Mechanisms regulating stem cell polarity and the specification of asymmetric divisions*

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Abstract

The ability of cells to divide asymmetrically to produce two different cell types provides the cellular diversity found in every multicellular organism. Asymmetric localization of cell-cell junctions and/or intrinsic cell fate determinants and position within specific environment ("niche") are examples of mechanisms used to specify cell polarity and direct asymmetric divisions. During development, asymmetric divisions provide the basis for establishment of the body axis and cell fate determination in a range of processes. Subsequently, asymmetric cell divisions play a critical role in maintaining adult stem cell populations, while at the same time generating an adequate number of differentiating daughter cells to maintain tissue homeostasis and repair. Loss of cell polarity, and consequently the potential for asymmetric divisions, is often linked to excessive stem cell self-renewal and tumorigenesis. Here we will discuss multiple factors and mechanisms that imbue cells with polarity to facilitate an asymmetric outcome to stem cell divisions, assuring self-renewal and maintenance of the stem cell pool.

*Edited by Gary Gilliland. Last revised February 17, 2009. Published March 31, 2009. This chapter should be cited as: Toledano, H. and Jones, D.L., Mechanisms regulating stem cell polarity and the specification of asymmetric divisions (March 31, 2009), StemBook, ed. The Stem Cell Research Community, StemBook, doi/10.3824/stembook.1.41.1, http://www.stembook.org.

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Asymmetric division is a property of stem cells that leads to the generation of two cells that can adopt different fates. One has the potential to renew stem cell identity and continue to divide in an asymmetric manner, whereas the other cell will differentiate along a specific lineage. In some cases, factors within the dividing mother cell lead to the differential segregation of cell fate determinants to give two distinct daughters upon division. In others, however, establishment of different fates is reinforced through signaling from neighboring cells. Ultimately, asymmetric divisions are regulated directly by genes that control the process of asymmetric cell division itself or determine the distinct cell fates of the two daughter cells.

1. *Drosophila* neuroblast divisions

Studies of the underlying mechanisms regulating asymmetric division of *Drosophila* neuroblasts (NBs) have contributed to the establishment of paradigms and identification of molecular components that control asymmetric division in more complex stem cell systems (Reviewed in Chia et al., 2008; Doe, 2008; Gonczy, 2008; Yu et al., 2006). NBs are neural stem/progenitor cells that are specified during embryogenesis and divide to generate the larval neurons. During larval and pupal stages, NB divisions resume to generate adult neurons. In the embryo, NBs divide perpendicular to the plane of the neuroepithelium to generate another (apical) NB and a smaller, basally located ganglion mother cell (GMC) that will differentiate into neurons or glia. The apical-basal polarity of the mother NB is inherited from its placement within the neuroepithelium and is coupled to differential distribution of cellular components. Lineage tracing analyses demonstrated that a single NB could give rise to a family of marked GMC/neuronal progeny, including the marked NB, confirming that NBs are asymmetrically dividing stem cells that generate multiple cell types (Doe, 2008; Schmid et al., 1999).

Several aspects of intrinsic polarity contribute to asymmetric division of NBs: **1.** Cell fate determinants are segregated to the basal cortex of the dividing NB, resulting in a disruption of the symmetry of the mother cell prior to division. **2.** The mitotic spindle is aligned along the apical-basal axis to ensure accurate segregation of these cell fate determinants to the appropriate daughter cell. **3**. Asymmetric positioning of the anaphase spindle results in daughter cells that will not only assume different fates but also differ in size. Such intrinsic cell polarity appears to be the major mechanism specifying asymmetric division of NBs; however, some studies suggest that extrinsic signals from the overlying epithelium also facilitate proper spatio-temporal localization of cell fate determinants (Lee et al., 2006). NBs that are still in contact with epithelial cells as they divide always produce GMCs opposite the site of epithelial-NB contact. In contrast, isolated NBs produce GMCs in random positions along the NB cortex. These data indicate that embryonic NBs respond to signals from the adjacent epithelium to specify correct spindle orientation and localization of cortical cell fate determinants.

Segregation of cell fate determinants to the daughter GMC is regulated by the reciprocal localization of four protein complexes: two complexes are localized to the apical cortex and two to the basal cortex (see Figure 1). The basal complexes, which will segregate to the GMC, asymmetrically localize three major cell fate determinants: Prospero, Brat, and Numb, which inhibit self-renewal and promote differentiation (Bowman et al., 2008). A key modulator of GMC differentiation is the homeodomain transcription factor Prospero (Doe et al., 1991; Hirata et al., 1995; Knoblich et al., 1995; Spana and Doe, 1995; Vaessin et al., 1991). *prospero* mutants fail to express many GMC-specific markers and exhibit axonal defects (Doe et al., 1991; Vaessin et al., 1991). The adaptor protein Miranda (Mira) binds the dsRNA binding protein Staufen, which in turn is bound to *prospero* mRNA. Mira also associates with Prospero protein and facilitates the asymmetric localization of the translational repressor Brain tumor (Brat; Bello et al., 2006; Betschinger et al., 2006; Lee et al., 2006). In *brat* mutants, Prospero fails to be partitioned to GMC resulting in NBs that fail to differentiate (Bowman et al., 2008; Lee et al., 2006). After segregation to the GMC, Mira is degraded, allowing the release of Pros, Staufen and Brat.

The second basal complex contains the Notch antagonist Numb and its binding partner, Partner of Numb (Pon). The adaptation of distinct cell fates by the daughter cell that contains Numb is thought to result largely from the ability of Numb to bind to the cytoplasmic domain of Notch (N) and antagonize N signaling in the GMC (Berdnik et al., 2002; Wang et al., 2006). However, the *numb* loss-of-function phenotype in embryonic NBs is not as severe as loss of *brat* or *pros*. Recent studies have demonstrated that Numb and N activities are associated with facilitating asymmetric stem/progenitor cell divisions in a number of other tissues (discussed below).

The evolutionary conserved PAR ('partition defective') proteins act as core components of the cell polarization machinery in animals ranging from *C. elegans* to humans (reviewed in Macara, 2004). The PAR complex, which contains Bazooka/Par-3, Par-6 and atypical Protein kinase C (aPKC), is the first to localize along the NB cell cortex

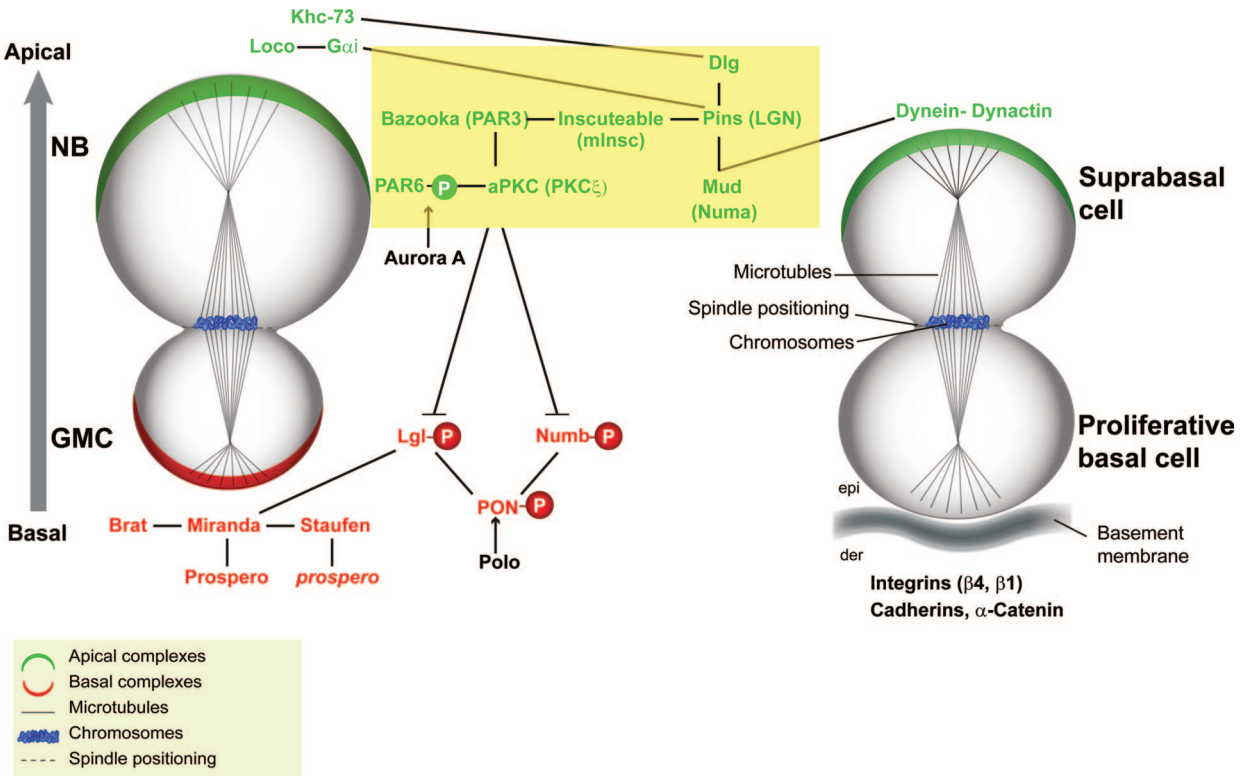

Figure 1. Asymmetric cell division in *Drosophila* larval neuroblasts (NBs; on the left) and mammalian epithelia (on the right). Schematic depiction of a polarized mother cell during anaphase. Apical protein complexes (green). Yellow box highlights evolutionarily conserved apical complexes that are important for polarity establishment and spindle positioning in these two systems. Cell fate determinants (red). Note that the *Drosophila* NB are apical, while progenitor cells within mammalian epithelia are located basally.

(see Figure 1). The PAR complex is primarily involved in recruiting the adaptor protein Inscuteable (Insc) to establish the polarity of the NB and facilitate proper spindle alignment by capturing one spindle pole at the apical cortex and aligning the spindle along the apical-basal axis (reviewed in Kraut et al., 1996; Parmentier et al., 2000; Roegiers and Jan, 2004; Schober et al., 1999; Yu et al., 2000). The PAR complex also plays a major role in cell fate specification by excluding the basal complexes from the apical cortex in part by phosphorylating and inactivating the tumor suppressor Lethal giant larva (Lgl), which is responsible for targeting Pon and Mira to the basal cortex (Betschinger et al., 2005; Betschinger et al., 2003). Phosphorylation of Lgl by aPKC leads to release of Lgl from the complex, which is replaced by Bazooka/Par-3, and ultimately a change in substrate specificity (discussed below; Wirtz-Peitz et al., 2008).

The second apical complex contains the GoLoco-motif protein Partner of Inscuteable (Pins), locomotion defects (Loco), and Gαi, a subunit of heterotrimeric G proteins (Parmentier et al., 2000; Schaefer et al., 2000; Yu et al., 2000; see Figure 1). Insc bridges the two apical complexes by binding to both Bazooka/Par-3 and Pins. Pins associates with mushroom body defective (mud), which is essential for proper spindle alignment, (Bowman et al., 2006; Izumi et al., 2006; Siller et al., 2006 and Discs Large (Dlg; Albertson and Doe, 2003; Peng et al., 2000) Dlg associates with the astral microtubule plus end protein Khc-73 to induce cortical polarity (Siegrist and Doe, 2005). The Pins complex mediates spindle formation and alignment to ensure that the cleavage plane is orthogonal to the apical-basal axis. Ultimately, the mitotic spindle is displaced toward the basal cortex, resulting in a longer spindle in the apical NB daughter.

In addition to the complexes that regulate asymmetric division of neuroblasts discussed above, recent data have demonstrated that components of the cell cycle machinery can also affect asymmetric protein localization and the specification of daughter cell fates. Analysis of embryos carrying dominant negative or hypomorphic alleles of *cdc2*, the kinase responsible for regulating the transition from G2 to mitosis, revealed a failure to properly localize both apical and basal complexes, resulting in symmetric NB divisions (Tio et al., 2001). Similarly, mutations in two other kinases

that were originally identified as centrosomal proteins, *aurora A* and *polo,* also result in symmetric NB divisions. In *aurora A* and *polo* mutant NBs, localization of Numb and Pon to the basal cortex is disrupted, and excessive NB self-renewal is observed at the expense of differentiating neurons. Overproliferation can be reversed, although not completely, by overexpression of wild type Numb (Lee et al., 2006; Wang et al., 2007; Wang et al., 2006). Likewise, reduction of N in *aurora A* and *polo* mutant NBs also suppresses overproliferation of neuroblasts.

Polo kinase phosphorylates Pon, which is important for proper localization of Numb (Wang et al., 2007). Furthermore, recent data have elucidated the mechanism by which Aurora A acts to segregate the fate determinant Numb to the GMC during mitosis. Aurora A phosphorylates Par-6, leading to activation of aPKC, phosphorylation of Lgl, and exchange of Bazooka/Par-3 for Lgl. The new complex has an altered substrate specificity, which enables aPKC to bind and phosphorylate Numb, followed by release of Numb to the basal cortex (Wirtz-Peitz et al., 2008). Therefore, mechanisms coupling cell cycle progression to asymmetric localization of Numb appear to exist to ensure N activation only in the neuroblast NB daughter to specify self-renewal.

Interestingly, a number of studies have demonstrated that mutations in factors that control asymmetric division of NBs lead to tumorigenesis. Basal cell fate determinants, such as Pros, Brat, Numb, Mira and Pon can act as tumor suppressors (Bello et al., 2006; Betschinger et al., 2006; Choksi et al., 2006; Lee et al., 2006; Wang et al., 2006). Homozygous mutations of any of these genes failed to correctly specify differentiated cells and produced supernumerary self-renewing NB daughters. Brain tissue from *mira*, *pros*, *numb, lgl*, *brat* or *pins* mutants that was transplanted into the abdomen of wild type flies exhibited metastatic behavior, undergoing massive proliferation and overgrowth, eventually killing the host (reviewed in (Beaucher et al., 2007; Caussinus and Gonzalez, 2005; Caussinus and Hirth, 2007). Similar studies showed that mutations of the apical complex components *dlg* and *lgl* caused malignant neoplastic tumors of the nervous system (Lee et al., 2006). Therefore, a failure to correctly specify cell fates, rather than a disruption in polarity, appears to be the primary cause of overproliferation and tumor formation. Taken together, these findings suggest a causal link between defects in NB asymmetric division and malignant overproliferation and underscore the critical need for precise regulation of asymmetric divisions of tissue stem cells.

2. *Drosophila* germline stem cells

Asymmetric division of germline stem cells (GSCs) in *Drosophila melanogaster* is highly regulated by proximity to external cues. In male GSCs, a fixed spindle is oriented perpendicular to a cluster of support cells, called the hub, that secrete the self-renewal factor Unpaired (Upd; reviewed in Yamashita et al., 2005). Upd is a cytokine-like molecule that binds the transmembrane receptor Domeless (Dome), resulting in activation of the highly conserved JAK-STAT signal transduction pathway, which is necessary for maintenance of germline and somatic stem cells in the testis (Kiger et al., 2001; Tulina and Matunis, 2001). Wild-type male GSCs divide with invariant asymmetry to generate a daughter cell that remains adjacent to the hub and retains stem cell identity and a daughter cell that is displaced away from the hub and initiates differentiation as a gonialblast. Therefore, self-renewal and differentiation of GSC daughters is tightly controlled by placement within a well-defined niche (reviewed in Jones and Wagers, 2008). Upd expression and secretion from the hub must be tightly regulated, as ectopic expression of Upd in early germ cells (Kiger et al., 2001; Tulina and Matunis, 2001) or activated forms of JAK in somatic cells (Leatherman and Dinardo, 2008) leads to hyperproliferation of stem cells and germ cell tumor formation.

As in NBs, spindle orientation in male GSCs is important for ensuring an asymmetric GSC division. However, few of the molecules that are involved in the control of spindle orientation in NBs have been demonstrated to play conserved roles in this system. Components of adherens junctions, such as the *Drosophila* orthologues of epithelial cadherin (DE-cad) and b-catenin (Armadillo, Arm) are enriched at the interface between GSCs and hub cells (Yamashita et al., 2003). Male GSCs and SSCs that are mutant for *shotgun*, the gene that encodes DE-cad, fail to self-renew and are not maintained, indicating that DE-cad is required for holding stem cells within the niche and close to self-renewal signals emanating from the hub (Voog et al., 2008). This clustering of adherens junctions has been proposed to create asymmetry within the stem cell and provide a scaffold to which the astral microtubule array is anchored (discussed below; Yamashita et al., 2003; Yamashita et al., 2007).

The *Drosophila* homolog of the adenamtous polyposis coli (APC) gene, dAPC2, also localizes to the interface between hub cells and GSCs, and mutations in dAPC2 result in mis-positioned centrosomes and mis-aligned spindles (Yamashita et al., 2003). Interaction between the GSC cortex and the spindle pole appears to be mediated by dAPC2 and the astral microtubules, as the percentage of mGSCs with mis-positioned centrosomes is significantly higher in testes from *APC2* mutants. Similarly, in mGSCs that are mutant for *centrosomin (cnn)*, the centrosomes cannot polymerize

microtubules, the link between one centrosome and the cortex cannot be established, and anastral misaligned spindles are assembled, leading to symmetric mGSC divisions (Yamashita et al., 2003). Interestingly, the number of GSCs with mis-positioned centrosomes always exceeds the number of cells with mis-oriented spindles, implying that a checkpoint is in place to ensure division only when the spindle is oriented properly (Yamashita et al., 2003). The frequency of mis-positioned centrosomes increases with age, and yet the checkpoint remains intact (Cheng et al., 2008). These data clearly demonstrate that correct spindle orientation is essential for maintaining the appropriate balance of stem and progenitor cells. It will be interesting to determine whether a similar checkpoint is present in other tissue stem cells that exhibit oriented divisions, potentially providing an important strategy to block cancer initiation in tissues maintained by stem cells.

Recent studies elegantly demonstrated that the mother centrosome is retained within the GSC daughter, while the newer daughter centrosome segregates to the gonialblast. Electron micrographs revealed that the centrosome proximal to the hub (mother centrosome) maintains a significant number of microtubules throughout the cell cycle. In addition, the majority of centrosomes examined were located close to adherens junctions between GSCs and hub cells, revealing a potential role for DE-cad in providing a scaffold within GSCs that could link the cell cortex to the centrosome via adherens junctions and robust astral microtubule arrays (Yamashita et al., 2007). Furthermore, GSCs mutant for *cnn* displayed randomized segregation of mother and daughter centrosomes, in cases when one of the centrosomes was actually found adjacent to the hub. Therefore, asymmetric inheritance of centrosomes is likely regulated by the same machinery that polarizes the GSC to facilitate proper spindle orientation. Whether loss of DE-cadherin in GSCs results in randomized spindle positioning or segregation of mother and daughter centrosomes, similar to loss of *cnn*, has not yet been determined.

Interestingly, centrosomes within dividing *Drosophila* larval NBs are also asymmetric. The “dominant” apical centrosome, which is retained within the NB, acts as a microtubule organizing center (MTOC) before its basal counterpart, which is inherited by the GMC daughter (Rebollo et al., 2007; Rusan and Peifer, 2007). Although both Polo kinase and Pins have been implicated in regulating this asymmetric centrosomal maturation, it is not clear what additional factors may mediate this novel centrosome cycle. Furthermore, it has not been determined whether the mother centrosome is always retained within the NB.

GSCs in the *Drosophila* ovary also divide asymmetrically to produce another GSC and a daughter cystoblast that initiates differentiation by undergoing four rapid divisions to generate interconnected 16-cell germline cysts. Only one of the cells within a cyst will become the oocyte, while the other 15 cells are nurse cells that support oocyte development.

As in male GSCs, the mitotic spindle in female GSCs is also oriented with respect to a group of somatic support cells, called cap cells, to which they are attached through adherens junctions (Song et al., 2002; Xie and Spradling, 2000). Adherens junctions are localized along the interface between GSCs and cap cells, and DE-cadherin is also required for maintenance of GSCs in the ovary (Song et al., 2002). However, a similar mechanism utilizing dAPC2, Cnn, and DE-cadherin to orient the mitotic spindle in female GSCs has not been identified. Instead, a special cytoplasmic organelle, known as the spectrosome (fusome in later germline cysts), is localized along the GSC cortex adjacent to cap cells and has been shown to play a role in orienting mitotic spindles in GSCs, cystoblasts, and developing cysts (Deng and Lin, 1997). Although spectrosomes are also found in male GSCs, they exhibit random placement along the GSC cortex (Yamashita et al., 2003) and are unlikely to play a role in mitotic spindle orientation. Limited space within the GSC niche in the ovary may play a significant role in the placement of daughter cells outside the influence of localized self-renewal factors, and recent studies have revealed that genetic programs are in place to regulate GSC competition for niche occupancy, including the levels of DE-cadherin (Jin et al., 2008). However, symmetric divisions of GSCs to replace lost stem cells can be observed within the ovary (Xie and Spradling, 2000), suggesting that the mechanisms regulating spindle orientation within female GSCs may be less “hard-wired” than those in place within male GSCs.

Lastly, the microRNA pathway has been implicated in regulating female GSC behavior and the onset of differentiation (Forstemann et al., 2005; Hatfield et al., 2005; Neumuller et al., 2008; Park et al., 2007). Components of the miRNA machinery, such as *dicer*-1, *loquacious*, or *AGO*-1, promote GSC self-renewal, as loss of these factors results in significant reduction in GSC proliferation and differentiating germline cysts. Conversely, Mei-P26, an inhibitor of miRNA biogenesis, is upregulated in cystocytes and germline cysts and plays a role in promoting differentiation. Interestingly, Mei-P26 and Brat share strong domain conservation and are considered members of the Trim-NHL protein family. Therefore, Trim-NHL proteins could act to specify asymmetric divisions by promoting differentiation of SC daughters in a variety of tissues maintained by stem cells (Neumuller et al., 2008).

3. Mammalian epidermal progenitor cells

Work on mouse embryos and in cultured skin cells demonstrated that progenitor cells at the base of the epidermis can replicate symmetrically to provide more stem cells, as well as asymmetrically, to generate a stratified epithelium. An asymmetric division produces another proliferative 'basal' cell that remains in contact with the basolateral membrane and one detached 'suprabasal' cell that is displaced apically toward the skin's surface (Lechler and Fuchs, 2005; Smart, 1970). Asymmetric divisions have also been observed within the basal layer of the esophageal epithelium (Seery and Watt, 2000). Many of the factors described above that specify polarity and regulate the asymmetric division of *Drosophila* NBs have orthologues in mammalian epithelial progenitors (see Figure 1). However, unlike during asymmetric divisions of NBs where the apical proteins remain in the NB stem cell, apical complexes form opposite the basement membrane and are segregated into the suprabasal cell that is poised for differentiation.

Basal progenitor cells are physically attached to the underlying basement membrane and contain adhesion molecules such as integrins and cadherins that are essential for spindle alignment (Lechler and Fuchs, 2005). Perpendicular divisions provide a natural mechanism for the unequal partitioning of the signaling molecules derived from the basement membrane into the two daughter cells. Mitotic cells with perpendicular spindles, representing asymmetric divisions, have an apical crescent of cortical LGN, the mammalian Pins orthologue. LGN binds Inscuteable (mInsc) and Par3 at the apical cortex of the basal cells. Similar to its *Drosophila* counterpart, LGN binds the Mud orthologue NuMa, which tethers spindles at the poles (Du et al., 2001). Furthermore, the aPKC orthologue, PKC$-\zeta$, also localizes to an apical crescent in basal cells (Lechler and Fuchs, 2005; see Figure 1).

Similar to what is observed in *Drosophila* NBs, studies suggest that asymmetric activation of the N pathway may be one mechanism utilized to ensure an asymmetric outcome to epithelial stem cell divisions. Suprabasal cells utilize the Notch intracellular domain (NICD) to promote differentiation (Blanpain et al., 2006). Furthermore, in cultured mammalian epithelial cells, Numb localizes primarily to the basolateral cortex as a result of PKC mediated phosphorylation, which results in its exclusion from the apical pole (Smith et al., 2007).

Although many of the molecular factors that specify asymmetric divisions appear to be conserved throughout evolution, at present the primary function of the apical proteins in mammalian epithelia appears to be to establish apico-basal polarity and determine spindle positioning, rather than to specify stem cell fate (reviewed in Lechler and Fuchs, 2005; Macara, 2004; Shin et al., 2007; Suzuki and Ohno, 2006). It is likely that attachment of basal cells to a basement membrane not only leads to a concentration of integrins but also growth factor receptors at the base of the cell that could influence stem cell maintenance and regulate stem cell behavior. Interestingly, the basal transcription factor p63 promotes epidermal proliferation (Mills et al., 1999; Yang et al., 1999) and also appears to be required for stratification (Senoo et al., 2007), as basal cells only divide symmetrically in the absence of p63 (Lechler and Fuchs, 2005). Therefore, much still remains to be uncovered regarding the regulation of epidermal stem cell behavior in the development and maintenance of adult skin. Future studies will likely lead to the identification of specific cell fate determinants and reveal the relative contributions of the environment and specific factors on cell fate and asymmetric divisions in the mammalian epidermis.

4. Asymmetry in other systems

Asymmetric stem cell divisions have been observed in a number of other tissues, although the factors that act to specify cell fate, mechanism(s) that orient mitotic spindles and the influence of extrinsic factors remain unknown. Interestingly, however, activation of the Notch signaling pathway and/or asymmetric segregation of Numb appears to be a common theme across a number of stem cell systems.

4.1. Drosophila intestinal stem cells

As described above for *Drosophila* NBs and mammalian epithelial progenitors, the Notch signalling pathway is also involved in mediating asymmetric division of intestinal stem cells (ISCs) in the midgut of adult flies (Ohlstein and Spradling, 2007). ISCs maintain the intestinal epithelium and generate both polyploid enterocytes as well as hormone-producing enteroendocrine cells (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006). ISCs reside along the basement membrane within clusters, or "nests," of 2–3 basally located diploid cells that are interspersed between polyploid enterocytes. Although all cells in the stem cell-containing nests are Notch^{+}, only the ISC directly contacts the basement membrane and stains positive for the Notch ligand Delta, whereas Notch signaling is activated exclusively in the daughter enteroblast. In other words, the ISCs signal through Delta to activate Notch target genes in enteroblasts (Ohlstein and Spradling, 2007). Uncovering how Notch signaling is blocked within the ISC to facilitate this asymmetric

division will likely reveal new paradigms for how stem cell self-renewal and maintenance is regulated. Interestingly, analysis of mitotic spindle orientation in dividing ISCs indicates that these stem cells divide non-randomly, such that the daughter ISC that remains adjacent to the basement membrane remains an ISC, while the daughter cell that is displaced away differentiates to form an enteroblast. However, the mechanism that regulates orientation of the mitotic spindle has not been characterized.

4.2. Hematopoietic stem cells

Although Notch signaling is not absolutely required for normal hematopoiesis (Mancini et al., 2005), N has also been implicated in regulating the fate of hematopoietic stem cells (HSCs) by blocking differentiation (Duncan et al., 2005). Duncan et al. found that transgenic mice carrying a Notch-responsive GFP reporter could be used to enrich for hematopoietic progenitors: GFP^+ cells contained approximately 40–60% HSCs, and expression of GFP was significantly downregulated in differentiating precursors (Duncan et al., 2005; Wu et al., 2007). Using this transgenic Notch reporter strain, real time imaging was used to visualize cultured hematopoietic precursor cell divisions. When precursor cells were grown under conditions known to maintain immature hematopoietic cells, symmetric renewing divisions (giving rise to two GFP^+ daughter cells) were primarily observed, whereas when the precursors were placed in differentiation–promoting conditions, the cells underwent primarily asymmetric (one GFP^+/one GFP^-) divisions or symmetric commitment divisions (two GFP^- cells).

Using these tools, the authors went on to show that different oncogenic, chromosomal translocations either affected proliferation and survival or influenced the pattern of divisions, providing evidence for how oncogenes, such as BCR-ABL, can lead to the transformation of hematopoietic progenitors. Although these data indicate that both intracellular factors and extrinsic cues are involved in regulating the outcome of hematopoietic precursor cell divisions, much remains to be done to elucidate the mechanisms by which such factors act to specify an asymmetric division. Given the fact that HSCs typically reside within a specialized niche within the bone marrow composed of osteoblasts (Calvi et al., 2003; Fleming et al., 2008; Zhang et al., 2003), vascular endothelial cells (Kiel et al., 2005; Sacchetti et al., 2007) and stromal reticular cells (Sugiyama et al., 2006), it will be particularly interesting to determine whether HSCs also divide with mitotic spindles oriented with respect to niche support cells.

4.3. Muscle stem cells

Satellite cells sustain production of myoblasts during postnatal growth and promote muscle repair after injury, effectively acting as muscle stem cells. Satellite cells reside beneath a basement membrane, adjacent to mature myofibers. They are normally quiescent but can be induced to enter the cell cycle upon injury. Asymmetric segregation of older (immortal) and younger DNA strands into different daughter cells was documented in a subset of dividing muscle-lineage cells during muscle growth and regeneration (Cairns, 1975; Conboy et al., 2007; Shinin et al., 2006). Furthermore, differential localization of proteins, such as Numb (Conboy and Rando, 2002; Shinin et al., 2006), and induced expression of differentiation genes, such as Myf5 (Kuang et al., 2007), within daughter cells *in vitro* and *in vivo* have also been reported, providing evidence that muscle progenitor cells can undergo asymmetric divisions. However, the canonical satellite cell pool is quite heterogeneous (Cerletti et al., 2008; Shinin et al., 2006). Characterizing the division of highly purified muscle progenitor cells (Cerletti et al., 2008), will facilitate identifying factors involved in the specification of daughter cell fates as well as the mechanism(s) that orient muscle satellite cell division in response to various environmental cues. It must be noted, however, that despite the ability of hematopoietic and muscle progenitor cells to divide asymmetrically, the relative importance of this mode of division in blood homeostasis and muscle regeneration has yet to be established.

4.4. Mammalian neural progenitors

Asymmetric divisions occur in the ventricular zone of the mammalian cerebral cortex and neuroepithelium of the vertebrate retina (reviewed in Gonczy, 2008). Symmetric divisions are primarily observed during early developmental stages presumably to increase the pool of neural progenitors, while asymmetric divisions occurred later on to generate differentiating neurons. Although spindle orientation is indicative of whether cell division will be asymmetric or symmetric in many systems, it is not yet clear to what extent spindle positioning and cell fate determination can be correlated in the developing vertebrate nervous system (Konno et al., 2008; Morin et al., 2007; Sanada and Tsai, 2005; Zigman et al., 2005). For example, reduction of mInsc led to disruption of spindle orientation and an increase in progenitor cells and neuronal defects in the retina (Zigman et al., 2005), whereas disruption of LGN activity lead to randomization of spindle orientation without disrupting daughter cell fate in the spinal cord neuroepithelium (Morin et al., 2007; see Figure 1). Similar to other systems, factors such as mInsc and LGN play conserved roles in regulating

spindle positioning in the vertebrate nervous system, while components of the N pathway have been found to influence cell fate decisions in this system (Chenn and McConnell, 1995; Petersen et al., 2004; Zhong et al., 1996; Zhong et al., 1997). However, Numb and Numblike may influence cell fate by mechanisms other than inhibition of N (Rasin et al., 2007; Zhou et al., 2007).

5. Conclusions

Investigating asymmetric cell divisions across species and in multiple stem cell systems has provided much insight into the various mechanisms utilized to generate cellular diversity and maintain adult stem cells. Work in invertebrate model systems provided paradigms for how both extrinsic signals and intrinsic factors both act to specify asymmetric divisions. Although there is now evidence that similar mechanisms are used in vertebrates, characterization of stem cells *in vivo*, isolation of pure populations of stem cells, and improvements in real time imaging will all facilitate studies aimed at determining the mechanisms that regulate asymmetric divisions in more complex mammalian stem cell systems.

Furthermore, studies into the mechanisms regulating asymmetric stem cell divisions have emphasized the importance of balancing the number of stem and progenitor cells. Although the correct balance is important during the establishment and maintenance of tissues, tight control of asymmetric divisions will be particularly critical during tissue repair, where an increase in the number of symmetric divisions may be required temporarily to increase the number of stem cells. Chronic injury or inflammation to a tissue might compromise the ability of stem cells to respond appropriately to repair damaged tissues and could eventually lead to the failure of stem cells to switch back to a mode of asymmetric divisions. An unregulated state of tissue repair could easily lead to the selection of stem cells that are resistant to normal growth control signals, a hallmark of cancer cells. Therefore, mechanisms that regulate asymmetric stem cell divisions will likely serve as potent strategies to block cancer initiation in multiple cell types and may provide new targets for anti-cancer therapeutics. Ultimately, identification of the factors involved in regulating adult stem cell behavior will be essential for maintenance and expansion of stem cells in culture, while maintaining the full scope of differentiation potential, as well as the directed differentiation of stem cells into specialized cell types for use in regenerative medicine.

6. Acknowledgements

The authors would like to thank Cecilia D'Alterio for help with the figure and Jones lab members, Terry Lechler, Uli Mueller, Amy Wagers, and an anonymous reviewer for comments on the manuscript. This work was supported by the Ellison Medical Foundation, the American Federation for Aging Research, and the NIH. We apologize to those colleagues whose work could not be referenced directly due to space constraints.

7. References

Albertson, R., and Doe, C.Q. (2003). Dlg, Scrib and Lgl regulate neuroblast cell size and mitotic spindle asymmetry. Nat Cell Biol *5*, 166–170.

Beaucher, M., Goodliffe, J., Hersperger, E., Trunova, S., Frydman, H., and Shearn, A. (2007). Drosophila brain tumor metastases express both neuronal and glial cell type markers. Dev Biol *301*, 287–297.

Bello, B., Reichert, H., and Hirth, F. (2006). The brain tumor gene negatively regulates neural progenitor cell proliferation in the larval central brain of Drosophila. Development *133*, 2639–2648.

Berdnik, D., Torok, T., Gonzalez-Gaitan, M., and Knoblich, J.A. (2002). The endocytic protein alpha-Adaptin is required for numb-mediated asymmetric cell division in Drosophila. Dev Cell *3*, 221–231.

Betschinger, J., Eisenhaber, F., and Knoblich, J.A. (2005). Phosphorylation-induced autoinhibition regulates the cytoskeletal protein Lethal (2) giant larvae. Curr Biol *15*, 276–282.

Betschinger, J., Mechtler, K., and Knoblich, J.A. (2003). The Par complex directs asymmetric cell division by phosphorylating the cytoskeletal protein Lgl. Nature *422*, 326–330.

Betschinger, J., Mechtler, K., and Knoblich, J.A. (2006). Asymmetric segregation of the tumor suppressor brat regulates self-renewal in Drosophila neural stem cells. Cell *124*, 1241–1253.

Blanpain, C., Lowry, W.E., Pasolli, H.A., and Fuchs, E. (2006). Canonical notch signaling functions as a commitment switch in the epidermal lineage. Genes Dev *20*, 3022–3035.

Bowman, S.K., Neumuller, R.A., Novatchkova, M., Du, Q., and Knoblich, J.A. (2006). The Drosophila NuMA Homolog Mud regulates spindle orientation in asymmetric cell division. Dev Cell *10*, 731–742.

Bowman, S.K., Rolland, V., Betschinger, J., Kinsey, K.A., Emery, G., and Knoblich, J.A. (2008). The tumor suppressors Brat and Numb regulate transit-amplifying neuroblast lineages in Drosophila. Dev Cell *14*, 535–546.

Cairns, J. (1975). Mutation selection and the natural history of cancer. Nature *255*, 197–200.

Calvi, L.M., Adams, G.B., Weibrecht, K.W., Weber, J.M., Olson, D.P., Knight, M.C., Martin, R.P., Schipani, E., Divieti, P., and Bringhurst, F.R., et al. (2003). Osteoblastic cells regulate the haematopoietic stem cell niche. Nature *425*, 841–846.

Caussinus, E., and Gonzalez, C. (2005). Induction of tumor growth by altered stem-cell asymmetric division in Drosophila melanogaster. Nat Genet *37*, 1125–1129.

Caussinus, E., and Hirth, F. (2007). Asymmetric stem cell division in development and cancer. Prog Mol Subcell Biol *45*, 205–225.

Cerletti, M., Jurga, S., Witczak, C.A., Hirshman, M.F., Shadrach, J.L., Goodyear, L.J., and Wagers, A.J. (2008). Highly efficient, functional engraftment of skeletal muscle stem cells in dystrophic muscles. Cell *134*, 37–47.

Cheng, J., Turkel, N., Hemati, N., Fuller, M.T., Hunt, A.J., and Yamashita, Y.M. (2008). Centrosome misorientation reduces stem cell division during ageing. Nature.

Chenn, A., and McConnell, S.K. (1995). Cleavage orientation and the asymmetric inheritance of Notch1 immunoreactivity in mammalian neurogenesis. Cell *82*, 631–641.

Chia, W., Somers, W.G., and Wang, H. (2008). Drosophila neuroblast asymmetric divisions: cell cycle regulators, asymmetric protein localization, and tumorigenesis. J Cell Biol *180*, 267–272.

Choksi, S.P., Southall, T.D., Bossing, T., Edoff, K., de Wit, E., Fischer, B.E., van Steensel, B., Micklem, G., and Brand, A.H. (2006). Prospero acts as a binary switch between self-renewal and differentiation in Drosophila neural stem cells. Dev Cell *11*, 775–789.

Conboy, I.M., and Rando, T.A. (2002). The regulation of Notch signaling controls satellite cell activation and cell fate determination in postnatal myogenesis. Dev Cell *3*, 397–409.

Conboy, M.J., Karasov, A.O., and Rando, T.A. (2007). High incidence of non-random template strand segregation and asymmetric fate determination in dividing stem cells and their progeny. PLoS Biol *5*, e102.

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

Doe, C.Q. (2008). Neural stem cells: balancing self-renewal with differentiation. Development *135*, 1575–1587.

Doe, C.Q., Chu-LaGraff, Q., Wright, D.M., and Scott, M.P. (1991). The prospero gene specifies cell fates in the Drosophila central nervous system. Cell *65*, 451–464.

Du, Q., Stukenberg, P.T., and Macara, I.G. (2001). A mammalian Partner of inscuteable binds NuMA and regulates mitotic spindle organization. Nat Cell Biol *3*, 1069–1075.

Duncan, A.W., Rattis, F.M., DiMascio, L.N., Congdon, K.L., Pazianos, G., Zhao, C., Yoon, K., Cook, J.M., Willert, K., Gaiano, N., and Reya, T. (2005). Integration of Notch and Wnt signaling in hematopoietic stem cell maintenance. Nat Immunol *6*, 314–322.

Fleming, H.E., Janzen, V., Lo Celso, C., Guo, J., Leahy, K.M., Kronenberg, H.M., and Scadden, D.T. (2008). Wnt signaling in the niche enforces hematopoietic stem cell quiescence and is necessary to preserve self-renewal in vivo. Cell Stem Cell *2*, 274–283.

Forstemann, K., Tomari, Y., Du, T., Vagin, V.V., Denli, A.M., Bratu, D.P., Klattenhoff, C., Theurkauf, W.E., and Zamore, P.D. (2005). Normal microRNA maturation and germ-line stem cell maintenance requires Loquacious, a double-stranded RNA-binding domain protein. PLoS Biol *3*, e236.

Gonczy, P. (2008). Mechanisms of asymmetric cell division: flies and worms pave the way. Nat Rev Mol Cell Biol *9*, 355–366.

Hatfield, S.D., Shcherbata, H.R., Fischer, K.A., Nakahara, K., Carthew, R.W., and Ruohola-Baker, H. (2005). Stem cell division is regulated by the microRNA pathway. Nature *435*, 974–978.

Hirata, J., Nakagoshi, H., Nabeshima, Y., and Matsuzaki, F. (1995). Asymmetric segregation of the homeodomain protein Prospero during Drosophila development. Nature *377*, 627–630.

Izumi, Y., Ohta, N., Hisata, K., Raabe, T., and Matsuzaki, F. (2006). Drosophila Pins-binding protein Mud regulates spindle-polarity coupling and centrosome organization. Nat Cell Biol *8*, 586–593.

Jin, Z., Kirilly, D., Weng, C., Kawase, E., Song, X., Smith, S., Schwartz, J., and Xie, T. (2008). Differentiation-defective stem cells outcompete normal stem cells for niche occupancy in the Drosophila ovary. Cell Stem Cell *2*, 39–49.

Jones, D.L., and Wagers, A.J. (2008). No place like home: anatomy and function of the stem cell niche. Nat Rev Mol Cell Biol *9*, 11–21.

Kiel, M.J., Yilmaz, O.H., Iwashita, T., Yilmaz, O.H., Terhorst, C., and Morrison, S.J. (2005). SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. Cell *121*, 1109–1121.

Kiger, A.A., Jones, D.L., Schulz, C., Rogers, M.B., and Fuller, M.T. (2001). Stem cell self-renewal specified by JAK-STAT activation in response to a support cell cue. Science *294*, 2542–2545.

Knoblich, J.A., Jan, L.Y., and Jan, Y.N. (1995). Asymmetric segregation of Numb and Prospero during cell division. Nature *377*, 624–627.

Konno, D., Shioi, G., Shitamukai, A., Mori, A., Kiyonari, H., Miyata, T., and Matsuzaki, F. (2008). Neuroepithelial progenitors undergo LGN-dependent planar divisions to maintain self-renewability during mammalian neurogenesis. Nat Cell Biol *10*, 93–101.

Kraut, R., Chia, W., Jan, L.Y., Jan, Y.N., and Knoblich, J.A. (1996). Role of inscuteable in orienting asymmetric cell divisions in Drosophila. Nature *383*, 50–55.

Kuang, S., Kuroda, K., Le Grand, F., and Rudnicki, M.A. (2007). Asymmetric self-renewal and commitment of satellite stem cells in muscle. Cell *129*, 999–1010.

Leatherman, J.L., and Dinardo, S. (2008). Zfh-1 controls somatic stem cell self-renewal in the Drosophila testis and nonautonomously influences germline stem cell self-renewal. Cell Stem Cell *3*, 44–54.

Lechler, T., and Fuchs, E. (2005). Asymmetric cell divisions promote stratification and differentiation of mammalian skin. Nature *437*, 275–280.

Lee, C.Y., Andersen, R.O., Cabernard, C., Manning, L., Tran, K.D., Lanskey, M.J., Bashirullah, A., and Doe, C.Q. (2006). Drosophila Aurora-A kinase inhibits neuroblast self-renewal by regulating aPKC/Numb cortical polarity and spindle orientation. Genes Dev *20*, 3464–3474.

Lee, C.Y., Robinson, K.J., and Doe, C.Q. (2006). Lgl, Pins and aPKC regulate neuroblast self-renewal versus differentiation. Nature *439*, 594–598.

Lee, C.Y., Wilkinson, B.D., Siegrist, S.E., Wharton, R.P., and Doe, C.Q. (2006). Brat is a Miranda cargo protein that promotes neuronal differentiation and inhibits neuroblast self-renewal. Dev Cell *10*, 441–449.

Macara, I.G. (2004). Par proteins: partners in polarization. Curr Biol *14*, R160–162.

Macara, I.G. (2004). Parsing the polarity code. Nat Rev Mol Cell Biol *5*, 220–231.

Mancini, S.J., Mantei, N., Dumortier, A., Suter, U., MacDonald, H.R., and Radtke, F. (2005). Jagged1-dependent Notch signaling is dispensable for hematopoietic stem cell self-renewal and differentiation. Blood *105*, 2340–2342.

Micchelli, C.A., and Perrimon, N. (2006). Evidence that stem cells reside in the adult Drosophila midgut epithelium. Nature *439*, 475–479.

Mills, A.A., Zheng, B., Wang, X.J., Vogel, H., Roop, D.R., and Bradley, A. (1999). p63 is a p53 homologue required for limb and epidermal morphogenesis. Nature *398*, 708–713.

Morin, X., Jaouen, F., and Durbec, P. (2007). Control of planar divisions by the G-protein regulator LGN maintains progenitors in the chick neuroepithelium. Nat Neurosci *10*, 1440–1448.

Neumuller, R.A., Betschinger, J., Fischer, A., Bushati, N., Poernbacher, I., Mechtler, K., Cohen, S.M., and Knoblich, J.A. (2008). Mei-P26 regulates microRNAs and cell growth in the Drosophila ovarian stem cell lineage. Nature *454*, 241–245.

Ohlstein, B., and Spradling, A. (2006). The adult Drosophila posterior midgut is maintained by pluripotent stem cells. Nature *439*, 470–474.

Ohlstein, B., and Spradling, A. (2007). Multipotent Drosophila intestinal stem cells specify daughter cell fates by differential notch signaling. Science *315*, 988–992.

Park, J.K., Liu, X., Strauss, T.J., McKearin, D.M., and Liu, Q. (2007). The miRNA pathway intrinsically controls self-renewal of Drosophila germline stem cells. Curr Biol *17*, 533–538.

Parmentier, M.L., Woods, D., Greig, S., Phan, P.G., Radovic, A., Bryant, P., and O'Kane, C.J. (2000). Rapsynoid/partner of inscuteable controls asymmetric division of larval neuroblasts in Drosophila. J Neurosci *20*, RC84.

Peng, C.Y., Manning, L., Albertson, R., and Doe, C.Q. (2000). The tumour-suppressor genes lgl and dlg regulate basal protein targeting in Drosophila neuroblasts. Nature *408*, 596–600.

Petersen, P.H., Zou, K., Krauss, S., and Zhong, W. (2004). Continuing role for mouse Numb and Numbl in maintaining progenitor cells during cortical neurogenesis. Nat Neurosci *7*, 803–811.

Rasin, M.R., Gazula, V.R., Breunig, J.J., Kwan, K.Y., Johnson, M.B., Liu-Chen, S., Li, H.S., Jan, L.Y., Jan, Y.N., Rakic, P., and Sestan, N. (2007). Numb and Numbl are required for maintenance of cadherin-based adhesion and polarity of neural progenitors. Nat Neurosci *10*, 819–827.

Rebollo, E., Sampaio, P., Januschke, J., Llamazares, S., Varmark, H., and Gonzalez, C. (2007). Functionally unequal centrosomes drive spindle orientation in asymmetrically dividing Drosophila neural stem cells. Dev Cell *12*, 467–474.

Roegiers, F., and Jan, Y.N. (2004). Asymmetric cell division. Curr Opin Cell Biol *16*, 195–205.

Rusan, N.M., and Peifer, M. (2007). A role for a novel centrosome cycle in asymmetric cell division. J Cell Biol *177*, 13–20.

Sacchetti, B., Funari, A., Michienzi, S., Di Cesare, S., Piersanti, S., Saggio, I., Tagliafico, E., Ferrari, S., Robey, P.G., Riminucci, M., and Bianco, P. (2007). Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. Cell *131*, 324–336.

Sanada, K., and Tsai, L.H. (2005). G protein betagamma subunits and AGS3 control spindle orientation and asymmetric cell fate of cerebral cortical progenitors. Cell *122*, 119–131.

Schaefer, M., Shevchenko, A., Shevchenko, A., and Knoblich, J.A. (2000). A protein complex containing Inscuteable and the Galpha-binding protein Pins orients asymmetric cell divisions in Drosophila. Curr Biol *10*, 353–362.

Schmid, A., Chiba, A., and Doe, C.Q. (1999). Clonal analysis of Drosophila embryonic neuroblasts: neural cell types, axon projections and muscle targets. Development *126*, 4653–4689.

Schober, M., Schaefer, M., and Knoblich, J.A. (1999). Bazooka recruits Inscuteable to orient asymmetric cell divisions in Drosophila neuroblasts. Nature *402*, 548–551.

Seery, J.P., and Watt, F.M. (2000). Asymmetric stem-cell divisions define the architecture of human oesophageal epithelium. Curr Biol *10*, 1447–1450.

Senoo, M., Pinto, F., Crum, C.P., and McKeon, F. (2007). p63 Is essential for the proliferative potential of stem cells in stratified epithelia. Cell *129*, 523–536.

Shin, K., Wang, Q., and Margolis, B. (2007). PATJ regulates directional migration of mammalian epithelial cells. EMBO Rep *8*, 158–164.

Shinin, V., Gayraud-Morel, B., Gomes, D., and Tajbakhsh, S. (2006). Asymmetric division and cosegregation of template DNA strands in adult muscle satellite cells. Nat Cell Biol *8*, 677–687.

Siegrist, S.E., and Doe, C.Q. (2005). Microtubule-induced Pins/Galphai cortical polarity in Drosophila neuroblasts. Cell *123*, 1323–1335.

Siller, K.H., Cabernard, C., and Doe, C.Q. (2006). The NuMA-related Mud protein binds Pins and regulates spindle orientation in Drosophila neuroblasts. Nat Cell Biol *8*, 594–600.

Smart, I.H. (1970). Variation in the plane of cell cleavage during the process of stratification in the mouse epidermis. Br J Dermatol *82*, 276–282.

Smith, C.A., Lau, K.M., Rahmani, Z., Dho, S.E., Brothers, G., She, Y.M., Berry, D.M., Bonneil, E., Thibault, P., and Schweisguth, F., et al. (2007). aPKC-mediated phosphorylation regulates asymmetric membrane localization of the cell fate determinant Numb. Embo J *26*, 468–480.

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

Spana, E.P., and Doe, C.Q. (1995). The prospero transcription factor is asymmetrically localized to the cell cortex during neuroblast mitosis in Drosophila. Development *121*, 3187–3195.

Sugiyama, T., Kohara, H., Noda, M., and Nagasawa, T. (2006). Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. Immunity *25*, 977–988.

Suzuki, A., and Ohno, S. (2006). The PAR-aPKC system: lessons in polarity. J Cell Sci *119*, 979–987.

Tio, M., Udolph, G., Yang, X., and Chia, W. (2001). cdc2 links the Drosophila cell cycle and asymmetric division machineries. Nature *409*, 1063–1067.

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

Vaessin, H., Grell, E., Wolff, E., Bier, E., Jan, L.Y., and Jan, Y.N. (1991). Prospero is expressed in neuronal precursors and encodes a nuclear protein that is involved in the control of axonal outgrowth in Drosophila. Cell *67*, 941–953.

Voog, J., D'Alterio, C., and Jones, D.L. (2008). Multipotent somatic stem cells contribute to the stem cell niche in the Drosophila testis. Nature *454*, 1132–1136.

Wang, H., Ouyang, Y., Somers, W.G., Chia, W., and Lu, B. (2007). Polo inhibits progenitor self-renewal and regulates Numb asymmetry by phosphorylating Pon. Nature *449*, 96–100.

Wang, H., Somers, G.W., Bashirullah, A., Heberlein, U., Yu, F., and Chia, W. (2006). Aurora-A acts as a tumor suppressor and regulates self-renewal of Drosophila neuroblasts. Genes Dev *20*, 3453–3463.

Wirtz-Peitz, F., Nishimura, T., and Knoblich, J. A. (2008). Linking cell cycle to asymmetric division: Aurora-A phosphorylates the Par complex to regulate Numb localization. Cell *135*, 161–173.

Wu, M., Kwon, H.Y., Rattis, F., Blum, J., Zhao, C., Ashkenazi, R., Jackson, T.L., Gaiano, N., Oliver, T., and Reya, T. (2007). Imaging hematopoietic precursor division in real time. Cell Stem Cell *1*, 541–554.

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

Yamashita, Y., Jones, D.L., and Fuller, M.T. (2003). Orientation of asymmetric stem cell division by the APC tumor suppressor and centrosome. Science *301*, 1547–1550.

Yamashita, Y.M., Fuller, M.T., and Jones, D.L. (2005). Signaling in stem cell niches: lessons from the Drosophila germline. J Cell Sci *118*, 665–672.

Yamashita, Y.M., Mahowald, A.P., Perlin, J.R., and Fuller, M.T. (2007). Asymmetric inheritance of mother versus daughter centrosome in stem cell division. Science *315*, 518–521.

Yang, A., Schweitzer, R., Sun, D., Kaghad, M., Walker, N., Bronson, R.T., Tabin, C., Sharpe, A., Caput, D., Crum, C., and McKeon, F. (1999). p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development. Nature *398*, 714–718.

Yu, F., Kuo, C.T., and Jan, Y.N. (2006). Drosophila neuroblast asymmetric cell division: recent advances and implications for stem cell biology. Neuron *51*, 13–20.

Yu, F., Morin, X., Cai, Y., Yang, X., and Chia, W. (2000). Analysis of partner of inscuteable, a novel player of Drosophila asymmetric divisions, reveals two distinct steps in inscuteable apical localization. Cell *100*, 399–409.

Zhang, J., Niu, C., Ye, L., Huang, H., He, X., Tong, W.G., Ross, J., Haug, J., Johnson, T., and Feng, J.Q., et al. (2003). Identification of the haematopoietic stem cell niche and control of the niche size. Nature *425*, 836–841.

Zhong, W., Feder, J.N., Jiang, M.M., Jan, L.Y., and Jan, Y.N. (1996). Asymmetric localization of a mammalian numb homolog during mouse cortical neurogenesis. Neuron *17*, 43–53.

Zhong, W., Jiang, M.M., Weinmaster, G., Jan, L.Y., and Jan, Y.N. (1997). Differential expression of mammalian Numb, Numblike and Notch1 suggests distinct roles during mouse cortical neurogenesis. Development *124*, 1887–1897.

Zhou, Y., Atkins, J.B., Rompani, S.B., Bancescu, D.L., Petersen, P.H., Tang, H., Zou, K., Stewart, S.B., and Zhong, W. (2007). The mammalian Golgi regulates numb signaling in asymmetric cell division by releasing ACBD3 during mitosis. Cell *129*, 163–178.

Zigman, M., Cayouette, M., Charalambous, C., Schleiffer, A., Hoeller, O., Dunican, D., McCudden, C.R., Firnberg, N., Barres, B.A., Siderovski, D.P., and Knoblich, J.A. (2005). Mammalian inscuteable regulates spindle orientation and cell fate in the developing retina. Neuron *48*, 539–545.