Subcellular Specialization and Organelle Behavior in Germ Cells

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ABSTRACT Gametes, eggs and sperm, are the highly specialized cell types on which the development of new life solely depends. Although all cells share essential organelles, such as the ER (endoplasmic reticulum), Golgi, mitochondria, and centrosomes, germ cells display unique regulation and behavior of organelles during gametogenesis. These germ cell-specific functions of organelles serve critical roles in successful gamete production. In this chapter, I will review the behaviors and roles of organelles during germ cell differentiation.

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Although all cells possess the same basic cellular components, such as a nucleus, cytoskeleton, and organelles, the organization, morphology, and/or behavior of these components within the cells varies depending on the cell type. Perhaps the most striking organization and reorganization of organelles is observed in germ cells as they undergo gametogenesis. In this chapter, I will review the behavior of organelles during oogenesis and spermatogenesis in *Drosophila*, focusing on discoveries made in the last quarter century since the last comprehensive reviews on oogenesis and spermatogenesis were published (Fuller 1993; Spradling 1993). An astounding amount of knowledge has accumulated since then, in part due to utilization of genomic, genetic, and molecular techniques that are improving day by day. At the same time, as we understand more about the processes of gametogenesis, we cannot help but notice the accuracy and insights of earlier studies on gametogenesis (King 1957; Fawcett *et al.* 1959; Counce 1963; Koch and King 1966, 1969; Koch *et al.* 1967; Mahowald 1968, 1971a,b; Mahowald and Strassheim 1970; Telfer 1975; Tokuyasu *et al.* 1977; Hardy *et al.* 1979, 1981).

As the behaviors of organelles are closely linked to the process of germ cell differentiation, I will first briefly summarize the stages of oogenesis and spermatogenesis, and then I will review how organelles change their morphology and behavior during the course of germ cell differentiation. Although the main focus of this chapter is organelle behavior and the cytoskeletal regulation underlying that behavior, the chapter is organized by the biological processes that take place during gametogenesis because each process often relies on multiple organelles.

Overview of Oogenesis

Oogenesis in *Drosophila* occurs within a unit called an ovariole, 16–20 of which compose an ovary (Spradling 1993). An

ovariole is an assembly line that yields mature eggs with the differentiation processes occurring in a spatiotemporal order along the axis of the ovariole. Each ovariole contains a germarium at the apical end followed by six to seven egg chambers in which ordered maturation occurs (Figure 1A). In the germarium, two to three germline stem cells (GSCs) reside in the stem cell niche formed by the terminal filament and cap cells (Figure 1A) (see Chapter 3 for details). Early germ cell division and development occurs in the germarium, which is subdivided into regions 1–3 based on the progression of cell division (Koch and King 1966; Koch *et al.* 1967; Spradling 1993). This is followed by 14 stages of oocyte development (King 1957). GSCs divide asymmetrically to produce one GSC and one cystoblast (CB). CBs then initiate their differentiation program, wherein they divide mitotically four times as cystocytes to yield a cyst containing 16 germ cells (region 1 of the germarium) (Figure 1, A and B). As the cytokinesis of these divisions is incomplete, they stay connected to each other via cytoplasmic bridges called ring canals (RCs) (Figure 1, B and C) (Brown and King 1964; Koch *et al.* 1967; Koch and King 1969). The newly-formed 16-cell cysts are found in region 2 of the germarium and these cysts are subsequently encapsulated by somatic follicle cells in region 3 of the germarium, which is also called a stage 1 egg chamber (Figure 1A). Follicle stem cells reside in the region 2a/b boundary, and their differentiating daughters encapsulate egg chambers as the cysts pass through the region (Nystul and Spradling 2007). Follicle cells continue to divide to encapsulate the growing nurse cell–oocyte complex.

Subsequently, the egg chamber buds off from the germarium (stage 2 egg chamber) and further progresses through the differentiation program (stages 2–14) (King 1957). Through

Figure 1 Oogenesis of *D. melanogaster*. (A) Overview of *Drosophila* oogenesis. Germ cells are shown in blue, except for oocytes, which are shown in yellow after oocyte fate determination. Structure of the germarium is detailed below. (B) Fusome and ring canal morphology in developing germline cysts in germarium. Upper panel: immunofluorescence image of germarium expressing Pavarotti-GFP (marking ring canals, green) stained for Add/Hts (fusome, red), Fas III (terminal filament and follicle cell membrane, red), and Vasa (germ cells, blue). Bottom panel: cyst formation. Fusome is indicated by orange lines, ring canal by green circles. Asterisks indicate the cystocyte that has inherited the larger amount of fusome during the first division and contains the highest number of ring canals within the cyst, possibly becoming the oocyte (yellow cell at 16-cell stage). (C) Ring canal in the developing egg chamber marked by F-actin (green) and Kelch (magenta). Reproduced from Hudson *et al.* (2015) with permission from Lynn Cooley and the Genetic Society of America. MT, microtubule; MTOC, MT-organizing center.

these stages, only one out of 16 interconnected cells within the cyst becomes specified as the oocyte and the remaining 15 cells differentiate as nurse cells, which support the differentiation of the oocyte. While nurse cells undergo polyploidization to support massive gene expression, oocytes undergo the meiotic program (e.g., homologous pairing and recombination) and their chromatin remains mostly quiescent. Materials (mRNAs, proteins, and organelles) that support early embryonic development are transported from the nurse cells into the oocyte where they are stored in a spatially organized manner.

Overview of Spermatogenesis

Like oogenesis, spermatogenesis is also organized in a spatiotemporal manner in the *Drosophila* testis (Fuller 1993). Eight to 10 GSCs reside at the apical tip of each testis, where

they attach to the hub cells that comprise the stem cell niche (see Chapter 3) (Figure 2, A and B). Male GSCs also divide asymmetrically to produce one GSC and one gonialblast (GB), the latter of which subsequently undergoes four mitotic divisions with incomplete cytokinesis to yield a cyst of 16 spermatogonia (SGs) (Tokuyasu *et al.* 1977; Hardy *et al.* 1979, 1981; Lindsley and Tokuyasu 1980). Upon completion of the mitotic divisions, SGs undergo meiotic S phase and G2 phase as spermatocytes (SCs). SCs grow in volume ~25 times while the meiosis-specific transcription program occurs. Unlike in females, where only 1 of 16 cells is fated to be passed on to the next generation, all 16 SGs/SCs are equivalent in their fate, and all SCs undergo meiosis to yield 64 sperm (Figure 2B). Cytokinesis during meiotic divisions is also incomplete, and the 64 spermatids at the end of meiosis are interconnected. These spermatids undergo dramatic

Figure 2 Spermatogenesis of *D. melanogaster*. (A) Phase contrast image of *D. melanogaster* testis (image: courtesy of Jaclyn Fingerhut). Spermatogenesis is organized in a spatiotemporal manner within the tubular testis. (B) Overview of *Drosophila* spermatogenesis. Fusome is indicated by orange lines, ring canal by green circles. (C) Ring canal and fusome morphology in SG cysts. Immunofluorescence image of testis expressing Pavarotti-GFP (ring canals, green) stained for Add/Hts (fusome, red) and Vasa (germ cells, blue). GB, gonialblast; GSC, germline stem cell; SC, spermatocyte; SG, spermatogonia.

morphological changes, starting as round spermatids and ending as fully elongated, mature sperm that are ~ 1.8 mm in length. At the very end of spermiogenesis, the interconnected sperm are individualized to release free, motile sperm into the seminal vesicle, ready for use in fertilization. The process of postmeiotic spermatogenesis (*i.e.*, spermiogenesis) is an example of some of the most dramatic changes in cell shape and specialization of organelles (see Chapter 6).

Centrosomes and the Spectrosome in Asymmetric Stem Cell Division

Asymmetric stem cell division is a mechanism by which stem cells balance self-renewal and differentiation, thereby preserving tissue homeostasis. Asymmetric stem cell division in the *Drosophila* male and female germlines is achieved within the context of the stem cell niche, a cellular microenvironment that influences stem cell identity (see Chapter 3). Asymmetric cell division is generally inseparable from the regulation of division orientation and cell polarity, which are precisely organized by the cytoskeleton. Therefore, cytoskeletal proteins [microtubules (MTs) and actins] play fundamental roles in asymmetric stem cell divisions.

Centrosomes consist of a pair of centrioles and pericentriolar materials. Centrosomes are the major MT-organizing centers (MTOCs) in many cell types, although acentrosomal MTOCs can be found in various cell types, such as differentiated epithelial cells in many organisms. *Drosophila* oogenesis and

spermatogenesis see a series of reorganizations of the cytoskeleton as the germ cells progress through asymmetric stem cell division, the mitotic divisions, meiosis, and finally terminal differentiation to yield highly specialized gametes ready for fertilization. During these processes, centrosomes play critical roles in many aspects of germ cell differentiation via their MTOC activity. Timely inactivation of centrosomes in the later stages of gametogenesis is also important for proper gamete production.

The spectrosome/fusome is a germline-specific, membranous organelle. In GSCs, this membranous organelle exhibits a spherical morphology and is called the spectrosome (Figure 1B and Figure 2B). A portion of the spectrosome is inherited by the differentiating daughters (GB in male, CB in female) during GSC division, and the spectrosome becomes branched, now called the fusome, running through the interconnected germ cells (Figure 1B and Figure 2B). Many cytoskeletal proteins (both MTs as well as actins) are localized to the spectrosome/fusome. Through its ability to host cytoskeletal proteins, the spectrosome/fusome contributes to centrosome positioning, which in turn helps to orient the mitotic spindle in a specific direction to allow for oriented division.

Orienting GSC divisions

Oogenesis and spermatogenesis start at the apical end of the gonad where GSCs reside in their niche (see Chapter 3). In the stem cell niche, GSCs are maintained in an undifferentiated

state to sustain continued production of differentiating germ cells. Both male and female GSCs divide asymmetrically to produce one stem cell and one differentiating cell (a GB in the male germline and a CB in the female germline), thereby balancing stem cell self-renewal and differentiation (Fuller and Spradling 2007). Through their ability to organize MTs, the centrosomes and spectroosome regulate asymmetric GSC division both in male and female GSCs. The degree to which GSCs rely on centrosomes vs. the spectroosome for asymmetric division varies in male vs. female GSCs.

In general, asymmetric cell division is achieved by orienting the spindle with respect to the preexisting polarity of the cell (e.g., asymmetrically-distributed intracellular fate determinants) or that of the cellular environment (e.g., position of the niche) (Morrison and Kimble 2006; Morrison and Spradling 2008; Inaba and Yamashita 2012). In the *Drosophila* testis, GSCs divide asymmetrically by orienting their spindles perpendicularly toward the hub cells, the major components of the stem cell niche (Yamashita *et al.* 2003) (Figure 3A). This spindle orientation enables the precise placement of one daughter cell inside the stem cell niche and the other outside of the niche, leading to an asymmetric outcome of the stem cell division. Spindle orientation is prepared during interphase through positioning of the centrosomes; the mother centrosome remains close to the hub–GSC junction, whereas the daughter centrosome migrates toward the other side of the GSC, leading to spindles that are oriented perpendicularly toward the hub cells (Yamashita *et al.* 2007). The mother centrosome nucleates slightly more astral MTs compared to the daughter centrosome. These astral MTs connect to the adherens junctions formed between the hub and the GSCs, thereby helping the mother centrosome anchor at the hub–GSC junction. Adherens junctions further recruit Apc2, which anchors the centrosomes to the adherens junctions (Yamashita *et al.* 2003; Wang *et al.* 2006; Inaba *et al.* 2010; Srinivasan *et al.* 2012; Liu *et al.* 2015). On the other hand, the daughter centrosome is free to migrate to the distal side of the GSC. Because of this arrangement, the GSCs consistently inherit the mother centrosome, whereas the GBs inherit the daughter centrosome. This led to the idea that the mother (or the daughter) centrosome may harbor fate determinants for stem cell self-renewal (or differentiation), although such molecules are yet to be discovered (Tajbakhsh and Gonzalez 2009). An ultrastructural analysis found that the mother centrosome always contains nine triplet MTs, whereas the daughter centrosome starts with nine doublet MTs, which gradually mature to become nine triplet MTs during the cell cycle (Gottardo *et al.* 2015), possibly explaining distinct behavior of the mother and daughter centrosomes. It was found that Klp10A, a MT-depolymerizing kinesin, specifically localizes to the GSC centrosomes but not the centrosomes of other cells such as SGs (Chen *et al.* 2016). Interestingly, in the absence of Klp10A, the GSC mother centrosome abnormally elongates, suggesting that Klp10A functions to counteract the mother centrosomes' inherent nature to grow (Chen *et al.* 2016). These results reveal the

presence of mechanisms that specifically operate on the mother centrosome in GSCs.

In female GSCs, it appears to be the spectroosome that predominantly orients the spindle and dictates asymmetric stem cell division (Figure 3B). Throughout most of the female GSC cell cycle, the spectroosome is localized to the apical side of the GSC, toward the cap cells (Deng and Lin 1997; Hsu *et al.* 2008). In spectroosome mutants, the mitotic spindle misorients (Deng and Lin 1997), suggesting that the spectroosome plays a central role in spindle orientation in female GSCs. The centrosomes also play a role in female GSC division: the small GTPase Rac, which regulates the actin cytoskeleton, is specifically activated at the niche (cap cell)–GSC interface, which in turn localizes Apc2 and positions the centrosome toward the cap cells, leading to a spindle oriented toward the cap cells (Lu *et al.* 2012). However, the GSC spindle correctly orients in the absence of functional centrosomes, suggesting that centrosomes likely play a secondary role (Stevens *et al.* 2007).

Female vs. male GSCs also differ in the behavior of the mother vs. daughter centrosomes; whereas the mother centrosome is always associated with the niche–GSC interface in male GSCs (Yamashita *et al.* 2007), it is the daughter centrosome in female GSCs that is associated with the niche and is inherited by the GSC (Salzmann *et al.* 2014). These results point to the presence of a precise developmental program that decides which centrosome goes to which cell. Interestingly, *Drosophila* neuroblasts also exhibit the same pattern as female GSCs; the stem cells inherit the daughter centrosome, while the mother centrosome is segregated to the differentiating cells (Conduit and Raff 2010; Januschke *et al.* 2011). Although the spectroosome does not appear to play a key role in spindle orientation in male GSCs, it may play a secondary role to orient the spindle. In the absence of functional centrosomes, the spectroosome in male GSCs shows a pattern of localization similar to that of female GSCs in which the spectroosome is at the apical side of the GSC and associates with the spindle pole proximal to the niche during mitosis, possibly anchoring and orienting the spindle in the absence of functional centrosomes (Yuan *et al.* 2012).

The centrosome orientation checkpoint ensures asymmetric stem cell division

In male GSCs, an additional mechanism called the centrosome orientation checkpoint (COC) ensures an asymmetric outcome following GSC division by monitoring centrosome position (Cheng *et al.* 2008) (Figure 3C). The COC functions specifically in GSCs but not in differentiating cells. The COC arrests GSCs prior to mitotic entry (*i.e.*, G2 phase) upon centrosome misorientation, thereby preventing GSCs from dividing with misoriented spindles that can result in symmetric divisions (Venkei and Yamashita 2015). A specialized structure, which is enriched for the polarity protein Bazooka (Baz)/Par-3, is formed between the hub and the GSCs and functions as a “docking site” for the centrosome. Docking is recognized as correct centrosome orientation and permits

Figure 3 Asymmetric GSC divisions. (A) Centrosome and spectrosome positioning during male GSC cell cycle. Hub is indicated with asterisks, GSCs with circle. Proximal centrosomes (mother) are indicated by arrowheads. Immunofluorescence images are apical tip of the testis stained for Spd-2 (centrosome, green), Fas III (hub, red), Add/Hts (spectrosome, red), and DAPI (blue). Modified from Venkei and Yamashita (2015) with permission from *Development*. (B) Centrosome and spectrosome positioning during female GSC cell cycle. GSCs (and connected cystoblasts) are indicated by dotted lines. Cap cells are indicated by asterisks. GSC–cap cell junction is indicated by solid lines. Arrowheads indicate proximal (daughter) centrosomes and arrows indicate the distal centrosomes. Reproduced from Salzmann *et al.* (2014) with permission from the American Society for Cell Biology. (C) COC in male GSCs. The polarity protein Baz/Par-3 localizes to the hub–GSC interface as a small “patch.” The centrosome “docking” to the Baz patch is likely interpreted as the correct centrosome orientation by COC, permitting mitotic entry. (D) Asymmetries during male GSC division. MT-nanotubes formed by GSCs reinforce niche–GSC signaling, while excluding GBs from the signal reception. Biased segregation in old/new histone H3 and in sister chromatids of sex chromosomes are observed. Midbody ring is stereotypically inherited by GBs. Baz, Bazooka; COC, centrosome orientation checkpoint; GB, gonialblast; GSC, germline stem cell; MT, microtubule.

mitotic entry (Inaba *et al.* 2015b) (Figure 3C). Par-1, a kinase that phosphorylates Baz (Krahn *et al.* 2009), is also an important component of the COC (Yuan *et al.* 2012). Par-1 localizes to the spectrosome, where Cyclin A, the cyclin required for mitotic entry, is sequestered in a Par-1-dependent manner (Yuan *et al.* 2012). Par-1-dependent sequestration of Cyclin A to the spectrosome is a key step to prevent precocious mitotic entry when the centrosomes are not correctly oriented (Yuan *et al.* 2012). It remains unclear how spectrosome-localized Par-1 may regulate Baz and how their interaction mediates the function of the COC.

Female GSCs also exhibit checkpoint-like arrest upon centrosome misorientation: the centrosome misorientation in Rac mutant GSCs does not lead to spindle misorientation due to cell cycle arrest in mitotic prophase (Lu *et al.* 2012). The cell cycle arrest in female GSCs is somewhat later than that in male GSCs (G2 phase), revealing a difference in the regulation of spindle orientation and cell cycle control in female vs. male GSCs.

Cellular asymmetries during asymmetric GSC division

Although the asymmetric outcome of GSC division can be mostly explained by the influence of the stem cell niche, many

GSC-intrinsic asymmetries have been reported, possibly contributing to asymmetric fates. For example, asymmetric segregation of old vs. new histone H3 is reported during the male GSC division, wherein the old histone H3 is preferentially inherited by the GSC (Figure 3D) (Tran *et al.* 2012; Xie *et al.* 2015). Also, sister chromatids of sex chromosomes are segregated in a biased manner during male GSC division (*i.e.*, a particular copy of sister chromatids of sex chromosomes has a much higher tendency to be retained by the GSCs compared to the other copy of the sister chromatids) (Figure 3D) (Yadlapalli and Yamashita 2013). Currently, it is unknown whether and how asymmetric histone inheritance and nonrandom sister chromatid segregation of sex chromosomes may be related to each other. Although it is tempting to speculate that these asymmetries confer distinct cell fates through segregation of unequal epigenetic information, the identity of this information remains entirely unknown.

In addition, the midbody ring, a remnant of the contractile ring that forms during cytokinesis, is stereotypically segregated during GSC division; the GBs almost always inherit the midbody ring in males (Figure 3D), whereas the GSCs inherit the midbody ring most of the time in females, showing a correlation

between inheritance of the daughter centrosome and inheritance of the midbody ring (Salzmann *et al.* 2014). The purpose of asymmetric midbody inheritance remains unknown, although the midbody is often inherited in a stereotypical pattern during stem cell division in other systems (Ettinger *et al.* 2011; Kuo *et al.* 2011).

Among these asymmetries described here, asymmetric sister chromatid segregation of sex chromosomes as well as midbody inheritance have been shown to depend on functional centrosomes, suggesting that a plethora of additional asymmetries may stem from asymmetry of the mother–daughter centrosomes.

MT-nanotubes and cytonemes reinforce the niche–stem cell signaling

As described above, the localized niche signaling and oriented cell division together control the asymmetric outcome of stem cell division. However, the mechanism that confines the niche-derived signaling ligands such that they are received in sufficient amounts only by the GSCs, thereby ensuring differentiation of daughter cells, remains poorly understood.

Recent studies have revealed the presence of previously unappreciated cellular protrusions that spatially limit the niche signaling and therefore reinforce the asymmetric outcome of GSC division. It was found that male GSCs form MT-based nanotubes (MT-nanotubes) that protrude into the hub cells (Figure 3D) (Inaba *et al.* 2015a). The surface of the MT-nanotubes serves as an exclusive interface, where the niche-derived ligand Dpp and GSC-derived Dpp receptor (Tkv) engage. This ensures the signal reception by GSCs, while limiting the access of differentiating cells to the ligands secreted by the niche.

Similarly, in the female GSC niche, cytonemes, which are also cellular protrusions but made of actin filaments, deliver the signaling ligand Hh from the cap cells to the escort cells, somatic cells that encapsulate germ cells (Rojas-Rios *et al.* 2012). Cytonemes are required for female GSC maintenance, indicating that precise delivery of Hh is critical for establishing the microenvironment that supports GSC identity.

Deviation from asymmetric stem cell divisions

Although GSC division is asymmetric most of the time in both females and males, symmetric GSC divisions can occur to increase GSC number. It has been shown that lost GSCs are efficiently replaced by symmetric GSC division. In females, upon loss of a GSC, division of the neighboring GSC yields two GSCs, where both daughters are attached to the cap cells and adopt a stem cell fate (Xie and Spradling 2000). In testes, a GB can crawl back to regain attachment to the hub, before the cytokinesis of GSC and GB becomes complete, yielding two GSCs from a single GSC division (Sheng and Matunis 2011). Symmetric GSC divisions are frequently observed when GSC number needs to increase, for example when the testis is recovering from protein starvation (Sheng and Matunis 2011), which is known to reduce GSC number (McLeod *et al.* 2010). It is interesting to note that GSC division remains oriented

even when the final outcome of the division is symmetric (Sheng and Matunis 2011). It is unknown how female GSCs achieve symmetric division (*e.g.*, changed spindle orientation or crawling-back of CBs).

In addition to symmetric GSC division, partially differentiated germ cells can revert back to a GSC identity (“dedifferentiation”) in both male and female germlines (Brawley and Matunis 2004; Kai and Spradling 2004; Cheng *et al.* 2008; Sheng *et al.* 2009). Dedifferentiation may compensate for GSC loss and contribute to tissue homeostasis. However, dedifferentiated male GSCs may be less functional, as their centrosomes are misoriented (perhaps because they had lost the mother centrosome when they had initiated differentiation) and divide less frequently due to COC (Cheng *et al.* 2008). Nonetheless, the fact that dedifferentiation occurs raises fundamental questions regarding the biological significance of asymmetries during GSC divisions such as the inheritance of the mother centrosome and old histones; if symmetric divisions and dedifferentiation can create GSCs from non-GSCs, what is the point of carefully programming spindle orientation and segregating certain components asymmetrically at all? The answer to this question likely requires much deeper understanding of asymmetric GSC division.

The Fusome and RCs Organize Germ Cell Cyst Formation

One prevalent characteristic of germ cells across many organisms from insects to mammals is their development as a cyst (Fawcett *et al.* 1959; Koch and King 1966). Germ cells destined to differentiate (cystocytes and SGs) first undergo multiple mitotic divisions, during which incomplete cytokinesis results in interconnected sister cells that share a common cytoplasm (Figure 1B and Figure 2B). In *Drosophila melanogaster*, the number of mitotic divisions is set precisely at four, yielding a cyst of 16 interconnected germ cells. In females, the connectivity of germ cells is critical for oocyte formation and development, wherein the oocyte collects cellular materials, such as RNA, protein, and organelles, from sister nurse cells. In contrast, there is no clear explanation as to why male germ cells develop as a cyst of interconnected germ cells. It was suggested that connectivity may help haploid germ cells retain access to a full genomic complement: Y chromosome-containing haploids have access to X-linked genes (Braun *et al.* 1989), and X chromosome-containing haploids have access to the Y chromosome-encoded male fertility factors (Bonaccorsi *et al.* 1988; Carvalho *et al.* 2000, 2001) through connectivity. However, the need to connect haploid germ cells does not explain the connectivity of premeiotic diploid cells. Moreover, in the panoistic ovaries of some insects, such as stoneflies, all germ cells develop as oocytes with no need for nurse cells, yet the germ cells are interconnected, dividing with incomplete cytokinesis during the early mitotic divisions (Telfer 1975; Pritsch and Buning 1989; Gottanka and Buning 1990; Buning 1993). These observations imply that germ cell connectivity serves an unappreciated purpose beyond oocyte differentiation and

haploid gamete complementation. Moreover, the presence of panoistic ovaries with germ cell connectivity in primitive insect species raises the possibility that germ cell connectivity might have originally evolved to serve this unappreciated purpose, with the nursing roles arising later. A recent study shed light on the potential importance of male germ cell interconnectivity. It was suggested that germ cells share the signal(s) that trigger germ cell death through the cytoplasmic connection in response to DNA damage (Lu and Yamashita 2017). This sharing kills all germ cells within the cyst, even if some germ cells are not sufficiently damaged to die on their own. This might confer a stringent genome protection mechanism that operates specifically in germ cells, while sparing somatic cells that are needed to build the organism but do not require the highest quality genomic DNA.

Intercellular connectivity within a cyst is supported by the fusome and RCs (Figure 1, B and C and Figure 2, B and C). RCs are stabilized cytoplasmic bridges derived from the contractile ring of cytokinesis. The fusome is a germ cell-specific membranous organelle derived from the spectrosome, as described above. During GSC division, a portion of the spectrosome is inherited by the differentiating daughter (CB or GB). This fragment of the spectrosome grows in size and branches to run through the interconnected germ cells during subsequent mitotic divisions, becoming known as the fusome. The fusome and spectrosome are essentially the same organelle differing mainly in their morphology; the spectrosome is spherical, whereas the fusome is branched. Although the composition of the spectrosome/fusome changes slightly during germ cell development (Lin *et al.* 1994; Lighthouse *et al.* 2008), how such changes in composition is related to functionality remains obscure. The spherical morphology of the spectrosome is often used to identify the stem cells, whereas the branched morphology of the fusome serves as an indicator of cyst development.

The fusome organizes cyst formation via spindle orientation and cell cycle synchronization

The major structural components of the fusome are *hu-li tai shao* (*hts*)/*adducin-like*, α - and β -*spectrin*, and *ankyrin* (Yue and Spradling 1992; Lin *et al.* 1994; de Cuevas *et al.* 1996). These proteins function as a skeletal scaffold on which a network of membranous tubules assembles. The network of membranous tubules increases in density as the fusome grows, with 16-cell cysts having higher densities of these tubules than younger cysts. Several lines of evidence suggest that these membranous tubules in the fusome likely derive from the ER. First, many ER components, such as TER94, Sec61 α , and the KDEL ER reporter, have been found in the fusome (Leon and McKearin 1999; Snapp *et al.* 2004). Second, photobleaching experiments have shown that the fusome is continuous with the ER compartment (Snapp *et al.* 2004). More recent studies confirmed the membranous nature of the fusome by showing that many vesicular components (*e.g.*, the ER, Golgi, and endosomes) are enriched in the fusome (Bogard *et al.* 2007; Roper 2007; Lighthouse *et al.* 2008). The localization of some components to the fusome is temporally regulated, with some

enriched in the early fusome and others enriched in the later fusome, revealing the dynamic nature of fusome composition. Although some degree of difference in fusome/spectrosome components is noted between females and males (Lin *et al.* 1994; Lighthouse *et al.* 2008), the functional relevance of these differences remains unknown.

CBs or GBs undergo four rounds of mitotic division to yield a cyst of 16 germ cells that are interconnected with stereotypical topology. To yield such a cyst, cystocyte/SG divisions must occur in synchrony and in the right orientation. The fusome and RCs play fundamental roles in these two aspects. First, the fusome anchors the mitotic spindle during cystocyte/SG divisions such that the new daughter cells are always displaced away from the existing cluster of cells. Second, the RCs and fusome together allow for the sharing of information among the germ cells within a cyst, ensuring synchronized mitoses (Yue and Spradling 1992). The fusome likely functions as a “selective barrier” to allow sharing of some components/information among germ cells while blocking the transport of others. For example, cell cycle information is clearly shared among germ cells within a cyst, but organelle movement is likely blocked by the fusome as mitochondrial and centrosome migration from the nurse cells to the oocyte only occurs after disintegration of the fusome in region 2b of the germarium (Mahowald and Strassheim 1970; Cox and Spradling 2003). Interestingly, in *hts* mutant male germlines, which lack the fusome, centrosomes move through RCs, resulting in SCs with too many or too few centrosomes, even though centrosomes do not normally move across RCs in male germ cells (Wilson 2005).

During cystocyte and SG divisions, one spindle pole is always associated with the fusome. Therefore, one daughter cell is placed away from the existing fusome (Lin and Spradling 1995; Deng and Lin 1997; McGrail and Hays 1997; Wilson 2005; Venkei and Yamashita 2015). Subsequently, the fusome and germ cells reorganize their relative positions, forming a tight cluster of germ cells connected by the fusome (Huynh and St Johnston 2004). The fusome is a major MT-organizing component in both the male and female germlines (McGrail and Hays 1997; Grieder *et al.* 2000), and the spectrosome/fusome is associated with a number of MT regulators and motors, enabling the spectrosome/fusome to anchor the mitotic spindle. For example, cytoplasmic dynein is associated with the fusome and is required for spindle orientation during cystocyte division (McGrail and Hays 1997; Liu *et al.* 1999). Orbit/Mast, a MT-associated protein, localizes to the fusome and the spindle during cystocyte divisions in the female germline, and is required for proper MT organization in the growing cyst (Mathe *et al.* 2003). The kinesin motor (Klp61F/BimC) shows a similar pattern, associating with the fusome in interphase and localizing to the spindle during mitosis in the male germline (Wilson 1999). Similarly, the MT–fusome linker *short stop*, a spectropodin homolog, is required for MT organization along the fusome, which is vital for oocyte formation (Roper and Brown 2004).

The fusome and RCs allow for synchronization of the cell cycle within a cyst. In the absence of the fusome, cell cycle

synchrony appears perturbed, and the number of germ cells within a cyst is no longer a power of two (Yue and Spradling 1992; de Cuevas *et al.* 1996). The fusome is associated with various cell cycle regulators such as Cyclin A, Cyclin B, Cyclin E, Cyclins' destruction machineries (SCF (Skp, Cullin, F-box) ubiquitin ligase component Cul1 and protease 19S-RP subunit S1), and Cdk1 (Lilly *et al.* 2000; Ohlmeyer and Schupbach 2003; Mathieu *et al.* 2013; Varadarajan *et al.* 2016). Overexpression of Cyclin A in germ cells causes an extra round of mitotic division, yielding cysts with 32 germ cells instead of 16 (Lilly *et al.* 2000). These studies suggest that the fusome, through its associated cell cycle regulators, has a strong influence on a cyst's mitotic divisions.

In addition to cell cycle synchronization, the fusome regulates incomplete cytokinesis. The central spindle complex components Survivin and Aurora B localize to the fusome and inhibit cytokinesis by antagonizing Cyclin B (Mathieu *et al.* 2013), although the fusome itself is not required for incomplete cytokinesis (Yue and Spradling 1992). Association of the fusome with cell cycle regulators indicates that the fusome does not function as a mere conduit for the cytoplasm; instead, it may function as a platform for cell cycle regulation, collecting information from each cell, integrating multiple inputs from all cells within the cyst, and transmitting a unified decision to all cells. Aforementioned photobleaching experiments demonstrated that the ER is shared among cystocytes (Snapp *et al.* 2004), and ER connectivity correlates with the ability of cystocytes to undergo synchronized mitoses, suggesting that shared ER might be the underlying mechanism of fusome-mediated information sharing within a cyst.

RC formation and maturation

The term RC refers to the cytoplasmic bridge/opening (*i.e.*, the space) through which materials are shared among germ cells within the cyst, but the term is often used to mean the cytoskeletal ring structure itself that stabilizes the canal/opening (Figure 1C) (Robinson and Cooley 1996; Mische *et al.* 2007). Early electron microscope studies revealed clear cytoplasmic openings connecting adjacent cells, characterized by electron dense material coating the inside of the plasma membrane (Fawcett *et al.* 1959; Brown and King 1964; Mahowald 1971a). This electron dense "outer rim" is further supported by the underlying "inner rim," which is less electron dense in electron microscope images.

RCs result from incomplete cytokinesis during germ cell division, and are characterized by a cytoskeletal ring structure that is derived from the contractile ring during cytokinesis. The fusome runs through the RCs, as described above. In the female germline, RCs grow in diameter from 0.5 to 7–10 μm , allowing for the movement of cytoplasmic contents from the nurse cells to the oocyte, including organelles such as mitochondria and centrosomes. Note that the mature RC ($\sim 10 \mu\text{m}$ in diameter) is a massive structure: as a reference, a GSC is $\sim 7 \mu\text{m}$ and could pass through a mature RC (Figure 1C). In the testis, RCs do not grow in diameter, but they do persist

until the very end of spermiogenesis, when they are finally discarded during sperm individualization (Hime *et al.* 1996).

In the female germline, RC growth and maturation follow a precise developmental program that coordinates with development of the fusome (Cooley 1998; Ong and Tan 2010). The first sign of RC maturation, when the contractile ring departs from its normal simple structure/composition, is the appearance of a phosphor-tyrosine epitope at the end of the third mitotic division in region 1 of the germarium. The outer rim of these early RCs contains contractile ring actin, anillin, and phosphor-tyrosine, and they have no inner rim (Theurkauf *et al.* 1992). After completion of the fourth mitosis, the outer rim maintains anillin and phospho-tyrosine, and the inner rim accumulates phosphor-tyrosine, F-actin, and Hts-RC, followed by recruitment of Kelch (Kel) (Xue and Cooley 1993). Subsequently, Anillin disappears from the RC (Robinson *et al.* 1994; Robinson and Cooley 1997b).

Hts-RC and Kel, together with F-actin, are the major components of RCs in the female germline (Figure 1C and Table 1). Hts-RC is a product of the *hts* gene, which produces a polypeptide that is subsequently cleaved to generate the Hts-RC and Hts-Fus proteins. Hts-RC localizes to the RCs, whereas Hts-Fus, which is often simply referred to as Hts or Adducin-like (Add), is a major component of the fusome, as described above. Cleavage of the Hts polypeptide is important for Hts-RC to localize to RCs (Petrella *et al.* 2007). Hts-RC is required for anchoring of the inner rim of the RC to the outer rim (Yue and Spradling 1992).

kel mutants show defective cytoplasmic transport (called the "dumpless" phenotype) from the nurse cells to the oocyte during late oogenesis due to abnormally high levels of actin filament bundling in the inner rim, which obstructs the lumen of the RCs (Xue and Cooley 1993; Tilney *et al.* 1996). Kel is an oligomeric protein (Robinson and Cooley 1997a) that likely functions as an adaptor protein for Cullin-RING ubiquitin ligase, which catalyzes target protein degradation. *cul-3* mutants show a similar phenotype as *kel* mutants, and *kel* and *cul-3* genes are required to disassemble the RC cytoskeleton to support expansion of the RC diameter (Hudson and Cooley 2010). Although Kel accumulates at abnormally high levels in *cul-3* mutants, it does not appear to be an essential target of *cul-3* degradation, as a nondegradable form of Kel is functional (Hudson *et al.* 2015). Functionally relevant target(s) of Cul-3-Kel E3 ligase have not yet been identified.

In addition to core/structural proteins described above, many other proteins are enriched at the RCs to regulate RC growth/maturation. Among these are Tec29/Btk29A and Src64, tyrosine kinases enriched at the RCs, that participate in a signaling cascade essential for RC growth. Src64 is required for the localization of Tec29/Btk29A to the RC, and mutations in either of these genes result in defective nurse cell transport, consistent with their function in RC development (Dodson *et al.* 1998; Guarnieri *et al.* 1998; Roulier *et al.* 1998; Lu *et al.* 2004). A few targets of this kinase cascade have been identified. First, Kel is phosphorylated in a *src64*-dependent manner to facilitate the exchange of actin monomers at the

Table 1 Genes that regulate ring canal formation

	Protein	Function/localization in female germline	Function/localization in male germline
Contractile ring components (known or likely to be common between male and female RC)	Anillin	Required for cytokinesis in general (Field <i>et al.</i> 2005).	Localized to RC (Hime <i>et al.</i> 1996).
	Peanut, Septin1, Septin2	Septins, required for cytokinesis.	Localized to RC (Hime <i>et al.</i> 1996).
	Pavarotti	Kinesin MKLP1. Contractile ring, RC (Minestrini <i>et al.</i> 2002).	Localized to RC (Salzmann <i>et al.</i> 2014).
	F-actin	Localizes to RC starting region 2a (Robinson <i>et al.</i> 1994; Tilney <i>et al.</i> 1996).	Contractile rings and spermatocyte fusome (Hime <i>et al.</i> 1996).
	Zipper	Myosin II heavy chain, required for cytokinesis in S2 cells (Rogers <i>et al.</i> 2003).	
	Spaghetti squash (Sqh)	Myosin II regulatory light chain. Required for cytokinesis. Required for nurse cell dumping (Wheatley <i>et al.</i> 1995).	Localized to RC Required for meiotic cytokinesis (Eikenes <i>et al.</i> 2013).
	Cindr	Required for cytokinesis in S2 cells. Component of RCs (Haglund <i>et al.</i> 2010).	Centralspindlin interactor. Localized to contractile ring and RC. Required for meiosis (Montembault <i>et al.</i> 2010).
	Nessum dorma	Localizes to RC, required for normal RC formation (Montembault <i>et al.</i> 2010).	Localized to RC and is required for RC formation (Miyachi <i>et al.</i> 2013).
	Orbit/CLASP	Localizes to RC and fusome. Required to recruit Anillin and Pavarotti to RC. RC occlusion (Mathe <i>et al.</i> 2003).	Required for Tyr-phosphorylation of RC and correct RC diameter (Eikenes <i>et al.</i> 2015b).
	Src64	Mediates Tyr-phosphorylation of Tec29/Btk29A and Kelch to support RC growth. Required for Tec29/Btk29A localization to RC. Regulates actin network at RC (Dodson <i>et al.</i> 1998; Guarnieri <i>et al.</i> 1998; Roulier <i>et al.</i> 1998; Kelso <i>et al.</i> 2002; Lu <i>et al.</i> 2004; O'Reilly <i>et al.</i> 2006).	
Mature RC components (common between male and female)	Tec29/Btk29A	Required for RC growth (Guarnieri <i>et al.</i> 1998; Roulier <i>et al.</i> 1998). Phosphorylates β -catenin/Armadillo around the RC (possibly contributes to disassembly of adherens junction around the RC to allow RC growth) (Hamada-Kawaguchi <i>et al.</i> 2015).	
	Mucin-D	RC component (Kramero and Kramerov 1999)	
Mature RC components (known or likely to be specific to female RC)	Kelch (Kel)	Required for RC development. Localizes to RC starting region 3 (Robinson <i>et al.</i> 1994). Bundles actin filaments (Robinson and Cooley 1997a; Kelso <i>et al.</i> 2002). Required to disassemble RC cytoskeleton (to support RC diameter growth). Mutants result in "small lumen" phenotype of RC (Robinson and Cooley 1997b; Hudson and Cooley 2010; Hudson <i>et al.</i> 2015).	Present in RC (Kramero and Kramerov 1999). Not present in male RC (Hime <i>et al.</i> 1996).
	Cullin-3 (Cul-3)	Binds to Kel and localizes to RC. Mutants result in "small lumen" phenotype of RC. Kel accumulates in cul-3 mutant RC (Hudson and Cooley 2010; Hudson <i>et al.</i> 2015).	
	Hts-RC	Required for RC development. Localizes to mature RC (starting region 2a) (Robinson <i>et al.</i> 1994; Petrella <i>et al.</i> 2007).	Not present in male RC (Hime <i>et al.</i> 1996).
	Cheerio	Filamin, F-actin cross-linking protein. Required for RC assembly. Localizes to RC (both inner and outer rims), required for localization of Kelch and Hts-RC (Robinson <i>et al.</i> 1997; Li <i>et al.</i> 1999; Sokol and Cooley 1999).	
	Arpc1, Arp3, Arpc3B	Arp2/3 complex. Required for RC expansion (Hudson and Cooley 2002).	
	α -Actinin	Actin cross-linking protein. Localizes to RC inner rim (Wahlstrom <i>et al.</i> 2004).	
	DmLipin	Lipin, Localizes to RC (Valente <i>et al.</i> 2010).	
	Cortactin	Localizes to RC. Required for RC growth. Dumping phenotype (Somogyi and Rorth 2004).	
	Akap200	PKA anchoring protein. Localizes to the outer rim of RC together with PKA-regulatory subunit II. Regulates RC size/stability (Jackson and Berg 2002).	

(continued)

Table 1, continued

	Protein	Function/localization in female germline	Function/localization in male germline
Required for RC maturation, not localizing to RC (or unknown localization)	dMYPT	Myosin-binding subunit of myosin phosphatase. Required for RC growth. Conversion of contractile ring to RC. Dumpyless phenotype (Tan <i>et al.</i> 2003; Ong <i>et al.</i> 2010).	
	Cappuccino	Formin homology family. RC formation (Jackson and Berg 1999).	
	Chickadee	Profilin. RC formation (Jackson and Berg 1999).	
	Capping protein (cpb)	Defective RC morphology (Ogienko <i>et al.</i> 2013).	
	Rings lost	RC growth, ubiquitin receptor and binds to 26S proteasome (Morawe <i>et al.</i> 2011).	
	Su(Hw)	Regulates RC through Src64 expression (Hsu <i>et al.</i> 2015).	
	Importin- α	Required for localization of Kelch to RC. Dumpyless phenotype due to RC occlusion (Gorjanacz <i>et al.</i> 2002, 2006).	
	Lark	Dumpyless phenotype. Required for RC actin organization, Hts-RC localization (McNeil <i>et al.</i> 2004).	
	Parcas	Negative regulator of Tec29/Btk29A. Mutants have extremely large RCs (Hamada-Kawaguchi <i>et al.</i> 2015).	
	Flapwing	Protein phosphatase 1. Mutant results in overconstriction of RC (Yamamoto <i>et al.</i> 2013).	

RC, ring canal.

RCs during their growth (Kelso *et al.* 2002). Second, Cortactin, another Src64 substrate, localizes to the RCs and regulates RC growth (Somogyi and Rorth 2004). Third, Tec29/Btk29A phosphorylates β -catenin/Armadillo to promote its release from adherens junctions; adherens junctions form around the RCs to “seal” juxtaposing plasma membranes between neighboring germ cells, and thus its release is likely required to allow RCs to grow (Fichelson *et al.* 2010; Hamada-Kawaguchi *et al.* 2015). As adherens junctions play an important role in anchoring the RCs to the plasma membrane during oocyte/nurse cell development (Loyer *et al.* 2015), the germ cells need to coordinate the assembly and disassembly of adherens junctions to allow RCs to grow without detaching from the plasma membrane. Many other RC components/regulators have been identified thus far (Table 1), although it remains unclear how these proteins are integrated into the core pathway.

Male RCs do not grow in diameter, staying $\sim 1\text{--}2\ \mu\text{m}$. The lack of stabilizing proteins likely reflects the lack of RC growth in the male germline. Female RC proteins, such as Kel and Hts-RC, are not detectably expressed in the male germline and do not localize to the male RCs (Hime *et al.* 1996). Male RCs also lack the robust array of F-actin seen in female RCs; however, most of the contractile ring components such as Septins (Sep1, Sep2, and Pnut), Pavarotti (kinesin MKLP1), Anillin, and Zipper (myosin II heavy chain) persist (Robinson *et al.* 1994; Hime *et al.* 1996; Eikenes *et al.* 2013) (Figure 2C and Table 1). Despite these differences, Src64-dependent regulation of RC maturation exists in the male germline and serves to regulate the actin cytoskeleton near the RCs (Eikenes *et al.* 2015b).

Although cytokinesis and abscission are normally the default last step of cell division in most somatic cells, a few lines of evidence suggest that stabilization of the contractile ring may be the default pathway in the germline and that the abscission is a GSC-specific add-on. In GSCs, which undergo complete abscission, the process of cytokinesis proceeds in two steps. After telophase, the progression of cytokinesis is blocked for an extended period of time, and then abscission resumes to complete cytokinesis (Lenhart and DiNardo 2015). This pausing implies that the first step is the default for germ cells and that the second step is an additional, specialized mechanism to complete cytokinesis specifically in the GSCs. It was shown that gain-of-function mutations in Aurora B kinase or Survivin, the components of the chromosome passenger complex, stabilize the contractile ring in the GSCs, resulting in a “stem cyst,” where GSCs fail to pinch off and continue their proliferation with incomplete cytokinesis just like SG cysts (Mathieu *et al.* 2013; Eikenes *et al.* 2015a; Matias *et al.* 2015). This suggests that sustained activity of Aurora B and/or Survivin may be the underlying molecular mechanism that allows the formation of germ cell cysts.

Oocyte Determination and Cyst Polarization

Female germ cell cysts undergo unique polarization events that are missing in the male germline. In contrast to the male germline, in which all germ cells differentiate into sperm, a

single cell out of 16 cystocytes is selected to become the oocyte and the remaining 15 germ cells differentiate as nurse cells in the female germline. This “symmetry breaking” requires the polarization of germ cells within the cyst. Oocyte determination is closely linked to fusome morphology, whereas RCs serve as an essential conduit to transport cellular components from the nurse cells to the oocyte.

Fusome inheritance and oocyte determination

Because of the way germ cells divide synchronously and in an oriented manner, as described above, the cyst contains 16 cells at the completion of the four mitotic divisions, among which two cells have four RCs, two have three RCs, four have two RCs, and eight have one RC (Figure 1B). The two cells that have four RCs initiate oocyte differentiation as “prooocytes,” and one of these two prooocytes eventually becomes the oocyte (Brown and King 1964). In region 2a of germarium, the two prooocytes (and sometimes a few other cystocytes) develop a synaptonemal complex, a protein complex that pairs homologous chromosomes, indicating meiotic entry. By the time the cyst enters region 3 of the germarium, the synaptonemal complex has disassembled in all but one prooocyte, clearly specifying this cell as the oocyte. Rearrangements among cells within the cyst position the oocyte at the most posterior side of the egg chamber.

Although it is difficult to conclusively determine which of the two cells with four RCs becomes the oocyte, it is likely that the fate of the future oocyte is determined when the CB divides to produce two cystocytes. The fusome is divided asymmetrically during the CB division; during CB mitosis, the fusome is associated with only one spindle pole, and upon CB division, one daughter inherits two-thirds of the fusome material while the other inherits the remaining one-third. Based on the observations made in the diving beetle *Dytiscus*, where the future oocyte is always larger than the sibling germ cells and inherits more of the fusome material (Telfer 1975), it was proposed that the cell that inherits more fusome material during the CB division is destined to become the oocyte (de Cuevas and Spradling 1998). Considering that the fusome organizes the MTs that play a critical role in the directional, motor-dependent transport that initiates oocyte fate determination (Grieder *et al.* 2000), it seems reasonable to assume that the cell with the largest amount of fusome becomes the oocyte, breaking the symmetry among cystocytes.

Although SGs show the same distribution of RC numbers within the cyst as in cystocytes (two SGs with four RCs and two SGs with three RCs etc.) and while some SGs have more fusome material than others, all SGs have the same fate to become sperm. It remains unclear whether fusome asymmetry in the male germline plays any role and/or whether there is any potential fate asymmetry among SGs/SCs.

The fusome and cyst polarity

In parallel with oocyte determination, the cyst undergoes MT polarization. During the cystocyte mitotic divisions, MT minus ends are found on the fusome (Grieder *et al.* 2000). Then,

MT minus ends are gradually concentrated to the center of the fusome in region 2 of the germarium, and by the time the cyst enters region 3, MT minus ends are enriched in the oocyte (Theurkauf *et al.* 1992). In sensory organ precursor cells, a slight asymmetry in the number of MT minus ends between two sister cells along the central spindle leads to fate asymmetry via asymmetric segregation of fate-determining SARA (Smad anchor for receptor activation) endosomes (Derivery *et al.* 2015). Similarly, it is possible that asymmetry in the number of MT minus ends due to asymmetric fusome segregation during CB division might trigger a cascade of MT polarization within the cyst, determining oocyte fate.

Par-1, an evolutionarily conserved kinase involved in MT organization and cell polarity, is localized to the fusome in proliferating cystocytes (Cox *et al.* 2001a; Huynh *et al.* 2001b). In *par-1* mutants, initial fate determination of the oocyte appears to be relatively normal, but oocyte fate cannot be maintained. Also, pharmacological perturbation of the MT cytoskeleton results in egg chambers with 16 nurse cells and no oocyte (Koch and Spitzer 1983; Theurkauf *et al.* 1993), demonstrating the critical role of MT organization in oocyte determination. Polarized transport of oocyte fate determinants, such as Orb and Cup, into the oocyte is mediated by the Egl-BicD-dynein complex, which walks along the MT cytoskeleton toward the minus ends, which are in the oocyte (Ran *et al.* 1994; Swan and Suter 1996; Mach and Lehmann 1997; Huynh and St Johnston 2000; Navarro *et al.* 2004). Thus, Par-1 may establish a polarized MT network within the cyst, along which Dynein-dependent transport machinery carries cargos that are essential for oocyte determination from the nurse cells to the oocyte. However, Dynein and its regulator Lis1 are also required for correct formation/branching of the fusome during cystocyte divisions (McGrail and Hays 1997; Liu *et al.* 1999; Bolivar *et al.* 2001), suggesting a mutual relationship between fusome integrity and MT organization.

The Baz(Par-3)/aPKC/Par-6 complex, a conserved complex regulating cell polarity, localizes to the ring structure that outlines the RCs during cystocyte divisions. This complex colocalizes with the adherens junction components E-cadherin and Armadillo/ β -catenin (Cox *et al.* 2001b; Huynh *et al.* 2001a). A mutation in any of these components results in cysts with 16 nurse cells and no oocyte, similar to *par-1* mutants. Typically, Par-1 and Baz/aPKC/Par-6 form mutually exclusive cortical domains for establishing cell polarity in broad systems including the *Caenorhabditis elegans* zygote and the *Drosophila* neuroblast (Suzuki and Ohno 2006; Prehoda 2009). It is unclear whether Par-1 and Baz/aPKC/Par-6 may have a similar antagonistic relationship during cystocyte divisions. However, later in the developing oocyte, Baz and Par-1 form mutually exclusive cortical domains (Par-1 on the posterior cortex and Baz on the anterior-lateral cortex), regulating MT polarity within the oocyte (Vaccari and Ephrussi 2002; Benton and St Johnston 2003).

In addition to the polarization of MTs within the cyst, it is important that the oocyte comes to occupy the most posterior position within the egg chamber. In region 2b of the germarium,

the future oocyte is located in the middle of the cyst but shifts to the posterior side of the cyst in region 3 as the cyst prepares to bud off from the germarium (Figure 1A). This oocyte positioning at the posterior side of the egg chamber is the first visible sign of egg chamber polarization and is critical for oocyte patterning, as follicle cell–germline interactions play essential roles in polarizing the oocyte, which in turn determines the body axes in the embryo (Ruohola *et al.* 1991; Gonzalez-Reyes and St Johnston 1994; Gonzalez-Reyes *et al.* 1995; Roth *et al.* 1995; Ray and Schupbach 1996). Oocyte fate leads to a higher expression of DE-cadherin (*Drosophila* E-cadherin) in the oocyte compared to the nurse cells, which cause it to be attracted to the posterior follicle cells that also have high levels of DE-cadherin expression, resulting in correct positioning of the oocyte at the posterior of the egg chamber (Godt and Tepass 1998; Gonzalez-Reyes and St Johnston 1998; Becam *et al.* 2005).

The polarity that is set up in early cysts through these processes prepares oocyte polarity and the embryonic axis as described below.

Development of the Oocyte by Cytoplasmic Transport and Storage

Once oocyte fate is determined, the oocyte further develops by collecting cellular materials supplied from the nurse cells by utilizing the polarized MT network that runs through the entire egg chamber (Figure 1A and Figure 4, A and B). Nurse cells undergo polyploidization and synthesize many of the materials (mRNAs and organelles) required for the oocyte to progress through oogenesis and early embryogenesis (King and Burnett 1959). Once transported into the oocyte, those materials have to be placed in the right location to establish embryonic patterning (Figure 1A and Figure 4, A and C). In particular, localized mRNAs and their spatiotemporally-regulated translation play a critical role in pattern formation in the oocyte/embryo. In the *Drosophila* oocyte, localized mRNAs are the foundation of development, defining the body axes (anterior–posterior and dorsal–ventral). The paradigmatic mRNA species that have driven the understanding of mRNA localization and translational control are *oskar* (*osk*), *gurken* (*grk*), *bicoid* (*bcd*), and *nanos* (*nos*) (Table 2). As is detailed in a series of excellent reviews (St Johnston 2005; Kugler and Lasko 2009; Martin and Ephrussi 2009), studies on these mRNAs have provided the framework for how mRNAs are localized to define cell polarity and determine cell fate. A recent genome-scale study identified over a 100 mRNA species that localize to the anterior or posterior of the oocyte (119 posterior mRNAs and 106 anterior mRNAs) (Jambor *et al.* 2015), arguing that localized mRNA might be a more prevalent means to establish cell polarity than previously appreciated.

Some mRNAs (*grk* and *osk*) are translated within the oocyte and the protein products are actively involved in establishing the polarity of the oocyte/embryo. Other mRNAs (*bcd* and *nos*) are translationally repressed until after fertilization (Gavis and

Lehmann 1994; Salles *et al.* 1994; Eichhorn *et al.* 2016), and these proteins form opposing protein gradients in the embryo to direct anterior and posterior patterning, respectively. *grk* mRNA localizes to the anterior–dorsal corner of the oocyte, where the translated Grk protein (a TGF α homolog) induces dorsal fate through interaction with the adjacent follicle cells (Figure 4C) (Neuman-Silberberg and Schupbach 1993, 1994, 1996; Roth *et al.* 1995). *bcd* mRNA is localized to the anterior of the oocyte, resulting in production of an anterior–posterior gradient of Bcd protein. Bcd is a morphogen that functions as a transcription factor and translational repressor to determine head and thorax development in the embryo (Figure 4C) (Driever and Nusslein-Volhard 1988a,b; Struhl *et al.* 1989; Dubnau and Struhl 1996; Rivera-Pomar *et al.* 1996). *osk* mRNA localizes to the posterior pole where Osk protein helps to define anterior–posterior polarity and specify pole plasm formation (Figure 4C) (Ephrussi *et al.* 1991; Ephrussi and Lehmann 1992). *nos* mRNA is localized to the posterior of the oocyte to produce Nos protein specifically at the posterior pole (Gavis and Lehmann 1992). Nos protein is a translational regulator that is essential for patterning the anterior–posterior body axis and for primordial germ cell development in the early embryo (Gavis and Lehmann 1992; Murata and Wharton 1995; Kobayashi *et al.* 1996; Dansereau and Lasko 2008).

There are several overarching key features about the polarized localization of fate determinants through mRNA localization as summarized below. mRNA-binding proteins that participate in these processes are listed in Table 2.

Transport by motors and anchoring after transport

osk, *bcd*, and *grk* mRNA are localized by directed transport of mRNAs from the nurse cells to the oocyte by motor proteins that move along MT tracks. This MT-dependent transport depends on Dynein and its cargo adaptors Egalitarian (Egl) and Bicaudal-D (Bic-D) (Table 2) (Ephrussi *et al.* 1991; Pokrywka and Stephenson 1991; Suter and Steward 1991; Li *et al.* 1994; Thio *et al.* 2000; Bullock and Ish-Horowicz 2001; Navarro *et al.* 2004; Weil *et al.* 2006; Clark *et al.* 2007). Once in the oocyte, the mRNA localization/transport mechanisms diverge so each mRNA can reach its distinct final localization (posterior for *osk*, anterior–dorsal corner for *grk*, and anterior for *bcd*). For example, *osk* mRNA switches to a kinesin-dependent mechanism to reach the posterior of the oocyte (Brendza *et al.* 2000; Cha *et al.* 2002; Gaspar *et al.* 2017). *osk* mRNA also requires Myosin-V for short-range transport near the oocyte cortex (Krauss *et al.* 2009). In contrast, transport of *grk* and *bcd* mRNAs inside the oocyte continues to rely on Dynein (Pokrywka and Stephenson 1991; MacDougall *et al.* 2003; Weil *et al.* 2006; Clark *et al.* 2007; Delanoue *et al.* 2007; Rom *et al.* 2007). The choice of motors correlates well with the MT polarity within the oocyte (see below).

nos mRNA does not show specific localization until nurse cell dumping occurs (Raff *et al.* 1990; Dalby and Glover 1992; Jongens *et al.* 1992; Wang *et al.* 1994; Nakamura *et al.* 1996). Dumping is a process during late oogenesis, where nurse cell

Figure 4 Polarization of oocyte through mRNA transport and localization. (A) Summary of mRNA transport and localization during *Drosophila* oogenesis. Reproduced from Becalska and Gavis (2009) with permission from Liz Gavis and *Development*. (B) The mechanism that polarizes MTs in developing oocytes. Shot anchors Patronin, a MT minus end-anchoring protein, to the actin cytoskeleton at the oocyte cortex except for the posterior, where their recruitment is inhibited by Par-1. This generates the gradient of MT nucleation along the oocyte cortex (higher at the anterior and lower at the posterior), which creates compartments of MT orientation (on average) within the oocyte. This MT orientation is sufficient to localize mRNAs. (C) Examples of localized fate determinants. Grk protein at the anterior–dorsal corner, *bcd* mRNA at the anterior, and *osk* mRNA at the posterior. Reproduced from Morais-de-Sa *et al.* (2014) and Vanzo and Ephrussi (2002) with the permission of Daniel St Johnston, Anne Ephrussi, and *Development*. MT, microtubule.

cytoplasm is nonselectively transported into the oocyte by a myosin-dependent contraction of the cortical actin that “squeezes” nurse cell contents into the oocyte. *nos* mRNA localization to the posterior pole is not mediated by direct transport along the MTs. Instead, *nos* mRNA is transferred to the oocyte by nurse cell dumping, and once in the oocyte, it is carried to the posterior by a diffusion-based mechanism that is facilitated by ooplasmic streaming, a kinesin-1-dependent process that causes directional “stirring” of the oocyte cytoplasm that mixes the existing cytoplasm and the incoming nurse cell cytoplasm (Figure 4A) (Forrest and Gavis 2003). The *nos* mRNA localization mechanism, which does not rely on directed transport, is inefficient and only 4% of total *nos*

mRNAs are localized at the posterior. The posterior concentration of *nos* mRNA is facilitated by the selective destabilization of unlocalized *nos* mRNA (Zaessinger *et al.* 2006).

Once mRNAs reach their final destination, they may be anchored in place. *osk*, *bcd*, and *nos* mRNA anchoring requires the actin cytoskeleton and its associated proteins (Wang *et al.* 1994; Polesello *et al.* 2002; Forrest and Gavis 2003; Babu *et al.* 2004; McNeil *et al.* 2004; Weil *et al.* 2008; Suyama *et al.* 2009). *grk* mRNA transport inside the oocyte and anchoring near the oocyte nucleus depend on dynein (MacDougall *et al.* 2003; Clark *et al.* 2007; Delanoue *et al.* 2007; Rom *et al.* 2007). In the case of *osk* mRNA, Long Osk, an isoform of the *osk* gene product, anchors *osk* mRNA, forming a positive

feed-forward loop to reinforce *osk* mRNA/Osk protein localization (Lehmann and Nusslein-Volhard 1986; Ephrussi *et al.* 1991; Ephrussi and Lehmann 1992; Breitwieser *et al.* 1996; Vanzo and Ephrussi 2002; Babu *et al.* 2004; Vanzo *et al.* 2007; Zimyanin *et al.* 2007; Suyama *et al.* 2009; Hurd *et al.* 2016). Short Osk, the other isoform of the *osk* gene product, anchors *nos* mRNA to the posterior pole and is sufficient for germ plasm formation (Ephrussi and Lehmann 1992; Smith *et al.* 1992; Markussen *et al.* 1995; Vanzo and Ephrussi 2002).

Organization of the cytoskeleton

mRNA transport and anchoring both rely on organized cytoskeletons in the nurse cells and oocyte. Motor proteins carry their cargo mRNAs to the right destination and the local actin cytoskeleton helps anchor mRNAs. To allow for the directed transport of *osk*, *grk*, and *bcd* mRNA from the nurse cells to the oocyte during the early stages of oogenesis (stage 2–6), MTs are organized by clustered centrosomes in the oocyte, which are formed by the migration of nurse cell centrosomes into the oocyte. This MTOC creates a MT array with the plus end extending into the nurse cells and the minus end anchored in the oocyte (Theurkauf *et al.* 1992, 1993). Therefore, minus end-directed dynein motors can carry cargos into the oocyte.

During mid oogenesis (stages 7–10), MTs are rearranged into acentrosomal tracks that are anchored to the oocyte cortex on their minus ends, forming an overall anterior-to-posterior gradient with the plus ends more concentrated on the posterior side of the oocyte (Theurkauf *et al.* 1992; Zimyanin *et al.* 2008; Parton *et al.* 2011) (Figure 4B). At this point, the centrosomes migrate to the antero-dorsal corner of the oocyte together with the nucleus; although they may participate in anchoring the nucleus (Zhao *et al.* 2012), the majority of MTs are no longer centrosomal. Polarization of MTs along the oocyte anterior–posterior axis requires several evolutionarily conserved polarity regulators such as Baz(Par-3)/Par-6/aPKC, Par-1, and Par-5/14-3-3 (Shulman *et al.* 2000; Tomancak *et al.* 2000; Huynh *et al.* 2001a,b; Benton *et al.* 2002; Benton and St Johnston 2003; Martin and St Johnston 2003; Doerflinger *et al.* 2006, 2010). Baz/Par-6/aPKC localizes to the anterior cortex of the oocyte, whereas Par-1 localizes to the posterior cortex, establishing mutually exclusive cortical domains. The substrate specificity subunit of SCF ubiquitin ligase, Slmb, regulates the establishment of mutually exclusive Par-6/aPKC anterior vs. Par-1 posterior cortical domains, likely by targeting Par-6/aPKC for degradation (Morais-de-Sa *et al.* 2014). MT nucleation (minus end anchoring) occurs at the anterior and lateral cortexes while it is inhibited specifically at the posterior cortex in a Par-1-dependent manner, resulting in overall MT orientation along the anterior–posterior axis (Parton *et al.* 2011). Short stop (Shot), the *Drosophila* spectraplakins protein, anchors MT minus ends to the actin cytoskeleton at the anterior/lateral cortex. Shot recruits patronin, a MT minus end-binding protein, which functions as a noncentrosomal MTOC, organizing the AP-polarized MTs (Nashchekin *et al.* 2016). Shot is localized to the anterior/lateral cortex in oocytes, and its localization to the posterior

side is inhibited in a Par-1-dependent manner (Nashchekin *et al.* 2016).

A recent study provided a simple computational model that can recapitulate many features of mRNA localization found in wild-type and mutant oocytes (Khuc Trong *et al.* 2015). By simply assuming that MT nucleation exists as a gradient along the cortex (high nucleation at the anterior and none at the posterior), MT arrangement as well as mRNA transport within the oocyte can be recapitulated through simulation (Figure 4B). Although MT orientation does not appear to be strongly polarized in individual oocytes, as is experimentally detected (Zimyanin *et al.* 2008), averaging many (simulated) oocytes reveals striking and stereotypical MT orientation patterns, which are compartmentalized within the oocyte in such a way that *osk* mRNA is transported to the posterior and *bcd* mRNA to the anterior.

Translational regulation of mRNAs

While mRNAs are being transported/localized, their translation must be strictly regulated; they must be repressed before arriving at the final destination and activated at the right developmental time and place. A plethora of mRNA-binding proteins have been identified that regulate the translation of these mRNAs, as summarized in Table 2. For example, translation of *osk* mRNA is repressed by inhibiting the eIF4G–eIF4E interaction, which is a critical step in translation initiation. Bruno, which binds to the 3′-UTR of *osk* mRNA, recruits Cup, which in turn disrupts the eIF4E–eIF4G interaction by binding to eIF4E (Wilhelm *et al.* 2003; Nakamura *et al.* 2004; Chekulaeva *et al.* 2006). In addition, ribonucleoprotein (RNP) granule formation also plays a critical role in translational regulation (see below) (Chekulaeva *et al.* 2006).

Cis-regulatory elements in mRNAs

mRNAs encode *cis*-regulatory information—typically in their 3′-UTRs, sometimes in their 5′-UTRs, and introns (before splicing)—which influences their behavior. These *cis*-regulatory elements, each bound by distinct sets of proteins, may regulate their transport (binding to motor proteins), translational repression during transport, anchoring, translational activation, and selective stabilization of correctly localized mRNAs, as well as selective destabilization of mislocalized mRNAs. Each mRNA species employs a combination of these mechanisms, resulting in the protein products localizing in the right place and at the right time. Moreover, alternative splicing can “edit” *cis*-regulatory elements, adding an additional layer of complexity to how mRNAs may be regulated (Horne-Badovinac and Bilder 2008). For example, splicing plays a critical role in regulating *osk* mRNA: the exon junction complex (EJC), which contains Y14/Tsunagi and Mago nashi, is deposited on *osk* mRNA upon splicing, which later plays a critical role in *osk* mRNA localization (Hachet and Ephrussi 2004).

RNA granule formation as a platform of mRNA regulation

mRNAs bound by various proteins for their regulation, such as motors for transportation or translational repressors for silencing, are further organized into RNP granules. RNP granule

Table 2 Regulators of oskar, gurken, bicoid, and nanos mRNAs

mRNA regulators	Function	References
Dynein (dhc, ddic1), Egl, Bic-D	Egl and Bic-D functions as a cargo-specific adaptor for Dynein to transport <i>osk</i> , <i>grk</i> , and <i>bcd</i> mRNAs from nurse cells to oocyte. Dynein carries the target mRNAs to the minus end of the MTs that are located in the oocyte, when Dynein/Egl/Bic-D-dependent mRNA transport happens (early/mid-oogenesis).	Ephrussi <i>et al.</i> (1991), Suter and Steward (1991), Li <i>et al.</i> (1994), Thio <i>et al.</i> (2000), Bullock and Ish-Horowitz (2001), MacDougall <i>et al.</i> (2003), Navarro <i>et al.</i> (2004), Clark <i>et al.</i> (2007), Rom <i>et al.</i> (2007), Jambor <i>et al.</i> (2014), Vazquez-Pianzola <i>et al.</i> (2017)
Kinesin I (Khc)	<i>osk</i> mRNA transport within oocyte to the posterior. This transport utilizes MTs, whose plus end is anchored at the posterior during mid-oogenesis.	Brendza <i>et al.</i> (2000), Cha <i>et al.</i> (2002)
pAbp	Poly(A)-binding protein. Stabilizes <i>osk</i> mRNA during transport. Translational activation of <i>grk</i> mRNA. Binds <i>bcd</i> mRNA. Interacts with Sqd and Cup.	Arn <i>et al.</i> (2003), Clouse <i>et al.</i> (2008), Jeske <i>et al.</i> (2011), Vazquez-Pianzola <i>et al.</i> (2011), McDermott <i>et al.</i> (2012)
Hrb27C/Hrp48	RRM (RNA recognition motif) protein. <i>osk</i> , <i>grk</i> mRNA localization. <i>osk</i> mRNA translational repression. Interacts with Sqd, Otu, Imp, Syp, and Cup.	Goodrich <i>et al.</i> (2004), Huynh <i>et al.</i> (2004), Yano <i>et al.</i> (2004), Geng and Macdonald (2006), McDermott <i>et al.</i> (2012)
Squid (sqd)	<i>osk</i> mRNA localization, translation. <i>grk</i> mRNA localization/anchoring, translation. <i>grk</i> mRNA translational repression in nurse cells. <i>grk</i> mRNA RNP. Interacts with Hrb27C, Syp, Cup, pAbp, and Imp.	Norvell <i>et al.</i> (1999), (2005), Goodrich <i>et al.</i> (2004), Steinhauer and Kalderon (2005), Geng and Macdonald (2006), Delanoue <i>et al.</i> (2007), Clouse <i>et al.</i> (2008), Caceres and Nilson (2009), McDermott <i>et al.</i> (2012), Weil <i>et al.</i> (2012)
IGF-II mRNA-binding protein (Imp)	Imp binds to <i>osk</i> mRNA via IE motifs within 3'-UTR, but is not required for <i>osk</i> mRNA localization. Imp binds to <i>grk</i> mRNA and contributes to its localization and translation. Interacts with Sqd. Hrb27C.	Geng and Macdonald (2006), Munro <i>et al.</i> (2006), McDermott <i>et al.</i> (2012)
Bruno (Bru)	Translational repression of <i>osk</i> mRNA. Derepression of translation upon posterior localization of <i>osk</i> mRNA. Translation of <i>grk</i> mRNA. Interacts with Vasa, Cup, Sqd, Me31B, and eIF4E1	Kim-Ha <i>et al.</i> (1995), Webster <i>et al.</i> (1997), Gunkel <i>et al.</i> (1998), Norvell <i>et al.</i> (1999), Filardo and Ephrussi (2003), Nakamura <i>et al.</i> (2004), Chekulava <i>et al.</i> (2006), Reveal <i>et al.</i> (2010), (2011), Kim <i>et al.</i> (2015)
Cup	Translational repression of <i>osk</i> mRNA by interacting with eIF4E and Bru. Translational repression of unlocalized <i>grk</i> and <i>nos</i> mRNAs. <i>osk</i> mRNA localization by recruiting Barentsz. Inhibit translation by blocking the binding of eIF4E and eIF4G, also by promoting mRNA deadenylation. Interacts with Sqd, pAbp, Smaug, Exu, Me31B, Yps, and Tral.	Wilhelm <i>et al.</i> (2003), (2005), Nakamura <i>et al.</i> (2004), Nelson <i>et al.</i> (2004), Clouse <i>et al.</i> (2008), Igreja and Izaurralde (2011), Jeske <i>et al.</i> (2011)
Smaug	Translational repression of unlocalized <i>nos</i> mRNA via binding to 3'-UTR of <i>nos</i> mRNA. <i>nos</i> mRNA decay by recruiting deadenylation complex CCR4-NOT. Binds to Cup to repress translation, and Osk protein to release translational repression of <i>nos</i> mRNA.	Smibert <i>et al.</i> (1996), (1999), Dahanukar <i>et al.</i> (1999), Nelson <i>et al.</i> (2004), Zaessinger <i>et al.</i> (2006), Jeske <i>et al.</i> (2011)
Ovarian tumor Syncrip	Localization and translational regulation of <i>osk</i> and <i>grk</i> . Interacts with Sqd and Hrb27C.	Goodrich <i>et al.</i> (2004) McDermott <i>et al.</i> (2012), McDermott and Davis (2013)
Me31B	P body component. Translational repression of <i>osk</i> mRNA (without affecting its transport to oocyte). Translational repression of <i>nos</i> mRNA. Interacts with Exu, Cup, Orb, Tral, and Yps.	Nakamura <i>et al.</i> (2001), Igreja and Izaurralde (2011), Jeske <i>et al.</i> (2011), Wong <i>et al.</i> (2011), McDermott <i>et al.</i> (2012)
Exuperantia(Exu)	<i>bcd</i> and <i>osk</i> mRNA localization. Exu associates with <i>bcd</i> mRNA within nurse cells, potentiating <i>bcd</i> mRNA to be transported to anterior cortex once in oocyte. Exu makes a complex with Yps and Me31B.	St Johnston <i>et al.</i> (1989), Pokrywka and Stephenson (1991), Wang and Hazelrigg (1994), Macdonald and Kerr (1997), Wilhelm <i>et al.</i> (2000), Cha <i>et al.</i> (2001), Nakamura <i>et al.</i> (2001), McDermott <i>et al.</i> (2012)

(continued)

Table 2, continued

mRNA regulators	Function	References
Orb	<i>osk</i> mRNA translation. <i>grk</i> mRNA RNP, translational activation of <i>grk</i> mRNA via polyadenylation of <i>grk</i> mRNA. Binds to Me31B, Bru, Cup, pAbp, and Wispy.	Chang <i>et al.</i> (2001), Castagnetti and Ephrussi (2003), Wong <i>et al.</i> (2011), Weil <i>et al.</i> (2012), Norvell <i>et al.</i> (2015), Davidson <i>et al.</i> (2016)
Trailer hitch (Tral)	<i>grk</i> mRNA localization. Binds to <i>nos</i> mRNA. Binds to Cup, Me31B, pAbp, and Yps.	Wilhelm <i>et al.</i> (2005), Snee and Macdonald (2009), Igreja and Izaurralde (2011), Jeske <i>et al.</i> (2011)
Yps	Binds to <i>osk</i> mRNA. Binds to Cup, Exu, Me31B, and Tral.	Wilhelm <i>et al.</i> (2000), (2003), (2005), Nakamura <i>et al.</i> (2004)
Wispy	Poly(A) polymerase that activates <i>grk</i> mRNA translation. Binds to Orb.	Wong <i>et al.</i> (2011), Cui <i>et al.</i> (2013), Norvell <i>et al.</i> (2015)
Pumilio	<i>bcd</i> mRNA deadenylation/translational repression. Binds and antagonizes pAbp.	Gamberi <i>et al.</i> (2002), Weidmann <i>et al.</i> (2014)
piRNA pathway components	piRNA components (<i>armi</i> , <i>aub</i> , <i>spn-E</i> , <i>mael</i> , <i>zuc</i> , and <i>squ</i>) are required for translational repression of <i>osk</i> mRNA. Additionally, <i>Aub</i> regulates <i>nos</i> mRNA localization independent of piRNA-mediated RNA silencing. <i>Aub</i> interacts with Rump. <i>Vasa</i> is required for <i>osk</i> , <i>nos</i> mRNA accumulation and <i>grk</i> mRNA translation.	Wang <i>et al.</i> (1994), Styhler <i>et al.</i> (1998), Tomancak <i>et al.</i> (1998), Cook <i>et al.</i> (2004), Lim and Kai (2007), Pane <i>et al.</i> (2007), Becalska <i>et al.</i> (2011), Vourekas <i>et al.</i> (2016).
Rumpelstiltskin (Rump)	<i>nos</i> , <i>osk</i> mRNA localization. Interacts with <i>Aub</i> .	Jain and Gavis (2008), Sinsimer <i>et al.</i> (2011)
Lost	<i>osk</i> , <i>nos</i> mRNA localization. Interacts with Rump.	Sinsimer <i>et al.</i> (2011)
Staufen (Stau)	<i>osk</i> , <i>bcd</i> mRNA localization. <i>osk</i> mRNA translation.	St Johnston <i>et al.</i> (1989), Ephrussi <i>et al.</i> (1991), Kim-Ha <i>et al.</i> (1991), Ferrandon <i>et al.</i> (1994), Micklem <i>et al.</i> (2000), Mhlanga <i>et al.</i> (2009), Laver <i>et al.</i> (2013)
PTB	<i>osk</i> mRNA localization and translational repression. Component of <i>osk</i> mRNA RNP. Binds to <i>grk</i> mRNA.	Besse <i>et al.</i> (2009), McDermott <i>et al.</i> (2012), McDermott and Davis (2013), Macdonald <i>et al.</i> (2016)
Barentsz (Btz)	<i>osk</i> mRNA transport.	van Eeden <i>et al.</i> (2001)
Bicoid stability factor (Bsf)	<i>bcd</i> mRNA stability and localization. <i>Osk</i> protein expression (through <i>osk</i> mRNA localization and translation).	Mancebo <i>et al.</i> (2001), Ryu and Macdonald (2015)
Rab11, Rbsn-5 (endocytic pathway)	<i>osk</i> mRNA transport, anchoring, and translation through organization of MTs at the posterior cortex.	Dollar <i>et al.</i> (2002), Tanaka and Nakamura (2008)
ESCRT-II complex (Vps22, Vps25, Vps36)	<i>bcd</i> mRNA localization (this function is independent of endosomal sorting).	Irion and St Johnston (2007)
Moesin	Anchoring of <i>osk</i> , <i>nos</i> mRNA at the posterior cortex via regulation of actin cytoskeleton.	Polesello <i>et al.</i> (2002)
Cappuccino, spire	<i>osk</i> mRNA anchoring via nucleation of F-actin downstream of Long Osk.	Chang <i>et al.</i> (2011)
Osk	Long Osk protein anchors <i>osk</i> mRNA and Short Osk protein at the posterior cortex via regulation of actin cytoskeleton. Short Osk is sufficient for pole plasm assembly, including <i>nos</i> mRNA localization and translation.	Wang and Lehmann (1991), Ephrussi and Lehmann (1992), Smith <i>et al.</i> (1992), Wang <i>et al.</i> (1994), Markussen <i>et al.</i> (1995), Rongo <i>et al.</i> (1995), Vanzo and Ephrussi (2002), Zaessinger <i>et al.</i> (2006)
Swallow	<i>bcd</i> mRNA localization (anchoring at the anterior cortex via actin cytoskeleton). Binds to Osk protein.	St Johnston <i>et al.</i> (1989), Pokrywka and Stephenson (1991), Schnorrr <i>et al.</i> (2000), Weil <i>et al.</i> (2008), (2010)
Didum (Myosin V)	Short-range transport of <i>osk</i> mRNA near the posterior cortex of oocyte.	Krauss <i>et al.</i> (2009)
Bic-C	<i>grk</i> mRNA and protein localization.	Snee and Macdonald (2009), McDermott <i>et al.</i> (2012)

Proteins involved in *osk*, *grk*, *bcd*, and *nos* mRNA regulation are organized based on their known physical interactions. However, it should be noted that not all of the proteins were shown to be in the same complex, and it is possible that proteins switch partners depending on the context and/or target mRNA. All of these proteins bind to mRNAs directly or indirectly. MT, microtubule; RNP, ribonucleoprotein.

formation is an essential part of RNA regulation (Weil 2014). For example, oligomerization of *osk* mRNA into RNP granules is critical for translational repression; Cup-mediated inhibition of *osk* mRNA translation is enhanced by the formation of *osk* RNP granules, which inhibit mRNAs from being accessed by ribosomes (Chekulaeva *et al.* 2006). RNP complex formation also enables mRNAs with partially defective *cis*-regulatory elements to localize normally by interacting with wild-type mRNAs within the granule; *osk* mRNAs that lack the first intron, which is critical for EJC loading and mRNA localization, can still be normally localized by interacting with endogenous, wild-type *osk* mRNA through their 3'-UTR (Hachet and Ephrussi 2004). PTB, a protein required for RNP assembly, is critical for this rescue *in trans*, suggesting that RNP assembly is a critical aspect of mRNA regulation (Macdonald *et al.* 2016).

mRNA localization during spermiogenesis

Postmeiotic spermatids also appear to utilize localized mRNAs. After meiosis, 64 spermatids elongate in synchrony to generate mature sperm. During this process, all of 64 spermatids must elongate in the same direction with their heads orienting toward the basal end of the testis (toward the seminal vesicle) and their tails toward the apical end. This process of polarized sperm elongation involves membrane polarization, requiring localized plasma membrane PtdIns (Phosphatidylinositol) (4, 5) P2 lipids, the exocyst complex, and Merlin/Nf2 (Dorogova *et al.* 2008; Fabian *et al.* 2010). During this process, Orb2, a translational regulator of CPEB (cytoplasmic polyadenylation element binding protein), shows polarized localization, concentrating on the tail side of the spermatid cyst and anchoring *orb2* and *apkc* mRNAs, resulting in polarized localization of their respective protein products (Xu *et al.* 2012, 2014). A handful of genes have been identified that are transcribed postmeiotically (although the vast majority of genes required for meiosis and spermiogenesis are transcribed in SCs), and these proteins show a localization pattern similar to that of *orb2* and *apkc* mRNAs (Barreau *et al.* 2008). Although the functions of these genes are not fully understood, they may regulate various aspects of spermatid cyst polarity, and their localization and translation may be under the control of Orb2.

Nuage as a Platform for the piRNA Pathway

Nuage, meaning “cloud” in French, is an electron dense, fibrous organelle that lacks a limiting membrane. It is consistently associated with the cytoplasmic side of the nuclear envelope (Mahowald 1962, 1968, 1971b, 2001; Counce 1963; Eddy 1975). Nuage represents a type of RNP granule specific to both male and female germ cells and shares many characteristics with the RNP granules found in somatic cells, such as processing bodies (P bodies) and stress granules. Nuage derives from germ granules/polar granules earlier in development. Whereas germ granules in embryos play key roles in mRNA regulation to specify germ

cell identity, nuage in adult germ cells is mainly involved in piRNA production.

The nuage in piRNA production and amplification

It is now widely accepted that the nuage concentrates the components of the piRNA pathway, which suppresses selfish elements such as transposons (Klattenhoff and Theurkauf 2008; Ghildiyal and Zamore 2009; Siomi *et al.* 2011). In the absence of piRNA pathway function, selfish elements such as transposons are derepressed in the germline, leading to germ cell loss and thus infertility both in the male and female germline. The piRNA pathway components that have been shown to localize to the nuage include Vasa, Aub, Ago3, Tejas, Qin/Kumo, Spinde-E, Krimper, Maelstrom, PAPI, Tudor, and Vreteno (Harris and Macdonald 2001; Findley *et al.* 2003; Brennecke *et al.* 2007; Lim and Kai 2007; Klattenhoff *et al.* 2009; Li *et al.* 2009; Malone *et al.* 2009; Nishida *et al.* 2009; Patil and Kai 2010; Handler *et al.* 2011; Liu *et al.* 2011; Zhang *et al.* 2011; Anand and Kai 2012; Xioli *et al.* 2014). As Chapter 2 heavily covers the function and mechanism of the piRNA pathway in transposon silencing, genome protection, and heterochromatin regulation, this chapter will mainly focus on the nuage as an organelle and describes the cellular and molecular mechanisms of its biogenesis in relation to germ cell development.

There is no clear linear hierarchy for the recruitment of the different nuage components (Findley *et al.* 2003; Lim and Kai 2007; Patil and Kai 2010; Handler *et al.* 2011; Liu *et al.* 2011; Ryazansky *et al.* 2016). A few components, such as Vasa and Spn-E, are clearly “upstream” as many other nuage components rely on these proteins for their nuage localization. In contrast, other components, such as Maelstrom, are clearly “downstream” as their localization depends on many other components but they themselves are not required for localizing other nuage components. However, the localization “epistasis” is not a linear one, and a full understanding will require further investigation.

piRNA production starts with transcription of piRNA precursors, which initially exist as long transcripts and are processed to generate primary piRNAs of ~26–31 nucleotides. These primary piRNAs, which are antisense to the mRNAs of selfish elements, are used to guide Aub, a PIWI family endonuclease, to recognize the complementary strand (*e.g.*, the sense strand of transposon mRNAs). Aub cleaves these sense strands to destroy the mRNAs of selfish elements and simultaneously generates secondary piRNAs. These sense strand secondary piRNAs become bound to Ago3, another PIWI family endonuclease, which also recognizes and cleaves the complementary sequence to generate more piRNAs. This feed-forward amplification of piRNA production is called the “ping-pong cycle” and distinguishes the piRNA pathway from other small RNA-mediated silencing pathways, *i.e.*, the siRNA- and miRNA-mediated pathways (Brennecke *et al.* 2007; Gunawardane *et al.* 2007; Klattenhoff and Theurkauf 2008). The nuage plays a critical role in this ping-pong cycle by localizing the participating proteins. The emerging picture is that

(1) PIWI proteins (Piwi, Aub, and Ago3) are symmetrically dimethylated on their arginine residues (sDMA) by the protein methyltransferase dPRMT5 (Kirino *et al.* 2009; Vagin *et al.* 2009). (2) Tudor domain proteins bind PIWI proteins after sDMA modification (Nishida *et al.* 2009). In the absence of dPRMT5, PIWI protein expression levels are markedly reduced in the developing egg chamber (Kirino *et al.* 2009). (3) Tudor domain proteins function to anchor PIWI proteins in the nuage (Kirino *et al.* 2009, 2010; Liu *et al.* 2011). The Tudor domain proteins characterized so far include PAPI, Tudor, Spn-E, Vreteno, Krimper, and Qin. Tudor is required for Aub localization to the nuage (Kirino *et al.* 2010) and PAPI is required for Ago3 localization to the nuage (Liu *et al.* 2011). Vreteno is required for PIWI stability and localization of Ago3 and Aub to the nuage (Handler *et al.* 2011). Systematic analysis of all Tudor domain-containing proteins has revealed their roles in the piRNA pathway (Handler *et al.* 2011).

A recent study further revealed the detailed mechanism of how nuage organization is related to the piRNA pathway; Aub is recruited to nuage in a piRNA-dependent manner, whereas Ago3 localizes to nuage independent of piRNAs but dependent on Krimper, a constitutive nuage component (Webster *et al.* 2015). Krimper mediates Aub–Ago3 interaction, thereby facilitating the ping-pong cycle (Webster *et al.* 2015). In a silkworm cell culture system, a similar complex is formed between Siwi and Ago3 (the Aub–Ago3 counterpart) for ping-pong amplification (Xiol *et al.* 2014). The heterotypic ping-pong cycle between Aub and Ago3 is critically important, because homotypic Aub–Aub ping-pong (due to the lack of Ago3 or Qin, which bridges Aub and Ago3) results in an excess of sense strand piRNAs, leading to a failure to silence selfish elements (Li *et al.* 2009; Zhang *et al.* 2011).

As described above, the nuage is characterized by its juxtaposition to the nuclear envelope. Recent studies indicate the functional relevance of this association. It was shown that Rhino (Rhi), an HP1 homolog specifically expressed in the germline, binds to piRNA clusters in the pericentromeric heterochromatin of the autosomes and regulates their transcription (Klattenhoff *et al.* 2009). Aub and Ago3 fail to localize to the nuage in *rhi* mutants, suggesting that piRNA precursor transcription is essential for nuage formation (Klattenhoff *et al.* 2009). Interestingly, Rhi and UAP56, a DEAD box RNA-binding protein implicated in RNA export, colocalize underneath the nuclear envelope, right across from the nuage. It appears that piRNA precursors that are transcribed in a Rhi-dependent manner are exported by UAP56 across the nuclear pore directly into the nuage, where they are processed to become primary piRNAs (Zhang *et al.* 2012). These results indicate that piRNA production, mediated by the coordinated action of pericentromeric heterochromatin, transcription of piRNA precursors from heterochromatin, nuclear export, and piRNA production, are spatially organized in germ cells.

A few components of the piRNA pathway highlight the relationship between nuage and mitochondria. Armitage, an RNA helicase required for primary piRNA production, shuttles between nuage and mitochondria together with Ago3.

Zucchini, likely the endonuclease required to generate primary piRNAs (Haase *et al.* 2010; Ipsaro *et al.* 2012; Nishimasu *et al.* 2012; Han *et al.* 2015; Mohn *et al.* 2015), is localized to the mitochondria (Huang *et al.* 2014). Why these components have to travel back and forth between the nuage and mitochondria is unknown. A proposed function of Zucchini and its mouse homolog mitoPLD phospholipase is to generate the signaling lipid phosphatidic acid, which might be required for activating nuage components (Watanabe *et al.* 2011; Nishimasu *et al.* 2012). Throughout an organism's life, nuage and related RNP granules are often associated with mitochondria, suggesting a functional significance for this association.

piNG body in SCs

In the *Drosophila* male germline, a main target of the piRNA pathway is a repetitive element called *stellate* (*ste*). *ste* is located on the X chromosome, and is repressed by the piRNA pathway through expression of an antisense piRNA cluster, encoded by *suppressor of stellate/su(ste)* on the Y chromosome (see Chapter 2 for details) (Livak 1990; Aravin *et al.* 2001, 2004). *ste* encodes a polypeptide that resembles the β subunit of casein kinase 2 (Bozzetti *et al.* 1995). *ste* overexpression results in the formation of crystals (protein aggregates) in germ cells, leading to a reduction in male fertility (Palumbo *et al.* 1994; Bozzetti *et al.* 1995). *ste* mRNA is targeted for degradation by the piRNA pathway (Nishida *et al.* 2007; Nagao *et al.* 2010). It is unknown whether Ste protein serves any biological function when expressed moderately, although it has been speculated that *ste* may cause meiotic drive, a phenomenon that causes the transmission of certain genetic elements in a distorted (non-Mendelian) ratio. However, definitive evidence for this is still lacking (Hurst 1992, 1996; Belloni *et al.* 2002).

In the male germline, nuage is associated with the nuclear envelope, as in the female germline. Primary SCs develop a considerably larger aggregation of nuage, called the piNG body (piRNA nuage giant body), which measures $> 2 \mu\text{m}$ in diameter (estimated to be ~ 50 times larger in volume than the regular nuage particles observed in GSCs and SGs) (Kibanov *et al.* 2011). The piNG body contains most nuage components, including Aub, Vasa, Ago3, Tud, and Spn-E, as well as the miRNA pathway component Ago1 (Kibanov *et al.* 2011). The piNG body develops in primary SCs, right about the time when *ste* mRNA is expressed, and conditions that perturb piNG body formation are associated with *ste* derepression (Kibanov *et al.* 2011). However, it remains unknown whether piNG body formation is essential for the function of the piRNA pathway.

Mitochondrial Differentiation During Germ Cell Differentiation

Mitochondria, often called the powerhouse of a cell, are responsible for the production of ATP and are thus essential in almost any cell type. As mitochondria are inherited exclusively from the mother and mitochondrial defects cause a plethora of pathologies, oogenesis must employ specialized

mechanisms that ensure the inheritance of high-quality mitochondria in sufficient quantities. On the other hand, although sperm are not responsible for mitochondrial quality/quantity in the next generation, sperm motility, and thus fertility, requires a large amount of energy, and therefore mitochondria of high quality and quantity. Accordingly, spermatogenesis also has a specialized program to reorganize the mitochondria best suited for ATP production.

Mitochondrial behavior in female germline

Mitochondria exhibit dynamic changes in their morphology and behavior during oogenesis. Mitochondrial distribution is equal, at least in quantity, in mitotic female GSCs (Cox and Spradling 2003). As cystocytes progress through the cyst-forming mitotic divisions in region 1 of the germarium, mitochondrial ATP synthase is required for them to differentiate beyond the four-cell stage (Teixeira *et al.* 2015) (Figure 5). Strikingly, this is independent of the ATP synthesizing function of ATP synthase. Instead, ATP synthase is required for mitochondrial crista maturation (increased lamellar formation within mitochondria) via dimerization to promote germ cell differentiation.

As germ cell cysts enter regions 2b and 3 of the germarium following the mitotic divisions, mitochondria undergo DNA replication (Hill *et al.* 2014). Mitochondrial DNA (mtDNA) replication is not observed at a high level in earlier stages. mtDNA replication depends on the functionality of the mitochondria, and mitochondria carrying a function-compromising mutation are selected out during this stage (Hill *et al.* 2014). Moreover, mtDNA replication occurs in mitochondria that aggregate near the fusome, and the fusome is required for mtDNA replication and mitochondrial movement into the oocyte. Nonfunctional mitochondria do not move near the fusome or replicate their mtDNA, highlighting the importance of the fusome in mitochondrial quality control (Cox and Spradling 2003; Hill *et al.* 2014). The mitochondria aggregated near the fusome enter the oocyte first in region 3 of the germarium to form the Balbiani body (Cox and Spradling 2003), although the remaining mitochondria also move into the oocyte later during nurse cell dumping (Hurd *et al.* 2016) (see below). However, the causal relationship between mtDNA replication, mitochondrial association with the fusome, mitochondrial movement into the oocyte, and mitochondrial functionality remains unknown.

Once these mitochondria move into the oocyte, they form the Balbiani body. The Balbiani body, observed in the oocytes of a wide range of organisms from insects to vertebrates (Kloc *et al.* 2004, 2014; Lei and Spradling 2016), is a large cytoplasmic aggregate of mitochondria, Golgi, ER, nuage, and other organelles, as well as certain mRNAs. The *Drosophila* Balbiani body also contains Golgi, ER, the centriole cluster, fusome material, and associated mRNAs (*e.g.*, *osk* and *orb* RNAs) (Cox and Spradling 2003). The Balbiani body is located on the posterior side of the oocyte during stage 6, but as MTs reorganize in the stage 7 egg chamber, Balbiani body-associated mitochondria disperse throughout the cytoplasm. Subsequently, as the egg chamber reaches stage 10b/11, a large number of mitochondria are transported from the nurse cells to the oocyte during nurse cell

dumping. MTs and kinesins are required for normal Balbiani body formation (Cox and Spradling 2006). Milton, a specific adaptor protein that connects mitochondria to kinesin motor proteins (Stowers *et al.* 2002), plays a critical role in forming the Balbiani body. Interestingly, when Balbiani body formation (mitochondrial association to the Balbiani body) was severely perturbed by mutation in the Milton gene, these oocytes still produced viable and fertile offspring (Cox and Spradling 2006). This suggests that Balbiani body-mediated mitochondrial inheritance does not have an immediate impact on oocyte's developmental potential.

A recent study showed that Long Osk, which is localized at the posterior cortex of oocytes, anchors mitochondria to the posterior of the oocyte through binding to the actin cytoskeleton (Hurd *et al.* 2016). This Osk-dependent trapping and enrichment of mitochondria occurs in the second phase of mitochondrial transport (*i.e.*, during nurse cell dumping), and enables PGCs to inherit a large number of mitochondria. In the absence of Long Osk, PGCs incorporate significantly fewer mitochondria, leading to formation of fewer PGSs and, ultimately, reduced fertility (Hurd *et al.* 2016). This indicates that Long Osk-dependent mitochondria anchoring is the major mechanism for mitochondrial inheritance to the next generation, and may explain the dispensability of Milton-dependent transport of mitochondria into oocytes during Balbiani body formation (Cox and Spradling 2006). During ooplasmic streaming, both mitochondrial populations, Balbiani body-associated mitochondria that entered the oocyte around stage 1 and those that entered the oocyte during nurse cell dumping, are likely mixed inside the ooplasm. This makes it unclear as to why a subset of mitochondria must enter the oocyte earlier (during Balbiani body formation) than others (during late oogenesis). However, it is possible that Long Osk traps a specific subset of mitochondria during ooplasmic streaming.

Mitochondrial specialization in spermatogenesis

During spermatogenesis, mitochondria play various roles, like in the female germline, and undergo dramatic reorganization to support the large capacity of ATP production required for sperm motility while preparing for uni-parental mitochondrial inheritance (*i.e.*, depletion of paternal mitochondria in the zygote).

First, during SG divisions, mitochondria play an important role in germ cell death (Yacobi-Sharon *et al.* 2013). A large amount of germ cells are eliminated before meiotic entry in *Drosophila* as well as in mammals (Allan *et al.* 1992). This germ cell death is distinct from apoptosis or necrosis, displaying a mixture of characteristics of both modes of cell death. Mitochondrial-associated proteins, such as the mitochondrial protease HtrA2/Omi, the mitochondrial serine/threonine protein kinase Pink1, Bcl2-related proteins, and endonuclease G, are required for germ cell death (Yacobi-Sharon *et al.* 2013).

During meiosis, mitochondria are segregated equally along the meiotic spindle. Right after the meiotic divisions, mitochondria start dramatically reorganizing by first aggregating

Figure 5 Mitochondrial behavior during oogenesis. During cystocyte divisions, cristae maturation, regulated by ATP synthase independent of its ability to synthesize ATP, is critical for differentiation of germ cells. After the formation of the 16-cell cyst, mitochondria are associated with the fusome (blue), and later they are transported into the oocyte, forming the Balbiani body. At this point, a subset of mitochondria remain in the nurse cells. During late oogenesis, the remaining mitochondria are transported into the oocyte during nurse cell dumping. Mitochondria are entrapped at the posterior cortex by the actin cytoskeleton, which is organized by Long Osk protein. These posteriorly-localized mitochondria will be passed on to the next generation (grandchild generation) by being incorporated by the pole cells of the embryos. GSC, germline stem cell.

and then fusing to generate two “mitochondrial derivatives,” which form a specialized mitochondrial structure called the Nebenkern [detailed in Chapter 6 and reviewed in Fabian and Brill (2012)]. Mitochondrial fusion is mediated by fuzzy onions (fzo), a founding member of the mitofusin class of proteins (Hales and Fuller 1997). Then, the Nebenkern elongates along the axis of the sperm tail, driving spermatid elongation (Noguchi *et al.* 2011).

Along with such drastic changes in morphology, growing spermatids also prepare for uni-parental transmission of mitochondria. The sole donor of mitochondria to the next generation is the mother, and thus no paternal mitochondria will be transmitted to the next generation. Despite the fact that mitochondria persist throughout spermatogenesis and become a major component of the mature sperm, providing energy for motility, their DNA is removed during spermiogenesis and mature sperm do not contain any mtDNA. Toward the end of sperm tail elongation, mtDNA is abruptly degraded by the activity of endonuclease G (DeLuca and O’Farrell 2012). This mechanism is further complemented by the removal of residual mtDNA during the process of individualization. As a result, even in the absence of endonuclease G, the mature sperm are still devoid of mtDNA (DeLuca and O’Farrell 2012). Moreover, right after fertilization, sperm-derived mitochondria are actively removed from the zygote via maternally provided components that resemble both autophagy and the endocytic pathways (Politi *et al.* 2014). These studies suggest that the uni-parental inheritance of mitochondria is a genetically-regulated, active mechanism.

Centriole Specialization During Late Gametogenesis

After the mitotic divisions as cystocytes or SGs, where centrosomes play a key role in spindle orientation, centrosomes undergo diverged specialization programs in the male and female germlines. Whereas centrosomes are eliminated in the

female germline before meiosis, the male germline prepares the first set of centrioles for the next generation by remodeling spermatid centrosomes.

Unique centrosome behavior during oogenesis

As described above, by the end of the mitotic divisions in the germarium, germ cell cysts contain a polarized MT network, which is organized by active centrosomes in the oocyte. In parallel, the centrosomes in the nurse cells are inactivated, leading to MT polarity with the minus ends residing within the oocyte and the plus ends extending into the nurse cells. Inactive centrosomes within the nurse cells move into the oocyte by the time the cyst reaches region 3 of the germarium (Koch and King 1969; Mahowald and Strassheim 1970). The underlying mechanism that maintains MTOC activity only on the oocyte centrosomes, while keeping the nurse cell centrosomes inactive, remains unknown. Interestingly, despite the essential role of MT organization in oocyte development and the clear role for centrosomes as MTOCs, oocytes are correctly specified and polarized following the complete loss of the centrioles (Stevens *et al.* 2007), indicating that centrosomes are not essential for oocyte determination or development, perhaps due to the presence of redundantly functioning parallel mechanism(s). Indeed, a more recent work suggested that acentrosomal MTOCs can form, at least for the process of nuclear migration in the oocyte (Zhao *et al.* 2012), explaining why the lack of centrioles may not have a drastic outcome.

At the beginning of midoogenesis (around stage 7), the oocyte nucleus migrates to the anterior corner of the oocyte in a MT-dependent manner, determining the future anterior–dorsal position of the embryo (Koch and Spitzer 1983). During oocyte nuclear migration, the active oocyte centrosomes are located at the posterior of the oocyte nucleus, and the growing MTs from the centrosome push the nucleus toward the anterior side of the oocyte (Zhao *et al.* 2012). After migration of the nucleus to the anterior–dorsal corner, the nucleus is anchored

in a dynein-dependent manner, as mutations in dynein and its associated proteins (Lis1 and Bic-D) result in mislocalization (falling off) of the oocyte nucleus after migration (Swan and Suter 1996; Swan *et al.* 1999; Lei and Warrior 2000; Duncan and Warrior 2002; Januschke *et al.* 2002; Zhao *et al.* 2012).

The oocyte eliminates its centrosomes during late oogenesis (stage 12/13), after the centrosomes have fulfilled their critical roles in organizing cyst polarity and oocyte axis formation. In most organisms studied to date, including *Drosophila*, female meiosis proceeds without centrosomes. The meiotic divisions in oocytes are acentrosomal (McKim and Hawley 1995), and chromosome segregation is achieved by noncentrosomal bundling of spindle MTs mediated by Msp and D-TACC, MT-regulating proteins (Cullen and Ohkura 2001). Acentrosomal meiosis, and therefore the lack of centrosomes in eggs, is speculated to be a mechanism to prevent the parthenogenic development of embryos. The very first centrosome in a newly fertilized zygote is provided by the sperm in the form of the basal body of the sperm axoneme (see below). When centrosome elimination is perturbed in oocytes due to artificially sustained Polo kinase activity, the extra centrosomes interfere with the zygotic divisions and lead to embryonic lethality, demonstrating the importance of centrosome elimination during oogenesis (Pimenta-Marques *et al.* 2016).

Centrosome specialization during spermatogenesis

In many species including *Drosophila*, only the mother donates mitochondria to the next generation and only the father donates centriole(s) to the next generation. The sperm-derived centrioles potentiate the zygote to undergo the first mitosis, by allowing the formation of the first spindle, which pulls the oocyte pronucleus toward the paternal pronucleus to join them into a single nucleus (Loppin *et al.* 2015). In some species, such as *C. elegans* and *Xenopus laevis*, the sperm carries a pair of centrioles, each of which become a centrosome after a single round of centrosome duplication in the zygote. These centrosomes serve as spindle poles to support the first zygotic division. On the contrary, *Drosophila* and human sperm appear to carry only a single centriole and potentially require an extra round of centriole duplication (Delattre and Gonczy 2004). However, it was recently shown that *Drosophila* sperm contains a proximal centriole-like (PCL) in addition to a centriole [called the “giant centriole” (GC)], which serves as the sperm basal body. PCL lacks the typical centriolar structure, but contains core centriolar proteins such as Asterless and Ana1, and closely associates with the GC (Blachon *et al.* 2009). During spermiogenesis, both the GC and the PCL undergo “centrosome reduction,” during which most centrosomal components are stripped off (Blachon *et al.* 2014). Centrosome reduction is a widespread phenomenon observed in a wide range of animals (Schatten 1994). In parallel with centrosome reduction, certain centriolar components, such as Poc1, become enriched on the PCL (Khire *et al.* 2016). Upon fertilization, the GC and PCL each immediately recruit maternally provided centrosomal proteins and serve as the two spindle poles for the first zygotic division

(Blachon *et al.* 2009, 2014). Centriole remodeling during spermiogenesis appears to be critical, as failure in centriole reduction and PCL reorganization results in reduced zygotic viability (Khire *et al.* 2015, 2016).

Y-Loop Lampbrush Chromosomes in Spermatocytes

A prevalent feature of SC development is the formation of the Y-loops, cytological manifestations of robust Y chromosome-associated gene transcription that form in the nucleoplasm of SCs (Bonaccorsi *et al.* 1988). In *Drosophila*, the Y chromosome is not required for male sex determination but is essential for male fertility. Nearly the entire 40 Mb of Y chromosome is heterochromatic, and it harbors only several genes (Carvalho *et al.* 2000, 2001; Vrbancin *et al.* 2008). The structure of the Y-loops has been heavily studied using *D. hydei* due to the prominence of the Y-loop structures, which enable cytological characterization (Kurek *et al.* 2000). Many *Drosophila* species have Y-loops, suggesting evolutionary conservation (Reugels *et al.* 2000; Piergentili 2007). Although less prominent than those in *D. hydei*, *D. melanogaster* SCs also develop Y-loops.

In *D. melanogaster*, Y-loops are formed by the transcription of three Y chromosome genes, *kl-5* (loop A), *kl-3* (loop B), and *ks-1* (loop C), with the three loops being spatially separated in the nucleoplasm (Bonaccorsi *et al.* 1988). *kl-5* and *kl-3* encode axonemal dynein heavy chains, and *ks-1* encodes ORY, a homolog of occludin (a tight junction component) (Carvalho *et al.* 2000, 2001). These and a few other Y chromosome genes possess an unusual gene structure: they have gigantic, satellite DNA-containing introns, resulting in gene sizes of ~4 Mb. However, the coding sequences of these genes are only ~3–15 kb, meaning that these genes get their size due to massive stretches of highly repetitive DNA in their introns (Kurek *et al.* 2000). These multi-megabase genes appear to be transcribed as a single unit containing all exons and introns (de Loos *et al.* 1984), suggesting that the transcription of these Y-loop genes likely takes the entire SC development stage (80–90 hr) to complete.

The Y-loops appear to be highly structured. RNA transcripts are associated with the core DNA/chromatin, which runs through the nucleoplasm, reminiscent of lampbrush chromosomes. RNA-binding proteins bind this lampbrush structure. A handful of Y-loop-binding proteins have been identified, the collection of which indicates a connection between Y-loops and RNA processing. For example, Boule, Pasilla, and RB97D specifically bind to loop C (Heatwole and Haynes 1996; Cheng *et al.* 1998; Redhouse *et al.* 2011). Boule, a member of the DAZL [deleted in azoospermia (DAZ)-like] family of RNA binding proteins, is required for meiotic progression in SCs (Cheng *et al.* 1998), and RB97D, a member of the RNA recognition motif family of RNA-binding proteins, is required for male fertility (Karsch-Mizrachi and Haynes 1993; Heatwole and Haynes 1996). A minor hnRNA-associated protein (recognized by antibody S5) localizes to loop A (Risau *et al.* 1983; Bonaccorsi *et al.* 1988). In addition, two other antibodies with unknown antigens have been identified to specifically bind to loop B or loops A/C (Bonaccorsi *et al.* 1988; Pisano *et al.* 1993).

The distinct localization of different sets of proteins to loops A, B, and/or C likely reflects that those Y-loop-binding proteins recognize specific sequences, such as satellite DNA transcripts. However, it remains unclear why Y-loops have to form in SCs. They might play a critical role in the expression of their corresponding gene. Alternatively, it has been suggested that the lampbrush-like structure itself may function as a platform for localizing proteins that are required in later stages of spermatogenesis (Hulsebos *et al.* 1984); a few proteins have been reported to localize to the Y-loops, which subsequently localize to the sperm tails. Androcam, a testis-specific calmodulin protein that is required for sperm motility, was shown to be expressed in SCs and localize to the Y-loop (loop B), then localize to elongating sperm tails (Lu and Beckingham 2000), where it functions as a light chain of Myosin VI to promote sperm individualization (Frank *et al.* 2006). Also, Boule, which binds to loop C, later translocates to the cytoplasm where it regulates the translation of Twine, the meiosis-specific Cdc25 phosphatase (Maines and Wasserman 1999), leading to the idea that the Y-loops may function to sequester proteins until they are needed.

A genetic screen was conducted to identify male sterile genes that affect Y-loop morphogenesis (Ceprani *et al.* 2008). Characterizing these mutants and genes may provide further insights into the role of Y-loops. Although Y-loops are only found in the genus *Drosophila*, some Y-loop proteins, such as Boule, have a clear functional homolog in mammals. Boule is a homolog of human DAZ and DAZL, and mutations in these genes display a strikingly similar meiotic arrest phenotypes in these two species (Reijo *et al.* 1995, 1996, 2000). Moreover, human *boule* can rescue the meiotic arrest phenotype of *Drosophila boule* mutants (Xu *et al.* 2003), suggesting functional conservation between these distant species.

Summary and Concluding Remarks

Gametogenesis is a goldmine of cell biology, exhibiting dramatic changes in the morphology and behavior of organelles and the cytoskeleton and telling us what cells can do. Through reviewing the processes of gametogenesis, I have summarized complex behaviors of organelles and the cytoskeleton in determining cell fates and polarizing cells.

Germ cells are the only cell type that is passed from one generation to the next, and their secret of immortality must lie somewhere within the processes of gametogenesis. We are still left wondering what constitutes the essence of immortality, and whether the unique cell biological features of gametogenesis may hold the key. Building upon what we have learned so far, future investigation may finally reveal the secret of germ cells' immortality.

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