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Regulation of asymmetric stem cell division: spindle orientation and the centrosome

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1. ABSTRACT

Asymmetric stem cell division, as a means of maintaining adequate numbers of stem cells, has attracted widespread attention from researchers in the stem cell biology field. Yet, the molecular and cellular mechanisms that govern asymmetric stem cell division remain poorly understood. Stem cells are not the only cell population that divides asymmetrically, and fortunately, great progress has been made in the understanding of asymmetric cell division during development, providing insight into strategies that stem cells may employ to divide asymmetrically. This review will summarize the importance of stem cell function and the role of asymmetric division in controlling stem cell behavior.

2. STEM CELL FUNCTIONS IN TISSUE HOMEOSTASIS, CANCER, AND TISSUE AGING

Throughout life, adult stem cells continuously supply highly differentiated but short-lived cells, such as blood cells, skin cells, intestinal epithelial cells, and sperm. The balance between the production of stem cells and differentiating cells is critical since an imbalance can lead to tumorigenesis (caused by stem cell overproliferation) or tissue degeneration (caused by stem cell depletion). Under homeostatic conditions, the production of new cells exactly compensates for lost cells, such that neither an increase nor a decrease in the net number of each cell type occurs. While an increase in cell number is required during development, the maintenance of cell number is essential

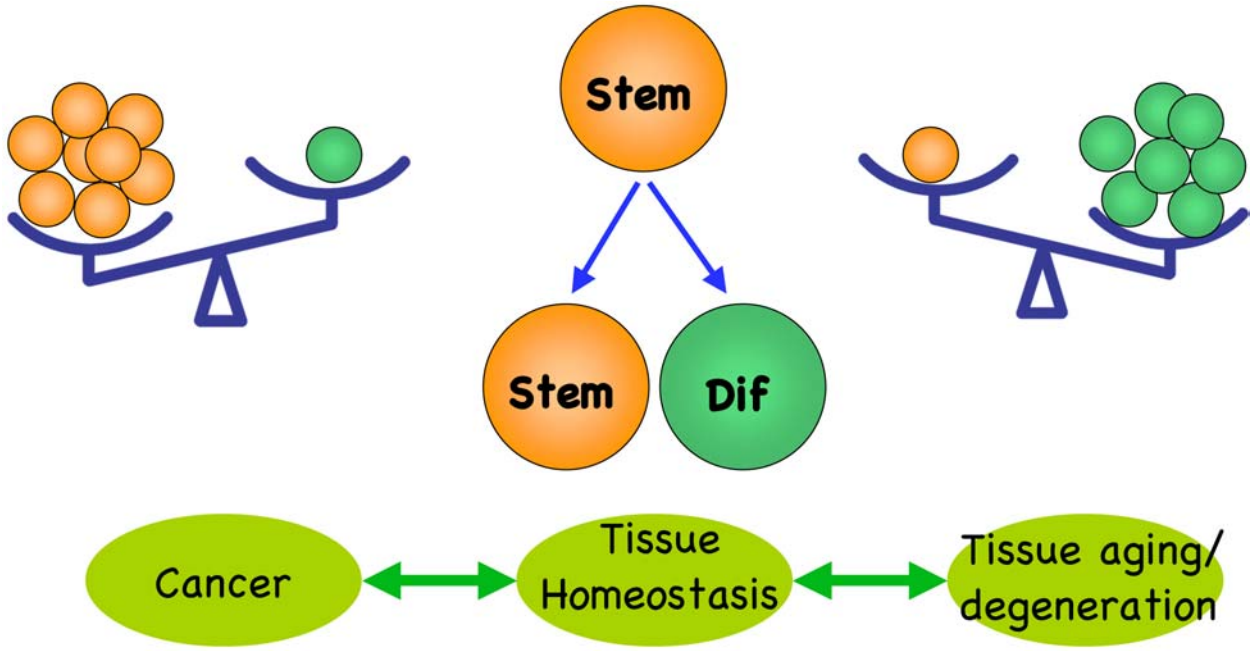

Figure 1. The balance of stem cell self-renewal and differentiation maintains tissue homeostasis. While an excess of stem cell self-renewal may lead to tissue hyperplasia and/ or tumorigenesis, an excess of differentiation may lead to tissue degeneration and/ or tissue aging. Asymmetric stem cell division, producing one stem cell and one differentiating cell, is a simple way to maintain the balance between stem cell and differentiated cell populations.

after the organ/tissue reaches its appropriate size in adulthood. Since adult stem cells are the source of most newly created cells, many human pathologies, such as cancer and age-related disorders, are speculated to result from the dysfunction and/or malfunction of stem cells (1-4) (Figure 1).

The intimate relationship between stem cells and cancer cells has been suspected for a long time, yet we do not fully understand how these two cell populations are related. Stem cells and cancer cells share several characteristics, such as a relatively undifferentiated state and the long-term capacity for proliferation. However, we do not know whether cancer cells originate directly from the stem cell population or whether other cell types acquire stem cell-like characteristics to become cancerous. Now, evidence suggests that both scenarios can happen depending on the type of cancer[5]. Reasons that are more practical have also prompted the study of stem cells and their relationship with cancer cells. Many cancer therapies that significantly decrease tumor mass, fail to cure patients, who eventually relapse with therapy-resistant disease. Cancer cells that are resistant to cancer therapy, but have the capacity to create new tumors, are thought to be responsible for relapses (5-7). Such 'seeding' cancer cells, now called cancer stem cells, share many characteristics with normal adult stem cells. For this reason, they are believed to originate from normal stem cells or alternatively, re-acquire certain stem cell characteristics via mutation. Indeed, essential characteristics of stem cells, such as proliferative capacity and a relatively undifferentiated state, could be easily exploited or modified to produce uncontrolled cell expansion. Thus, an understanding of normal stem cell behavior is essential for the efficient therapeutic targeting of cancer stem cells (8).

There is compelling evidence to suggest that a decline in stem cell function contributes to tissue aging (2-4, 9-13). In many systems, including mouse hematopoietic stem cells and muscle stem cells (satellite cells), stem cell functionality (i.e., the ability to reconstitute the tissue upon transplantation) diminishes when the donor organism becomes older, while the number of stem cells does not significantly decrease (and in fact, sometimes increases) (10, 14). The mechanisms responsible for diminished stem cell function remain largely unidentified. Intrinsic changes in stem cell populations may underlie a decline in stem cell function, such as in hematopoietic stem cells (15). In multiple mammalian tissues, the expression of the cell cycle inhibitor (Cdk inhibitor) and tumor suppressor, Ink4a, increases with age within the stem cell compartment, and this increase occurs concurrent with a decline in stem cell function (16-18). The age-dependent decline in stem cell function is partially rescued in Ink4a null mice, suggesting that an up-regulation of Ink4a during aging contributes to the functional decline of stem cells. This observation also suggests that increasing tumor suppressor function contributes to tissue aging in Ink4a mice. In mouse hematopoietic stem cells, p21 is reported to control stem cell quiescence and maintain the stem cell reservoir, the depletion of which leads to premature exhaustion of the stem cell pool (19). It has been recently reported that the expression of Cdk inhibitors, p15, p16, p21, and p27, increases with age in a TGF-β dependent manner in

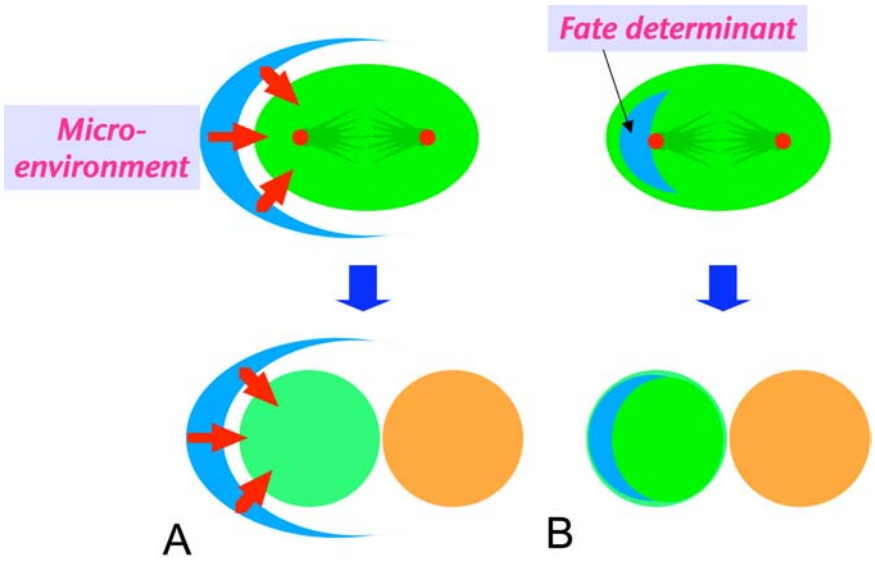

Figure 2. Two mechanisms of asymmetric cell division. Cells divide asymmetrically either by the extrinsic or intrinsic fate determinants. In the case of extrinsic fate determinants (or microenvironment), the daughter cells are placed in different microenvironment, so that the two daughters take on different fates (A). In the case of intrinsic fate determinants, such determinants are asymmetrically localized within a cell and subsequently segregate differentially into the two daughter cells so that the two daughters take on different fates (B).

satellite cells, reducing the regenerative capacity of muscle (20). Nonetheless, it is unclear whether the Cdk inhibitors accumulate in aged stem cells because these cells have ceased to proliferate for other reasons or whether their accumulation causes stem cells to stop proliferating. In addition, the mechanisms responsible for the up-regulation of cell cycle inhibitors and their relationships to normal stem cell function are unknown.

In other cases, extrinsic changes in the stem cell microenvironment or systemic environment may account for a decline in stem cell function, as in satellite cells and *Drosophila* germ line stem cells (21-23). In the case of satellite cells, the aging effects in the systemic environment appear to be dominant, since the exposure of satellite cells from aged animals to younger systemic environments rejuvenates the satellite cells and promote their proliferation through reactivation of Notch signaling (21).

3. ASYMMETRIC DIVISION AS A MEANS OF TISSUE HOMEOSTASIS

During development, once the stem cell population reaches the desired size (that of adult tissues), tissue homeostasis favors the preservation of stem cell number, while providing a source of new differentiated cells to compensate for cell loss. One simple way to accomplish this equilibrium is for stem cells to divide asymmetrically, producing one stem cell and one differentiating cell, so that the stem cell number does not change as a result of the production of differentiated cells.

Asymmetric division is a common theme during development and is not limited to stem cell populations. Indeed, the basic molecular architecture appears to be conserved among many asymmetrically dividing cells, from yeast to humans and from embryonic cells to stem cells. Cells divide asymmetrically through 1) intrinsic fate determinants and/or 2) extrinsic fate determinants. In the case of extrinsic fate determinants, cell division itself may be symmetric, but asymmetric placement of the two daughter cells into different environments leads to asymmetric fate determination (Figure 2a). In the case of intrinsic determinants, fate determinants are restricted to a small area within the cell (e.g., a part of the membrane or centrosome), causing them to be segregated into only one daughter upon cell division (Figure 2b). Examples of cells that use these two distinct strategies are summarized below.

3.1. Asymmetric cell division by intrinsic fate determinants

Many cell types divide asymmetrically and produce two daughter cells with distinct fates by segregating fate determinants unequally during mitosis. The two best-studied examples of this are *Drosophila* neuroblasts and early *C. elegans* embryos. Although many differences exist between these two cell types, including the fate and developmental stage, they use similar mechanisms to divide asymmetrically.

Drosophila neuroblasts produce one neuroblast and one ganglion mother cell (GMC), which divides once more before terminal differentiation. In *Drosophila* neuroblasts, a cortical 'crescent' forms at the apical and basal side of the cell, with each dictating different aspects of asymmetric division (Figure 3a) (24). The apical crescent contains the Par-3/Par-6/aPKC complex, inscuteable protein, and the Pins/Mud/Gαi complex. The primary function of these protein complexes is to induce cellular asymmetry, but not cell fate *per se*. These apical

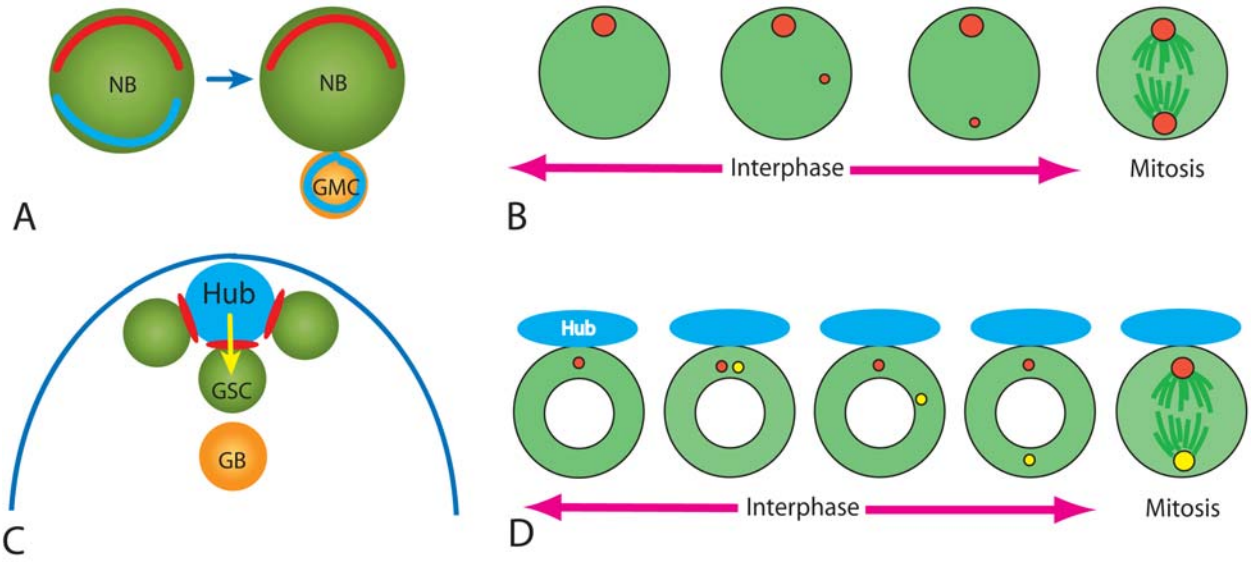

Figure 3. Asymmetric stem cell division in the *Drosophila* male germ line stem cells (GSCs) and neuroblasts (adapted from Yamashita and Fuller (66). The *Drosophila* neuroblasts divide asymmetrically by segregating fate determinants asymmetrically within the cell. The apical crescent (red) containing aPKC/Baz (Par3)/Par6 and Pins/Mud/Gai directs the formation of the basal crescent and orients the mitotic spindle. The basal crescent (blue) contains Numb, Pon, Miranda, and Prospero that either promotes or allows differentiation. The larval neuroblast spindle is oriented by stereotypical positioning of centrosomes, in a mechanism very similar to that of GSCs. One larger centrosome with higher MTOC activity stays at the apical side, while the smaller centrosome migrates toward the opposite side of the neuroblast. In contrast to the larval neuroblasts, embryonic neuroblasts orient the mitotic spindle by a programmed rotation of the metaphase spindle. The *Drosophila* male GSCs divide asymmetrically within the context of the stem cell niche (hub), which secretes a signaling ligand, Upd, to activate the JAK-STAT pathway within GSCs. GSCs are physically attached to the hub cells via adherens junction (red) to support efficient signaling between the hub and the GSCs. GSCs divide asymmetrically within the context of the niche-stem cell signaling, by orienting centrosomes with respect to the hub cells. The mother centrosome (red dot) always localizes close to the hub, while the daughter centrosome (yellow dot) migrates away from it, thereby setting up a perpendicular orientation of the mitotic spindle. Reproduced with permission from the Journal of Cell Biology.

proteins are required for forming the basal crescent, orienting the spindle, and inducing spindle asymmetry (the apical half of the spindle is larger than the basal half, which leads to a larger neuroblast and a smaller GMC). The basal crescent contains Numb, Pon, Miranda, Prospero, and Brat. In many cell types, Numb acts as a repressor of the Notch pathway, although this has not been demonstrated in neuroblasts. In neuroblasts, loss-of-function mutations of Numb lead to over proliferation and a tumor phenotype (25-26), suggesting that Numb represses the neuroblast (stem cell) fate or promotes differentiation. Interestingly, recent studies have demonstrated that larval neuroblasts use differential MTOC activity of the centrosomes to organize the spindle orientation (Figure 3b) (27-28) (see below for details).

The asymmetry of early *C. elegans* embryos also involves the Par-3/Par-6/aPKC complex [29], suggesting that the mechanisms underlying asymmetric division are widely conserved among many cell types, including stem cells. This protein complex localizes to the anterior side of the fertilized embryo, counteracting the functions of the posterior-localized Par-1 and Par-2 proteins (29). It is worth noting that the Par-3/Par-6/aPKC complex is found in symmetrically dividing, polarized cells, such as *Drosophila* embryonic epithelial cells (30). Thus, the primary function of the Par-3/Par-6/aPKC complex may be to set up the polarity of cells, while other (perhaps cell-type specific) proteins may serve to ”interpret” cell polarity and then orient the spindle appropriately.

3.2. Asymmetric cell division by extrinsic fate determinants

The other strategy for asymmetric cell division is to place two daughter cells into different microenvironments that in turn dictate their fates. In case of stem cells, such microenvironment is called the stem cell niche. Increasing number of adult stem cells has been reported to reside within the niche (31-33). The niche provides signals that are required for stem cell identity and protect cells from differentiation. Because of its critical requirement for stem cell identity, the niche functions not only to maintain the stem cell pool, but also to limit its size, thus acting as a safe-guard against cancer (1). The niche can be sufficient to maintain a constant number of stem cells, as exemplified by the *Caenorhabditis elegans* germ line. Somatic cells, known as distal tip cells (DTCs), project thin processes that surround a population of germ cells and maintain stem cell identity. DTCs provide LAG-2 ligand, which activates the GLP-1 Notch receptor within germ cells to specify stem cell identity (34). Interestingly, the size of the germ line stem cell (GSC) population

appears to be controlled stochastically. That is, stem cells can divide either symmetrically or asymmetrically, and the size of the area within the DTC processes determines the number of stem cells.

In other cases, such as the male and female germ lines of *Drosophila melanogaster*, the niche is used in combination with the regulation of division orientation to maintain homeostasis. In these systems, asymmetric stem cell division is used to maintain stem cell number, while producing a differentiating cell. The GSCs interact with the niche supporting cells (hub cells for male GSCs, cap cells for female GSCs) via an E-cadherin-based adherens junction, which serves as a mechanical support for the stem cell-niche interaction (35-36) (Figure 3c). *Drosophila* female GSCs divide asymmetrically, as dictated by the orientation of the mitotic spindle, in the context of the stem cell niche. The terminal filament and cap cells secrete the BMP signaling ligand, Dpp. Dpp acts on neighboring GSCs to repress the differentiation program (37). A subcellular organelle, known as the spectrosome, always localizes to the apical side of female GSCs and anchors one spindle pole in order to orient the mitotic spindle (38).

In the male, hub cells secrete the signaling ligand, Upd (Unpaired), which activates the JAK-STAT pathway within GSCs and somatic stem cells (cyst progenitor cells (CPCs)) to maintain stem cell identity (39-41) (Figure 3c). GSCs within this Upd-JAK-STAT signaling microenvironment orient their mitotic spindles perpendicular to the hub, which ensures that one stem cell daughter stays close to the hub and maintains stem cell identity, while the other is displaced away from the hub and becomes committed to differentiation (36). The spindle orientation is established well before mitosis occurs through the positioning of the centrosomes. The mother centrosome remains close to the hub, while the daughter centrosome migrates away from the hub (Figure 3d) (42). Electron microscopic analysis has revealed that the mother centrosome harbors many microtubules (MTs) that anchor it to the adherens junction between the hub and GSCs, whereas the daughter centrosome has only a few MTs (42). This observation is consistent with extensive cell biological evidence showing differential microtubule organizing center (MTOC) activity between mother and daughter centrosomes (43). Consistent with the idea that astral MTs anchored to the mother centrosome are responsible for the stereotypical positioning of centrosomes, loss-of-function mutations in the centrosomin (cnn) gene, which encodes a protein that anchors astral MTs to centrosomes, results in random centrosome positioning and mother-daughter choice (36, 42). Cnn is a coiled-coil protein that is one of the major components of pericentriolar material (PCM) (44-47). In the absence of the Cnn protein, most PCM components fail to localize to centrosomes and, as a result, these centrosomes anchor interphase astral MT arrays very inefficiently. Thus, the centrosome misorientation phenotype observed in *cnn* mutant GSCs is presumed to be the result of a failure to link the mother (proximal) centrosome to the hub-GSC adherens junction, due to a lack of astral MTs. We have also proposed that such astral MTs emanating from the mother (proximal) centrosome are tethered to the hub-GSC adherens junction in an Apc2 protein-dependent manner. Apc2 co-localizes with E-cadherin and β-catenin (Armadillo) at the hub-GSC adherens junction, and loss-of-function *apc2* mutations result in centrosome misorientation in GSCs. The Apc2 protein is a homolog of the mammalian APC (adenomatous polyposis coli) tumor suppressor and is known to bind both MTs and the adherens junction component, β-catenin. Although intriguing, it is unknown whether the mother or daughter centrosome is associated with any fate determinants that direct either stem cell identity or differentiation. It is worth noting that in early mollusk embryos, fate-determining mRNAs associate with only one centrosome to dictate asymmetric cell fate (48).

4. LESSONS FROM BUDDING YEAST

Budding yeast is an excellent model system in which to study asymmetric cell division. Although budding yeast is a unicellular organism, many aspects of its division are asymmetric. Thus, available data from yeast can provide important insights into the molecular and cellular mechanisms underlying asymmetric division and mitotic spindle orientation, as well as the mechanisms that safeguard against the failure of these processes.

4.1. Parallels between asymmetric division of budding yeast and multicellular organisms

A mother yeast cell produces a smaller bud cell (asymmetric cell size), and mating type switching only occurs in the mother cell (asymmetric cell fate). Such asymmetry is controlled by elaborate cellular mechanisms, such as microtubule-cortex interactions and asymmetric segregation of fate determinants (49). In budding yeast, mRNA for the transcriptional repressor and mating type switch inhibitor, Ash1, is predominantly concentrated in the daughter cell (bud), leading to mating type switching only in the mother cell. Ash1 mRNA is asymmetrically localized through directed transport on the actin cytoskeleton. Cis-acting sequences within the Ash1 mRNA are recognized by the RNA binding protein, She2p, which binds to the adapter protein, She3p. She3p, in turn, connects Ash1 mRNA to the Myo4 motor protein (50).

Correct spindle orientation is also important for budding yeast division. Since a budding yeast cell forms a bud before nuclear division occurs, spindles must be aligned along the mother-bud axis so that the two divided nuclei are segregated into the mother and bud cells. The molecular mechanism underlying mitotic spindle orientation is strikingly similar to that of male GSC spindle orientation. The mother spindle pole body (SPB, yeast counterpart to the centrosome) has a more robust astral MT array, as in *Drosophila* male GSCs, and in unperturbed budding yeast cells, is normally directed toward the bud (Figure 4). These astral MTs are captured and stabilized by the bud tip cortex in a Kar9p-dependent manner, guiding the mother SPB to the bud tip (51-52). Kar9 is proposed to be an ortholog of APC, suggesting a conserved link of centrosomes (SPB)-astral microtubules-Apc2 (Kar9p)-the cell cortex (52).

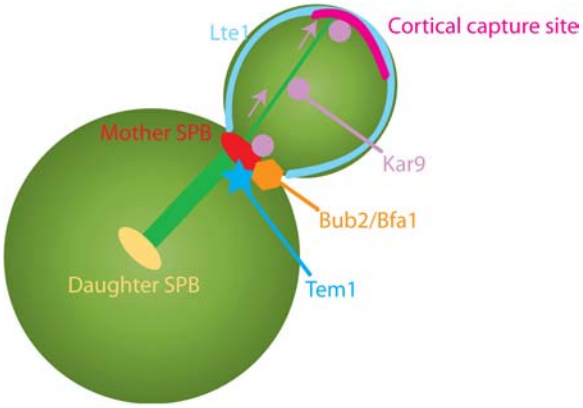

Figure 4. The mechanism of spindle orientation and spindle position checkpoint in budding yeast (adapted fromYamashita and Fuller (66). The mother SPB is recruited to the bud in a Kar9p –dependent manner and anchored to the cortical capture site at the bud tip. The spindle position checkpoint monitors the presence of the bud-oriented (normally mother) SPB in the bud. The spindle position checkpoint inhibits the activation of the mitotic exit network (MEN) in response to incorrect positioning of the bud-oriented SPB. Reproduced with permission from the Journal of Cell Biology.

4.2. Backup mechanisms to ensure asymmetric division— the orientation checkpoint in budding yeast

As described above, asymmetric cell division is an elaborate process during which cells establish cell polarity (intrinsic or extrinsic), coordinate polarity with the cell division plane, and divide asymmetrically. A disruption at any step may result in a failure of asymmetric cell division (i.e., two nuclei in the mother cell and no nucleus in the bud or symmetric cell division where the division needs to be asymmetric), prompting the question of whether safeguards exist. Again, insights come from budding yeast; if cells sense a failure, then cell cycle progression is arrested or delayed to give the cell enough time to correct the failure. Namely, budding yeast have a spindle position checkpoint (SPOC) to ensure the correct segregation of nucleus into the mother cell and the daughter cell. This should not be confused with the spindle assembly checkpoint. The spindle assembly checkpoint ensures that each of the two spindle poles (and subsequently the two daughter cells) inherits an equal and correct amount of chromosomes. On the other hand, the spindle position checkpoint ensures that the correctly assembled spindle is appropriately positioned with respect to the mother-daughter axis, which is important given that budding yeast form a daughter cell (bud) before nuclear division occurs. Even when the spindle is perfectly formed and competent to segregate genetic material equally, cells would not be able to survive if the nuclear division occurred within the mother cell, as this would result in a polyploid mother cell and an anucleate daughter cell. Thus, the spindle must be aligned with the mother-daughter axis, so that each cell receives one nucleus.

The major component of the yeast spindle position checkpoint is the Bub2p/Bfa1p GAP (GTPase activating protein) protein complex (53). This protein complex localizes to the daughter-bound SPB and responds to misalignment (or incorrect positioning) of the spindle within the cell. Kin4 protein kinase is a positive regulator of Bub2p/Bfa1p (54-56), but the mechanism responsible for its activation by spindle misalignment is not well understood. The downstream target of the Bub2p/Bfa1p protein complex is the Tem1p GTPase, a key regulator of the mitotic exit network (MEN). The Bub2p/Bfa1p complex maintains the Tem1p GTPase in an inactive state (GDP-bound form) in response to spindle misalignment, thereby inhibiting mitotic exit when the spindle is incorrectly aligned. Tem1p activates a signaling cascade involving Cdc14p and other MEN proteins to allow cell cycle progression. The spindle position checkpoint is a very effective mechanism that links information about SPB position to cell cycle progression, and it is essential for oriented cell division in the context of pre-formed cell polarity. Interestingly, components of the MEN and spindle position checkpoint, such as Tem1p, Bub2p, and Bfa1p, localized to the bud-bound SPB (ie. mother SPB in unperturbed cells) and responds to misalignment (or incorrect positioning) of the spindle within the cell (56-60) (Figure 4). Lte1p, the guanine nucleotide exchange factor for the GTPase, Tem1p, localizes specifically to the bud cortex, and Tem1p-Lte1p interactions appear to participate in the spindle position/ orientation checkpoint. When the mother SPB enters the bud, Lte1p activates Tem1p, which then activates the MEN to enable cell cycle progression. In contrast, when one spindle pole does not successfully move into the bud, the SPB-localized Bub2p/Bfa1p complex prevents activation of Tem1p, delaying mitotic exit.

Whether higher (multicellular) eukaryotes have a checkpoint mechanism similar to that of budding yeast is currently unclear. The development and homeostasis of multicellular organisms would be particularly sensitive to perturbations in asymmetric cell division. For example, a failure in asymmetric division during early development can lead to the loss of one cell type or even an entire organ. In addition, an increase in symmetric divisions, at the expense of asymmetric stem cell division, may lead to either stem cell depletion or stem cell over proliferation. The latter case is particularly relevant to tumorigenesis or tissue hyperplasia. Thus, it is plausible that a similar orientation checkpoint exists in multicellular organisms to ensure an asymmetric outcome of the division.

Recently, we have found that, in *Drosophila* male GSCs, mitotic entry is delayed in response to centrosome misorientation (61). Since the correct orientation of centrosomes is essential for proper spindle orientation and asymmetric stem cell division, such delays appear to ensure the asymmetric outcome of the division, a failure of which may lead to symmetric stem cell division and defective tissue homeostasis. We propose that a checkpoint exists to monitor correct centrosome orientation to prevent mitosis with misoriented division plane. Although the molecular mechanism by which stem cells sense incorrect centrosome positioning awaits further investigation, it is tempting to speculate that a mechanism similar to the SPOC operates in stem cell populations of higher organisms, including

humans, a failure of which leading to tumorigenesis or other late onset pathologies. Since a failure in such a checkpoint would lead to occasional misoriented divisions, but not a failure in division itself (which would lead to embryonic lethality or other early onset problems), the genes responsible for monitoring asymmetric division would be susceptible to mutation that is carried over generations.

Due to the mechanism that delays mitotic entry upon centrosome misorientation, male GSCs do not divide symmetrically with misoriented spindle. This is in contrast to female GSCs that can reorient the mitotic spindle in response to the loss of a neighboring GSC and divide symmetrically to replenish the stem cell pool (62). Yet, the decline in net stem cell number is much milder than expected from the actual half life of each GSC in male germ line, suggesting that there must be a mechanism to replenish the stem cell pool during aging (63). Then, how male GSCs are replenished, if symmetric stem cell divisions do not occur? We found that the lost GSCs are rather likely replenished by dedifferentiation of spermatogonia (61), another proposed mechanism for stem cell replenishment (64-65).

Our laboratory has also found that the number of GSCs with misoriented centrosomes substantially increases with age, leading to an overall decrease in stem cell division in aged tissue (61). We speculate that such cell cycle delay (due to the mechanism to delay cell cycle in response to centrosome misorientation) leads to an age-related decline in spermatogenesis. This might be the underlying mechanism of the overall cell cycle changes that were observed in GSCs from aged testes, as reported recently. First, Wallenfang *et al.* described a decreased cell cycle index measured by BrdU incorporation in GSCs from aged flies (63). Boyle *et al.* (23) observed that Cyclin E accumulates with age, which might suggest GSCs arrest in a particular cell cycle stage (although it does not necessarily suggest that they are in G1 phase, since it has been reported that the GSCs have distinctive kinetics of cyclin fluctuation during cell cycle, co-expressing multiple Cyclins at a time (66)). It is tempting to speculate that the mechanism that ensures asymmetric stem cell division (which could function as a potential tumor suppressor mechanism) eventually leads to decreased stem cell division, resulting in decreased tissue homeostasis in aged tissue.

5. CONCLUSIONS

Asymmetric cell division is an elaborate mechanism that is essential for almost all organisms from yeast to humans. Asymmetric stem cell division is crucial for the development and tissue homeostasis of multicellular organisms (66). Understanding asymmetric stem cell division may enable manipulation of the cell division mode, making it possible to grow adult stem cells in culture for therapeutic purposes. Such understanding will come from the study of not only stem cells, but also very simple organisms, such as yeast.

6. ACKNOWLEDGEMENTS

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Abbreviations: APC: adenomatous polyposis coli, Cdk: cyclin-dependent kinase, cnn: centrosomin, CPCs: cyst progenitor cells, DTCs: distal tip cells, ganglion mother cell (GMC), GSC: germ line stem cell, JAK-STAT: Janus kinases-signal-Transducers and Activators of Transcription, MTs: microtubules, MTOC: microtubule organizing center, MEN:mitotic exit network, PCM: pericentriolar material, SPB:spindle pole body, SPOC: spindle position checkpoint, Upd: Unpaired

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