Leading Edge
Review

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Mechanisms of Asymmetric Stem Cell Division

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Stem cells self-renew but also give rise to daughter cells that are committed to lineage-specific differentiation. To achieve this remarkable task, they can undergo an intrinsically asymmetric cell division whereby they segregate cell fate determinants into only one of the two daughter cells. Alternatively, they can orient their division plane so that only one of the two daughter cells maintains contact with the niche and stem cell identity. These distinct pathways have been elucidated mostly in *Drosophila*. Although the molecules involved are highly conserved in vertebrates, the way they act is tissue specific and sometimes very different from invertebrates.

A hallmark of all stem cells is the ability to simultaneously generate identical copies of themselves but also to give rise to more differentiated progeny. Work mostly done in the fruitfly, *Drosophila*, has suggested two different mechanisms by which this can be achieved (Horvitz and Herskowitz, 1992) (Figure 1). When an intrinsic mechanism is used, regulators of self-renewal are localized asymmetrically during mitosis so that they are inherited by only one of the two daughter cells (Betschinger and Knoblich, 2004; Yu et al., 2006). Already in interphase, cells which undergo such intrinsically asymmetric divisions use apical-basal or planar polarity of the surrounding tissue to set up an axis of polarity. As they enter mitosis, this axis is used to polarize the distribution of protein determinants and to orient the mitotic spindle so that these determinants are inherited by only one of the two daughter cells. Alternatively, the stem cell is in close contact with the stem cell niche and depends on this contact for maintaining the potential to self-renew (Li and Xie, 2005). By orienting its mitotic spindle perpendicularly to the niche surface, it ensures that only one daughter cell can maintain contact with the stem cell niche and retain the ability to self-renew. In contrast to intrinsically asymmetric cell divisions, which usually follow a predefined developmental program, niche-controlled stem cell divisions offer a high degree of flexibility. Occasionally, the stem cell can divide parallel to the niche, thereby generating two stem cells to increase stem cell number or to compensate for occasional stem cell loss. For this reason, niche mechanisms are more common in adult stem cells, whereas intrinsically asymmetric divisions predominate during development.

Collectively, both types of cell division are referred to as asymmetric cell division. An asymmetric cell division is defined as any division that gives rise to two sister cells that have different fates—a feature that can be recognized by differences in size, morphology, gene expression pattern, or the number of subsequent cell divisions undergone by the two daughter cells (Horvitz and Herskowitz, 1992). Although some stem cells—in particular hematopoietic and embryonic stem (ES) cells—do not quite fit this definition when kept in culture, it is safe to assume that most, if not all, stem cells undergo asymmetric cell divisions when they are in their natural environment.

In *Drosophila*, neuroblasts and ovarian stem cells are well-studied examples for the intrinsic and extrinsic mode of asymmetric cell division, respectively. Although these simple categories may not apply as exclusively to mammalian stem cells and both pathways seem to be combined in some cell types, they provide a conceptual framework that will help us to understand the complexity of mammalian stem cell biology. Below, I describe the anatomy and molecular machineries of asymmetric cell division in *Drosophila* neuroblasts and ovarian germline stem cells and use neural, muscle, and hematopoietic stem cells as examples to illustrate the similarities and differences in higher organisms (see Table 1 for a summary of the model systems described).

Asymmetric Protein Segregation in *Drosophila*

Drosophila *Neural Precursor Cells*

Drosophila sensory organ precursor (SOP) cells and neuroblasts (the progenitors of the peripheral and central nervous system, respectively) are well-studied examples of intrinsically asymmetric cell divisions (Figure 2). SOP cells give rise to the four cells present in external sensory organs (Figure 2A). Although they are not stem cells, SOP cells have revealed many of the fundamental principles for asymmetric cell division. This is mainly due to their simple and highly reproducible lineage: SOP cells delaminate from a polarized epithelium and then divide into an anterior pIIb and a posterior pIIa cell. After SOP division, pIIa and pIIb divide once more to generate the two outer and the two inner cells of the organ, respectively. Asymmetry in all of these divisions is generated by different levels of Notch activity in the two daughter cells (Schweisguth, 2004; Le Borgne et al., 2005). It is thought that SOP cells inherit epithelial planar polarity and use it to segregate regulators of the Notch signaling pathway into one of the two daughter cells.

In contrast to SOP cells, *Drosophila* neuroblasts undergo multiple rounds of stem cell-like divisions (Figure 2B). During each division, they give rise to a large cell that retains neuroblast properties and a smaller cell that is called the ganglion mother cell (GMC) and divides only once more to generate two differentiating neurons. Neuroblasts come in two flavors; embryonic neuroblasts give rise to the relatively simple nervous system of

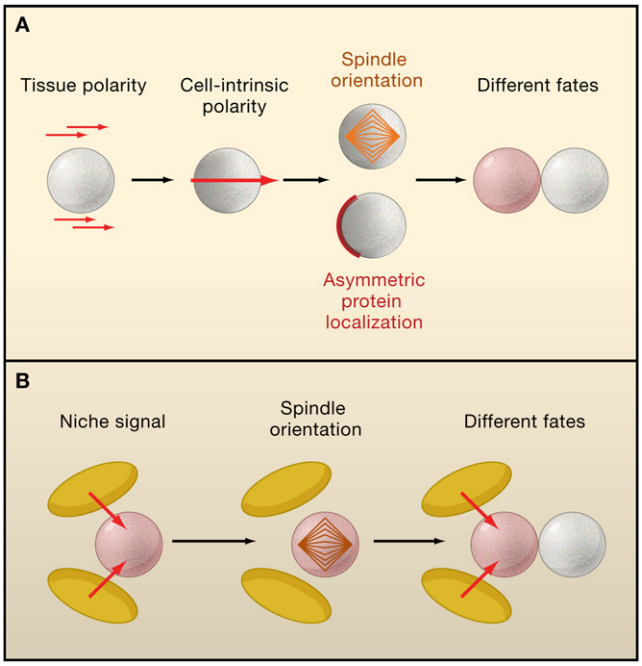

Figure 1. Extrinsic and Intrinsic Regulation of Stem Cell Self-Renewal

(A) Stem cells can set up an axis of polarity during interphase and use it to localize cell fate determinants asymmetrically in mitosis. Orientation of the mitotic spindle along the same polarity axis ensures the asymmetric segregation of determinants into only one of the two daughter cells.

(B) Stem cells may depend on a signal coming from the surrounding niche for self-renewal. By orienting their mitotic spindle perpendicularly to the niche surface, they ensure that only one of the two daughter cells continues to receive this signal and maintains the ability to self-renew.

the larva. They are specified within a monolayered epithelium called the ventral neuroectoderm and delaminate from the epithelium to undergo repeated rounds of asymmetric division along the apical-basal axis. It is thought that certain aspects of epithelial polarity are inherited by the neuroblasts and used to polarize the first mitotic division. Although the reproducible position and the relatively simple lineages of embryonic neuroblasts have made them a valuable system to discover basic principles of asymmetric division, their restricted self-renewal capacity limits their usefulness as a true stem cell model. Mainly for this reason, the field has recently begun to focus on larval neuroblasts.

Larval neuroblasts generate the thousands of neurons found in the central nervous system of an adult fly. Unlike embryonic neuroblasts, which become smaller with each division, larval neuroblasts regrow back to their original size after each division and can divide hundreds of times (Ito and Hotta, 1992; White and Kankel, 1978) (Figure 2B). Several types of larval neuroblasts can be distinguished based on their position within the larval central nervous system (Figure 2C). In the ventral nerve chord, 30 ventral nerve chord neuroblasts per hemisegment divide repeatedly along the apical-basal axis to form the neurons of the thoracic and abdominal ganglia (Truman and Bate, 1988). In each of the two brain lobes, approximately 85 central brain neuroblasts give rise to most of the neurons present in the adult brain (Ito and Hotta, 1992). Central brain neuroblasts are heterogeneous in cell cycle length and regulation of self-renewal. In particular, a group of less than 10 dorso-posterior (DP) neuroblasts seems to be particularly susceptible to mutations in tumor suppressor genes (Betschinger et al., 2006). Compared to other central brain neuroblasts, these precursors generate many more progeny and they might even have a different lineage in which GMCs divide more than once. It is worth noting that much of the earlier experiments on *Drosophila* larval neuroblasts did not distinguish between these subgroups and might have to be reinvestigated.

In addition to the central brain neuroblasts, the fly brain contains the mushroom body and optic lobe neuroblasts. In each brain hemisphere, four mushroom body neuroblasts give rise to 2500 neurons called Kenyon cells that form the learning and memory centers (Ito and Hotta, 1992; Ito et al., 1997). To generate this large number of neurons, they start dividing much earlier than central brain neuroblasts and proliferate throughout most of the pupal stages of development. Whereas mushroom body and central brain neuroblasts are already specified during embryogenesis and simply reactivate their proliferation programs during larval stages, optic lobe neuroblasts follow a distinct program of neurogenesis (Egger et al., 2007). They arise from two multilayered neuroepithelia called the inner- and outer-proliferation centers (White and Kankel, 1978). Neuroepithelial cells divide symmetrically in parallel to the epithelial surface. Neuroblasts are generated on the rims of these epithelia. They lose their adherens junctions, turn on neuroblast markers, and start dividing asymmetrically and perpendicularly to the epithelial plane. Following a canonical neuroblast lineage, optic lobe neuroblasts give rise to the neurons in the visual processing centers of the fly brain.

Segregating Determinants

The different fate of the two neuroblast daughter cells is thought to be induced by the unequal segregation of several proteins into one of the two daughter cells (Figure 3). Due to their combined activity in specifying daughter cell fate, these proteins are referred to as segregating determinants. Because determinant segregation can even occur in individual cultured neuroblasts, it is thought to be governed by a cell-intrinsic machinery (Broadus and Doe, 1997) (note, however, that partially redundant extrinsic cues exist as well—see below and Siegrist and Doe, 2006). Before mitosis, the proteins Par-3, Par-6, atypical PKC (aPKC), Inscuteable, Pins, Gαi, and Mud (see below for their individual functions) accumulate on the apical side of the cell cortex (Betschinger and Knoblich, 2004; Suzuki and Ohno, 2006; Goldstein and Macara, 2007). Although they are preferentially inherited by the apical daughter cell, which remains a neuroblast, they are not thought to influence cell fate directly. Instead, they induce the asymmetric localization of cell fate determinants to the opposite, basal side of the cell and their segregation into the basal GMC. Below, I discuss those determinants for which functions in *Drosophila* neural stem cells have been shown.

The first segregating determinant was called Numb and was actually identified in SOP cells (Rhyu et al., 1994). In *numb* mutants, both daughter cells of the SOP assume the fate of the cell that normally does not inherit the Numb protein. Conversely,

Table 1. Model Systems for Asymmetric Cell Division

Mother Cell	Daughter Cell Types	Polarity Cue	Mechanism of Unequal Fate Specification
Drosophila sensory organ precursor cell	Four cell types forming external sensory organs: socket, hair, sheath, neuron	Planar polarity	Asymmetric segregation of Numb results in differential Notch regulation
Drosophila neuroblast	Neuroblast, ganglion mother cell	Epithelial polarity	Asymmetric segregation of Numb, Prospero, and Brat results in self-renewal versus cell-cycle exit
Drosophila ovarian germline stem cell	Stem cell, cystoblast	Niche architecture	Diffusible signal (Dpp and Gbb) from stem cell niche
Mouse brain progenitor cells	Progenitor cell, neuron (occasionally: intermediate/basal progenitor)	Apical-basal polarity of neuro-epithelium	Unidentified segregating determinant or apical membrane compartment or basal fiber
Mouse muscle satellite cells	Stem cell ($Myf5^-$), committed progenitor ($Myf5^+$)	Unclear, maybe integrin contact with basal lamina	Segregating determinant (Numb), signal from basal lamina or both
Mouse hematopoietic stem cell	Hematopoietic stem cell, committed progenitor	Signal from stem cell niche (blood vessel or osteoblast)	Different levels of Notch signaling (maybe induced by Numb segregation)
Mouse T-lymphocytes	Effector T cell, memory T cell	Immunological synapse	Unequal segregation of Numb, CD8 and Interferon γ receptor

numb overexpression results in the transformation into the opposite cell fate. Numb acts as a tissue-specific repressor of the Notch pathway (Le Borgne et al., 2005; Schweisguth, 2004). It binds to the endocytic protein α-Adaptin (Berdnik et al., 2002) and might control the intracellular trafficking of Notch intermediates. When Numb is mutated in the larval brain, the mutant neuroblasts overproliferate and form a tumor-like phenotype (Lee et al., 2006a; Wang et al., 2006). Lineage analysis shows that this is due to occasional divisions in which a neuroblast still divides into a larger and a smaller daughter cell but both daughter cells eventually show the gene expression and proliferation pattern of a neuroblast. Similar (but not identical) brain phenotypes are observed upon mutation of other segregating determinants and have made *Drosophila* neuroblasts an ideal model system to investigate the biology of cancer stem cells (Figure 4, see below) (Gonzalez, 2007).

Like Numb, the transcription factor Prospero (Pros) segregates asymmetrically in neuroblasts. Although Pros is already present in neuroblasts, it only enters the nucleus once asymmetrically segregated into the GMC (Betschinger and Knoblich, 2004). When Pros is mutated in embryonic neuroblasts, the GMC continues to express neuroblast markers and undergoes multiple rounds of division (Choksi et al., 2006). Several cell-cycle regulators including Cyclins A and E and Cdc25 (*string* in *Drosophila*) are upregulated and may be responsible for this phenotype (Li and Vaessin, 2000). In larval neuroblasts, mutations in Pros cause stem cell-derived tumors (Betschinger et al., 2006; Lee et al., 2006c; Bello et al., 2006). Pros contains a homeodomain and binds upstream of over 700 genes many of which are involved in neuroblast self-renewal or cell-cycle control. However, Pros can also induce the expression of neural differentiation genes indicating that it can act both as a transcriptional activator and inhibitor (Choksi et al., 2006).

More recently, a third important regulator of neuroblast self-renewal has been identified (Lee et al., 2006c; Bello et al., 2006; Betschinger et al., 2006). This protein is called Brat and was previously shown to act as an inhibitor of ribosome biogenesis and cell growth (Frank et al., 2002). Brat is a member of a new conserved protein family that is characterized by the presence of a C-terminal NHL domain, a coiled-coil region and an N-terminal Zinc binding B-box (Slack and Ruvkun, 1998). In *Drosophila*, Brat, Mei-P26, and Dappled are members of this family. Given that all three proteins act as tumor suppressors, growth control might be a common function of this protein family. During embryogenesis, Brat cooperates with Pros to specify GMC fate. Although only a small subset of GMCs is affected in *pros* mutants, *pros/brat* double mutants show an almost complete loss of all GMCs (Betschinger et al., 2006). In larval brains, *brat* causes the formation of stem cell-derived tumors consisting almost entirely of large cells expressing neuroblast markers. This has led to the hypothesis that Brat might inhibit cell growth in one of the two neuroblast daughter cells to prevent self-renewal and induce terminal differentiation. The molecular mechanism by which Brat regulates cell growth and cell fate is currently unknown. Brat has a second function in specifying the anterior-posterior body axis and for this function, it binds to Nanos and Pumilio to repress translation of the posterior identity gene *hunchback* (Sonoda and Wharton, 2001). In neuroblasts, however, neither the phenotypes nor the expression patterns of Nanos, Pumilio or Hunchback suggest that Brat acts in a similar manner. Instead, Brat was suggested to be a transcriptional activator of Pros (Lee et al., 2006c; Bello et al., 2006) because *brat* mutant tumors are Pros negative and overexpression of Pros can rescue tumor formation in *brat* mutants. However, this hypothesis neither explains why *brat* enhances the *pros* null mutant phenotype in embryonic neuroblasts nor why it regulates cell growth even in tissues that do not express Pros. Given that *brat* tumors arise specifically in DP neuroblasts (see above), lower expression levels of Pros in these cells would also explain why the *brat* tumors are Pros

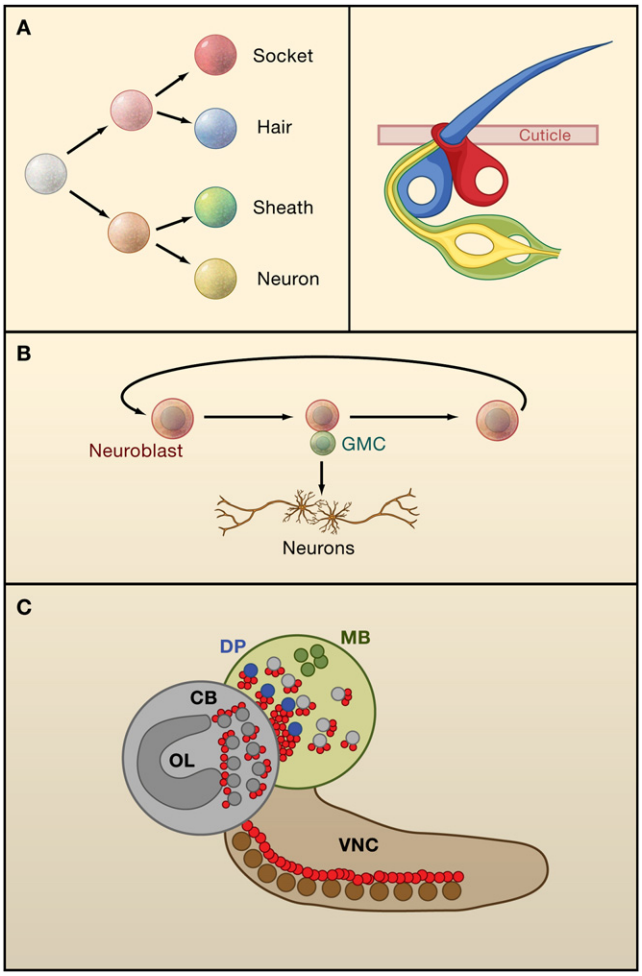

Figure 2. *Drosophila* Neural Precursor Cells

(A) Sensory organ precursor cells generate the four cells of external sensory organs in two consecutive rounds of asymmetric cell division.

(B) Neuroblasts divide into one self-renewing daughter cell and one ganglion mother cell (GMC), which divides only once more into two differentiating neurons. Cellular growth is restricted to the self-renewing daughter cell.

(C) The *Drosophila* larval brain contains ventral nerve chord (VNC, brown), optic lobe (OL, gray), mushroom body (MB, green), and central brain (CB, gray) neuroblasts, (GMCs and neurons, red). A group of dorso-posterior (DP, blue) central brain neuroblasts generates more daughter cells and is particularly susceptible to tumor formation.

negative and why these cells are particularly susceptible to loss of other tumor suppressors. Clearly, more experiments including the identification of functional binding partners are necessary to determine how Brat acts. In fact, *brat* orthologs were found to be essential for RNA interference in *Caenorhabditis elegans* (Kim et al., 2005) and regulation of micro RNAs could be another very exciting function for this protein family.

Adaptor Proteins

The asymmetric segregation of Pros, Brat, and Numb is mediated by two adaptor proteins called Miranda and Pon (Partner of Numb) (Betschinger and Knoblich, 2004). Miranda is a coiled-coil protein that binds to Pros and Brat. Miranda also binds to the RNA binding protein Staufen which in turn transports *pros* RNA but is not required for cell-fate specification in neuroblasts. Like the determinants, Miranda localizes asymmetrically and segregates into one of the two daughter cells in dividing neuroblasts. In Pros, Brat, or Staufen mutants, Miranda localization is unaffected. In *miranda* mutants, however, all three determinants are uniformly cytoplasmic and segregate equally into both daughter cells. Thus, Miranda acts as an obligatory molecular adaptor that connects Pros, Brat, and Staufen to the machinery for asymmetric protein localization.

The adaptor protein for Numb is a coiled-coil protein called Pon. Pon binds to Numb but—unlike Miranda—is not strictly required for asymmetric localization of its binding partner. In Pon mutant neuroblasts, Numb localization is delayed in metaphase. Although Numb eventually localizes asymmetrically in anaphase and telophase, this leads to a defect in self-renewal (Wang et al., 2007). Thus, Pon assists the asymmetric localization of Numb but is not required during late stages of mitosis. Therefore, the machinery for asymmetric localization seems to act on both Numb and Pon.

Setting up Polarity

Both the orientation of stem cell division as well as the polarized distribution of cell fate determinants follows an axis of polarity that is already determined in interphase. The molecular correlate of this axis is the asymmetric distribution of the proteins Par-3 (Bazooka in *Drosophila*), Par-6 and aPKC (Suzuki and Ohno, 2006; Goldstein and Macara, 2007). These proteins were originally found in *C. elegans* and are involved in essentially all biological processes that involve cell polarity. Par-6 is a small protein containing one PDZ domain and an N-terminal PB1 domain through which it interacts with a similar domain on the protein kinase aPKC. Par-6 also contains a CRIB domain with which it binds Cdc42, a small GTPase that is critical for Par-6 localization to the cell cortex (Atwood et al., 2007). Par-3 is a large protein containing three PDZ domains that can also bind to aPKC, although this interaction seems to be less stable and highly dynamic. In *Drosophila* embryos, the three proteins localize apically in the neurogenic ectoderm where they are required for apical-basal polarity. Their apical localization is maintained during neuroblast delamination and therefore, they localize to the apical neuroblast cell cortex—opposite to where the determinants concentrate in mitosis. In mutants for any of the three proteins, the others are delocalized, cell fate determinants are uniformly distributed around the cell cortex, and mitotic spindles orient randomly. Thus, the Par-proteins provide positional information for several processes that occur during asymmetric cell division.

Asymmetric Protein Localization

The key substrate for aPKC is called Lethal (2) giant larvae (Lgl) (Plant et al., 2003; Yamanaka et al., 2003; Betschinger et al., 2003). Lgl is a cytoskeletal protein that is required in epithelial cells for specifying the basolateral domain and for restricting aPKC, Par-3 and Par-6 to the apical domain. Although it binds tightly to aPKC and Par-6, it is not concentrated apically and is uniformly cortical instead. In embryonic neuroblasts Lgl is not necessary for spindle orientation or the apical localization of aPKC, Par-3 or Par-6. However, it is essential for recruiting cell fate determinants to the cell cortex and for their asymmetric localization during mitosis (Peng et al., 2000; Ohshiro et al.,

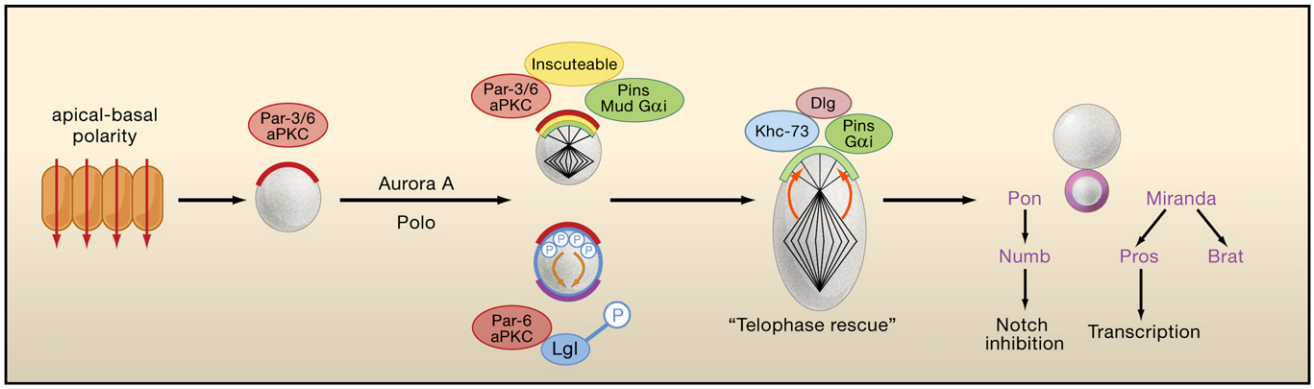

Figure 3. Intrinsically Asymmetric Divisions in *Drosophila* Neuroblasts

Epithelial apical-basal polarity is used to establish the asymmetric accumulation of Par proteins (Par-3, Par-6, aPKC, red) to the apical cortex. Upon entry into mitosis and the activation of Aurora-A and Polo kinases, the mitotic spindle is oriented by the microtubule binding protein Mud. Mud is recruited apically by Pins and Gαi (green), which in turn associate with Inscuteable (yellow) and Par-3. The asymmetric localization of cell fate determinants (purple) to the cortex opposite Par-3/6 and aPKC requires the phosphorylation of Lgl (blue) by aPKC. Late in mitosis, astral microtubules of the mitotic spindle can redirect cortical polarity (orange arrows) through the telophase rescue pathway, which involves the kinesin Khc-73 (blue) and the protein Discs-large (Dlg, pink). Ultimately, the cell fate determinants Numb, Pros, and Brat (purple) segregate into the small daughter cell with the help of their adaptor proteins Pon and Miranda. In this cell, Numb represses Notch signaling, and Pros regulates transcription; the function of Brat is unknown.

2000). This may be different in larval neuroblasts where aPKC is slightly mislocalized and mitotic spindles are sometimes abnormal (Albertson and Doe, 2003; Lee et al., 2006b) in *lgl* mutants. However, these overall polarity defects are unlikely to explain the highly penetrant phenotypes in determinant localization that are observed in *lgl* mutants. Thus, Lgl seems to play an important role in the asymmetric localization of cell fate determinants during mitosis.

Lgl is phosphorylated by aPKC on three conserved serines in a region that separates the N-terminal β-propellers and the less conserved C-terminal region of the protein (Betschinger et al., 2003). Phosphorylation induces an intramolecular interaction of the N- and C-termini that might prevent Lgl binding to the actin cytoskeleton (Betschinger et al., 2005). As overexpression of activated aPKC yields a phenotype that resembles *lgl* loss of function, this phosphorylation event seems to inactivate the Lgl protein. This has led to a model where apically localized aPKC restricts Lgl activity to the basal side of the neuroblast. Because Lgl is responsible for recruiting cell fate determinants to the cell cortex, this model could explain their asymmetric localization in neuroblasts. Lgl binds both to myosin II and to proteins involved in exocytosis (Wirtz-Peitz and Knoblich, 2006). It could therefore act either by regulating myosin (Barros et al., 2003) or vesicular trafficking but direct evidence for either of these two mechanisms in *Drosophila* is currently missing. Thus, the molecular mechanism by which Lgl acts in neuroblasts remains enigmatic.

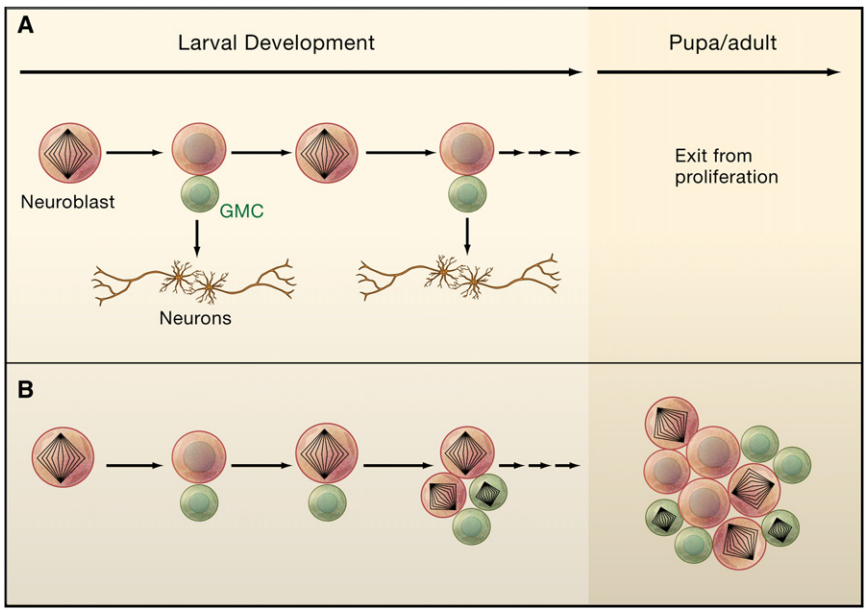

Figure 4. *Drosophila* Neuroblasts Can Become Tumor Stem Cells

(A) Normally, neuroblasts divide into one neuroblast daughter (red), which continues to grow, and one GMC (green), which stops cell growth and divides only once more into two neurons (brown). During pupal stages, all neuroblasts stop proliferating, and no mitotic activity exists in adult fly brains.

(B) Neuroblasts that lack any of the tumor suppressor genes Brat, Prospero, or Numb or have defects in their asymmetric segregation give rise to tumors. They still divide asymmetrically, but the mutant GMCs do not produce neurons. Instead, they regrow and continue to proliferate like neuroblasts. During pupal stages, these cells do not stop proliferating. Thus, defects in asymmetric cell division lead to the formation of a cell type that proliferates like a neuroblast but is immortal and no longer responds to the hormonal signals that inhibit proliferation during pupal development.

How cell fate determinants localize asymmetrically in mitosis remains the biggest mystery of asymmetric cell division. Although early models have proposed that actin-myosin dependent transport along the cell cortex is responsible, this cortical transport model is incompatible with more recent photobleaching experiments that have determined the dynamics of asymmetric protein localization (Mayer et al., 2005). Such experiments failed to detect any directional lateral mobility of segregating determinants. Instead, the determinants exchange rapidly between cortex and cytoplasm. Therefore, it is their cortical affinity that varies between the apical and basal side. Direct binding of segregating determinants to Lgl would provide a simple explanation for this variation. However, none of the determinants bind to Lgl. Moreover, the total amount of determinants at the cell cortex does not change upon Lgl overexpression indicating that Lgl is not rate limiting and therefore cannot be the cortical binding site itself. More likely, the segregating determinants localize asymmetrically because their interaction with a cortical binding site is either inhibited by aPKC or activated by Lgl (or any one of their binding partners).

In addition to Lgl, aPKC can also phosphorylate segregating determinants directly. Numb can be phosphorylated by aPKC on three serine residues at the N-terminus of the protein (Smith et al., 2007). Phosphorylation releases Numb from the cell cortex into the cytoplasm. The phosphorylation sites are in the region that is required for asymmetric localization of Numb and are located within a stretch of positively charged amino acids that might target Numb to the plasma membrane by interacting with phospholipids. Thus, in an alternative model for Numb localization, aPKC could release Numb from the apical neuroblast cortex by directly phosphorylating the protein. How Lgl would fit into such a model, however, is currently unclear.

Spindle Orientation

To ensure asymmetric segregation of cell fate determinants, the orientation of the mitotic spindle needs to be coordinated with their asymmetric localization. In embryonic neuroblasts, this coordination is achieved by a protein called Inscuteable (Kraut et al., 1996). Inscuteable localizes apically by binding to Par-3 and recruits an additional protein called Pins into the apical complex. The C-terminal half of Pins contains three so-called GoLoco domains which in turn bind to the heterotrimeric G protein subunit Gαi. Gαi binding serves two purposes (Nipper et al., 2007): Binding to the first GoLoco domain recruits Pins to the plasma membrane, presumably to facilitate its apical concentration. Upon Gαi binding to the second and third GoLoco domains, Pins changes its conformation: It switches from an inactive state where the N-terminus interacts with the GoLoco domains to an active state where the N-terminus binds an additional protein called Mud (Siller et al., 2006; Izumi et al., 2006; Bowman et al., 2006). Mud is the *Drosophila* homolog of the microtubule and dynein binding protein NuMA. Although the precise mechanism by which Mud interacts with the mitotic spindle are unclear, this cascade of protein interactions suggests a simple model in which the apical concentration of Mud provides a docking site for astral microtubules which, in turn, attracts one of the spindle poles to orient the mitotic spindle.

Consistent with this view, mutations in Mud cause overproliferation in larval central brain and mushroom body neuroblasts, presumably because the misoriented spindles lead to missegregation of cell fate determinants. Compared to *brat* mutants, however, these overproliferation phenotypes are very mild. This is due to "telophase rescue," a phenomenon that occurs in many mutants affecting asymmetric cell division and describes the correction of asymmetric protein localization defects during late stages of mitosis. Even in mutants that lead to a complete delocalization of determinants in metaphase, the asymmetry is rescued in anaphase and telophase and eventually, proteins are correctly segregated into only one daughter cell. Telophase rescue is mediated by an interaction of the mitotic spindle with the overlying cell cortex (Siegrist and Doe, 2005). The kinesin Khc-73 localizes to plus ends of astral microtubules and binds to an adaptor protein called discs large (Dlg) at the cell cortex. Dlg binds to Pins and together, these interactions lead to a clustering of the polarity complexes over the spindle pole and a polarization of the cell cortex in the direction of the mitotic spindle. In *mud* mutants, the misoriented mitotic spindle can use this mechanism to reorient cortical polarity during late mitosis and thereby rescue determinant segregation in many (but not all) neuroblasts.

Recent live imaging experiments (Rebollo et al., 2007; Rusan and Peifer, 2007) have suggested that Insc, Pins and Gαi act differently in embryonic and larval neuroblasts. Embryonic neuroblasts set up their mitotic spindle parallel to the overlying epithelium. In metaphase, the spindle rotates into an apical-basal orientation in an Insc dependent manner (Kaltschmidt et al., 2000). This rotation can occur in both directions suggesting that the two centrosomes have equal potential to become the apical spindle pole. In larval neuroblasts, however, it is always the old centrosome that is closest to the Insc/Pins/Gαi complex. Its position is fixed in a Pins dependent manner whereas the new centrosome first migrates randomly within the cell and only later gets fixed at the basal pole. As a result, the spindle is already set up in its correct orientation.

Cell-cycle Control

Neuroblast polarity is set up in interphase, but cell fate determinants only localize asymmetrically during mitosis. How is their subcellular localization coupled to cell-cycle progression?

Entry into mitosis is triggered by activation of the kinase Cdc2. Whereas Cdc2 is essential for mitosis in general, the kinases Aurora A, Aurora B, and Polo are only required for a subset of mitotic events (Meraldi et al., 2004; Barr et al., 2004). Aurora A has a key function in centrosome maturation and spindle formation, Aurora B acts in cytokinesis and Polo regulates many mitotic events including the spindle checkpoint, centrosome maturation and cytokinesis. Recent results have shown that Aurora A and Polo are both also required for the asymmetric localization of Numb. In *aurora-A* mutant SOP cells (Berdnik and Knoblich, 2002) or neuroblasts (Lee et al., 2006a; Wang et al., 2006), Numb fails to localize asymmetrically although the asymmetry of Par-3 or Insc is unaffected. In SOP cells, this leads to cell-fate transformations in the external sensory organ lineage whereas in larval neuroblasts, mutations in Aurora A cause the formation of stem cell-derived tumors. The situation is complicated by the fact that Aurora A is also required for correct orientation of the mitotic spindle. Although the spindle orientation defects can be explained because Aurora A phosphorylates D-TACC (Barros et al., 2005)—a centrosomal protein required for stabilization of astral microtubules—the molecular basis for the Numb

localization defects are unclear. Aurora A might be required for aPKC to phosphorylate Lgl or Numb but the critical substrate is unknown. In any case, the spindle orientation and Numb localization defects together lead to neuroblast overproliferation in Aurora A mutants and qualify the kinase as a tumor suppressor protein.

Tumor formation is also observed in Polo mutants (Wang et al., 2007). Like Aurora A, Polo is required for spindle orientation and the asymmetric localization of Numb and Pon but not for the initial polarization of the neuroblast. The critical substrate of Polo kinase for spindle orientation is a centrosomal protein called Asp (Barr et al., 2004). Polo also phosphorylates the Numb binding adaptor protein Pon in a domain responsible for its asymmetric localization and this is how it can promote Numb asymmetry (Wang et al., 2007). As for Aurora A, however, it is unclear whether the spindle orientation or Numb localization defects are responsible for tumor formation.

Neuroblasts as a Cancer Stem Cell Model

It has recently become clear that many tumors are maintained by a small fraction of so-called cancer stem cells that give rise to all the other cells present in the tumor (Reya et al., 2001). This hypothesis has enormous therapeutic implications but also raises the possibility that defects in stem cell lineages might be among the earliest lesions that lead to tumor formation. *Drosophila* neuroblasts have emerged as a model system that recapitulates the transition from a normal stem cell to a tumor stem cell upon mutation of genes involved in asymmetric cell division (Caussinus and Gonzalez, 2005). Normally, neuroblasts undergo a predefined proliferation pattern and stop dividing during pupal stages. Exit from mitotic proliferation still occurs when brain tissue is transplanted to adult flies indicating that it follows a cell intrinsic program. In mutants for any of the genes *pins*, *numb*, *pros*, or *brat*, however, transplanted neuroblasts overproliferate and form large metastasizing tumors that can be serially propagated for years. With the notable exception of Pins, this tumor formation correlates with the neuroblast overproliferation that is observed in the corresponding mutants. Thus, genes involved in asymmetric cell division suppress tumor formation in *Drosophila* neuroblasts.

What could be the mechanism of tumor formation? Neuroblasts still divide asymmetrically in *numb*, *pros* or *brat* mutants but eventually the smaller GMC grows to the size of the neuroblast and proliferates in a stem cell-like fashion (Figure 4). Although this cell-fate transformation can explain the increased number of larval neuroblasts observed in these mutants, it cannot account for the apparent immortalization of the mutant cells (Caussinus and Gonzalez, 2005) and their continued proliferation during adult stages (Lee et al., 2006c; Bello et al., 2006). Thus, besides being transformed back into a neuroblast, the mutant GMCs must undergo other changes that make them insensitive to the hormonal signals that stop cell growth and proliferation at the end of larval development. Identifying how mutant *Drosophila* neuroblasts escape those controls may teach us something about the transition from normal to tumor-initiating stem cells that may occur in human tumors.

Niche-Dependent Asymmetric Cell Divisions

Drosophila ovaries and testes, the female and male reproductive organs, are among the best understood model systems for stem cell biology in any organism. Unlike neuroblasts, germline stem cells (GSCs) control self-renewal via short range diffusible signals coming from surrounding cells (see Review by S.J. Morrison and A.C. Spradling, page 598 of this issue). *Drosophila* germline stem cells have been extensively covered (Li and Xie, 2005; Fuller and Spradling, 2007) and the similarieties and differences between the male and female germline have recently been described in an excellent review (Fuller and Spradling, 2007). Therefore, I will only summarize the principle mechanisms below, focusing on the most recent results obtained in ovaries, which are slightly better understood than the male germline.

In each germarium (the region of the ovary that contains the stem cells), 2-3 GSCs are surrounded by an equal number of cap cells, which form the stem cell niche. GSCs and cap cells are connected by adherens junctions that contain both β-catenin and DE-cadherin. Removal of either one of these proteins from the GSCs results in stem cell loss suggesting that niche adhesion is essential for stem cell maintenance (Song et al., 2002). The cap cells secrete the two BMP ligands, Dpp and Gbb (Fuller and Spradling, 2007), which cooperate to activate the type I and type II BMP receptors Tkv and Sax expressed by the stem cells. Both ligands are required to trigger a signal transduction cascade that results in transcriptional repression of a gene called *Bam*. Upon division of the stem cell, one of the two daughter cells loses direct contact with the niche, no longer receives the BMP signal and initiates Bam transcription. Bam initiates a characteristic differentiation program in the cystoblast: It undergoes four rounds of incomplete transit amplifying divisions to form a cyst of interconnected cells, which downregulate cell-cycle genes and exit mitotic proliferation. Eventually, one of the cells becomes the oocyte whereas the others undergo multiple rounds of endoreplication to become support cells. In *Bam* mutants, cystoblasts fail to initiate this differentiation program. Instead, they continue to proliferate like stem cells leading to the formation of an ovarian tumor.

The ovarian GSC niche also contains a second somatic cell type called the escort stem cells (ESCs) (Decotto and Spradling, 2005; Fuller and Spradling, 2007). ESCs are located within a sheath that surrounds the germarium. They are in contact with the cap cells and ensheath the GSCs with cytoplasmic extensions. Like the GSCs, they divide asymmetrically in a stem cell-like fashion. Their division is coordinated with the rate of cyst production and their daughters, the escort cells (ECs), wrap their processes around cystoblasts and individual cysts and move together with them away from the niche. Ultimately, they undergo apoptosis after the cysts exit from mitotic proliferation. ESCs require the transcriptional activator STAT, which is the nuclear target of the JAK-STAT signal transduction pathway. When STAT is removed from the ESCs, the germarium loses its shape and GSCs are rapidly lost. Conversely, when the JAK-STAT ligand Unpaired is overexpressed in ESCs, their number is increased and GSCs increase their division rate leading to the occasional formation of germline stem cell tumors (Decotto and Spradling, 2005). Thus, in addition to the Dpp signal from the cap cells, the GSCs seem to require an additional signal that is provided by the ESCs. In turn, ESCs and ECs require a signal from the GSCs that is transmitted via the EGF receptor pathway (Schulz et al., 2002) indicating that reciprocal

signaling occurring between ESCs and GSCs controls proliferation in the ovarian stem cell niche.

In contrast to neuroblasts, which follow a stereotyped lineage, ovarian stem cells are fairly dynamic in their regulation of symmetric versus asymmetric division. GSCs are randomly lost from the niche with a half life of approximately 4–5 weeks. Upon GSC loss, the remaining GSCs can divide parallel to the cap cells and thereby generate two daughter cells, which maintain niche contact and stem cell identity (Xie and Spradling, 2000). When all GSCs are induced to differentiate, for example by transiently overexpressing *Bam*, however, symmetric divisions cannot make up for the stem cell loss. In such cases, transit amplifying cells can de-differentiate into functional stem cells (Kai and Spradling, 2004). For this, cyst cells complete cytokinesis and the resulting single cells repopulate the stem cell niche. In aging flies, however, these stem cell homeostasis mechanisms become less efficient and the number of GSCs declines (Pan et al., 2007). This is attributed to a loss of E-cadherin from the stem cell-niche junction or a decrease in JAK-STAT signaling (Boyle et al., 2007). Stem cell loss can be reversed by overexpressing superoxide dismutase (SOD) thereby reducing oxygen radical damage in the stem cell or in the niche, suggesting that it is not a systemic phenomenon but that stem cells decline with age in a cell autonomous manner (Pan et al., 2007). Thus, the presence of a morphologically defined niche can regulate stem cell numbers over a long time and provides mechanisms to correct occasional defects that are not available for intrinsically asymmetric divisions.

Although much better understood in ovarian stem cells, niche dependent mechanisms may play a role in neuroblasts as well. When kept in cell culture, embryonic neuroblasts can reorient their division axis in response to surrounding epithelial cells (Siegrist and Doe, 2006). The in vivo-correlate of this cell culture phenomenon may be a signal coming from the overlying epidermis that orients neuroblast divisions along the apical-basal axis. What this signal might be and how it integrates with the cell-intrinsic polarity, however, is currently unclear. In larval neuroblasts, a glycoprotein called Anachronism is secreted by surrounding glia cells and has an inhibitory influence on mitotic activity (Ebens et al., 1993). On the other hand, ovarian stem cell divisions also show some signs of intrinsic asymmetry: A spectrin rich organelle called the spectrosome is inherited preferentially by the stem cell (Deng and Lin, 1997). In mutants that have no spectrosome, stem cells divide with misoriented mitotic spindles but the asymmetry of the division itself is unaffected (Deng and Lin, 1997) suggesting that intrinsic mechanisms do not play a major role in ovarian stem cells. Thus, it seems like neuroblasts and ovarian stem cells use distinct mechanisms to divide asymmetrically.

A number of other stem cell lineages have recently been discovered in adult flies. Stem cells are present in the adult gut (Ohlstein and Spradling, 2006, 2007), in the malpighian tubules (Affolter and Barde, 2007; Singh et al., 2007; Micchelli and Perrimon, 2006) and in the hematopoietic system (Krzemien et al., 2007; Mandal et al., 2007). How asymmetric cell division is controlled in these lineages is currently unclear. It will be interesting to determine whether extrinsic or intrinsic mechanisms exist or whether combinations of these apparently distinct pathways are employed.

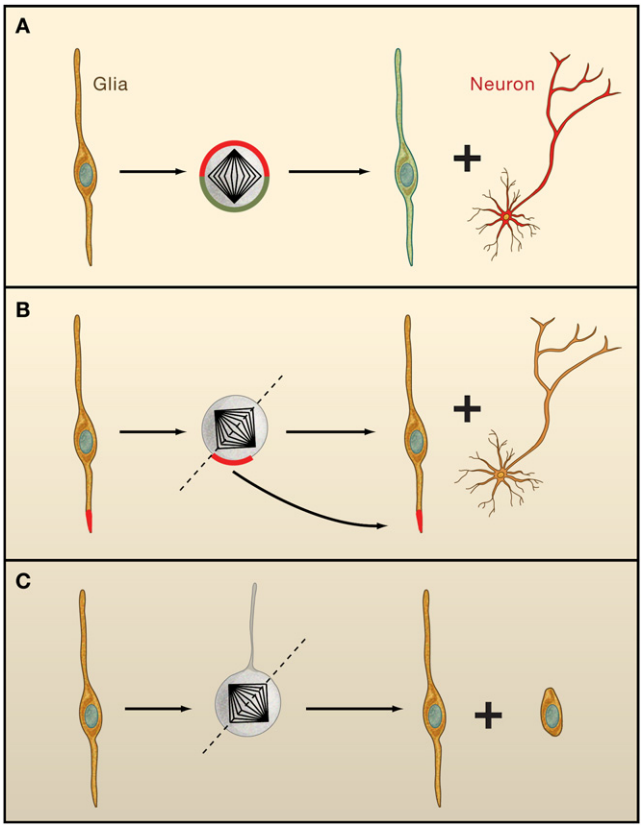

Figure 5. How Mammalian Neural Progenitors May Divide Asymmetrically

(A) Segregating determinants inherited by the apical (green) or basal (red) daughter cells induces a radial glia (green) or neuronal (red) fate.

(B) A narrow apical domain (red) is asymmetrically inherited even in oblique divisions that deviate only slightly from a vertical division plane.

(C) The basal fiber is retained during mitosis and inherited by only one daughter cell.

Asymmetric Cell Division in the Mammalian Brain

Asymmetric cell division is a key feature of mammalian stem cells as well. The mechanisms by which this asymmetry is generated, however, are much less clear. Niches have been identified for many types of mammalian stem cells but the precise interactions between the stem cells and their niches are only starting to become clear. Adult stem cells usually have extremely long cell-cycle times or are entirely quiescent making the analysis of asymmetric cell division in these adult cells enormously complicated. Therefore, most of what we know about asymmetric cell division in mammals is derived from more rapidly dividing embryonic progenitor cells. One of the most intensely studied models is the developing brain. Brain progenitors reside in a polarized environment and are thought to use this polarity to generate identical copies of themselves but also other cells that differentiate into neurons. In addition, they achieve the remarkable task of switching from entirely symmetric divisions during early development to asymmetric divisions during later development. Below, I summarize the various models that have been proposed for how asymmetry is achieved during neurogenesis in the mouse forebrain (Figure 5).

Mouse Neurogenesis

The mammalian brain develops from a pseudostratified neuroepithelium which invaginates from an area called the neural plate (Gotz and Huttner, 2005). Several distinct stages of neurogenesis can be distinguished. At embryonic day E8, neural plate cells display all the features of a polarized epithelium, contain tight and adherens junctions and function as a diffusion barrier. At E9, when the neural tube is closed and the neural stem cell marker nestin appears, some epithelial characteristics are lost (Gotz and Huttner, 2005): Tight junction markers disappear and the neuroepithelium no longer acts as a diffusion barrier. However, apical adherens junctions are retained and certain transmembrane proteins like Prominin are exclusively apical indicating that apical and basolateral membrane domains are still present. The nuclei of these early neuroepithelial cells move up and down the apical-basal axis in a cell cycle-dependent fashion—a phenomenon that is called interkinetic nuclear migration. Mitosis always occurs at the apical-most position and the mitotic spindle is oriented parallel to the epithelial surface resulting in symmetric divisions that expand the progenitor pool.

Between embryonic day E10 and E11, neuroepithelial cells start to express characteristic features of glia cells (Mori et al., 2005). They accumulate glycogen granules, express the astrocyte-specific glutamate receptor (GLAST), the brain lipid binding protein (BLBP) and in other organisms—but not in mice—also the glia marker glial fibrillary acidic protein (GFAP). From this time on, the progenitor cells are referred to as radial glia cells. Like the neuroepithelial cells, radial glia cells span the entire neuroepithelium and extend processes to the apical ventricular lumen and the basal pial surface. They continue interkinetic nuclear migration but nuclear migration is now restricted to the so-called ventricular zone which is the apical-most part of the epithelium. It is thought that radial glia cells are the progenitors of most—if not all—the neurons in the mammalian neocortex.

Although the first differentiating neurons can already be identified at E10, the peak of neurogenesis is at E14–E15. Neurons exit the cell cycle within the ventricular zone and then migrate along the fibers of the radial glia cells into the more basal area of the neuroepithelium, where their differentiation occurs. Most of these neurons are generated from asymmetric cell divisions of the radial glia cells, which occur exclusively at the apical most edge of the ventricular zone. Although the majority of these divisions (around 60% in slice cultures at E17–E19 calculated from Noctor et al., 2004) are stem cell-like and generate one radial glia cell and one differentiating neuron, minor fractions generate two proliferating radial glia cells or two terminally differentiating cells. At later stages of neurogenesis, an increasing fraction of asymmetric divisions gives rise to the so-called basal progenitor cells (also called intermediate progenitors). These are progenitors that lose their glia identity and migrate to the basal side of the ventricular zone, where they form the subventricular zone and undergo at least one more symmetric division generating two terminally differentiating neurons. Over time, the subventricular zone becomes a significant second area of neurogenesis that contains 25% or 50% of the dividing progenitor cells in the dorsal and ventral telencephalon, respectively. How are these various modes of cell division regulated and how is asymmetry established within mammalian neural progenitors?

Unlike neuroepithelial cells, radial glia can divide either parallel or perpendicular to the epithelial surface (note that the term "orientation of a division" describes the orientation of the cleavage furrow in mammalian systems, whereas it refers to spindle orientation in *Drosophila* and I will follow these conventions). Several studies have provided evidence for a correlation between spindle orientation and the asymmetry of radial glia division. In the ferret cerebral cortex, horizontal divisions are preferentially asymmetric, whereas vertical divisions tend to be symmetric (Chenn and McConnell, 1995). When imaged in organotypic slice culture, the fraction of divisions that are not vertical follows the pattern of neurogenesis with a maximum of 50% at E14 and a decline at later stages (Haydar et al., 2003). Together with the observation that mitotic spindles rotate more actively during horizontal divisions, this has led to the hypothesis that—like in *Drosophila*—an active reorientation of the mitotic spindle into an apical-basal orientation is responsible for the asymmetry of vertebrate neural progenitor divisions. However, many of these non-vertical divisions actually occur in an oblique orientation and do not result in an unequal segregation of the apical and basal membrane domains (see below). Furthermore, experiments in fixed brain sections actually determined a strong predominance of vertical divisions at all stages of neurogenesis (Stricker et al., 2006) and therefore the number of horizontal divisions is too low to explain the high fraction of asymmetry in mouse neural progenitor cell division. This was confirmed in more recent live imaging studies in which the number of horizontal divisions is also shown to be much lower than previously thought (Konno et al., 2008). Below, I discuss the various mechanisms that have been suggested to cause asymmetric cell division in the mouse brain.

Molecular Conservation of Asymmetric Cell Division

Although the precise correlation between the orientation and the asymmetric outcome of mammalian brain progenitor cell divisions remains unclear, many experiments have demonstrated that the orientation of the mitotic spindle does influence the fate of the daughter cells. Like in *Drosophila*, spindle orientation requires heterotrimeric G proteins and their binding parners Pins and Inscuteable (Sanada and Tsai, 2005; Zigman et al., 2005; Konno et al., 2008). When heterotrimeric G-proteins are inhibited or when the Pins homolog AGS-3 is targeted by RNAi, the number of vertical divisions in radial glia cells increases. As a result, the number of asymmetric progenitor divisions is reduced. Instead, progenitors divide symmetrically to generate two differentiating neurons. Thus, in radial glia cells, active orientation of the mitotic spindle is required for asymmetric but not for symmetric division. It should be noted, though, that a recent study (Konno et al., 2008) has suggested that spindle orientation might be important for the position of the daughter cells but not for neuronal production rate. Clearly, more work is needed to resolve these apparently contradictory results.

Many other molecules regulating asymmetric cell division in flies are involved in mammalian neurogenesis as well. As in *Drosophila*, the proteins Par-3 (also called ASIP in vertebrates), Par-6 and aPKC (PKCζ and PKCλ in vertebrates) localize apically in neural progenitor cells and are concentrated in adherens junctions (Manabe et al., 2002). When only one of the two aPKC isoforms is mutated, adherens junctions are lost but epithelial

polarity is maintained and neurogenesis proceeds normally (Imai et al., 2006). Upon depletion of Par-3 or Par-6, however, progenitor cells are more likely to exit the cell cycle and differentiate (Costa et al., 2008). Conversely, overexpression of Par-6 increases the number of symmetric divisions and the proliferation potential. Mutations in the basolateral protein Lgl, on the other hand, cause overproliferation of neural progenitor cells and the formation of rosette-like structures resembling primitive neuroectodermal tumors (Klezovitch et al., 2004). Taken together, these results suggest that molecules in the apical domain promote proliferation whereas the basolateral domain has an inhibitory role (Figure 5A). Although this is similar to *Drosophila*, the underlying mechanism may actually be different: Overproliferation can also be induced by activated β-catenin, a protein that is essential for epithelial polarity but is not involved in asymmetric segregation of determinants (Chenn and Walsh, 2002).

Segregating Determinants

Although the segregating determinants that were found in flies are conserved in mice, none of them has so far been shown to act as a segregating determinant in the mammalian brain. The Pros homolog Prox-1 is a potential tumor suppressor and is expressed in certain brain regions but does not seem to segregate asymmetrically (Dyer et al., 2003). The Brat homologs TRIM2, TRIM3, and TRIM32 are highly expressed in the brain but their role in brain development remains to be determined. Staufen has a conserved role in RNA transport but does not seem to be involved in asymmetric cell division in vertebrates. Most work so far has been done on the mammalian homologs of Numb. Numb has two mouse homologs which are called Numb and Numblike. Both proteins inhibit Notch signaling and act redundantly in brain development (Petersen et al., 2002). Although Numblike is a cytoplasmic protein, Numb is concentrated apically in the developing neocortex (Zhong et al., 1996). This apical concentration has led to the hypothesis that Numb might be asymmetrically inherited during horizontal, but not vertical divisions and could be responsible for their symmetric versus asymmetric outcome. Early reports on the Numb knockout phenotype were consistent with a role as segregating determinant: When both Numb and Numblike are deleted in progenitor cells, early neurons emerge in the expected patterns but progenitors are progressively depleted resulting in a stop of neurogenesis (Petersen et al., 2002). However, because Numblike does not show the asymmetric segregation that has been described for Numb, its contribution to this phenotype is hard to explain. Furthermore, when a later Cre line is used to remove the conditional allele, neural progenitors actually overproliferate instead of being lost (Li et al., 2003). Finally, deleting *numb* does not affect stem cell maintenance in the adult brain where neural stem cells also undergo asymmetric cell divisions (Kuo et al., 2006). Instead, ependymal cells in the stem cell niche lose their epithelial markers and stem cell proliferation is only indirectly affected. Fortunately, a recent study has resolved this paradox by demonstrating that the real function of Numb in brain development might actually be to maintain adherens junctions in radial glia cells (Rasin et al., 2007). Immunoelectron microscopy analysis of Numb localization shows that what was thought to be an apical crescent is actually generated by the apical endfeet of interphase radial glia cells that surround the mitotic progenitors. Within the mitotic cell itself, the apical domain is actually very narrow and most of the Numb protein is basolateral—consistent with its localization in *Drosophila*. Numb is actually concentrated on vesicles near adherens junctions and might regulate the trafficking of E-cadherin. In its absence, adherens junctions are lost from radial glia cells and this may explain the morphological defects observed in the mutant mice. Thus, Numb regulates epithelial polarity but might not actually be a segregating determinant in mouse neural progenitor cells (although it does play this role in other cell types—see below). It is therefore currently unclear whether the mechanism for asymmetric cell division in neural precursors is conserved between *Drosophila* and vertebrates. Brat remains as the only *Drosophila* protein for which a role as a segregating determinant could be conserved in vertebrates and it will be exciting to determine its functional conservation.

It is possible, however, that proteins segregate asymmetrically in vertebrate neural progenitors that have not been implicated in asymmetric cell division in flies. In fact, the EGF receptor shows a polarized distribution in dividing mouse neural progenitors (Sun et al., 2005) and is sometimes preferentially inherited by one of the two daughter cells. However, this asymmetric segregation is only seen in a fraction of the progenitors and can by no means explain all asymmetric divisions. Furthermore, EGFR also localizes asymmetrically in the subventricular zone where cells are supposed to divide symmetrically. In cell culture, however, the daughter cell inheriting EGFR expresses different markers and responds differently to EGF than its sibling suggesting that EGFR asymmetry might well contribute to fate specification in the developing mouse brain.

Structural Asymmetry of Mammalian Neural Precursor Divisions

Mammalian neural precursor divisions are morphologically highly asymmetric. A number of visible subcellular structures are asymmetrically inherited and could contribute to asymmetric fate specification. These include the apical adherens junctions and the apical membrane domain as well as the apical and basal processes that are characteristic features of radial glia cells.

When compared to other epithelial cells, the apical membrane domain of radial glia cells is very narrow due to their highly elongated morphology (Gotz and Huttner, 2005; Rasin et al., 2007). As a consequence, the apical domain could be asymmetrically inherited not only in horizontal divisions but also in oblique divisions in which the cleavage furrow only slightly deviates from a perfect vertical orientation (Figure 5B). A detailed cytological study has revealed that the orientation of the cleavage plane is actually a very poor predictor of the symmetric or asymmetric inheritance of the apical domain (Kosodo et al., 2004). This might explain why the number of asymmetric divisions is so much higher than the fraction of clearly horizontal divisions. Moreover, when the expression of a cell-cycle regulator called Tis21 is used as an indicator for cell-cycle exit of one of the two daughter cells, many terminal neurogenic divisions are actually correlated with an asymmetric inheritance of the apical domain. It is interesting that the apical domain actually becomes progressively narrower over the time of neurogenesis. This has been attributed to a release of parts of this domain into the lateral ventricle during each progenitor division (Dubreuil et al., 2007) and could explain

why the fraction of asymmetric divisions increases with time. So far, no cell fate determinant is known that resides in the small apical domain. However, a scenario where an apical receptor is stimulated by a ligand contained within the ventricle would be quite plausible. Identification of such a receptor would strongly support this interesting model for asymmetric cell division in the mammalian brain. It should be noted, however, that a recent live imaging study (Konno et al., 2008) failed to confirm the asymmetric inheritance of the apical domain during asymmetric progenitor division and therefore some aspects of this interesting hypothesis might need to be reinvestigated.

During the peak time of neurogenesis, mouse brain progenitors have the morphology of radial glia cells and extend an apical process to the ventricular surface and a basal process to the basement membrane at the pial surface. Although the apical process disappears during mitosis, elegant live imaging studies in slice culture have shown that the basal process is actually maintained throughout division. As a consequence, the process is inherited by one of the two daughter cells even in divisions with a vertical cleavage plane (Figure 5C). This morphological asymmetry of the two daughter cells could explain why most radial glia divisions are asymmetric although only a small fraction has a horizontal cleavage plane. Two scenarios have been proposed (Fishell and Kriegstein, 2003): Either, the radial fiber is inherited by the future neuron which uses the fiber to translocate to the cortex (Miyata et al., 2001). In this case, the radial glia cell would have to develop a new fiber after each division. Alternatively, the fiber could go with the radial glia cell and the neuron could use the fiber of its sibling cell for migration to the cortex (Noctor et al., 2001). Life imaging support exists for both models but so far, a cell-fate-determining function for the fiber remains to be demonstrated.

Thus, the precise mechanism by which neural stem and progenitor cells self-renew and generate neurons at the same time, is unclear. Like in *Drosophila*, Par-3/6 and aPKC are involved and heterotrimeric G-proteins with their binding partners orient the mitotic spindle. Whether—as in *Drosophila*—unequal fates are generated by segregating determinants or whether the asymmetric inheritance of the apical domain or the radial fiber are responsible, is currently unclear. Of course, combinations of these scenarios are possible, for example if the radial fiber would transport a segregating determinant or if the apical domain regulates the response to an extracellular signal. Given that adult neural stem cells have glia identity as well (Doetsch, 2003) it is quite likely that the principles found during development are applicable to adult neurogenesis as well.

Asymmetric Cell Division in Nonneuronal Stem Cells

Satellite Cells

Satellite cells are a population of muscle cells that is important for homeostasis of muscle tissue and for regeneration after injury (Cossu and Tajbakhsh, 2007; see Review by D.J. Laird et al., page 612 of this issue). Since satellite cells can both self-renew and contribute to the syncytium they appear to have all the characteristic features of a stem cell. Due to their position on the surface of the muscle fiber beneath the basement membrane and their characteristic expression of the marker Pax7, satellite cells can easily be analyzed by standard immunofluorescence technologies. Satellite cells can be transplanted, isolated by FACS sorting and imaged in real time in cultured myofibers and therefore have become a valuable system to study stem cell biology.

Recent studies have provided more evidence that satellite cells are actually a heterogeneous population where all cells express Pax7 but only a fraction also expresses the myogenic transcription factor Myf5 (Kuang et al., 2007). In vitro lineage analysis shows that the Myf5-positive satellite cells arise from the Myf5-negative cells. Upon transplantation, only the Myf5-negative cells contribute significantly to the satellite cell compartment whereas the $Myf5^+$ cells preferentially undergo terminal differentiation. Thus, the $Pax7^+$ $Myf5^-$ cells are true stem cells whereas the $Pax7^+$ $Myf5^+$ cells are more committed progenitors.

Immunofluorescence and live imaging studies indicate that $Myf5^+$ cells arise from $Myf5^-$ cells through asymmetric cell division. Although most satellite cell divisions are planar and symmetric giving rise to two $Pax7^+$ $Myf5^-$ or two $Pax7^+$ $Myf5^+$ cells, these asymmetric divisions are oriented perpendicularly to the muscle fiber and create one cell that loses contact with the basement membrane, expresses Myf5 and becomes a committed progenitor. It is conceivable that integrin mediated contact with the basement membrane is essential for satellite cells to maintain their stem cell character. Consistent with this, knockout studies and molecular analysis of human diseases have shown that integrins are essential to maintain muscle function. In addition, several experiments have suggested that the Notch pathway plays an important role in maintaining the satellite cell population (Luo et al., 2005). When Notch is inhibited, satellite cells are not maintained and undergo premature differentiation. Conversely, overactivation of the Notch pathway inhibits muscle cell differentiation. Upon muscle injury, an initial burst of Notch signaling during satellite cell activation is followed by a decline of Notch signaling as the daughter cells become fusion competent to regenerate the muscle. Two potential mechanisms could regulate Notch signaling in satellite cells: First, the receptor Notch-3 is highly expressed in the $Pax7^+$ $Myf5^-$ population but much lower in the $Pax7^+$ $Myf5^+$ population. Given that the Notch ligand Delta-1 is more abundant in committed progenitors, these cells could signal back to the $Pax7^+$ $Myf5^-$ cells to maintain their uncommitted state. Second, the Notch inhibitor Numb is asymmetrically segregated during satellite cell division. Numb overexpression can inhibit Notch signaling and induce committment of the progenitor cells and it is conceivable that asymmetric segregation of Numb is a primary mechanism to establish asymmetric cell divisions of muscle stem cells. In *Drosophila*, Numb is not only involved in neurogenesis but plays a very similar role during muscle development (Carmena et al., 1998; Park et al., 1998). It is thought that its localization mechanism and downstream pathway are identical in the two tissues. Therefore, the data from satellite cells suggest that Numb has a conserved role in controlling the lineage of muscle progenitors.

Asymmetric Cell Division in the Hematopoietic System

The hematopoietic system certainly represents one of the best understood stem cell lineages. However, we know very little about how choices between self-renewal and commitment are made within this system, maybe because it is mostly studied on isolated cells. Hematopoietic stem cells (HSCs) are often seen in close proximity with osteoblasts or endothelial cells

of blood vessels and both have therefore been suggested to act as the stem cell niche (Kiel et al., 2005). Whether a signal from these cell types is required for self-renewal and if so, what that signal would be, is currently unclear. Interestingly, recent live imaging experiments have revealed exciting aspects of intrinsic asymmetry within the HSC population (Wu et al., 2007; Schroeder, 2007). These experiments used a GFP-reporter for the Notch signaling pathway, which is active within HSCs but downregulated during differentiation. They revealed that HSCs can divide symmetrically or asymmetrically resulting in daughter cells with equal or unequal levels of Notch signaling. Numb is present on HSCs and segregates into one of the two daughter cells during the asymmetric divisions. Although Numb is not strictly required in HSCs (Wilson et al., 2007), it might act redundantly with other factors to inhibit Notch in one of the two daughter cells. Given that Notch signaling is known to be essential for maintaining the undifferentiated state of hematopoietic stem cells (Duncan et al., 2005), those asymmetric cell divisions will probably form one stem cell and one more committed daughter cell. It is interesting that the balance between symmetric and asymmetric divisions depends on the environment: When cultured on an osteoblastic cell line, HSCs undergo mostly asymmetric divisions whereas a generic stromal cell line induces mostly symmetric divisions. Thus, HSCs may control self-renewal through a stem cell niche that regulates the balance between symmetric and asymmetric stem cell divisions.

Asymmetric cell divisions are not only found in HSCs but also at later stages of hematopoiesis. When T-lymphocytes encounter their antigen, they form a synapse-like connection with the antigen-presenting cell. It is now known that this encounter is followed by an asymmetric cell division giving rise to one effector T cell and one memory T cell that maintains the selected T cell lineage and has certain stem cell-like features. A beautiful recent study has turned this into the best understood asymmetric cell division in vertebrates that shows a striking conservation of the machinery used in *Drosophila* neuroblasts (Chang et al., 2007). Upon antigen contact, the centrosome moves close to the immunological synapse together with the transmembrane protein CD8 and the integrin LFA-1. The induced polarization of the T cell results in asymmetric distribution of aPKC to the cell pole opposite the immunological synapse. During mitosis, this results in the asymmetric segregation of Numb into the proximal daughter cell. Together with Numb, also CD8, the interferon γ receptor and other factors segregate into the proximal daughter cell and it is unclear which of these is responsible for determining effector fate. However, the strikingly similar localization patterns suggest that—as in *Drosophila* neuroblasts—Numb induces differentiation whereas aPKC promotes a more stem cell-like state in the respective daughter cells.

Future Directions

In recent years, the extrinsic and intrinsic mechanisms that control asymmetric cell division in *Drosophila* neuroblasts and oocytes have largely been worked out. How they control cell growth and cell-cycle progression, however, is less clear. Do stem cells use the same pathways that control organ size and proliferation in other tissues and control them differently in self-renewing and differentiating daughter cells? Or is there a stem cell-specific machinery that controls growth and proliferation in a variety of stem cell lineages? Why does stem cell proliferation get out of control when asymmetric cell division is compromised? What are the molecular events that occur when such a compromised stem cell becomes unresponsive to growth control signals? And finally, to what extent are the mechanisms discovered in *Drosophila* also used in vertebrates? The striking conservation of the machinery for asymmetric cell division has raised hopes for an easy answer to this burning question. It is now clear, however, that these hopes were premature. It seems likely that this machinery acts in a tissue-specific manner and the way it acts needs to be analyzed in each case. The striking discovery of stem cell lineages in the adult *Drosophila* gut (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006), malpighian tubules (Singh et al., 2007) and hematopoietic system (Mandal et al., 2007) might lead to the identification of entirely new mechanisms for stem cell control. Given the speed at which the field has moved so far, I am sure we will not have to wait for too long.

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