underlying asymmetric cell division have been studied in organisms ranging from bacteria, yeast, worms (*Caenorhabditis elegans*) and flies (*Drosophila*) to mammals (Betschinger and Knoblich 2004).

Here we review the asymmetric features of germline cell division, from the mitosis of germline stem cells (GSCs) to the extrusion of polar bodies after the meiotic divisions. As asymmetric divisions of germline cells have been particularly well documented in *Drosophila*, we focus on this model organism and compare it with data gathered in other organisms whenever possible.

2 Asymmetric Germline Stem Cell Division During *Drosophila* Gametogenesis

Stem cells are characterized by both their ability to make more stem cells, a process called self-renewal, and their capacity to generate specialised cells forming organs (Fuchs and Segre 2000). While symmetric division is required for the expansion of a stem cell population (after an injury for example), asymmetric division allows the simultaneous production of two cell types, a new stem cell and a cell fated to differentiate (Morrison and Kimble 2006). Germline stem cells have been described in organisms where gametes are produced throughout the entire life, such as vertebrate males and *Drosophila* males and females (Li and Xie 2005; Zhao and Garbers 2002). Asymmetric germline stem cell (GSC) division has been documented mainly in *Drosophila* so far and has been shown to rely on extrinsic factors.

More precisely, a micro-environment of somatic cells called a niche controls the maintenance of the GSC fate, by providing specific signals, and the orientation of the GSC division (Wong et al. 2005).

In *Drosophila*, GSCs are located at the anterior apex of the ovary and of the testis (Wong et al. 2005). In the ovary, two to three GSCs are closely associated with somatic cells called cap cells (Fig. 1A). Anterior to theses cells lie the terminal filament cells and posterior to the cap cells are located the inner sheath cells. In the testis, seven to nine GSCs directly contact the hub cells, which are equivalent to the female cap cells (Fig. 1B). GSC divisions are asymmetric since the two daughter cells adopt different fates. The daughter cell which stays in contact with the cap cells in the female or with the hub cells in the male becomes a GSC, which maintains the stem cell pool, while the posterior daughter cell becomes a cystoblast in the female or a gonioblast in the male. After several mitotic and meiotic divisions, cystoblasts and gonioblast differentiate into cysts giving rise to gametes. Asymmetric GSC division thus permits GSC self-renewal to occur simultaneously with the production of a differentiating cell.

2.1 Extrinsic Features of Asymmetric Germline Stem Cell Division in *Drosophila*

Somatic cells play a fundamental role in controlling asymmetric GSC division. Both the orientation of the mitotic spindle and daughter cell fate determination are dependent upon the surrounding somatic cells. Adherens and gap junctions have been shown to be present at the interface between GSCs and cap/hub cells (Fig. 1A,B) (Gilboa et al. 2003; Song et al. 2002). Loss of function of a major component of adherens junctions, E-Cadherin, leads to a loss of GSCs and to sterility, showing the critical role played by somatic cells-GSC contact for the maintenance of the GSCs (Song et al. 2002). In the male, adherens junctions also orient GSC divisions along the anterior-posterior (A-P) axis by anchoring one centrosome to the anterior cortex. Anchorage occurs through the recruitment of the Adenomatous polyposis coli proteins APC1 and APC2 and of Centrosomin to the adherens junction, which aligns the GSC mitotic spindle along the A-P axis (Yamashita et al. 2003). The anterior daughter cell generated after mitosis stays in contact with the cap/hub cells and adopts a GSC fate while its posterior sibling differentiates as it contacts other somatic cells called escort cells in the female and cyst progenitor cells in the male (Decotto and Spradling 2005). Cyst progenitor cells have been shown to repress the GSC fate and to promote the gonioblast fate via the epidermal growth factor (EGF) pathway (Kiger et al. 2000; Tran et al. 2000). Thus, the asymmetry of GSC division is promoted extrinsically as both fates are induced by two types of specialised somatic cells.

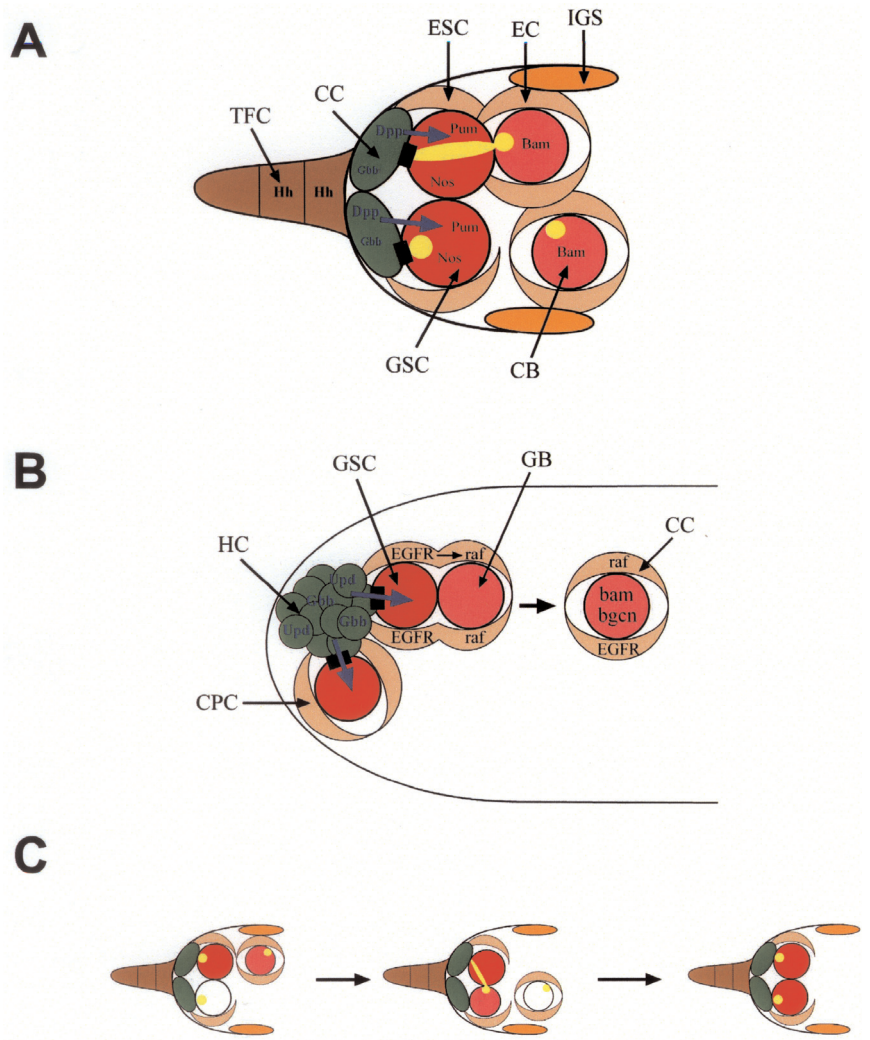

Fig. 1. A–C Asymmetric divisions of germline stem cells: **A** *Drosophila* ovarian niche: germline stem cells (GSC, *in red*) are attached to somatic cap cells (CC, *in green*) by adherens junctions (*black square*). GSCs are surrounded by Escort Stem Cells (ESC). GSCs divide asymmetrically to give rise to another stem cell and to a cystoblast (CB, *in pink*) anteriorly. Cystoblasts are surrounded by somatic Escort Cells (EC). The asymmetry of the division can be visualized by the asymmetric partitioning of the spectrosome (*yellow*). During interphase, the spectrosome is localized close to the adherens junctions in the GSCs. It anchors one pole of the mitotic spindle during mitosis. CCs maintain the GSC fate by secreting Decapentaplegic (Dpp, *in blue*) and Glass Bottom Boat (Gbb, *in blue*). GSCs express nanos (nos) and pumillio (pum), while CBs express bag-of-marbles (bam). Terminal follicle cells (TFC, *brown*) express hedgehog (hh). Inner germarial sheath cells (IGS) are *in*

In contrast with the implication cyst progenitor cells and possibly escort cells in promoting cyst differentiation, anterior somatic cells (hub and cap cells) play a key role in maintaining the GSC fate by providing a specific microenvironment. This cellular environment, together with the signals emitted by these anterior somatic cells compose what is called a niche that controls the balance between GSC self-renewal and differentiation (Li and Xie 2005; Ohlstein et al. 2004; Spradling et al. 2001). A true niche is able to keep its properties even in the absence of stem cells and must be capable of reprogramming differentiated cells into stem cells. Such has been shown to be the case for the niches found in the *Drosophila* ovary and testis. More precisely, when differentiation of all female GSCs into 4- 8- or 16-cells cysts is triggered by the transient over-expression of the cystoblast-specific gene *bag-of-marbles* (*bam*), the cap cells induce the cyst cells that are in contact to de-differentiate into GSCs (Kai and Spradling 2004). The cells located at the apex of the ovary thus form a true niche which is able to reprogram differentiated cells (cyst cells) into GSCs. In the male, similar experiments have shown that hub cells also form a niche within the testis; not only are hub cells required for GSC maintenance but these cells are also able to reprogram spermatogonies into GSCs (Brawley and Matunis 2004). Asymmetric GSC division thus results from the combination of two categories of extrinsic signals, signals provided by anterior somatic cells, which promote the GSC fate, and signals emitted by posterior somatic cell, which induce differentiation.

What are the signals promoting the GSC vs differentiated cell fate? As mentioned earlier, the EGF pathway promotes gonioblast differentiation in the male germline: loss of function of the EGF receptor or of the downstream effector *raf* leads to an increase in the number of GSCs (Fig. 1B) (Kiger et al. 2000; Tran et al. 2000). Conversely, signals promoting GSC fate have been described in both male and female niches. Niche cells emit several proteins which diffuse within the extracellular space and are received by target cells (GSCs) (Fig. 1A,B). Three signalling pathways have

Fig. 1. (*Cont'd*) *orange*; **B** *Drosophila* testis niche: germline stem cells (GSC, *in red*) are attached to somatic hub cells (HC, *in green*) by adherens junctions (*black square*). GSCs are surrounded by cyst progenitor cells (CPC). GSCs divide asymmetrically to give rise to another stem cell and to a gonioblast (GB, *in pink*) posteriorly. Gonioblasts are surrounded by somatic Cyst Cells (CC). HCs maintain the GSC fate by secreting Unpaired (Upd, *in blue*) and Glass Bottom Boat (Gbb, *in blue*). Activation of the EGF pathway in CCs represses the GSC fate in favour of the GB fate; **C** symmetric stem cell division in the ovarian niche: when a GSC (*white*) directly differentiates as a CB, the neighbouring GSC divides orthogonally to the antero-posterior axis, so that both daughter cells remain in the niche. Cells from the niche induce both cells to become GSCs. This division is thus symmetric even though the spectrosome is asymmetrically partitioned (*yellow*)

been shown to be important for GSC maintenance: 1) the Bone morphogenetic protein (BMP) pathway, activated by the ligands Decapentaplegic (Dpp) and Glass bottom boat (Gbb), 2) the Janus kinase and signal transducer and activator of transcription (JAK-STAT) pathway, activated by the Unpaired (Upd) ligand which binds the Domeless receptor and acts upstream of the Hopscotch kinase and the STAT92E transcriptional activator, and 3) the Hedgehog (Hh) pathway (Wong et al. 2005). In female flies, Dpp is expressed by the cap cells and plays an essential role in GSC maintenance (Xie and Spradling 1998). The involvement of the JAK-STAT pathway is more indirect as it acts within the recently identified escort cells which then regulate the progeny of the GSCs (Decotto and Spradling 2005). The situation is different in male flies since it is the Upd and Gbb ligands expressed by the hub cells that both play a key function for GSC maintenance (Kawase et al. 2004; Kiger et al. 2001; Tulina and Matunis 2001).

In the ovary, the BMP pathway has been dissected in a detailed fashion and the main molecular players implicated in signal reception, transduction and target gene regulation have been identified. In female GSCs, Dpp and Gbb bind the Punt and Thick vein receptors, which phosphorylate the DNA-binding protein Mothers against Dpp (Mad). Activation of Mad via its phosphorylation promotes its binding to the Medea protein and induces the Mad-Medea complex to localize to the nucleus. Nuclear Mad-Medea complexes bind a specific regulatory sequence of the cystoblast-specific gene *bam*, which inhibits its transcription and therefore maintains the GSC fate (Chen and McKearin 2005; Song et al. 2004). Conversely in cystoblasts, no BMP signal is received, Mad is not activated, *bam* is transcribed, the GSC-specific RNA-binding proteins Nanos (Nos) and Pumillio (Pum) are inhibited and differentiation is triggered (Szakmary et al. 2005).

In *Drosophila*, GSCs divide asymmetrically due to the major influence of the environment. Upon GSC division, mitotic spindle orientation is controlled by the niche and once division is completed, GSC vs cystoblast (or gonioblast) fate decision depends on the position of the daughter cells relative to the niche. The daughter cell which stays in the niche receives signals promoting the GSC fate while its sibling receives signals inducing differentiation as it is positioned outside the niche.

2.2 Intrinsic Features of Asymmetric Germline Stem Cell Division in *Drosophila*

In addition to the extrinsic factors controlling asymmetric GSC division, one intrinsic asymmetry has also been described upon mitosis, the asymmetric inheritance of a structure present in GSCs called a spectrosome (Huynh 2005; Lin et al. 1994). The spectrosome is made of vesicles attached by cytoskeleton proteins such as α- and β-spectrin, ankyrin and the *Drosophila* homologue of adducin: Hu-li-tai shao (Hts), an actin and spectrin-binding

protein (de Cuevas et al. 1996; Lin et al. 1994; Yue and Spradling 1992). In the adult, the spectrosome is stably anchored at the anterior side of the GSC, in contact with the adherens junctions between the GSC and the overlying cap (hub) cells (Song et al. 2002). During female GSC division, one pole of the mitotic spindle is anchored by the spectrosome thus orientating the division along the A-P axis of the germarium (Deng and Lin 1997). The cystoblast is then produced toward the posterior and the renewed GSC stays at the anterior of the germarium. At the end of telophase, a transient cytoplasmic bridge called ring canal forms between the GSC and the cystoblast. New spectrosome material accumulates in this ring canal and the initial spectrosome elongates from the anterior side of the GSC to fuse with the new spectrosome (de Cuevas and Spradling 1998; Deng and Lin 1997). The cytoplasmic bridge is then severed and one-third of the spectrosome is inherited by the cystoblast where it forms the fusome (see below) while two-thirds remain in the GSC, marking this division as clearly asymmetric (Deng and Lin 1997; Lin and Spradling 1997). The spectrosome relocalizes to the anterior side of the GSC, while the fusome takes a spherical shape at one end of the cystoblast. The function of this asymmetrically distributed organelle regarding GSC vs cystoblast (or gonioblast) cell fate determination remains to be explored. The spectrosome appears important for asymmetric GSC division as it is involved in the anchoring of the mitotic spindle (Deng and Lin 1997). However asymmetric inheritance of the spectrosome upon GSC division does not seem to be sufficient to trigger asymmetric fate decision. When a GSC divides perpendicular to the anterior-posterior axis relative to the niche, both daughter cells become GCSs (Fig. 1C). The division is thus symmetric, despite the asymmetrical behaviour of the fusome (Xie and Spradling 2000). There is so far no evidence of an intrinsic factor which would be sufficient to control the GSC vs differentiated cell fate decision.

Extrinsic factors thus play the major role in establishing asymmetry during GSC division in *Drosophila*. Interestingly, the presence of a niche maintaining GSCs has also been demonstrated in *C. elegans* and in vertebrates (Wong et al. 2005; Zhao and Garbers 2002). As extrinsic signals also regulate GSC fate maintenance in these organisms, the choice between asymmetric and symmetric GSC division depends on the orientation of the mitotic spindle. In *Drosophila*, GSC divisions are generally oriented along the A-P axis. This leads to the formation of daughter cells exposed to different environments, which triggers asymmetric fate decision. Only in cases where a GSC is missing does a neighbour GSC divide symmetrically, perpendicular to the A-P axis, which generates two GSCs. In *C. elegans* and in vertebrates, the orientation of the GSC mitotic spindle appears stochastic. These divisions can be either symmetric, if both daughter cells remain in the niche, or asymmetric in cases where one of the daughter cells exits the niche. Asymmetric vs symmetric fate decision is thus thought to be controlled by proximity to the niche rather than by programmed asymmetric GSC divisions (Lin 1997; Morrison and Kimble 2006).

3
Asymmetric Cell Division During *Drosophila* Oogenesis: Importance of the Fusome for the Specification and Polarisation of the Female Gamete

The differentiating cell generated after GSC division undergoes several rounds of divisions, leading to an amplification of the germline cell population. These divisions are symmetric in males and ultimately generate spermatozoids. In females of some insects, this amplification step takes place but only one gamete is produced per cystoblast. More precisely, in *Drosophila*, the one cell cystoblast goes through four rounds of synchronous mitosis with incomplete cytokinesis to form a cyst of 16 germline cells, which are interconnected by ring canals (Spradling 1993). All the cells within the cyst share the same cystoplasm, but they differ regarding their number of ring canals; for example only two cells, called pro-oocytes, have four ring canals. Once the 16 cell cyst has formed, one of the two pro-oocytes differentiates into the oocyte while the other fifteen cyst cells become nurse cells. An asymmetric fate decision is thus taken within the 16 cell cyst so that only one cell adopts the oocyte fate (Huynh and St Johnston 2004).

Two main models have been proposed to explain how the oocyte is selected. One model is based on the symmetrical behaviour of the two cells with four ring canals (pro-oocytes), and proposes that there is a competition between the two pro-oocytes to become the oocyte (Carpenter 1975, 1994). The "winning" cell would become the oocyte, while the "losing" cell would revert to the nurse cell fate. This process would thus rely on an extrinsic mechanism, such as cell-cell communication within the cyst. However, the factor that could control this fate decision has remained elusive. A second model suggests that the choice of the oocyte is biased by the establishment of some intrinsic asymmetry as early as the first cystoblast division, which is maintained until the overt differentiation of the oocyte (Lin and Spradling 1995; Theurkauf 1994). The formation of a germline-specific organelle called the fusome strongly supports this second model.

The fusome is a large cytoplasmic structure and is an important feature of cyst development both in *Drosophila* and in *Xenopus* (Kloc et al. 2004; Telfer 1975). In *Drosophila* this structure derives from the spectrosome and links all the cells of the cluster through the ring canals. The fusome is made of a continuous network of interconnected ER-derived tubules kept together by components of the sub-membranous cytoskeleton, such as α-spectrin, β-spectrin, and Hts (de Cuevas et al. 1996; Lin et al. 1994; Snapp et al. 2004; Yue and Spradling 1992). The fusome also contains microtubules and microtubules associated proteins (Grieder et al. 2000; Roper and Brown 2004). It was found in *Drosophila* ovaries that, in the absence of the fusome, cells of the same cluster divide asynchronously and fail to specify an oocyte, despite the presence of ring canals (de Cuevas

et al. 1996; Lin et al. 1994; Yue and Spradling 1992). This demonstrates a key role of the fusome as a channel of communication between the cells for the synchronisation and differentiation of the germline cyst. Interestingly, the fusome is asymmetrically inherited upon the four rounds of cystoblast division. This asymmetric behaviour of the fusome is likely to be essential for oocyte fate determination.

The first mitosis of the cystoblast is very similar to the GSC division (Fig. 2). One pole of the mitotic spindle is anchored by the spherical fusome (de Cuevas and Spradling 1998; Deng and Lin 1997); a new fusome "plug" forms into the arrested furrow at the other end of the cell and comes to fuse with the "original" fusome. However, in contrast to the GSC division, cytokinesis is incomplete and both cells remain linked by a stable ring canal. Furthermore, although it is not known how the plug and the "original" fusome move to fuse together, the "original" fusome does not seem to elongate from one side of the cystoblast, as in the GSC (de Cuevas and Spradling 1998). Cystoblast division is asymmetric as one cell contains the "original" fusome plus half of the plug, whereas the other cell only retains the other half of the fusome plug. At the next division, the two mitotic spindles again orient with one pole close to the fusome and new fusome plugs form in the two ring canals situated at opposite ends (de Cuevas and Spradling 1998; Lin and Spradling 1995; McGrail and Hays 1997; Storto and King 1989). The fusome plugs then move with their ring canal to fuse with the central fusome, which thus remains in the two previous cells. This asymmetric behaviour of the fusome is then repeated in the next two divisions. The oldest cell, therefore, retains the original fusome and accumulates four halves of fusome plugs. Thus, this cell has more fusome than all the other cells and can be identified throughout the divisions. The current model suggests that this cell will become the oocyte (see below). Once the 16 cells-cyst is formed, the fusome starts to break down and disappears. The behaviour of the *Drosophila* fusome during asymmetric cystoblast division has important consequences on the formation and polarisation of the female cyst (de Cuevas et al. 1997; Huynh and St Johnston 2004).

First, upon cystoblast division, the fusome anchors one pole of each mitotic spindle (Deng and Lin 1997; Lin and Spradling 1995). This orientation of the divisions ensures that one cell inherits all the previous ring canals, while a new one is formed at the opposite end of the cell and thus branched off the central fusome. By orienting the mitotic spindle, the fusome therefore directly controls the asymmetry of the division as one cell inherits the old ring canals while the other does not. This orientation also leads to an invariant pattern of interconnections between the cells, with the two central or oldest cells having n ring canals (n being the number of cystoblast divisions), their daughter cells n–1, etc. This pattern is important for the polarisation of the cyst as the oocyte always arises from one of the two cells with the greatest number (n) of ring canals (Buning 1994).

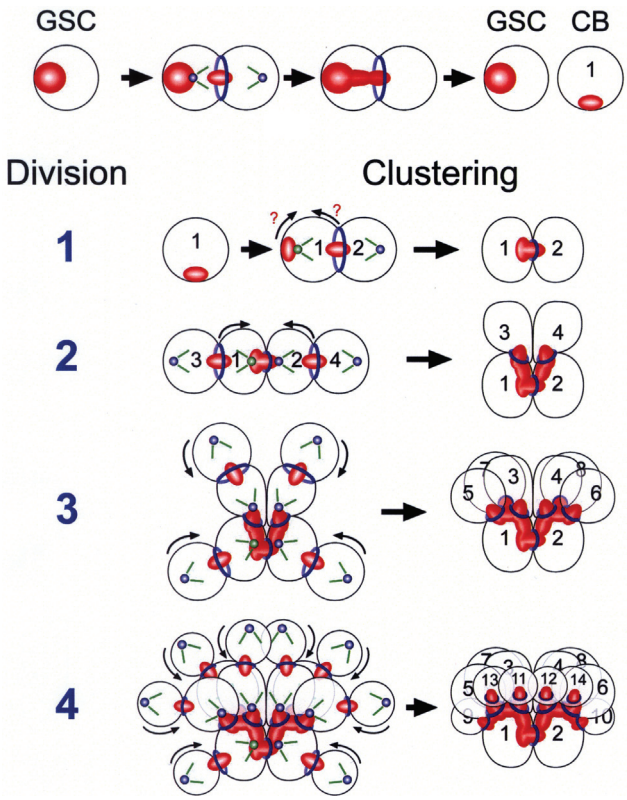

Fig. 2. Asymmetric divisions of female germ cells and formation of the fusome. The spectrosome (*red*) of the GSC anchors one pole of the mitotic spindle and orients the division along the anterior-posterior axis. A new fusome plug forms into the transient ring canal. The spectrosome elongates to fuse with the plug. The cytoplasmic bridge is then severed and one-third of the spectrosome/fusome is inherited by the cystoblast while two-third remains in the GSC, marking this division as clearly asymmetric. The spectrosome/fusome (*red*) of the cystoblast (1) interacts with one of the centrosomes (*green and blue spheres*) to anchor one pole of the mitotic spindle (*green lines*), during the first incomplete division. 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 divisions 2, 3 and 4. 1) One pole of each mitotic spindle is anchored by the fusome. 2) A new fusome plug forms into each ring canal. 3) The ring canals move centripetally for the fusome plugs to fuse with the central fusome (*black arrows*). This behaviour has several crucial consequences: 1) cystocyte (1) has more fusome than the other cystocytes; 2) the same centrosome (*green sphere*) could be inherited by the cystocyte (1) from division 1 through division 4; and 3) the fusome always marks the anterior of cystocyte (1), after the clustering of the ring canals

Second, the formation of the fusome provides the strongest evidence in support of an intrinsic mechanism controlling asymmetric fate descision within the cyst (de Cuevas and Spradling 1998). Indeed, the asymmetric inheritance of the "original fusome" during the cyst divisions could play a role in determining which cell will adopt the oocyte fate. This model is supported by analogy with the diving beetle *Dytiscus*, in which oogenesis is very similar to *Drosophila* (Giardina 1901; Telfer 1975). A *Dytiscus* cyst is formed of 15 nurse cells and one oocyte, resulting from four incomplete and synchronous divisions of a cystoblast. However, unlike *Drosophila*, the oocyte can be distinguished as early as the two-cell stage because it contains a large ring of highly amplified rDNA. Moreover, the cell that inherits that ring of rDNA, also inherits the fusome. This also suggests that the early selection of the oocyte could be a general feature among insects (de Cuevas and Spradling 1998; Grieder et al. 2000). Unfortunately, in *Drosophila* most of the fusome has already degenerated by the time oocyte-specific proteins such as BicD or Orb accumulate in a single cell. However, the preferential accumulation of the centrosomes and *oskar*, and *orb* mRNAs in one cell can be detected earlier in cystoblast development, and this is always the cell with the most fusome (Lilly and Spradling 2003; Grieder et al. 2000). This is particularly obvious in *egl* and *BicD* mutants, in which the fusome perdures longer, and where the centrosomes clearly accumulate in the cell with the largest piece of fusome remnant (Bolivar et al. 2001). These data strongly suggest that the "original" fusome marks the future oocyte, in support of an intrinsic mechanism underlying oocyte fate determination. 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 by the asymmetric inheritance of the fusome upon division.

What is the link between the asymmetric inheritance of the fusome and the selection of the oocyte? The simplest model is that an oocyte determinant 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 (Theurkauf 1994). Consequently, oocyte determinants could cosegregate with this centriole. Such a mechanism has been shown to mediate the segregation of *dpp* and *eve* mRNAs into specific cells during the asymmetric divisions of the early *Ilyanassa obsoleta* embryo (Lambert and Nagy 2002). 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 towards this cell. Consistent with the second model, it has been suggested that although the fusome starts to degenerate, it acts as a matrix to organise the restriction of oocyte-specific proteins, centrioles and meiosis to a single cell by multiple pathways. However, what molecular mechanisms regulate the different pathways and how these pathways interpret the fusome polarity remains unknown.

4
Asymmetric Meiotic Cell Division Leading to the Formation of Unequal Sized Daughter Cells

Meiosis is a succession of two particular cell divisions without an intervening replication (S) phase, leading to the formation of haploid cells, the gametes, in diploid organisms. In the first division, homologous chromosomes become paired and exchange genetic material (via crossing over) before moving away from each other into separate daughter nuclei (reductional division, MI). Sister chromatids separate during the second division (equational division, MII), giving rise to haploid cells. As such, these two divisions are asymmetric since they generate cells with different genetic pools. Here, we focus on female meiotic maturation, which presents an additional asymmetric feature, the formation of unequal sized daughter cells. Before these divisions, the oocyte is arrested in meiotic prophase I in most animal species. A hormonal stimulus usually triggers the reinitiation of meiotic maturation. The timing of fertilization is, however, species-specific (Fig. 3). During female meiotic maturation, both MI and MII produce a small cell called the polar body and a large cell, the oocyte. The polar bodies eventually degenerate while the oocyte conserves the entire maternal stores accumulated during oogenesis.

4.1
Meiotic Spindle Positioning

The size asymmetry observed during MI and MII depends on the localisation and on the orientation of the meiotic spindle. More precisely, size asymmetry results 1) from the migration of the spindle to the periphery of the oocyte and 2) from spindle alignment with an axis perpendicular to the overlying cell cortex. The combination of these two steps minimizes the size of the polar bodies by positioning the plane of division close to the cortex. If either of these two steps is impaired, daughter cell size asymmetry becomes reduced or abolished. Cortical localisation of the spindle is achieved either

Fig. 3. (*Cont'd*) GVBD and formation of an MI spindle. The spindle migrates in an actin-dependent manner to the closest cortical region. This region then defines the animal pole. An actin cap forms above the "cortical" pole of the MI spindle and the first polar body is extruded. Fertilization causes the MI spindle to rotate 90° and leads to the extrusion of the second polar body; **D** in *C. elegans*: the GV is displaced from the center of the oocyte before GVBD. After GVBD, the MI spindle migrates in a microtubules-dependent manner to the cortex, where it stays parallel to it. Fertilization elicits a 90° rotation of the MI spindle and the subsequent expulsions of two polar bodies

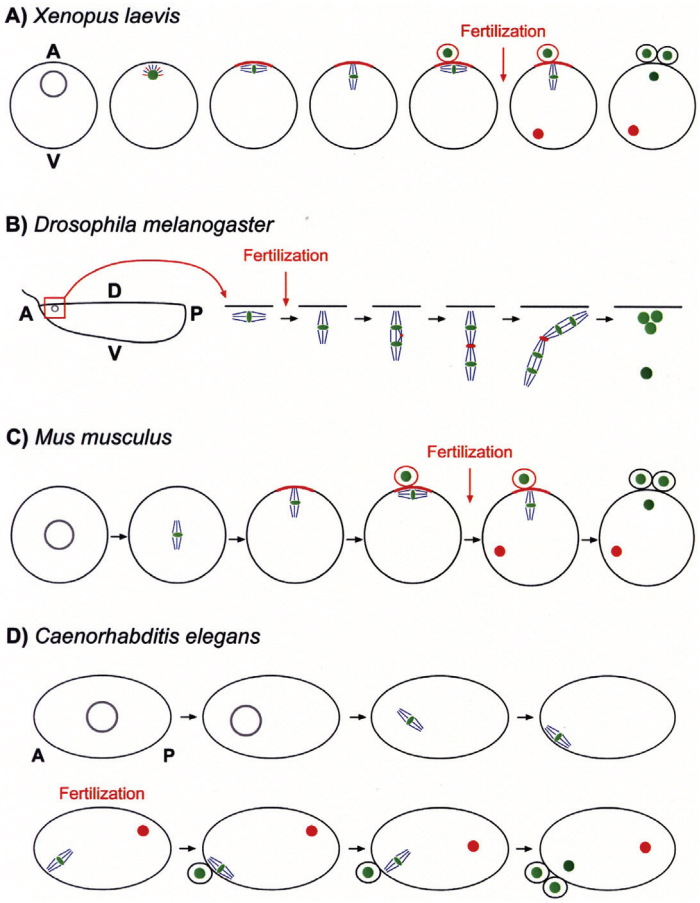

Fig. 3. A–D Asymmetric divisions during meiosis. In most animal species, the oocyte is arrested in meiotic prophase I. A hormonal stimulus usually triggers the reinitiation of meiotic maturation. The timing of fertilization is species specific. The GV is visualized as a *grey circle*, chromosomes are in *green*, microtubules in *blue*, actin in *red. Red dots* are male pronuclei after fertilization and *dark green dots* are female pronuclei after completion of meiotic maturation: **A** in *Xenopus laevis*: the GV is asymmetrically localized close to the animal pole. Reinitiation of meiosis maturation [illegible] GVBD and migration of the chromosomes surrounded by the MTOC-TAM [illegible] metaphase I spindle then elongates parallel to the cortex. An actin cap forms above the spindle. The spindle rotates 90°, before expulsion of the first polar body. Fertilization triggers the expulsion of the second polar body and the disappearance of the actin cap; **B** in *Drosophila melanogaster*: the GV is localized asymmetrically at the dorso-anterior corner. After reinitiation of meiosis, the MI spindle lies parallel to the cortex. Fertilization induces a 90° rotation of the MI spindle and two successive mitosis without cytokinesis. The inner most nucleus becomes the female pronucleus (*dark green*); **C** in *Mus musculus*: the GV is localized centrally in the oocyte arrested in prophase I. Reinitiation of meiosis induces

by the migration of the oocyte nucleus arrested in prophase I (germinal vesicle, GV, stage) or by the migration of the MI spindle, or by both when the breakdown of the GV (GVBD) occurs while it is migrating (Fig. 3). Depending on the species, spindle positioning perpendicular to the overlying cell cortex occurs either directly after migration or requires an extra 90° rotation as described below.

In some species of fishes, amphibian, worms, sea urchin and sea cucumber, the GV is already asymmetrically localized toward the animal pole (Gard 1991; Miyazaki et al. 2005). In *Xenopus*, reinitiation of meiosis triggers the breakdown of the nuclear envelope (GVBD) and the migration of the chromosomes associated with a dense array of microtubules and actin microfilaments, the MTOC-TMA (Microtubule-Organizing-Center-Transient-Microtubules-Array). Once migration is completed, the MI spindle lies parallel to the cell cortex and rotates 90° to extrude the first polar body; second polar body extrusion occurs following a similar spindle rotation (Fig. 3A).

In starfish and fly oocytes, the GV is positioned just beneath the cell cortex, which defines the future position of the meiotic spindle (Endow and Komma 1997; Miyazaki et al. 2000). This localisation occurs long before meiosis is reinitiated. After re-entry into meiosis, two polar bodies are sequentially extruded in starfish. In *Drosophila* the situation is atypical (Endow and Komma 1997, 1998; Skold et al. 2005; Tavosanis et al. 1997; Theurkauf and Hawley 1992). The MI spindle first lies parallel to the cell cortex and later rotates 90° (Fig. 3B). Although MI progresses up to anaphase, no polar body is extruded. The MII spindles assemble during MI anaphase and have a shared pole that forms within the MI spindle. Both MII spindles are aligned as a tandem, the inner most nucleus becomes the female pronucleus while the three nuclei next to the cortex assemble into a rosette like structure and degenerate.

In *Ciona*, *C. elegans*, amphibians, some molluscs and mammals, reinitiation of meiosis leads to germinal vesicle breakdown (GVBD) and to the formation the MI spindle, which then moves towards the cell cortex (Brunet and Maro 2005; Maro and Verlhac 2002; Pielak et al. 2004; Prodon et al. 2006). In the mouse (Fig. 3C), the MI spindle is assembled around the chromosomes, generally in the center of the oocyte, and migrates towards the cortex (Longo and Chen 1985; Verlhac et al. 2000). Experiments carried in oocytes cultured in vitro show that, once the MI spindle is assembled, its axis defines the path of migration (Verlhac et al. 2000). Migration proceeds towards the nearest part of the cell cortex, following the axis of the spindle, and the first polar body is extruded. After cytokinesis, the MII spindle forms parallel to the overlying cell cortex and cycle arrests in metaphase. MII resumes upon fertilization, the spindle undergoes a 90° rotation and the second polar body is extruded. In *C. elegans* (Fig. 3D), the situation is somewhat different since the MI spindle forms at the periphery of the oocyte and migrates only a short distance following a path perpendicular to the spindle axis (Albertson and Thomson 1993; Yang et al. 2003, 2005). Once the MI spindle has reached the cortex, meiotic maturation

stops until fertilization, which triggers a 90° rotation of the spindle and the sequential extrusion of two polar bodies.

4.1.1 Molecular Mechanisms

During mitosis, movement and orientation of the spindle have been shown to occur through astral microtubules that emanate from centriole-containing centrosomes. However, oocytes rarely have centrioles and in most examined species, female meiotic spindles are devoid of centrosomes and lack astral microtubules. In oocytes, GV or spindle migration and anchoring has been described to rely mainly on microtubules and/or on actin microfilaments, depending on the considered species.

In mice, actin microfilaments are not required for GVBD or MI spindle assembly, but play an essential role regarding spindle migration, as shown by drug treatment (Longo and Chen 1985). In addition, microtubule depolymerization experiments have demonstrated that, in the mouse oocyte, meiotic chromosome migration can occur without microtubules. The lack of microtubules results in the scattering of the chromosomes but migration to the cell cortex is not impaired (Longo and Chen 1985). Therefore microfilaments appear to be the essential factor involved in spindle relocalisation (Sun and Schatten 2006). In agreement with a central role of actin for spindle migration, loss of function of Formin-2, a straight actin filament nucleator, has been shown to lead to defects in MI spindle positioning and to the absence of polar body extrusion (Leader et al. 2002). More precisely, it was recently shown that in Formin-2 mutant oocytes, the MI spindle completely fails to migrate and that the late steps of cytokinesis are impaired (Dumont et al., in press). The *mos*/mitogen activated protein kinase pathway was also shown to be required for MI spindle migration (Verlhac et al. 2000). Interestingly, in *mos*-/- oocyte, polar body extrusion occurs despite the lack of spindle migration. This is due to an 'anaphase rescue' mechanism involving abnormal elongation of the anaphase spindle. Finally, four members of the PAR proteins family have been described to localize on the MI and MII spindles, PAR1, PAR3, PAR4 and PAR6 (Duncan et al. 2005; Moore and Zernicka-Goetz 2005; Szczepanska and Maleszewski 2005; Vinot et al. 2004). The PAR proteins have been shown to control cortical polarity and asymmetric cell division in a wide range of organisms and cell types (Betschinger and Knoblich 2004), suggesting that they could play an important role in the regulation of asymmetric meiotic division. Interestingly, during asymmetric mitotic division, the PAR proteins establish a cortical polarisation that is later interpreted by the spindle, whereas in the mouse oocyte, PAR proteins are first localized on the spindle and only later at the cell cortex (see below), indicating that spindle migration and cortical polarisation could be coupled through a PAR-dependent mechanism. Consistent with this hypothesis, the specific enrichment of PAR6 to the leading pole of the migrating MI

spindle suggests that PAR6 could control spindle migration and interaction with the cell cortex (Vinot et al. 2004). However, in the absence of functional data, one can only speculate about the putative function(s) of the PAR proteins. Interestingly, a recent study has shown that an upstream regulator of the PAR3/PAR6/aPKC complex, the Rho GTPase Cdc42, is required for correct spindle morphology and migration (Na and Zernicka-Goetz 2006). When a dominant negative form of Cdc42 is injected into oocytes, the MI spindle does not migrate, instead it elongates and no polar body is formed. In addition, aPKC, which is normally localized at the spindle poles, becomes located on the whole length of the spindle. The authors indicate that Cdc42 could act via two pathways, controlling respectively spindle morphology and spindle migration.

The lack of microtubule requirement for chromosome migration in the mouse oocyte is in contrast with the situation in *Drosophila*, *Xenopus* and *C. elegans* where microtubules and their associated motors play a key function for GV/spindle migration and anchoring (Gard 1991, 1992; Gard et al. 1995; Januschke et al. 2006; Theurkauf et al. 1992, 1993; Yang et al. 2003, 2005). In *Drosophila*, GV migration depends on a complex network of microtubules as indicated by microtubule depolymerization experiments (Januschke et al. 2006; Theurkauf et al. 1992, 1993). In addition, disruption of molecular motors directed either towards the plus-ends or the minus-ends of the microtubules have been shown to lead to defective GV migration (Januschke et al. 2002). Anchorage of the GV to the oocyte cortex was also demonstrated to require microtubules and the Lis1/dynein complex (Swan et al. 1999). In *Xenopus*, the microtubule, microfilament and intermediary filament networks play an important role controlling asymmetric meiotic division (Gard 1991, 1992; Gard and Klymkowsky 1998). A myosin (Myo10) has been shown to be critical for nuclear anchoring, spindle assembly and anchoring to the cortex by integrating the actin microfilaments and microtubule cytoskeletons (Weber et al. 2004). In *C. elegans*, depletion of microtubules blocks GV and MI spindle migration. In addition, both a microtubule severing enzyme and a microtubule associated kinesin have been involved in the translocation of the MI spindle to the oocyte cortex (Yang et al. 2003, 2005).

In all cases described above, GV or spindle migration creates or reinforces an asymmetry within the oocyte as chromosomes are relocalized to an off-centre position. Coincidently, a second asymmetry is also established, at the cortex of the oocyte.

4.2 Cortical Asymmetry

It is interesting to note that, concomitantly with MI spindle migration, the oocyte cortex becomes asymmetric. In the mouse, before entry into meiosis, at the germinal vesicle (GV) stage, the oocyte cortex presents microvilli

in a uniform fashion. After germinal vesicle breakdown (GVBD), the cortical region above the migrating MI spindle becomes gradually devoid of microvilli and enriched in actin (Brunet and Maro 2005; Longo and Chen 1985; Maro et al. 1984; Sun and Schatten 2006). In addition, cortical granules become excluded from the area surrounding the MII spindle (Deng et al. 2003). The role of the cortical reorganisation observed during mouse meiosis is unclear. The actin rich domain could be involved in anchoring the meiotic spindle and/or chromosomes; this domain has also been suggested to prevent sperm entry next to the female pronucleus. It is noteworthy that actin enrichment above the meiotic spindle is a general feature of meiotic maturation as it has been described in ascidian, *Tubifex*, *Xenopus*, pig, horse, cow and human oocytes, suggesting an essential function (Kim et al. 1997, 1998; Pickering et al. 1988; Sardet et al. 2002; Tremoleda et al. 2001).

Experiments carried in the mouse indicate that chromosomes are involved in remodelling the overlying oocyte cortex by a mechanism acting at a distance that remains to be determined (Maro et al. 1986). Upon meiotic spindle depolymerization, using colcemid, the chromosomes become scattered in subcortical zones. Interestingly, under these experimental conditions, actin microfilaments and myosinIIA accumulate at the cortex overlying or adjacent to each chromosome mass. Thus, cortical remodeling occurs in a chromosome-dependent and microtubule-independent mechanism, possibly involving direct interaction between chromosomes and microfilaments (Longo and Chen 1985). PAR proteins have also been reported to localize asymmetrically during *Xenopus*, mouse and *C. elegans* meiosis (Duncan et al. 2005; Nakaya et al. 2000; Sonneville and Gonczy 2004; Vinot et al. 2004; Wallenfang and Seydoux 2000). In *Xenopus*, it has been shown that 2–3 h after GVBD, aPKC together with PAR3 (ASIP) become specifically localized at the animal cortex of the oocyte, while the vegetal side becomes devoid of these proteins (Nakaya et al. 2000). Possibly acting in concert with PAR proteins, Cdc42 has been involved in controlling asymmetric meiotic cell division in the frog (Ma et al. 2006). Cdc42 is activated at the spindle pole-cortical contact side immediately before polar body formation and inhibition of Cdc42 leads to a failure to extrude a polar body. Interestingly, the Cdc42 cortical activity zone is circumscribed by a cortical RhoA activity zone (Bement et al. 2005). RhoA is also a small GTPase required for both the accumulation of cortical actin during assembly and for the actomyosin contractility of the furrow during cytokinesis (Glotzer 2005). In the *Xenopus* oocyte, concentration of active RhoA depends on microtubules but not on actin and is required for cytokinesis. The authors thus propose that the complementary pattern of Cdc42 and RhoA activities could be an evolutionary conserved process that couples spindle positioning to asymmetric cytokinesis (Ma et al. 2006). In the mouse, both PAR3 and PAR6 have been shown to localize as a crescent overlying the meiotic spindle (Duncan et al. 2005; Vinot et al. 2004). The crescent of PAR3 is present during both MI and MII while the crescent

of PAR6 is detected only during MII. The cortical localization of PAR3 and PAR6 is not dependent on microtubules but requires (at least for PAR3) microfilaments. The function of these two proteins during meiosis is not known, but it is interesting to note that PAR3 is restricted to a subdomain of the actin-rich cortical region which is surrounded by a zone of accumulation of the phosphorylated form of MARCKS (the myristoylated alanine-rich C-kinase substrate protein) (Michaut et al. 2005). Similarly to the situation in *Xenopus*, it has been proposed that the circumferential localization of p-MARCKS could define the position of the contractile ring responsible for polar body abscission, while PAR3 localized centrally and at the ends of the MI and MII spindle could facilitate anchoring the spindle within the forming polar body (Duncan et al. 2005). Finally, in a fashion reminiscent of what was described in mouse oocytes, a crescent of PAR proteins (PAR1 and PAR2) has been shown to be present at the cell cortex overlying the *C. elegans* female pronucleus when progression through meiosis is blocked or delayed (Sonneville and Gonczy 2004; Wallenfang and Seydoux 2000). Altogether, these data indicate that the formation of a cortical crescent of PAR proteins above the meiotic spindle could be a conserved feature involved in the control of polar body emission.

5 Conclusions and Perspectives

Many steps of gametogenesis rely on asymmetric cell division throughout the animal kingdom, but the molecular mechanisms underlying these asymmetric divisions are yet to be further explored. In *Drosophila*, several signalling pathways have been shown to regulate GSC self-renewal vs differentiation in a non-autonomous fashion; however the question whether intrinsic factors could also control asymmetric GSC division remains open. The processes leading to the selection of the oocyte after the mitotic divisions of the insect cystoblast also remain poorly understood. The asymmetric segregation of the fusome upon cystoblast division is likely to play an important function in the selection of the oocyte and future work should shed light on molecules associated with the fusome that are involved in oocyte fate determination. Finally, the mechanisms leading to the asymmetric meiotic division are still far from clear. The presence of PAR proteins on the meiotic spindle and/or at the cell cortex of oocytes of many different species raises the exciting possibility that PAR proteins could play a conserved role in the control of polar body extrusion. Considering the essential roles of PAR proteins throughout evolution in cell polarisation, mitotic spindle positioning and anchoring and in the establishment of mitotic spindle asymmetry (Betschinger and Knoblich 2004), it is tempting to speculate that similar PAR-dependent mechanisms are involved during asymmetric meiotic division.

Acknowledgements We are grateful to Sophie Louvet-Vallée and Marie-Hélène Verlhac for critical reading of the manuscript. P.F. is an A.R.C post-doctoral fellow and J.R.H. is funded by the C.N.R.S. and A.R.C.

References

Albertson DG, Thomson JN (1993) Segregation of holocentric chromosomes at meiosis in the nematode, Caenorhabditis elegans. Chromosome Res 1:15–26

Bement WM, Benink HA, von Dassow G (2005) A microtubule-dependent zone of active RhoA during cleavage plane specification. J Cell Biol 170:91–101

Betschinger J, Knoblich JA (2004) Dare to be different: asymmetric cell division in Drosophila, C. elegans and vertebrates. Curr Biol 14:R674–R685 Bolivar J, Huynh JR, Lopez-Schier H, Gonzalez C, St Johnston D, Gonzalez-Reyes A (2001) Centrosome migration into the Drosophila oocyte is independent of BicD and egl, and of the organisation of the microtubule cytoskeleton. Development 128:1889–1897

Brawley C, Matunis E (2004) Regeneration of male germline stem cells by spermatogonial dedifferentiation in vivo. Science 304:1331–1334

Brunet S, Maro B (2005) Cytoskeleton and cell cycle control during meiotic maturation of the mouse oocyte: integrating time and space. Reproduction 130:801–811

Buning J (1994) The insect ovary: ultrastructure, previtellogenic growth and evolution. Chapman and Hall, New York

Carpenter A (1975) Electron microscopy of meiosis in Drosophila melanogaster females. I Structure, arrangement, and temporal change of the synaptonemal complex in wild-type. Chromosoma 51:157–182

Carpenter A (1994) Egalitarian and the choice of cell fates in Drosophila melanogaster oogenesis. In: Marsh J, Goode J (eds) Germline development. Wiley, Chichester, pp 223–246

Chen D, McKearin D (2005) Gene circuitry controlling a stem cell niche. Curr Biol 15:179–184

Cox RT, Spradling AC (2003) A Balbiani body and the fusome mediate mitochondrial inheritance during Drosophila oogenesis. Development 130:1579–1590

de Cuevas M, Lee JL, Spradling AC (1996) α-Spectrin is required for germline cell division and differentiation in the *Drosophila ovary*. Development 124: 3959–3968

de Cuevas M, Spradling AC (1998) Morphogenesis of the Drosophila fusome and its implications for oocyte specification. Development 125:2781–2789

de Cuevas M, Lilly MA, Spradling AC (1997) Germline cyst formation in Drosophila. Ann Rev Genet 31:405–428

Decotto E, Spradling AC (2005) The Drosophila ovarian and testis stem cell niches: similar somatic stem cells and signals. Dev Cell 9:501–510

Deng W, Lin H (1997) Spectrosomes and fusomes anchor mitotic spindles during asymmetric germ cell divisions and facilitate the formation of a polarized microtubule array for oocyte specification in Drosophila. Dev Biol 189:79–94

Deng W, Lin H (2001) Asymmetric germ cell division and oocyte determination during Drosophila oogenesis. Int Rev Cytol 203:93–138

Deng M, Kishikawa H, Yanagimachi R, Kopf GS, Schultz RM, Williams CJ (2003) Chromatin-mediated cortical granule redistribution is responsible for the formation of the cortical granule-free domain in mouse eggs. Dev Biol 257: 166–176

Dumont J, Million K, Sunderland K, Rassinier P, Lim H, Leader B, Verlhac M-H (in press) Formin-2 is required for spindle migration and for the late steps of cytokinesis in mouse oocytes. Dev Biol

Duncan FE, Moss SB, Schultz RM, Williams CJ (2005) PAR-3 defines a central subdomain of the cortical actin cap in mouse eggs. Dev Biol 280: 38–47

Endow SA, Komma DJ (1997) Spindle dynamics during meiosis in Drosophila oocytes. J Cell Biol 137:1321–1336

Endow SA, Komma DJ (1998) Assembly and dynamics of an anastral:astral spindle: the meiosis II spindle of Drosophila oocytes. J Cell Sci 111(17): 2487–2495

Fuchs E, Segre JA (2000) Stem cells: a new lease on life. Cell 100:143–155

Gard DL (1991) Organization, nucleation, and acetylation of microtubules in Xenopus laevis oocytes: a study by confocal immunofluorescence microscopy. Dev Biol 143:346–362

Gard DL (1992) Microtubule organization during maturation of Xenopus oocytes: assembly and rotation of the meiotic spindles. Dev Biol 151:516–530

Gard DL, Klymkowsky MW (1998) Intermediate filament organization during oogenesis and early development in the clawed frog, Xenopus laevis. Subcell Biochem 31:35–70

Gard DL, Cha BJ, Schroeder MM (1995) Confocal immunofluorescence microscopy of microtubules, microtubule-associated proteins, and microtubule-organizing centers during amphibian oogenesis and early development. Curr Top Dev Biol 31:383–431

Giardina A (1901) Origine dell'oocite e delle cellule nutrici nel Dytiscus. Int Mschr Anat Physiol 18:417–484

Gilboa L, Forbes A, Tazuke SI, Fuller MT, Lehmann R (2003) Germ line stem cell differentiation in Drosophila requires gap junctions and proceeds via an intermediate state. Development 130:6625–6634

Glotzer M (2005) The molecular requirements for cytokinesis. Science 307:1735–1739

Grieder NC, de Cuevas M, Spradling AC (2000) The fusome organizes the microtubule network during oocyte differentiation in *Drosophila*. Development 127:4253–4264

Huynh JR (2005) Fusome as a cell-cell communication channel of Drosophila ovarian cyst: Landes Biosciences, http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=eurekah.chapter.64665

Huynh JR, St Johnston D (2004) The origin of asymmetry: early polarisation of the Drosophila germline cyst and oocyte. Curr Biol 14:R438–R449

Jan Y, Jan L (1998) Asymmetric cell division. Nature 392:775–778

Januschke J, Gervais L, Dass S, Kaltschmidt JA, Lopez-Schier H, St Johnston D, Brand AH, Roth S, Guichet A (2002) Polar transport in the Drosophila oocyte requires Dynein and Kinesin I cooperation. Curr Biol 12:1971–1981

Januschke J, Gervais L, Gillet L, Keryer G, Bornens M, Guichet A (2006) The centrosome-nucleus complex and microtubule organization in the Drosophila oocyte. Development 133:129–139

[Au1] Kai T, Spradling A (2004) Differentiating germ cells can revert into functional stem cells in Drosophila melanogaster ovaries. Nature 428:564–569

Kawase E, Wong MD, Ding BC, Xie T (2004) Gbb/Bmp signaling is essential for maintaining germline stem cells and for repressing bam transcription in the Drosophila testis. Development 131:1365–1375

Kiger A, White-Cooper H, Fuller M (2000) Somatic support cells restrict germline stem cell self-renewal and promote differentiation. Nature 407:750–754

Kiger AA, Jones DL, Schulz C, Rogers MB, Fuller MT (2001) Stem cell self-renewal specified by JAK-STAT activation in response to a support cell cue. Science 294:2542–2545

Kim NH, Chung KS, Day BN (1997) The distribution and requirements of microtubules and microfilaments during fertilization and parthenogenesis in pig oocytes. J Reprod Fertil 111:143–149

Kim NH, Chung HM, Cha KY, Chung KS (1998) Microtubule and microfilament organization in maturing human oocytes. Hum Reprod 13:2217–2222

Kloc M, Bilinski S, Dougherty MT, Brey EM, Etkin LD (2004) Formation, architecture and polarity of female germline cyst in Xenopus. Dev Biol 266:43–61

Knoblich JA (2001) Asymmetric cell division during animal development. Nat Rev Mol Cell Biol 2:11–20

Lambert JD, Nagy LM (2002) Asymmetric inheritance of centrosomally localized mRNAs during embryonic cleavages. Nature 420:682–686

Leader B, Lim H, Carabatsos MJ, Harrington A, Ecsedy J, Pellman D, Maas R, Leder P (2002) Formin-2, polyploidy, hypofertility and positioning of the meiotic spindle in mouse oocytes. Nat Cell Biol 4:921–928

Li L, Xie T (2005) Stem cell niche: structure and function. Annu Rev Cell Dev Biol 21:605–631

Lin H (1997) The tao of stem cells in the germline. Ann Rev Genet 31:455–491

Lin H, Spradling AC (1995) Fusome asymmetry and oocyte determination in *Drosophila*. Dev Gen 16:6–12

Lin H, Spradling A (1997) A novel group of pumilio mutations affects the asymmetric division of germline stem cells in the Drosophila ovary. Development 124:2463–2476

Lin H, Yue L, Spradling A (1994) The Drosophila fusome, a germline-specific organelle, contains membrane skeletal proteins and functions in cyst formation. Development 120:947–956

Longo FJ, Chen DY (1985) Development of cortical polarity in mouse eggs: involvement of the meiotic apparatus. Dev Biol 107:382–394

Ma C, Benink HA, Cheng D, Montplaisir V, Wang L, Xi Y, Zheng PP, Bement WM, Liu XJ (2006) Cdc42 activation couples spindle positioning to first polar body formation in oocyte maturation. Curr Biol 16:214–220

Maro B, Johnson MH, Pickering SJ, Flach G (1984) Changes in actin distribution during fertilization of the mouse egg. J Embryol Exp Morphol 81:211–237

Maro B, Verlhac MH (2002) Polar body formation: new rules for asymmetric divisions. Nat Cell Biol 4:E281–E283

Maro B, Johnson MH, Webb M, Flach G (1986) Mechanism of polar body formation in the mouse oocyte: an interaction between the chromosomes, the cytoskeleton and the plasma membrane. J Embryol Exp Morphol 92:11–32

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

Michaut MA, Williams CJ, Schultz RM (2005) Phosphorylated MARCKS: a novel centrosome component that also defines a peripheral subdomain of the cortical actin cap in mouse eggs. Dev Biol 280:26–37

Miyazaki A, Kamitsubo E, Nemoto SI (2000) Premeiotic aster as a device to anchor the germinal vesicle to the cell surface of the presumptive animal pole in starfish oocytes. Dev Biol 218:161–171

Miyazaki A, Kato KH, Nemoto S (2005) Role of microtubules and centrosomes in the eccentric relocation of the germinal vesicle upon meiosis reinitiation in sea-cucumber oocytes. Dev Biol 280:237–247

Moore CA, Zernicka-Goetz M (2005) PAR-1 and the microtubule-associated proteins CLASP2 and dynactin-p50 have specific localisation on mouse meiotic and first mitotic spindles. Reproduction 130:311–320

Morrison SJ, Kimble J (2006) Asymmetric and symmetric stem-cell divisions in development and cancer. Nature 441:1068–1074

Na J, Zernicka-Goetz M (2006) Asymmetric positioning and organization of the meiotic spindle of mouse oocytes requires CDC42 function. Curr Biol 16:1249–1254

Nakaya M, Fukui A, Izumi Y, Akimoto K, Asashima M, Ohno S (2000) Meiotic maturation induces animal-vegetal asymmetric distribution of aPKC and ASIP/PAR-3 in Xenopus oocytes. Development 127:5021–5031

Ohlstein B, Kai T, Decotto E, Spradling A (2004) The stem cell niche: theme and variations. Curr Opin Cell Biol 16:693–699

Pickering SJ, Johnson MH, Braude PR, Houliston E (1988) Cytoskeletal organization in fresh, aged and spontaneously activated human oocytes. Hum Reprod 3:978–989

Pielak RM, Gaysinskaya VA, Cohen WD (2004) Formation and function of the polar body contractile ring in Spisula. Dev Biol 269:421–432

Prodon F, Chenevert J, Sardet C (2006) Establishment of animal-vegetal polarity during maturation in ascidian oocytes. Dev Biol 290:297–311

Roper K, Brown NH (2004) A Spectraplakin is enriched on the fusome and organizes microtubules during oocyte specification in Drosophila. Curr Biol 14:99–110

Sardet C, Prodon F, Dumollard R, Chang P, Chenevert J (2002) Structure and function of the egg cortex from oogenesis through fertilization. Dev Biol 241:1–23

Skold HN, Komma DJ, Endow SA (2005) Assembly pathway of the anastral Drosophila oocyte meiosis I spindle. J Cell Sci 118:1745–1755

Snapp EL, Iida T, Frescas D, Lippincott-Schwartz J, Lilly MA (2004) The fusome mediates intercellular endoplasmic reticulum connectivity in Drosophila ovarian cysts. Mol Biol Cell 15:4512–4521

Song X, Zhu CH, Doan C, Xie T (2002) Germline stem cells anchored by adherens junctions in the Drosophila ovary niches. Science 296:1855–1857

Song X, Wong MD, Kawase E, Xi R, Ding BC, McCarthy JJ, Xie T (2004) Bmp signals from niche cells directly repress transcription of a differentiation-promoting gene, bag of marbles, in germline stem cells in the Drosophila ovary. Development 131:1353–1364

Sonneville R, Gonczy P (2004) Zyg-11 and cul-2 regulate progression through meiosis II and polarity establishment in C. elegans. Development 131:3527–3543

Spradling A (1993) Developmental genetics of oogenesis. In: Bate M, Martinez-Arias A (eds) The development of *Drosophila melanogaster*. Cold Spring Harbor Laboratory Press, New York, pp 1–70

Spradling A, Drummond-Barbosa D, Kai T (2001) Stem cells find their niche. Nature 414:98–104

Storto P, King R (1989) The role of polyfusomes in generating branched chains of cystocytes during Drosophila oogenesis. Dev Genet 10:70–86

Sun QY, Schatten H (2006) Regulation of dynamic events by microfilaments during oocyte maturation and fertilization. Reproduction 131:193–205

Swan A, Nguyen T, Suter B (1999) Drosophila Lissencephaly-1 functions with Bic-D and dynein in oocyte determination and nuclear positioning. Nat Cell Biol 1:444–449

Szakmary A, Cox DN, Wang Z, Lin H (2005) Regulatory relationship among piwi, pumilio, and bag-of-marbles in Drosophila germline stem cell self-renewal and differentiation. Curr Biol 15:171–178

Szczepanska K, Maleszewski M (2005) LKB1/PAR4 protein is asymmetrically localized in mouse oocytes and associates with meiotic spindle. Gene Expr Patterns 6:86–93

Tavosanis G, Llamazares S, Goulielmos G, Gonzalez C (1997) Essential role for gamma-tubulin in the acentriolar female meiotic spindle of Drosophila. EMBO J 16:1809–1819

Telfer W (1975) Development and physiology of the oocyte-nurse cell syncitium. Adv Insect Physiol 11:223–319

Theurkauf W (1994) Microtubules and cytoplasm organization during Drosophila oogenesis. Dev Biol 165:352–360

Theurkauf WE, Hawley RS (1992) Meiotic spindle assembly in Drosophila females: behavior of nonexchange chromosomes and the effects of mutations in the nod kinesin-like protein. J Cell Biol 116:1167–1180

Theurkauf W, Smiley S, Wong M, Alberts B (1992) Reorganization of the cytoskeleton during Drosophila oogenesis: implications for axis specification and intercellular transport. Development 115:923–936

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

Tran J, Brenner T, DiNardo S (2000) Somatic control over the germline stem cell lineage during *Drosophila* spermatogenesis. Nature 407:754–757

Tremoleda JL, Schoevers EJ, Stout TA, Colenbrander B, Bevers MM (2001) Organisation of the cytoskeleton during in vitro maturation of horse oocytes. Mol Reprod Dev 60:260–269

Tulina N, Matunis E (2001) Control of stem cell self-renewal in Drosophila spermatogenesis by JAK-STAT signaling. Science 294:2546–2549

Verlhac MH, Lefebvre C, Guillaud P, Rassinier P, Maro B (2000) Asymmetric division in mouse oocytes: with or without Mos. Curr Biol 10:1303–1306

Vinot S, Le T, Maro B, Louvet-Vallee S (2004) Two PAR6 proteins become asymmetrically localized during establishment of polarity in mouse oocytes. Curr Biol 14:520–525

Wallenfang MR, Seydoux G (2000) Polarization of the anterior-posterior axis of C. elegans is a microtubule-directed process. Nature 408:89–92

Weber KL, Sokac AM, Berg JS, Cheney RE, Bement WM (2004) A microtubule-binding myosin required for nuclear anchoring and spindle assembly. Nature 431:325–329

Wong MD, Jin Z, Xie T (2005) Molecular mechanisms of germline stem cell regulation. Annu Rev Genet 39:173–195

Xie T, Spradling AC (1998) Decapentaplegic is essential for the maintenance and division of germline stem cells in the Drosophila ovary. Cell 94:251–260

Xie T, Spradling AC (2000) A niche maintaining germ line stem cells in the Drosophila ovary. Science 290:328–330

Yamashita YM, Jones DL, Fuller MT (2003) Orientation of asymmetric stem cell division by the APC tumor suppressor and centrosome. Science 301:1547–1550

Yang HY, McNally K, McNally FJ (2003) MEI-1/katanin is required for translocation of the meiosis I spindle to the oocyte cortex in C elegans. Dev Biol 260: 245–259

Yang HY, Mains PE, McNally FJ (2005) Kinesin-1 mediates translocation of the meiotic spindle to the oocyte cortex through KCA-1, a novel cargo adapter. J Cell Biol 169:447–457

Yue L, Spradling A (1992) hu-li tai shao, a gene required for ring canal formation during Drosophila oogenesis, encodes a homolog of adducin. Genes Dev 6:2443–2454

Zhao GQ, Garbers DL (2002) Male germ cell specification and differentiation. Dev Cell 2:537–547

Author Queries:

[Au1]: Spradling. AC or spradling. A. Please check. throughout reference.