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Polarity in Stem Cell Division: Asymmetric Stem Cell Division in Tissue Homeostasis

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Many adult stem cells divide asymmetrically to balance self-renewal and differentiation, thereby maintaining tissue homeostasis. Asymmetric stem cell divisions depend on asymmetric cell architecture (i.e., cell polarity) within the cell and/or the cellular environment. In particular, as residents of the tissues they sustain, stem cells are inevitably placed in the context of the tissue architecture. Indeed, many stem cells are polarized within their microenvironment, or the stem cell niche, and their asymmetric division relies on their relationship with the microenvironment. Here, we review asymmetric stem cell divisions in the context of the stem cell niche with a focus on *Drosophila* germ line stem cells, where the nature of niche-dependent asymmetric stem cell division is well characterized.

Asymmetric cell division allows stem cells to self-renew and produce another cell that undergoes differentiation, thus providing a simple method for tissue homeostasis. Stem cell self-renewal refers to the daughter(s) of stem cell division maintaining all stem cell characteristics, including proliferation capacity, maintenance of the undifferentiated state, and the capability to produce daughter cells that undergo differentiation. A failure to maintain the correct stem cell number has been speculated to lead to tumorigenesis/tissue hyperplasia via stem cell hyperproliferation or tissue degeneration/aging via a reduction in stem cell number or activity (Morrison and Kimble 2006; Rando 2006). This necessity changes during development. The stem cell pool requires expansion earlier in development, whereas maintenance is needed later to sustain tissue homeostasis.

There are two major mechanisms to sustain a fixed number of adult stem cells: stem cell niche and asymmetric stem cell division, which are not mutually exclusive. Stem cell niche is a microenvironment in which stem cells reside, and provides essential signals required for stem cell identity (Fig. 1A). Physical limitation of niche "space" can therefore define stem cell number within a tissue. Within such a niche, many stem cells divide asymmetrically, giving rise to one stem cell and one differentiating cell, by placing

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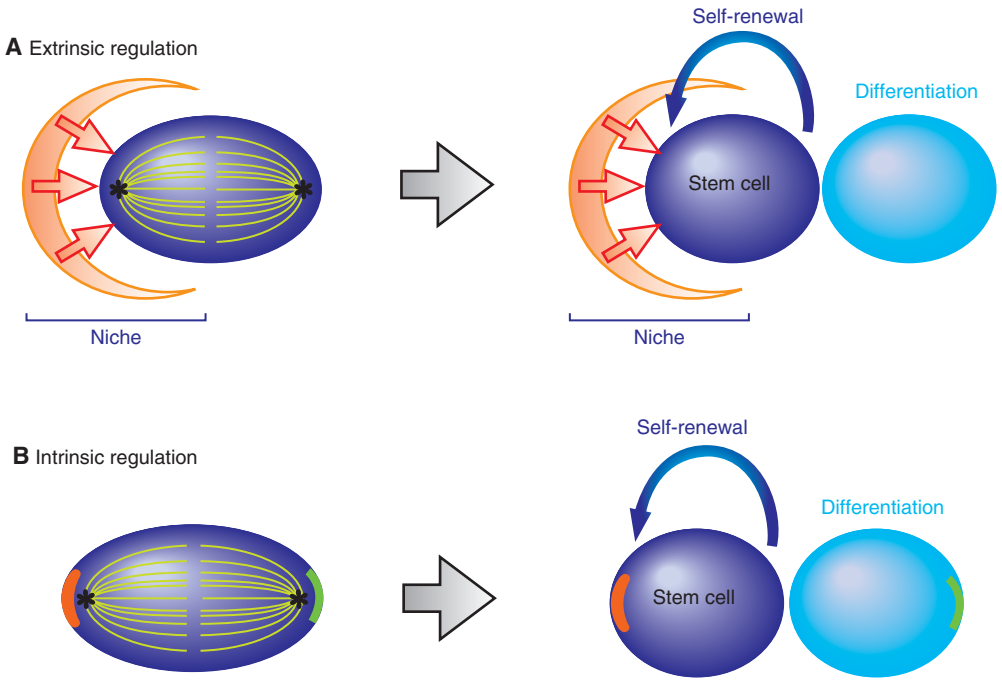

Figure 1. Mechanisms of asymmetric stem cell division. (*A*) Asymmetric stem cell division by extrinsic fate determinants (i.e., the stem cell niche). The two daughters of stem cell division will be placed in distinct cellular environments either inside or outside the stem cell niche, leading to asymmetric fate choice. (*B*) Asymmetric stem cell division by intrinsic fate determinants. Fate determinants are polarized in the dividing stem cells, which are subsequently partitioned into two daughter cells unequally, thus making the division asymmetrical. Self-renewing (red line) and/or differentiation promoting (green line) factors may be involved.

one daughter inside and another outside of the niche, respectively (Fig. 1A). Nevertheless, some stem cells divide asymmetrically, apparently without the niche. For example, in *Drosophila* neuroblasts, cell-intrinsic fate determinants are polarized within a dividing cell, and subsequent partitioning of such fate determinants into daughter cells in an asymmetric manner results in asymmetric stem cell division (Fig. 1B) (see Fig. 3A and Prehoda 2009).

In this review, we focus primarily on asymmetric stem cell divisions in the *Drosophila* germ line as the most intensively studied examples of niche-dependent asymmetric stem cell division. We also discuss some examples of stem cell division outside *Drosophila*, where stem cells are known to divide asymmetrically or in a niche-dependent manner.

DROSOPHILA MALE GERM LINE STEM CELLS

Signaling in the Male Germ Line Stem Cell (GSC) Niche

The *Drosophila* male and female germ lines have served as ideal model systems for studying the regulation of stem cell behavior and asymmetric stem cell division controlled by the microenvironment or stem cell niche. *Drosophila* male germ line stem cells (GSCs) reside in a stem cell niche whose major components are hub cells and cyst stem cells (CySCs; historically called cyst progenitor cells) (Fig. 2A). Hub cells are generally thought to be postmitotic, attached to the apical wall of the testis, and consist of 8–16 somatically derived cells (Hardy et al. 1979). These hub cells are surrounded by 7–12 GSCs, such that each one is physically attached to the hub and encapsulated

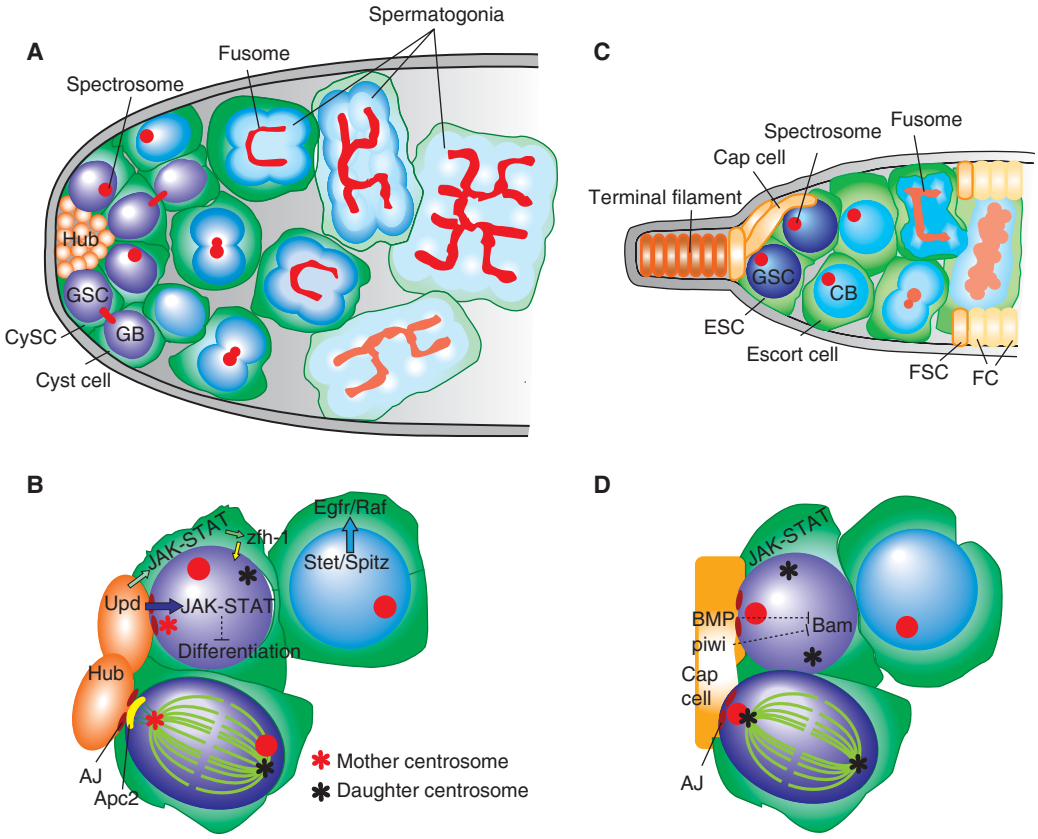

Figure 2. The anatomy of the *Drosophila* germ line stem cell (GSC) niche and asymmetric stem cell division. (*A*) Male GSC niche: GSCs and cyst stem cells (CySCs) are attached to hub cells via adherens junctions (AJs). GSCs divide asymmetrically to self-renew and produce a gonialblast (GB) that initiates the differentiation program. The GB further undergoes four synchronous, transit-amplifying divisions to yield 16 spermatogonia interconnected by the fusome. The spectrosome is a spherical version of the fusome observed in GSCs. A pair of CySCs encapsulates the GSCs and provides signals required for GSC identity. Similar to GSCs, CySCs divide asymmetrically to self-renew and produce cyst cells. Cyst cells exit the cell cycle, a pair of which encapsulates the GB and spermatogonia to promote differentiation. (*B*) Asymmetric stem cell division of male GSC by centrosome orientation: Upd ligand is secreted from hub cells to activate the JAK-STAT pathway in GSCs and CySCs to specify their stem cell identity. The Zfh-1 transcription factor controls CySC identity. Together with hub cells, CySCs dictate GSC identity. The mitotic spindle is oriented toward the hub cells via positioning of the centrosome. Spectrosomes in male GSCs are not oriented with respect to the hub cells during interphase. EGFR signaling ensures the encapsulation of germ cells by cyst cells. (*C*) Female GSC niche: GSCs are attached to the cap cells via adherens junctions. GSCs divide asymmetrically to self-renew and produce a cystoblast (CB) that initiates differentiation. The CB divides four times to give rise to 16 germ cells interconnected by the fusome, only one of which becomes an oocyte, whereas the remaining 15 cells become nurse cells. Escort stem cells (ESCs) encapsulate the GSC, while their daughters, escort cells, encapsulate the developing germ cells. Escort cells are later replaced by follicle cells, which are daughters of follicle stem cells (FSCs). (*D*) Asymmetric stem cell division of female GSC by the spectrosome: BMP signaling and Piwi controls GSC identity via niche-GSC interaction. The mitotic spindle is oriented toward the cap cells via anchoring of one spindle pole to the spectrosome, which localizes consistently to the apical side of GSCs. (Figures are adapted and modified from Fuller and Spradling 2007.)

by a pair of CySCs, which also maintain contact with the hub via cell processes. Male GSC division produces one GSC and one gonialblast, which then undergoes transit-amplifying divisions as spermatogonia (Fig. 2A). Spermatogonia become spermatocytes, which commit to meiosis and further differentiate into spermatids. Similar to GSCs, CySCs also divide asymmetrically, producing one CySC and one cyst cell. A pair of CySCs encapsulates a GSC, whereas a pair of cyst cells encapsulates developing gonialblast, spermatogonia, and spermatocytes. Cyst cells that encapsulate these developing germ cells do not divide, and differentiating germ cells undergo transit-amplification as well as further development (i.e., meiosis and spermiogenesis) within the cyst encapsulated by a pair of cyst cells.

The interaction between germ cells and somatic cells is important for their normal functions. Self-renewal of GSCs and CySCs is specified by the JAK-STAT signaling pathway, which is activated by the ligand Unpaired (Upd) following secretion by hub cells (Fig. 2B) (Kiger et al. 2001; Tulina and Matunis 2001; Leatherman and Dinardo 2008). Consistently, overexpression of Upd in germ cells (GSCs and spermatogonia) or somatic cells (CySCs and cyst cells) hinders proper differentiation and results in tumor-like accumulation of undifferentiated, stem-like cells, whereas mutants of *JAK* or *STAT* lead to a rapid loss of GSCs and CySCs in a cell-autonomous manner (Kiger et al. 2001; Tulina and Matunis 2001; Leatherman and Dinardo 2008). In addition, BMP signaling, which plays an important role in female GSC identity (see the following discussion), is implicated in stem cell self-renewal and/or proliferation of spermatogonia (Shivdasani and Ingham 2003; Kawase et al. 2004; Schulz et al. 2004), suggesting a conserved mechanism of stem cell self-renewal/differentiation in both sexes. Because Upd acts within a short range (Harrison et al. 1998), only cells closely attached to the hub maintain their stem cell identity, and those displaced away from the hub initiate differentiation. Indeed, it has been demonstrated that cell adhesion between GSCs and hub cells, as well as between CySCs and hub cells, is required for stem cell maintenance, presumably by keeping cells together to take advantage of short-range self-renewal signals. The majority of adherens junctions in GSCs are clumped at the hub-GSC interface. These include the *Drosophila* epithelial cadherin (DE-cadherin) and the β-catenin homolog Armadillo (Yamashita et al. 2003). Consistently, loss-of-function mutant clones of *shotgun*, which encodes DE-cadherin, result in a rapid loss of GSCs and CySCs in a cell autonomous manner, suggesting a role for DE-cadherin in stem cell maintenance (Voog et al. 2008). As described in more detail in the following discussion, GSC mitotic spindle is oriented toward the hub-GSC interface, playing a key role in determining daughter cell positions with respect to the niche, and thus their fates.

In addition to regulatory signals from hub cells, the soma–germ communication between GSCs and CySCs (and/or spermatogonia and cyst cells) also plays an important role in specifying the fate of a germ cell (i.e., stem cell self-renewal vs. commitment to differentiation) (Fig. 2B). Loss-of-function mutations in the epidermal growth factor receptor (EGFR) or its downstream mediator Raf in cyst cells leads to hyperproliferation of cells with GSC or gonialblast characteristics (Kiger et al. 2000; Tran et al. 2000). Furthermore, mutation in germ cells of either *stet*, a component of the EGFR signaling pathway, or *spitz*, an EGFR ligand, leads to a failure in the encapsulation of germ cells by cyst cells, causing defects in germ cell differentiation (Schulz et al. 2002; Sarkar et al. 2007). EGFR signaling appears to control germ cell–somatic cell (CySCs or cyst cells) attachment by modulating a balance between the Rac1 and Rho1 small GTPases. Strikingly, Sakar et al. demonstrated that overexpression of a dominant negative form of Rho1 in somatic cells rescues the *spitz* mutant phenotype, allowing somatic cells to encapsulate germ cells successfully (Sarkar et al. 2007). This implies that EGFR signaling regulates germ cell identity primarily by controlling cell–cell interactions. Moreover, these results indicate that EGFR signaling does not provide

the self-renewal signal per se, but allows other self-renewal signaling to be transduced by placing germ and soma in close contact.

It was recently discovered that CySCs play an instructive and dominant role in GSC specification. Although overexpression of an active form of JAK kinase in the germ line does not cause GSC or CySC tumors, such expression in cyst cells results in tumorigenesis (Leatherman and Dinardo 2008). Zfh-1, a transcription factor whose expression is restricted to CySCs and quickly down-regulated in cyst cells, was identified as a downstream target of the JAK-STAT pathway. Indeed, overexpression of Zfh-1 in cyst cells leads to the hyperproliferation of CySCs, which in turn results in hyperproliferation of GSCs (Leatherman and Dinardo 2008). Taken together, these data demonstrate that CySCs play an instructive role in specifying GSC identity to the germ cells they encapsulate. These studies illuminate the complexity of cell–cell interactions in cell fate determination, in particular the interaction between two stem cell populations.

Asymmetric Stem Cell Division in the Niche: Spindle Orientation by the Centrosome

Theoretically, the limited availability in space provided by the short-range self-renewal signals that specify stem cell identity should be sufficient to sustain a fixed number of stem cells without depletion or hyperproliferation. However, GSCs appear to have adopted additional internal machinery to tightly regulate the balance between their self-renewal and differentiation.

The mitotic spindle is the macromolecular machinery involved in segregating chromosomes into two daughter cells. The spindle orientation determines the cleavage plane orientation (reviewed by Eggert et al. 2006), and thus delimits the spatial arrangement of the two daughter cells. The centrosome is an essential component of the cellular machinery that directs mitotic spindle formation and determines its orientation (reviewed by Kellogg et al. 1994). The asymmetric behavior of centrosomes plays a critical role in proper asymmetric stem cell division in *Drosophila* GSCs (Yamashita et al. 2003; Yamashita et al. 2007) and neuroblasts (Rebollo et al. 2007; Rusan and Peifer 2007).

In male *Drosophila* GSCs, the mitotic spindle is oriented perpendicular to the hub-GSC interface, leading to asymmetric division with the daughter cells inside and outside the niche (Fig. 2B) (Yamashita et al. 2003; Yamashita et al. 2007). Specifically, the mother centrosome is located near the apical cortical region adjacent to the hub cells. The newly duplicated daughter centrosome moves toward the distal end by the onset of mitosis. The mother centrosome may be captured and remains anchored by interphase astral microtubules via adherens junctions such as DE-cadherin and Armadillo formed at the hub-GSC interface. The unusually early centrosome separation in male GSCs, which occurs right after centrosome duplication, rather than at the G2/M transition, suggests that GSCs may be exploiting the difference in microtubule-anchoring activity between mother and daughter centrosomes before the daughter centrosome matures to anchor microtubules. Consistent with the idea that the mother centrosome is anchored to the hub-GSC interface by microtubules, loss-of-function mutants of Centrosomin (Cnn) exhibit dysfunctional centrosome orientation and randomization of the mother–daughter choice. Cnn is an integral component of the pericentriolar material (PCM) and is required for anchoring many centrosomal components onto centrosomes (Megraw et al. 1999; Vaizel-Ohayon and Schejter 1999; Megraw et al. 2001; Megraw et al. 2002). Indeed, electron microscopy has revealed that the mother centrosome tends to have more interphase astral microtubules, some of which span from the PCM area to adherens junction (Yamashita et al. 2007). In addition, homologs of adenomatous polyposis coli (Apc1 or Apc2) are required for centrosome orientation in GSCs (Yamashita et al. 2003). Apc proteins associate with microtubule plus ends and the actin cytoskeleton, and play important roles in cellular adhesion (McCartney et al. 1999; Yu et al. 1999; McCartney et al. 2001;

Akong et al. 2002a; Akong et al. 2002b; Hamada and Bienz 2002). In GSCs, Apc1 colocalizes with spindle poles and Apc2 localizes to the hub-GSC interface, the latter of which may bind both microtubules and the adherens junction component β-catenin, thus linking the centrosome and adherens junction (Yamashita et al. 2003). It is currently unknown if these Apc genes are required for GSC self-renewal or not.

It should be noted that the asymmetry of GSC division solely relies on the asymmetric microenvironment (i.e., inside vs. outside of the niche-signaling range), because germ line cells that initiated differentiation (spermatogonia in testis and cystocytes in ovary) can revert or "dedifferentiate" into GSCs once they are brought back to the stem cell niche (Brawley and Matunis 2004; Kai and Spradling 2004). Once established during development, this asymmetric microenvironment sustains tissue homeostasis.

How such tissue architecture is established during development is a fascinating but less understood process. In *Drosophila* male germ line, this process appears to occur during embryonic development when primordial germ cells (PGCs, precursors of GSCs) and the somatic gonadal precursors (SGPs, precursors of hub, CySC, and cyst cells) coalesce (DeFalco et al. 2003; Wawersik et al. 2005). PGCs that happen to be juxtaposed to the SGP/hub cells acquire GSC identity, whereas other PGCs directly undergo differentiation. Concomitantly, cell–cell contacts (presumably adherens junctions as in adult flies) are formed between GSCs and the SGP/hub cells, and as soon as GSC fates are established, their divisions are oriented and asymmetric (Tanentzapf et al. 2007). The establishment of GSC asymmetry by interacting with the niche component cells occurs in female germ line as well (Asaoka and Lin 2004).

Asymmetric Stem Cell Division and Aging

Stem cell maintenance is important for tissue homeostasis. A decline in either stem cell number or proliferation may lead to tissue degeneration associated with disease or aging. Indeed, a decline in stem cell number attributable to changes in both the stem cells themselves and the stem cell niche has been reported in male and female germ line stem cells (Boyle et al. 2007; Pan et al. 2007). In addition to these, we have demonstrated that changes in stem cell orientation precede a decline in stem cell number in the male germ line, leading to tissue aging. As *Drosophila* males age, GSCs with misoriented centrosomes accumulate progressively (~40% of total GSCs at day 30), when a decrease in stem cell number is still subtle. Misoriented GSCs do not undergo mitosis until the centrosome orientation is corrected (Cheng et al. 2008). This combination of a slowdown in cell division (i.e., G2/M transition) in misoriented GSCs with an increase in misoriented GSCs is speculated to cause a decline in spermatogenesis with age. The fact that GSCs resume cell cycle progression on correction of the centrosome orientation implies that there might be a novel checkpoint mechanism to monitor centrosome orientation and ensure an asymmetric outcome of stem cell division.

Remarkably, some misoriented GSCs originate from dedifferentiation (Cheng et al. 2008). Throughout *Drosophila* adulthood, individual GSCs are lost at a certain rate (Xie and Spradling 1998; Wallenfang et al. 2006). Dedifferentiation of partially differentiated germ cells was proposed as a mechanism to replenish the stem cell pool (Brawley and Matunis 2004; Kai and Spradling 2004). Such dedifferentiated GSCs were found to have a high incidence of centrosome misorientation (Cheng et al. 2008). Therefore, whereas dedifferentiation may play a key role in maintaining GSC number with age, reduced mitotic activity because of misorientation results in less GSC division and thus reduced production of differentiating germ cells (i.e., spermatogonia). These results suggest that a decline in stem cell activity comes from an imperfect yet compensating activity of the tissue to replenish the stem cell pool (Spradling 2008). It should be noted that when germ cells commit to differentiation, they lose the mother centrosome that stays in the GSCs. Thus, dedifferentiated GSCs do not possess the very old mother centrosomes, even

though they may have a mother centrosome that has undergone multiple cell cycles since they committed to differentiation. Thus, one can speculate that the high frequency of centrosome misorientation in dedifferentiated GSCs may reflect the fact that they do not have the "very old" centrosomes or the "centrosomal Eve" that was generated early during embryogenesis (Yamashita and Fuller 2008). Additional research is required to elucidate whether any physical differences between the "centrosomal Eve" in native GSCs and the "not-so-old mother centrosome" in dedifferentiated GSCs exist.

DROSOPHILA FEMALE GERM LINE STEM CELLS

Signaling in the Female GSC Niche

The niche of female GSCs shares numerous similarities with male GSCs in both structure and signaling. Female GSCs and their niche reside in a structure called the germarium, located at the anterior tip of each ovariole, the individual egg-producing unit of the ovary (also see Roth and Lynch 2009). This niche is composed of three kinds of somatic cell populations: terminal filament (TF) cells at the tip of the germarium, cap cells at the base of the terminal filament cells, and escort stem cells (ESCs) (Fig. 2C) (Decotto and Spradling 2005). A few ovarian GSCs, typically two to three, are tightly associated with five to seven cap cells via adherens junctions and receive short-range signals secreted from the niche cells (i.e., cap cells and TF) for self-renewal. Loss of adherens junctions between cap cells and GSCs causes the stem cells to migrate away from the cap cells and undergo differentiation (Song and Xie 2002). Between four to six ESCs are also anchored to the cap cells and contact GSCs. ESCs are believed to be the female counterpart of CySCs in males, both of which encapsulate GSCs and contribute to their fate via JAK-STAT signaling (Decotto and Spradling 2005; Leatherman and Dinardo 2008). Similar to CySCs in males, ESCs also produce daughter cells called escort cells, which encapsulate the differentiating germ line cysts until they undergo apoptosis and are replaced by follicle cells.

The BMP signaling pathway is the best understood mechanism shown to be both necessary and sufficient for the maintenance of female GSCs. Two BMP-like ligands, dpp and gbb, are secreted from cap cells, which then activate the signaling pathway in GSCs and prevent them from differentiating by repressing the transcription of Bam, a key differentiation-promoting factor (Fig. 2D) (Chen and McKearin 2003a; Chen and McKearin 2003b; Song et al. 2004; Chen and McKearin 2005). Loss of GSCs is observed in dpp mutants because of premature differentiation and slower division rates. Dpp overexpression completely blocks differentiation of cystoblasts, resulting in GSC-like tumors (Xie and Spradling 1998). Transient induction of Bam expression by heatshock, either in the second instar larval ovariole, where dpp is activated in the entire germ cell compartment, or in an adult ovariole, where dpp is ectopically expressed in somatic cells, induces differentiation of GSCs (Ohlstein and McKearin 1997). More interestingly, the induced cystocytes gradually undergo dedifferentiation over time and are converted into functional germ line stem cells in the presence of dpp in the surrounding somatic cell population, demonstrating the dominance of the niche in maintaining GSCs (Kai and Spradling 2004). Fs(1)Yb (Yb)-Piwi-mediated signaling is also required for GSC maintenance and was initially thought to be BMP-independent because dpp overexpression did not rescue Piwi mutant defects or show similar effects as Yb or Piwi overexpression. Yb exerts its function by regulating Piwi expression in TF cells and cap cells (King and Lin 1999; King et al. 2001). Piwi is involved in germ line development, stem cell identity, epigenetic regulation, and transposon silencing in many systems. Piwi binds to a complex class of small noncoding RNAs called Piwi-interacting RNAs (piRNAs) to execute its functions (Lin 2007; Ghildiyal and Zamore 2009; Lin and Yin 2009). In Yb-null mutant females, GSCs lose their ability to self-renew by either differentiating into germ line cysts without any division or

undergoing a limited number of abnormal cell divisions. Piwi mutants phenocopy Yb mutants. Piwi and Yb overexpression increases GSC-like or cystoblast-like germ cells (King and Lin 1999; King et al. 2001). Surprisingly, two recent reports demonstrate that BMP- and Piwi-mediated signaling converge to act synergistically to silence Bam (Chen and McKearin 2005; Szakmary et al. 2005). Interestingly, the JAK-STAT pathway is also found to be essential in ESCs to maintain female GSC identity (Decotto and Spradling 2005). How these different signaling pathways regulate GSC self-renewal cooperatively remains to be addressed.

Asymmetric Stem Cell Division in the Niche: Spindle Orientation by the Spectrosome

Similar to male GSCs, female GSCs undergo asymmetric cell division to maintain the balance between self-renewal and differentiation. Normally, a GSC divides asymmetrically to produce one daughter that stays associated with the cap cells to retain stem cell identity and another that moves away from the cap cells to become a cystoblast, which commits to differentiation. The cystoblast undergoes four additional rounds of mitoses with incomplete cytokinesis to form an interconnected 16-cell cyst. In contrast to male GSCs, whose spindle orientation is set up by centrosome positioning during interphase, the spindle of a female GSC is oriented through anchorage of one spindle pole to the spectrosome, a germ cell specific subcellular organelle, which is always located on the apical side of the GSC (Fig. 2D) (Deng and Lin 1997; also see Roth and Lynch 2009). Eliminating the spectrosome randomizes spindle orientation in the dividing female GSC (Deng and Lin 1997). Cytoplasmic dynein has been shown to be involved in coupling the mitotic spindle to the membrane skeletal proteins in the spectrosome/fusome (McGrail and Hays 1997). In agreement with the distinct mechanisms for orienting the mitotic spindle in male versus female GSCs (the centrosome vs. spectrosome, respectively), spectrosome positioning in male GSCs is random during interphase (Yamashita et al. 2003), and centrosome positioning is reported to be random during interphase in female GSCs (Stevens et al. 2007). Also, whereas female GSCs have been observed to undergo symmetric stem cell division on elimination of neighboring GSCs (Xie and Spradling 2000), male GSCs rarely undergo these events (Cheng et al. 2008), further suggesting distinct mechanisms for stem cell orientation and asymmetric stem cell division.

The molecular identity of the spectrosome/fusome has yet to be fully resolved. Nevertheless, this structure is always referred to as a membranous compartment associated with membrane skeletal proteins and microtubules with vesicle trafficking function similar to the ER and/or Golgi (Deng and Lin 1997; Roper and Brown 2004; Snapp et al. 2004; Roper 2007; Lighthouse et al. 2008). In addition, cyclin A has been reported to localize to the spectrosome/fusome, controlling the number of germ cell divisions before entering mitosis and implying a cell cycle role for the fusome (Lilly et al. 2000).

OTHER *DROSOPHILA* STEM CELLS AND STEM CELL POLARITY

Until recently, the *Drosophila* adult body, except for the gonad, was regarded as mostly postmitotic without much necessity for replenishment or adult stem cells. However, recent studies revealed many somatic stem cells in *Drosophila* adult tissues, including midgut (Micchelli and Perrimon 2006; Ohlstein and Spradling 2006; Ohlstein and Spradling 2007; Lin et al. 2008), hindgut (Takashima et al. 2008), and malpighian tubule stem cells (Singh et al. 2007). Midgut stem cells divide with an oriented spindle (relatively fixed orientation with respect to basement membrane) (Ohlstein and Spradling 2007); however, whether this spindle orientation is used as a means for asymmetric division or for any other aspects of stem cell biology remains to be determined. Underlying muscle cells that associate with midgut stem cells control stem cell identity via wingless signaling (Lin et al. 2008), and midgut stem cells can respond to injury to repair the damaged tissue quickly (Amcheslavsky et al. 2009).

This illuminates a striking parallel between *Drosophila* and mammalian intestinal stem cells (Casali and Batlle 2009).

Recent characterization of ovarian follicle stem cells revealed a novel type of stem cell microenvironment (Nystul and Spradling 2007). Follicle stem cells (FSCs) produce follicle cells that encapsulate developing oocyte and nurse cells, taking the place of escort cells after they undergo apoptosis. Each ovariole appears to contain exactly two FSCs residing in separate niches on opposite sides of the germarium (Fig. 2C). The most striking feature of FSCs is the apparent lack of fixed niche components. Instead, the location of FSCs appears to be determined relative to the location of other cells. Further investigation is required to determine if FSCs maintain a polarity toward any of their interacting cells. Although several signaling pathways, including the Hedgehog, Wingless, and BMP pathways, as well as adherens junctions, have been implicated in FSC maintenance (Forbes et al. 1996; Song and Xie 2002; Song and Xie 2003; Kirilly et al. 2005), it is still unknown how these signals crosstalk or which cells provide polarity cues to FSCs. In spite of the progress made thus far in understanding *Drosophila* adult stem cells, the polarity and asymmetric division of stem cells remain to be elucidated.

VERTEBRATE STEM CELLS: HOW MUCH CAN WE APPLY LESSONS FROM *DROSOPHILA*?

Although many stem cell populations have been identified and the number continues to grow, only a few of them have been characterized in terms of their polarity/asymmetry and relationship with their niche. In many cases, the presence and/or nature of the stem cell niche itself is still ambiguous. Below, we focus on some stem cell populations outside *Drosophila*, where the cell polarity and/or mitotic spindle orientation is known.

Mammalian (mouse) Neuroepithelial Cells

All neurons and macroglial cells of the mammalian central nervous system are derived from neuroepithelial (NE) cells, which function as stem cells. During development, NE cells shift from proliferative, symmetric divisions to neurogenic, asymmetric divisions. This asymmetry derives from the inheritance of a tiny apical surface area (apical cortex), which contains Cadherin (i.e., the cell that inherited this apical cortex retains stem cell potential). Because this apical cortex is very small, spindle orientation does not need to be strictly parallel or vertical with respect to the apical surface to determine a symmetric versus asymmetric outcome, respectively (Fig. 3B) (Kosodo et al. 2004). Indeed, NE cells frequently divide asymmetrically with an almost parallel spindle with only a slight tilt, unequally segregating the apical cortex into two daughters. This is in clear contrast to the *Drosophila* neuroblast, where the drastic spindle orientation (horizontal vs. vertical) is a determinant for asymmetric stem cell division (Fig. 3A) (for more details, see Yu et al. 2006 and Chia et al. 2008). In the normal *Drosophila* neuroblasts, the mitotic spindle is formed perpendicularly with respect to the apical and basal "crescents" that contain fate determinants and complexes that regulate spindle orientation, leading to asymmetric division (Fig. 3A). In contrast, in the neuroectoderm cells, from which neuroblasts derive, and in the mutant neuroblasts defective in spindle orientation, the mitotic spindle is formed in parallel to these crescents, resulting in symmetric divisions.

These observations in the *Drosophila* highlight the correlation between spindle orientation and cell fates, and raise the question as to how mammalian NE cells divide symmetrically versus asymmetrically in spite of their narrow range of spindle orientations. Yet, a shift from symmetric (proliferative) to asymmetric (neurogenic) divisions occurs during development, clearly implying that there must exist a mechanism that determines symmetric versus asymmetric division. Interestingly, disruption of the function of LGN, a homolog of Pins required for spindle orientation in the *Drosophila* neuroblasts, leads to dramatic randomization of spindle orientation with a wider range of spindle orientation than normally

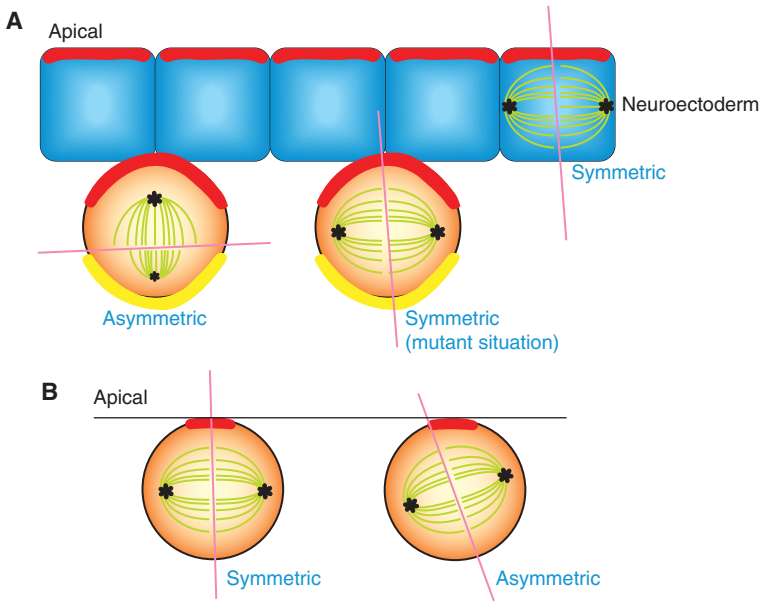

Figure 3. Spindle orientation and asymmetric division in *Drosophila* and mouse neuronal stem cells. (*A*) In *Drosophila* neuroblast, spindle is oriented perpendicular to the apical crescent (red line) that contains the Baz (Par3)-Par6-aPKC complex, as well as Pins, Insc, and Gαi, leading to asymmetric stem cell division. The apical crescent is required for spindle orientation, the basal crescent formation and spindle size asymmetry (i.e., the apical half is larger than the basal half). The basal crescent (yellow line) contains fate determinants that promote/allow differentiation such as Numb, Miranda, and Prospero. In the mutants that are defective in spindle orientation, the apical and basal crescents are bisected into two daughters, leading to symmetric stem cell division. Neuroectoderm cells (from which neuroblasts are derived) also have the apical complex, except for Insc. They divide symmetrically by orienting mitotic spindle parallel to the apical crescent. Ectopic expression of Insc in these cells result in the recruitment of Pins to the apical cortex, leading to perpendicular spindle orientataion (Yu et al. 2000). (*B*) In mammalian (mouse) neuroepithelial cells, the mode of cell division shifts from symmetric to asymmetric during development. The stem cell identity is determined by inheritance of a tiny apical cortex containing cadherin (red line), and thus mitotic spindle does not have to tilt significantly to divide asymmetrically.

observed in mammalian NE cells (Konno et al. 2008). This raises the intriguing possibility that a "parallel" spindle is the result of an elaborate regulation of spindle orientation, and that a slight tilt in orientation within a small range is actively controlled to dictate symmetric versus asymmetric NE divisions.

Interestingly, recent studies found another asymmetry during NE cell divisions: Prominin-1, or CD133, localizes to the midbody ring of dividing NE cells with an interesting asymmetry, correlated with the mode of stem cell division. Prominin-1 is expressed in many adult stem cells and cancer stem cells, although its function remains unclear. Before the neurogenic stage of development, when NE cells are proliferating by symmetric divisions, the Prominin-1-containing midbody ring is excluded from both daughters, being released into the extracellular space (Dubreuil et al. 2007; Farkas and Huttner 2008). Once neurogenesis begins later in development, NE cells start dividing asymmetrically and the Prominin-1-containing ring is inherited by the stem cell (NE cell), whereas the daughter fated to differentiate is devoid of this ring. This strong correlation implies that the Prominin-1-containing midbody ring might be somehow involved in stem cell behavior such as stem cell potential or asymmetric stem

cell division. Although intriguing, further work is required to examine this hypothesis.

Mammalian Skin Stem Cells

Skin stem cells reside within the basal layer of the epidermis and contact the basement membrane. These cells tend to divide symmetrically with parallel spindles relative to the basement membrane when the tissue (i.e., skin surface area) is increasing in size during development. Then, they undergo asymmetric division by shifting the spindle orientation from parallel to perpendicular once stratification starts later in development and during tissue homeostasis (Lechler and Fuchs 2005). The basement membrane provides key features such as integrin-mediated focal adhesion and growth factors. Thus, asymmetric inheritance of contact with the basement membrane automatically dictates asymmetric stem cell division and stratification of epidermal cells. It was further shown that these epidermal cells control spindle orientation in a similar manner to *Drosophila* neuroblasts. LGN, mInsc, the mammalian homolog of *Drosophila* Insc, and Par3 are localized asymmetrically in the dividing epidermal stem cells (Lechler and Fuchs 2005).

Conclusion: Parallels between Mammalian and *Drosophila* Stem Cells

The two examples above provide a very nice parallel between the mammalian and *Drosophila* stem cell systems. First, spindle orientation is the main theme for determining symmetric or asymmetric stem cell division regardless of whether it is controlled by intrinsic or extrinsic cues. The mechanism of how spindle orientation is determined varies from one system to another. These two mammalian examples discussed above resemble *Drosophila* neuroblasts in that these stem cells appear to use spindle rotation, which presumably allows for greater flexibility in the decision about symmetric versus asymmetric stem cell division. However, such flexibility may leave room for stem cells to divide symmetrically without regulation, leading to tumorigenesis. It should be noted that all of mammalian examples represent stem cells in a developing tissue at the transition from symmetric to asymmetric stem cell division. Therefore, it would be interesting to investigate how stem cell division is controlled in mammals once they are established as adult stem cells, where stereotyped asymmetric stem cell division would be favored. *Drosophila* GSC studies have focused on the adult stage, and thus may represent a more stereotyped mechanism to ensure asymmetric stem cell divisions.

Asymmetric stem cell division is fundamental to tissue homeostasis, and requires elaborate mechanisms to establish and maintain cell asymmetry in the context of the resident tissue. Recent discoveries from *Drosophila* and mammalian tissues have enriched our knowledge about how stem cells divide asymmetrically. In general, stem cells reside in their special microenvironment (or niche) that specifies stem cell identity. Resident stem cells are often closely associated with the niche component via cell–cell contact, such as adherens junctions. In such a context of tissue architecture and cell–cell signaling, stem cells are polarized such that they orient mitotic spindles to divide symmetrically or asymmetrically. Although our knowledge on stem cell division is far from comprehensive, the parallels between *Drosophila* and mammalian systems are striking, suggesting that asymmetric stem cell division is an evolutionarily conserved process. Further studies investigating the parallels between these model organisms and humans will lead to a better understanding of the mechanism of stem cell division, and of diseases that are caused by dysfunctional stem cell division.

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