Cell Stem Cell
Perspective

Asymmetric Stem Cell Division: Precision for Robustness

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Asymmetric cell division (ACD) produces two daughter cells with distinct fates or characteristics. Many adult stem cells use ACD as a means of maintaining stem cell number and thus tissue homeostasis. Here, we review recent progress on ACD, discussing conservation between stem and non-stem cell systems, molecular mechanisms, and the biological meaning of ACD.

Introduction

Asymmetric stem cell division is a mechanism that balances stem cell self-renewal and differentiation through the production of one stem cell and one differentiating cell. It is a simple way of maintaining the stem cell population without increasing it and is thus thought to be a vital mechanism for tissue homeostasis and tumor suppression. Recent studies have revealed that many stem cells have the capacity to divide asymmetrically, whereas other stem cell populations are proposed to divide symmetrically with the decision of self-renewal versus differentiation being determined stochastically. The molecular machineries that achieve asymmetric stem cell division are remarkably conserved among many stem cell populations and with many other non-stem cell systems that divide asymmetrically. Here we summarize recent progress in asymmetric stem cell division and discuss its implications. To facilitate new lines of perspective, readers will be directed to appropriate reviews for discussion on recurring themes.

Asymmetric Cell Division Is a Conserved Feature of Essentially All Organisms

Although asymmetric cell division (ACD) has been studied extensively in the context of developmental biology and stem cell biology, ACD is not unique to stem cells, to multicellular organisms, or even to eukaryotes. In fact, bacterial and yeast cells divide asymmetrically, as do many cells in developing embryos. The realization that ACD is not a unique feature of multicellular organisms provides us with a novel perspective on a few points. (1) Did cellular asymmetry arise as a strategy for cells to divide asymmetrically? (2) Is some level of asymmetry inevitable in any cell division? (3) If so, was such inherent asymmetry utilized to achieve ''intended'' asymmetry later during evolution?

Many bacteria species are known to divide asymmetrically, with the most prominent example being *Caulobacter crescentus*. *Caulobacter* develops a stalk that attaches to the substrata, such as the surface of a plant, prior to DNA replication. Upon division, the ''mother'' retains this stalk and remains in place, while the ''daughter'' develops flagella to swim away from the stalked mother cell (Figure 1A) (Goley et al., 2009; Laub et al., 2007). Asymmetric division in *Caulobacter* is associated with a number of asymmetries that differentiate the mother cell from the daughter, including distinct cell cycle progression and subcellular architecture. In *Mycobacterium*, the cell that inherits the old pole upon division has a growth advantage over the one that inherited the new pole, leading to deterministic heterogeneity in elongation rate. It has been shown that *Caulobacter* and *E. coli* age in a similar way to yeast cells (described below), producing mother cells that gradually ''age'' with decreased growth/division rates and increased incidence of death (Ackermann et al., 2003; Stewart et al., 2005). Such studies clearly demonstrate that even unicellular organisms divide asymmetrically.

Interesting questions arise as to whether such asymmetry has evolved to achieve a ''good'' outcome (e.g. one daughter cell inheriting something that confers advantages to it) or to avoid a ''bad'' outcome (e.g. harmful materials being excluded from one cell by sacrifice of the other cell). On one hand, in *Caulobactor*, the cell cycles and fates of the two daughters are differentially regulated through the asymmetric activation of distinct transcription factors. This suggests that asymmetric division in *Caulobactor* is, at least in part, for segregation of ''the good.'' However, aging of the mother cell in *E. coli* as a result of inheritance of protein aggregates (Lindner et al., 2008) suggests that the asymmetry also functions to exclude ''the bad'' and protect the cell from harmful materials. While the perception of ''good'' versus ''bad'' can be vague or confusing, this distinction is important when speculating how ACD has evolved and will be revisited several times in this Perspective.

Budding yeast, *Saccharomyces cerevisiae*, is a unicellular eukaryote that divides asymmetrically (Figure 1B): the mother cell is larger, older, and able to switch mating type, while the daughter is smaller, rejuvenated, and unable to switch mating type. Ash1, a repressor of expression of the HO endonuclease required for mating type switching, is restricted to the bud (daughter) cell, leading to mother-cell-specific HO expression (Amon, 1996). Asymmetric localization of Ash1 protein to the bud cell nucleus is achieved by polarized localization of Ash1 mRNA through the function of actin and myosin (Long et al., 1997). In addition to the ''fate determinant'' Ash1, budding yeast also segregate other factors asymmetrically, many of which are suspected to cause aging. Factors that are known or suspected to cause aging in yeast cells include: (1) extrachromosomal

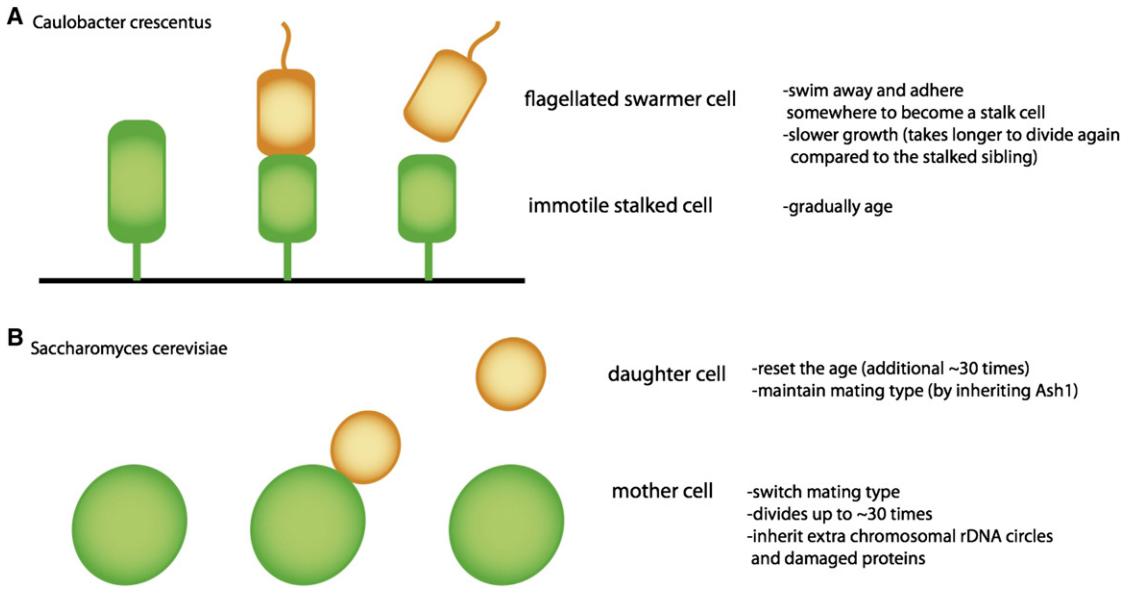

Figure 1. Asymmetric Division of Unicellular Organisms

(A) ACD of *Caulobacter crecentus*. The stalked cell stays attached to the substrata, whereas its daughter develops flagella to swim away from the mother. The daughter eventually attaches to the substrata by developing a stalk to repeat the cycle.

(B) ACD of *Saccharomyces cerevisiae*. The daughter cell buds off from the mother cell. The daughter cell asymmetrically inherits Ash1, which suppress the expression of HO endonuclease, thereby suppressing mating type switching, whereas the mother cell undergoes mating type switching due to the lack of Ash1. The mother cell can typically divide up to 30 times. The newborn daughter is rejuvenated and can divide approximately another 30 times. Toward the end of the mother's life, the division becomes more symmetric, and the daughter cells are not as rejuvenated as those born at earlier divisions but are born somewhat aged. Sporulation (gametogenesis) can reset the aging process (Unal et al., 2011).

rDNA circles (ERCs), which result from intrachromosomal recombination at the rDNA locus and are confined to the mother cell; and (2) damaged proteins such as carbonylated proteins and protein aggregates, which are known to segregate into the mother cells (Henderson and Gottschling, 2008).

In multicellular organisms, the significance of asymmetric division is more profound. Whereas failure of ACD in yeast or bacteria may yield organisms less fitted (such as daughter cells with a shorter lifespan), a failure in ACD during early development of multicellular organisms will probably yield no organisms at all (death prior to birth). The best-studied example of asymmetric division in non-stem cells is found in zygotic development of *Caenorhabditis elegans*, in which a series of ACDs governed by evolutionarily conserved Par genes create diverse cell types of the organism (Gonczy and Rose, 2005; Munro and Bowerman, 2009). The molecular mechanisms used in this system are widely conserved in many other asymmetrically dividing cells, as well as in cellular polarization processes in a broad range of systems without being necessarily linked to ACD (St Johnston and Ahringer, 2010).

Examples of Asymmetric Stem Cell Divisions

As described above, ACD is not an invention of stem cells, and the molecular players governing ACD of stem cells and non-stem cells are strikingly similar. In recent years, however, interest in ACD in the context of stem cell biology has increased considerably due to its contribution to tissue homeostasis through the balancing of stem cell self-renewal and differentiation.

The fundamental mechanisms underlying ACD are largely divided into two types, intrinsic regulation and extrinsic regulation, although the distinction between these is not necessarily straightforward. In "intrinsic" mechanisms, the fate determinants are asymmetrically segregated into two daughter cells, leading to asymmetric outcome of the division. In "extrinsic" mechanisms, two daughters of the division are placed in distinct microenvironments that confer distinct fates. In both cases, the oriented mitotic spindle with respect to fate-determining factors/components is often utilized to achieve an asymmetric outcome of the division.

Without doubt, the best-studied example of such stem cells are *Drosophila* neuroblasts (Figure 2A). Type I neuroblasts divide asymmetrically to give rise to another neuroblast and a ganglion mother cell (GMC), which divides once more to generate neurons. Type II neuroblasts also divide asymmetrically in a very similar manner to type I neuroblasts, but generate transit-amplifying populations that increase the number of neurons per neuroblast division (Bello et al., 2008; Boone and Doe, 2008; Bowman et al., 2008). The mitotic spindle of the dividing neuroblast aligns along the polarity axis determined by Baz(Par-3)/Pa-r6/aPKC and Pins/Gαi complexes, which asymmetrically localize to the "apical" cortex of the neuroblast (Knoblich, 2008; Morin and Bellaïche, 2011; Siller and Doe, 2009; Yu et al., 2006). The spindle orientation dictated by these complexes in turn compartmentalizes fate-determining molecules such as Numb and Miranda. Inheritance of Numb biases the response of the daughter cells to Notch signaling, whereas Miranda functions as a scaffolding protein that segregates the fate determinants Prospero, Brat, and Staufen proteins into GMCs. Prospero is a transcription factor responsible for the expression of GMC genes, and Brat is a translational repressor

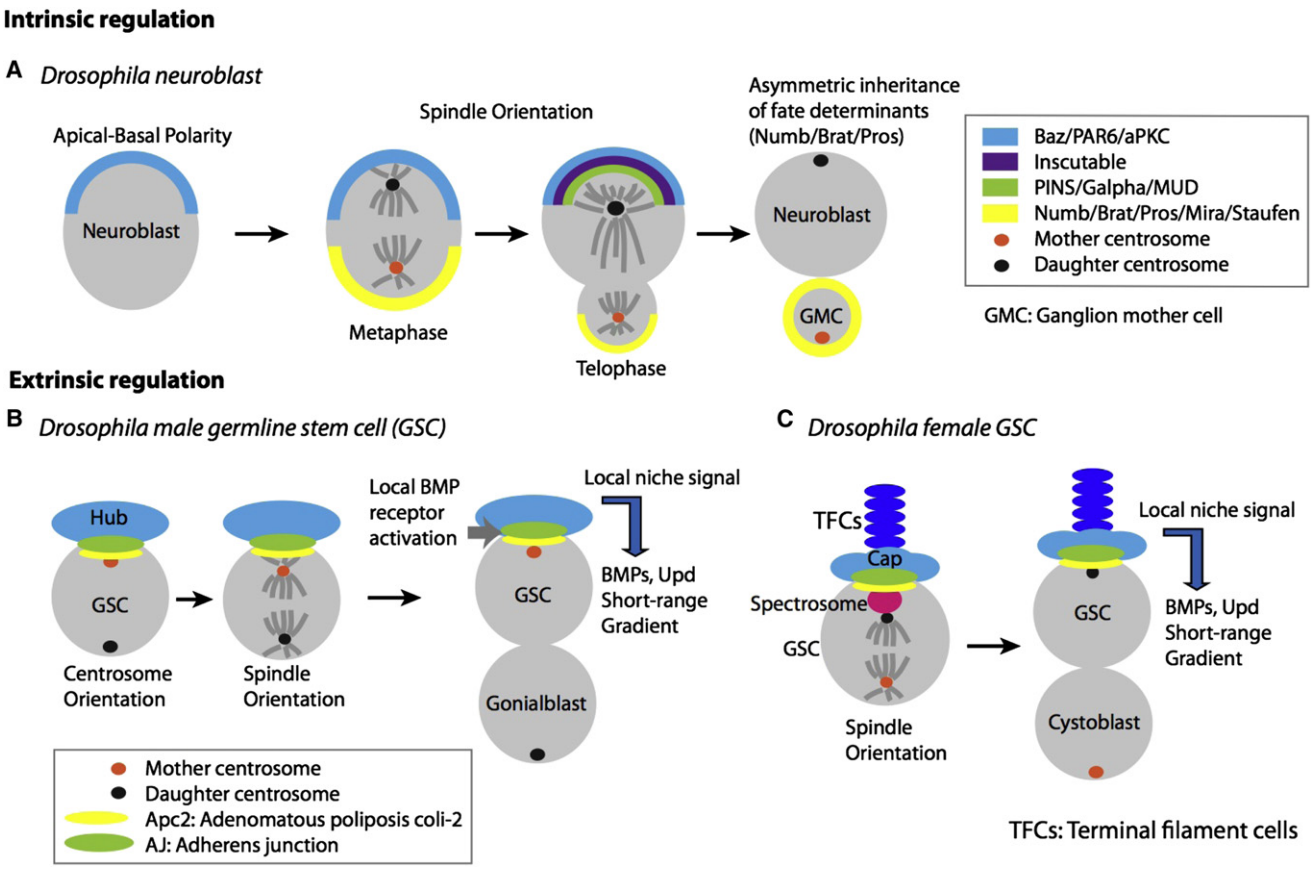

Figure 2. Asymmetric Stem Cell Division in *Drosophila*

(A) ACD of *Drosophila* neuroblast. The Baz/Par-6/aPKC complex polarizes the cell, and the PINS/Ga/MUD(NuMA) complex orients the spindle. Basally localized proteins such as Numb, Brad, Prospero (Pros), Staufen, and Miranda contribute to fate asymmetry (see text for detail). Neuroblasts inherit the daughter centrosome upon division.

(B) ACD of *Drosophila* male GSCs. Hub cells and cyst stem cells (not shown in the diagram) constitute the male GSC niche. Local signaling of Upd and BMP ligands creates the niche space. Male GSCs adhere to the hub cells through adherens junctions, toward which the spindle is oriented. The mother centrosome is inherited by GSCs upon division.

(C) ACD of *Drosophila* female GSCs. Cap cells and terminal filament cells constitute the female GSC niche. Local signaling of Upd and BMP ligands creates the niche space. Female GSCs adhere to the cap cells through adherens junctions. The spectrosome and the centrosome are oriented toward the adherens junction. The daughter centrosome is inherited by GSCs upon division.

that is required for suppression of neuroblast identity in GMCs, thereby promoting differentiation. Staufen is an mRNA binding protein that is responsible for segregating Prospero mRNA, reinforcing asymmetric fates between the neuroblast and GMC. Two reports in this issue of *Cell Stem Cell* show that a mouse homolog of Staufen, Staufen2, is asymmetrically segregated during asymmetric division of neural stem cells to confer asymmetric cell fates in a manner similar to *Drosophila* Staufen, thus promoting neural differentiation (Figure 3A) (Kusek et al., 2012; Vessey et al., 2012). These studies further identified cargo mRNAs for Staufen2, such as Trim32, Prox1, Pumilio 2, and DDX1, providing further insights into how Staufen might contribute to fate determination.

The best-studied examples of extrinsic regulation of (or microenvironment/niche-dependent) asymmetric stem cell division are the male and female germline stem cells (GSCs) of *Drosophila* (Figure 2B). At the apical tip of the gonad, hub cells (in testis) and cap cells and terminal filament cells (in ovary) contribute to the creation of a stem cell niche by providing short-range signals. These niche cells secrete the ligand Upd, which activates the JAK-STAT pathway, and the ligand Dpp, which activates bone morphogenetic protein (BMP) signaling. Although it was once thought that Upd has a dominant role in the male niche and Dpp has a dominant role in the female niche, recent studies suggest that both contribute significantly to the maintenance of the GSCs in both sexes (Decotto and Spradling, 2005; Leatherman and Dinardo, 2010; Zheng et al., 2011). GSCs align their spindle perpendicularly toward the hub or cap cells, placing one daughter cell in direct contact with the niche while displacing the other daughter one cell diameter away from the niche. This results in fate asymmetry between two daughter cells (self-renewal versus differentiation). In the case of niche-dependent asymmetric stem cell division, the niche component automatically provides a "reference point" for spindle orientation, in addition to providing self-renewing factors. Indeed, adherens junctions containing E-cadherin (Song and Xie, 2002; Yamashita et al., 2003) are formed between both male and female GSCs and their niche cells (hub cells and cap cells, respectively): while

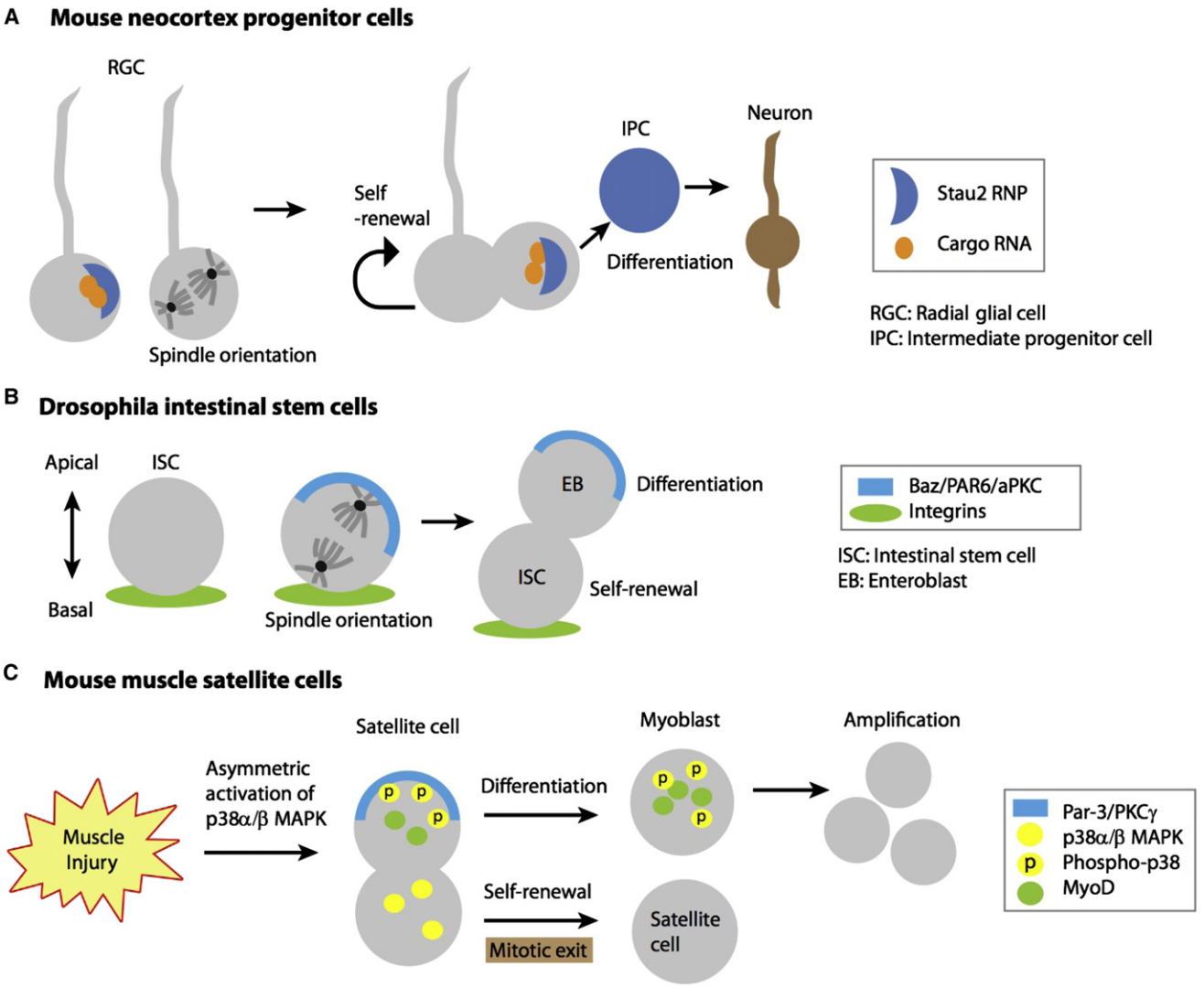

Figure 3. Recently Discovered Examples of Asymmetric Stem Cell Division

(A) Mouse radial glial progenitor cells (RGCs) divide asymmetrically. The Par complex is involved in ACD, and the mother centrosome is inherited by the RGC. Recent reports show the involvement of Staufen2 in the segregation of fate-determining mRNAs into differentiating cells.

(B) *Drosophila* intestinal stem cells divide asymmetrically through activity of the Par complex (Baz/Par-6/aPKC), which is linked to the basement membrane via integrins.

(C) Mouse satellite cells divide asymmetrically through the Par complex, which dictates asymmetric activation of the p38α/β MAPK.

the adherens junction reinforces the niche signaling through attachment of stem cells to the niche cells, it probably also provides the cue for cell polarity (see below).

Together, these studies have provided a framework for asymmetric stem cell division, in which fate-determining factors (intrinsic or extrinsic) are partitioned into two daughter cells asymmetrically through programmed orientation of the mitotic spindle.

Mechanisms of Cortical Polarization

The most upstream event in ACD appears to be polarization of the cell. Irrespective of whether ACD relies on extrinsic or intrinsic determinants, cells need to polarize the cell cortex such that the division plane is specified in a way that yields unequal daughter cells. This cortical polarization can then be used to create further asymmetry by localizing fate determinants and/or by specifying the division plane, which is often achieved by specifying the mitotic spindle orientation. In cases where ACD relies on extrinsic information such as the stem cell niche, the interface between the stem cells and the niche components can easily provide a specialized cortical area toward which cells can polarize.

In cases of ACD that rely on intrinsic fate determinants, cells have no reference point in relation to tissue architecture. This seems to be the case for the *Drosophila* larval neuroblasts, where intrinsic fate determinants are the major (if not sole) factors that instruct fate asymmetry. Indeed larval neuroblasts do not appear to have particular polarity with respect to the tissue yet maintain their cortical polarity through "memory" of the division plane from the last mitosis, provided by the astral

microtubules (Januschke and Gonzalez, 2010). This suggests that cells are capable of creating and maintaining cortical polarity even when there is no reference point from extracellular structures. In the case of *Drosophila* embryonic neuroblasts, the cells appear to orient toward the cell-cell junction provided by neuroepithelial cells (Siegrist and Doe, 2006), suggesting that cells that undergo ACD primarily through intrinsic fate determinants may still use extrinsic cues (if present) as an additional guide. However, other studies have strikingly shown that the activation of Lkb1 (a homolog of *C. elegans* Par-4) is sufficient to polarize mouse intestinal cells that are cultured in isolation (Baas et al., 2004), suggesting that cells have the potential to intrinsically polarize the cell cortex in preparation for asymmetric ACD, even in the complete absence of extrinsic polarity cues.

Mechanisms of Oriented Cell Division

In animal cells, the division plane is almost exclusively determined by the spindle orientation. Once cortical polarity is determined as described above, the specialized cell cortex can dictate the orientation of division by attracting a spindle pole or the centrosome. The basic mechanisms of spindle orientation are conserved in stem and non-stem populations and extensive studies in *Drosophila* neuroblast (stem cell) and *C. elegans* zygotes (non-stem cell), as well as comparison between these two systems, have contributed tremendously to our understanding of spindle orientation mechanisms. Because the molecular mechanisms of spindle orientation during ACD have been thoroughly covered in many recent reviews, we will only briefly summarize the common theme here. In both *Drosophila* neuroblasts and *C. elegans* zygotes, the evolutionarily conserved ''polarity cassette'' consisting of Par-3(Baz)/Par-6/aPKC plays a major role in polarizing the cells (Cowan and Hyman, 2004; Lesage et al., 2010; Morin and Bellaïche, 2011). The Par complex plays a crucial role in orchestrating spindle orientation and cortical polarization through its localization to the cell cortex in a polarized manner as a crescent. This polarization can in turn create a complementary cortical domain opposite the Par complex crescent through a mutual exclusion process. For example, in *Drosophila* neuroblasts, aPKC phosphorylates Lgl protein to exclude it to the opposite side of the cell, thus promoting differentiation (Betschinger et al., 2003). A new study in this issue of *Cell Stem Cell* indicates that a similar mechanism of spindle orientation operates in the asymmetric division of *Drosophila* intestinal stem cells (Figure 3B) (Goulas et al., 2012). In intestinal stem cells, the cortical polarization of Par-3(Baz)/Par-6/aPKC and the spindle orientation are placed in the new context of tissue polarity of intestinal epithelium, where intestinal stem cells attach to the basement membrane via integrin. Although this study reveals the evolutionarily conserved nature of the Par complex in ACD, it contrasts with the fate determining mechanism employed by mouse intestinal stem cells, where neutral competition of symmetrically dividing stem cells appears to maintain the stem cell population (Snippert et al., 2010). It is also interesting to note that *Drosophila* intestinal stem cells do not undergo obligative ACD but can divide symmetrically or asymmetrically (Goulas et al., 2012; O'Brien et al., 2011), which might provide flexibility in fate determination depending on changing demands on adult tissues.

It is unknown whether the Baz/Par-6/aPKC complex plays a role in spindle orientation in male and female GSCs, although Baz was shown to localize at the male GSC-hub interface (Leatherman and Dinardo, 2010). Male and female GSCs orient their mitotic spindles toward the hub or cap cells, respectively. In male GSCs, the stereotypical positioning of the mother and daughter centrosomes during interface prepares the orientation of the mitotic spindle (Figure 2B) (Yamashita et al., 2003, 2007). Adherens junctions formed between the hub and GSCs function as a reference point to which the mother centrosome anchors (Inaba et al., 2010). E-cadherin at the adherens junction functions at least in part through recruitment of Apc2, which connects the adherens junction to the centrosome via microtubules. Female GSCs are also anchored to the cap cells through adherens junctions (Figure 2C) (Song and Xie, 2002). In female GSCs an apically localized spectrosome, an ER-like membranous organelle, anchors the mitotic spindle (Deng and Lin, 1997). However, a recent report also shows stereotypical positioning of the centrosome that involves the function of Rac and Apc2 (Lu et al., 2012), suggesting a greater similarity between male and female GSCs than previously appreciated.

There is now ample evidence that many other stem cells utilize similar mechanisms to divide asymmetrically. In mouse epidermal stem cells, mitotic spindles are oriented either parallel or perpendicular to the basal membrane, resulting in symmetric stem cell expansion or asymmetric stem cell division (Lechler and Fuchs, 2005; Williams et al., 2011). This process involves the Par complex for cell polarization and the NuMA-dynein complex for spindle orientation. Involvement of the Par complex in asymmetric stem cell division is also conserved in mouse neural progenitor cells (Bultje et al., 2009; Costa et al., 2008). Furthermore, satellite cells, stem cells for muscle repair, were shown to utilize the Par complex for asymmetric division through asymmetric activation of p38α/β MAPK (Figure 3C) (Troy et al., 2012). Upon injury, satellite cells divide asymmetrically, during which Par-complex-mediated asymmetric activation of p38α/β MAPK and MyoD allows only one daughter to continue cell division to repair the muscle, while the other daughter retreats back into quiescence, thus preserving the satellite cell population. Together, these studies highlight the conserved nature of ACD and spindle orientation throughout evolution.

Asymmetric Segregation of Fate Determinants, Garbage, and Beyond

While the machinery that achieves ACD is largely conserved as described above, the actual carrier of information that results in asymmetric fates through its asymmetric segregation may vary from system to system. Many examples of asymmetrically segregated materials have been reported to date. Among them, clear fate determinants such as Prospero, Brat, and Numb are the easiest to understand with respect to how they contribute to ACD.

In addition, some asymmetrically dividing stem cells have been shown to segregate the centrosome asymmetrically. In such cases, either the mother or daughter centrosome is segregated stereotypically into the stem cells upon division. For example, in *Drosophila* male GSCs and mouse radial glial progenitor cells, the mother centrosomes are consistently retained by the stem cells upon division (Wang et al., 2009;

Yamashita et al., 2007). In contrast, in *Drosophila* neuroblasts, the daughter centrosome is consistently inherited by the stem cells (Conduit and Raff, 2010; Januschke et al., 2011). Unlike the asymmetric segregation of clear fate determinants, the contribution of asymmetric centrosome segregation to asymmetric fates is unclear. Nonetheless, in NIH 3T3 cell culture system, the cell that contains the mother centrosome has been shown to develop primary cilia faster than the daughter-centrosome-containing cell, leading to different sensitivity to Hh signaling (Anderson and Stearns, 2009). This suggests that the centrosome asymmetry might directly influence cell fates.

Furthermore, it has been speculated that the mother centrosome might anchor certain strands of the sister chromatids, resulting in their nonrandom segregation into daughter cells (Tajbakhsh and Gonzalez, 2009). This possibility has been interpreted in two ways: either segregation of error-free template strand or segregation of distinct epigenetic information. The so-called "immortal strand hypothesis" proposes that stem cells may retain template DNA strands to avoid the accumulation of replication-induced mutations. This is a highly controversial topic within the field, with seemingly opposing reports even in the same cell types (Lansdorp, 2007; Rando, 2007; Tajbakhsh, 2008). Although further investigations will be required to settle this issue, the exclusion of error-containing DNA strands into the differentiating daughter reflects the idea of segregating "the bad." On the contrary, the asymmetric sister chromatid segregation as a means of segregating distinct epigenetic information reflects the idea of segregating "the good," positively influencing the cell fate. Other "bad" things such as protein aggregates have been reported to segregate asymmetrically during stem cell division in association with the centrosome (Rujano et al., 2006) (see below).

Recent studies revealed another interesting asymmetry during cell division. In some cell types, the midbody ring, the remnant of the contractile ring, is inherited by one daughter of the division and excluded from the other daughter (Gromley et al., 2005). In recent studies, such midbody ring inheritance was correlated to cell fates during stem cell division and the age of the centrosome that the cell inherits (Ettinger et al., 2011; Kuo et al., 2011). Because the midbody ring is a remnant of the contractile ring, it has been postulated to be "garbage" of cell division. However, the precisely determined mode of midbody ring inheritance in certain stem cell populations implies that it is regulated for some purpose(s). It is possible that the midbody ring is indeed garbage, and cells are carefully removing their garbage so that it is excluded from the cells that should be most protected. Alternatively, the midbody ring might be a carrier of molecules that determine or modulate cell behavior. These two ideas are not necessarily mutually exclusive, since midbody inheritance might have evolved as a mechanism of removing the garbage, but could subsequently have acquired an additional function to carry useful information.

Fine-Tuning the Asymmetric Outcome

In niche-dependent (i.e., extrinsic cue-dependent) asymmetric stem cell division, the asymmetric outcome relies on asymmetric placement of the daughter cells into two distinct microenvironments, one that supports self-renewal and another that promotes differentiation. Therefore, defining the niche space is of paramount importance for understanding ACD. Although the niche signal has long been regarded as "local," recent studies of the *Drosophila* GSC niche have begun to elucidate how such localness is achieved. For example, localized distribution of the heparin sulfate glycoproteins Dally and Dally-like was shown to define the niche space by regulating the distribution of niche-derived BMP ligands (Dpp or Dpp/Gbb heterodimers) (Hayashi et al., 2009). Similarly, Dally's potential interaction partner, magu, was also shown to regulate the Dpp gradient (Zheng et al., 2011). Consistent with the localness of Dpp signaling, Tkv activation was shown to be limited to the hub-GSC interface using an activation sensor system (Michel et al., 2011). Although BMP ligands are known to be long-range morphogens in both imaginal discs and embryos, the range of these factors could be tightly limited to create a niche of just a single cell diameter's width.

Another issue in fine-tuning the ACD is how oriented division is ensured. Since the niche space can be as narrow as one cell diameter, the placement of daughter cells in the right microenvironments requires fine precision. While the molecular mechanisms that achieve ACD have been intensively investigated, little is known about what happens if those mechanisms fail for any reason. It is well established that the succession of events during the cell division cycle is ensured by various checkpoint mechanisms. The checkpoint mechanism that specifically monitors the correct spindle orientation has been most intensively studied in budding yeast, where it is termed the "spindle position checkpoint (SPOC)" (Caydasi et al., 2010; Pereira and Yamashita, 2011). Stem cells also appear to have such a mechanism that is dedicated to ensuring asymmetric outcome of the division. As described above, spindle orientation of *Drosophila* male GSCs is predetermined during interphase by stereotypical positioning of the centrosomes (Yamashita et al., 2003, 2007). GSCs without stereotypical centrosome positioning do not enter mitosis until the correct centrosome positioning is reacquired, suggesting the presence of a checkpoint that monitors centrosome positioning before entry into mitosis (Cheng et al., 2008; Yuan et al., 2012). In dividing *Drosophila* neuroblasts, incorrect spindle orientation with respect to the localization of fate determinants in metaphase is typically corrected through repolarization of the cortex by the time cells reach telophase ("telophase rescue"), often resulting in a normal asymmetric outcome (Lu et al., 1998; Peng et al., 2000; Schober et al., 1999; Wodarz et al., 1999). Although it is currently unclear whether metaphase neuroblasts with incorrect spindle orientation arrest in the cell cycle to correct the polarity, the existence of telophase rescue strongly suggests that neuroblasts also possess a checkpoint to ensure asymmetric outcome. It is to yet be determined whether the checkpoint mechanisms in *Drosophila* male GSCs and neuroblasts are similar to each other and/or to SPOC in the budding yeast.

Some Stem Cells Divide Symmetrically—or Do They?

Certain stem cells are thought to divide symmetrically. Such symmetric division would be critically important when expansion of the stem cell population is needed, such as when tissues increase in size during development or after injury. For example, using live observation and lineage tracing experiments, mouse spermatogonial stem cells were convincingly

shown to undergo symmetric divisions, with stochastic fate decision between self-renewal and differentiation (Klein et al., 2010; Nakagawa et al., 2007, 2010). Similarly, mouse intestinal stem cells were shown to undergo stochastic self-renewal of Lgr5+ stem cells based on neutral competition (Snippert et al., 2010). However, it was recently proposed that Bmi1+ expressing stem cells might be upstream of Lgr5+ stem cells in the hierarchy (Tian et al., 2011), and thus it is possible that Bmi1+ stem cells are dividing asymmetrically to give rise to Lgr5+ stem cells that divide symmetrically. Using short-term lineage tracing, Poulson and Lechler (2010) confirmed that mouse epidermal stem cells undergo both symmetric and asymmetric stem cell divisions. A recent report on esophageal progenitors further added an example of progenitor cells switching between asymmetric and symmetric divisions (Doupé et al., 2012).

There is now ample evidence that stem cells undergo symmetric stem cell division. However, as described above, there are cellular asymmetries inherent to any cell division, such as asymmetries in centrosome age, midbody inheritance, and chromosome strands. Although such inherent asymmetry may not confer any phenotypical asymmetry to stem cells, it is worth speculating how symmetric a "symmetric division" may actually be. Also, it is possible that symmetrically divided cells may acquire distinct fates later on (thus ultimately resulting in ACD) due to stochastic decision or influence from complex tissue contexts. Taken together, the definitions of asymmetric versus symmetric division may not be straightforward, and we have to be aware of the possibility that each decision may involve complex combinations of many factors.

It is easy to envision symmetric stem cell division in which "the good," such as fate determinants, is equally partitioned into two daughter cells. The cell that is undergoing symmetric division could adjust the division plane such that both daughters inherit the self-renewing factors. In *Drosophila* neuroblasts of a spindle orientation mutant, the ratio of opposing fate determinants (self-renewing versus differentiating) was found to determine the fate of daughter cells, and thus spindle orientation with respect to polarization of fate determinants is sufficient to determine asymmetric versus symmetric division (Cabernard and Doe, 2009). However, in a mutant background, this clearly points to a potential mechanism for how stem cells might handle the choice of symmetric versus asymmetric division. In the case of mouse neural progenitors, the symmetry versus asymmetry choice appears to be precisely and temporally regulated. During early development, neural epithelial cells divide symmetrically to expand the pool size. Then, at around embryonic days E10–11, these cells become radial glial progenitor cells and switch to asymmetric division to yield one progenitor and one differentiating cell. During division of these cells, the stem cell marker CD133 is associated with the midbody. When cells are dividing symmetrically as neural epithelial cells, the midbody containing CD133 is released into the extracellular fluid, whereas asymmetrically dividing neural progenitors retain the midbody (Farkas and Huttner, 2008). It was recently shown that the capacity for midbody release correlates highly with stemness (Ettinger et al., 2011), implying that the midbody-associated factor or factors indeed contribute to creating asymmetry, and that this is prevented by release of the midbody when cells are symmetrically dividing.

In contrast to segregation of "the good," segregation of "the bad," such as damaged proteins or mutated DNA strands, is not fully compatible with the idea of symmetric stem cell divisions. As described above, many cells have been reported to asymmetrically segregate "the bad," thus protecting one cell at the expense of the other. But when a stem cell divides symmetrically, where do those "bad" things go? One possibility is that the protein aggregates are still segregated asymmetrically into one cell, in which case the daughter cell that inherited the protein aggregates would have a shorter lifespan and might not be able to continue self-renewal as many times as its sibling. If so, even if the "symmetrically" divided stem cells behave similarly for a while, the division is not genuinely symmetric in that one daughter is destined for earlier death than the other. A second possibility is that the protein aggregates are now segregated symmetrically, in which case both daughters would age somewhat by dividing symmetrically, and thus this is not a really "self-renewing" division. In yeast, if asymmetric segregation of ERCs is perturbed, both the mother and bud cells age equally, shortening the lifespan of the newly born daughter. It is possible that symmetric stem cell division is a mechanism that responds to a profound need to increase stem cell number (such as after injury) at the expense of complete self-renewal of the stem cell characteristics. If so, symmetric division might not be as mighty as previously thought. The third possibility is that the symmetrically dividing stem cells can actually remove protein aggregates, so that both daughters completely rejuvenate after the division. However, this would raise a further question: if cells have the ability to remove "the bad" at all, why don't they do so all the time? This further leads to the question of whether "the bad" is really bad. It is possible that the protein aggregates are actually used to count the number of cell divisions so that non-stem cells would not divide too many times. In that sense, segregation of the bad is actually good in terms of controlling the number of cell divisions. Currently, there is no experimental evidence supporting any one of these possibilities over the others, revealing the fact that we are far from understanding nature's strategies and the purpose of ACD. Problems in addressing these questions partly lie in our lack of ability to identify potential asymmetries in seemingly symmetrically dividing stem cells. Even if both daughters of a stem cell division express stem cell markers and divide a few times in a stem-cell-like manner, it does not exclude the possibility that one daughter is less proficient as a stem cell than the other over the long term.

Concluding Remarks

Asymmetric stem cell division is a carefully regulated process with a fundamental importance in tissue homeostasis. However, despite enormous progress in this field, many unanswered questions remain. While it is clear that evolutionarily conserved protein complexes participate in cell polarization and spindle orientation leading to ACD, future investigation is needed to elucidate how this evolutionarily conserved core process is integrated into the local context of each cell and how overall tissue homeostasis is maintained as a result of ACD and its regulatory mechanisms.

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