

Drosophila Neuroblast Asymmetric Cell Division: Recent Advances and Implications for Stem Cell Biology

Minireview

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Asymmetric cell division is an evolutionarily conserved mechanism widely used to generate cellular diversity during development. *Drosophila* neuroblasts have been a useful model system for studying the molecular mechanisms of asymmetric cell division. In this minireview, we focus on recent progress in understanding the role of heterotrimeric G proteins and their regulators in asymmetric spindle geometry, as well as the role of an Inscuteable-independent microtubule pathway in asymmetric localization of proteins in neuroblasts. We also discuss issues of progenitor proliferation and differentiation associated with asymmetric cell division and their broader implications for stem cell biology.

Asymmetric cell division is one of the primary mechanisms used by progenitor cells to generate neuronal diversity in *Drosophila* central and peripheral nervous systems (CNS and PNS, respectively). The study of its underlying mechanisms started about 12 years ago with the discovery that Numb functions as an intrinsic cell-fate determinant that is segregated into only one of the two daughter cells during neural precursor division (Rhyu et al., 1994). Since then, largely through the efforts of a handful of labs, considerable insights into the inner workings of this fundamental pathway have been gained. In this minireview, we will discuss recent progress in this field. Before doing so, we will first start with a brief overview of the “state of the field” until just a couple of years ago.

In the *Drosophila* embryonic CNS, each abdominal segment of the ventral nerve cord (VNC) consists of approximately 700 neurons and 60 glial cells that have distinct fates and morphologies. These neurons and glia are together derived from roughly 60 progenitor cells called neuroblasts (NBs). To generate the diversity of cell fates from a single precursor cell, NBs undergo programmed asymmetric cell division. During *Drosophila* embryogenesis, NBs are specified from a monolayer of epithelial cells called the neuroectoderm through a process of lateral inhibition. Once specified, NBs delaminate from the epithelium layer and enter mitosis. During prophase of the cell cycle, two centrosomes migrate

laterally to opposite sites of the NB with parallel alignment to the epithelium layer. At metaphase, the mitotic spindle rotates by 90° to be oriented along the apical-basal axis. Upon cytokinesis, each NB gives rise to two daughter cells with distinct size and fate. The larger apical daughter cell maintains a NB identity and continues to divide in a stem-cell-like fashion, whereas the smaller basal daughter cell becomes a ganglion mother cell (GMC) which undergoes terminal division to generate two postmitotic neurons or glia (Figure 1; also reviewed by Betschinger and Knoblich [2004]; Chia and Yang, 2002; Jan and Jan, 2001).

The apical-basal polarity of an epithelial cell is established through an evolutionarily conserved protein complex consisting of Bazooka (Baz, the fly homolog of *C. elegans* Par-3), Par-6, and atypical PKC (aPKC), also known as the Par complex. This protein complex is maintained in the specified NB and utilized by the NB to preserve its cellular polarity. When a NB delaminates from the epithelium, it forms a cellular structure called the “apical stalk,” which is in contact with the surrounding epithelial cells (Figure 1). The Par complex in the apical stalk colocalizes with the NB-specific protein Inscuteable (Insc), establishing an apical-basal polarity in the delaminating NB. During mitosis, the Insc/Par complex localizes to the apical cortex as a crescent and recruits another evolutionarily conserved protein complex, comprised of Partner of Inscuteable (Pins) and the heterotrimeric G protein subunit G α i, that maintains apical-basal polarity. This sequential recruitment model explains how apical-basal polarity of a NB is first established and subsequently maintained during the first round of asymmetric cell division (Parmentier et al., 2000; Schaefer et al., 2000; Yu et al., 2000).

The primary roles of the two protein complexes are distinct: the Pins/G α i complex is mainly involved in spindle orientation (metaphase NBs align their spindles perpendicular to the epithelium layer), whereas the Par complex appears to play a primary role in the basal localization of cell-fate determinants (Wodarz et al., 1999; Schober et al., 1999; Izumi et al. 2004). The apical Par complex directs the basal localization and segregation of cell-fate determinants, such as Prospero (Pros) and Numb and their adaptor proteins, Miranda (Mira) and Partner of Numb (Pon) through two cortically localized tumor suppressors, Discs large (Dlg) and Lethal (2) giant larvae (Lgl) (Ohshiro et al., 2000; Peng et al., 2000; Betschinger et al., 2003). Dlg and Lgl are primarily involved in localizing basal proteins and have only a mild effect on the crescent formation of the apical proteins (Ohshiro et al., 2000; Peng et al., 2000; Siegrist and Doe, 2005), although Dlg can also interact with apical protein Pins (Bellaiche, et al., 2001). Phosphorylation of Lgl by apically localized aPKC leads to Lgl inactivation and prevents its association with the apical cortex, while non-phosphorylated Lgl at the basal cortex is in an active state and recruits cell-fate determinants (Betschinger et al., 2003). The phosphorylation state of Lgl also restricts myosin II activity to the apical cortex, which results in the “push” of cell-fate determinants to the basal

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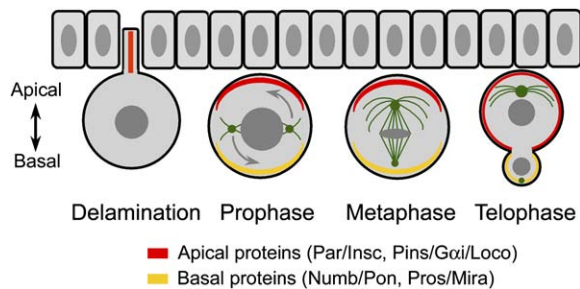


Figure 1. Asymmetric Cell Division of *Drosophila* NB

A schematic representation of NB delaminating from epithelium. As the NB is delaminating from the epithelium, it forms an apical stalk, which is in contact with the surrounding epithelial cells. During prophase, Par/Insc/Pins/Gai proteins (red crescent) are asymmetrically localized at the apical cortex, whereas Numb/Pon/Pros/Mira (yellow crescent) proteins are localized at the basal cortex. The two centrosomes and the mitotic spindle (green) are aligned parallel to the surface of epithelium. During metaphase, the mitotic spindle rotates 90° to become aligned perpendicular to the epithelial layer. During anaphase/telophase, asymmetric spindle geometry is generated, which divides the neuroblast into two daughter cells of distinct fate and size: a large apical NB and a smaller basal GMC. The basal daughter GMC preferentially inherits cell-fate determinants Numb and Pros.

cortex (Barros et al., 2003). In contrast to the function of Myosin II in excluding cell-fate determinants from the apical cortex, Myosin VI (also termed Jaguar) positively regulates basal localization and segregation of Mira/Pros via vesicle transport machinery (Petritsch et al., 2003).

For the remainder of this minireview, we will focus on four aspects of NB asymmetric cell division that have been highlighted by recent studies. We will first discuss how heterotrimeric G proteins are activated in a receptor-independent manner. Next, we will explore how asymmetric spindle geometry is generated during anaphase/telophase and what factors orient mitotic spindle along the apical-basal axis. And finally, we return to a fundamental question of how proliferation and differentiation are coordinated during asymmetric cell division and the implications of these studies for mammalian stem cell biology. A review of another area of considerable recent progress, namely, the role of endocytic pathway in asymmetric cell division of *Drosophila* sensory organ precursors (SOPs) can be found elsewhere (Somers and Chia, 2005).

Receptor-Independent Activation of Heterotrimeric G Proteins in *Drosophila* NBs

In the canonical model of heterotrimeric G protein signaling, extracellular signals can be transduced to targets within the cell through a seven-pass transmembrane receptor called G protein coupled receptor (GPCR). In the inactive state, the GDP-GαGβγ heterotrimer associates with GPCR. Upon ligand binding, GPCR acts as a guanine nucleotide exchange factor (GEF) to stimulate release of GDP from the Gα subunit, which in turn is converted to the GTP bound form. GTP-Gα dissociates from the Gβγ complex and each activates its downstream effectors. G protein signaling is attenuated through the hydrolysis of GTP to GDP by the intrinsic GTPase activity of Gα. The hydrolysis rate is accelerated by GTPase-activating

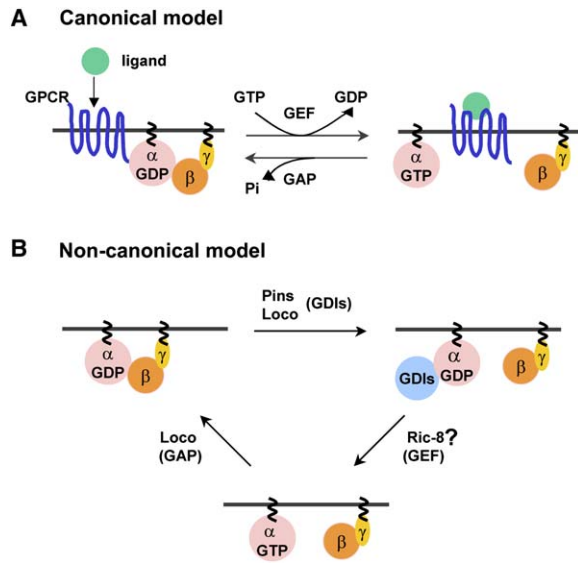


Figure 2. Canonical and Noncanonical Models of Heterotrimeric G Protein Signaling

(A) In the canonical model, upon ligand binding, a seven-pass transmembrane protein, GPCR, can act as a GEF to activate heterotrimeric G proteins. In the *Drosophila* NBs, heterotrimeric G proteins can also be activated in a receptor-independent manner.

(B) In a possible noncanonical cycle, two GDIs, Pins, Loco, and a putative cytosolic GEF, Ric-8, may act synergistically to activate heterotrimeric G proteins. The attenuation of G proteins may be mediated by Loco acting as a GAP.

proteins (GAPs), which typically contain regulator of G protein signaling (RGS) domain(s) (Figure 2A).

Heterotrimeric G proteins can also be activated through a receptor-independent mechanism by cytosolic proteins containing GoLoco/GPR domains, a mechanism employed in *Drosophila* NB asymmetric cell division. GoLoco/GPR domain proteins can interact with GDP-Gα and compete with Gβγ for GDP-Gα binding. In this capacity, GoLoco/GPR domain proteins act as guanine nucleotide dissociation inhibitors (GDIs) to dissociate GDP-Gα from Gβγ dimer. In the absence of ligand binding to GPCR, this mechanism may initiate the noncanonical heterotrimeric G protein signaling pathway. In *Drosophila*, the GoLoco/GPR domain-containing proteins Pins and Loco act as GDIs for Gai (Schaefer et al., 2001; Yu et al., 2005). pins, loco, or Gai single mutants display similar phenotypes in spindle geometry and daughter cell size difference. Simultaneous inactivation of Pins and Loco results in a much more severe disruption of spindle geometry and daughter cell size difference, similar to Gβ 13F or Gγ1 mutants which remove β and γ subunits of heterotrimeric G proteins respectively, suggesting that Pins and Loco may act redundantly to dissociate the inactive heterotrimer in *Drosophila* NBs (Yu et al., 2005; Figure 2B). Loco also contains the RGS domain that shows GAP activity for Gai, indicating that Loco may act as a GAP to hydrolyze GTP-Gai to GDP-Gai (Yu et al., 2005; Figure 2B).

A question remains as to how GDP-Gai is converted to GTP-Gai in the absence of GPCRs acting as receptor GEFs. One candidate molecule that performs this function could be the evolutionally conserved Ric-8. Mammalian Ric-8A can act as a cytosolic GEF for GoLoco

bound myristoylated $G\alpha_i$ (Tall and Gilman, 2005). In nematodes, Ric-8 exhibits GEF activity for only one of two redundant $G\alpha$ proteins implicated in the asymmetric cell division of one-cell zygotes (Afshar et al., 2004; Afshar et al., 2005). In *Drosophila*, Ric-8 is required for the membrane-targeting of heterotrimeric G proteins (David et al., 2005; Hampoelz et al., 2005; Wang et al., 2005). However, it remains to be determined whether *Drosophila* Ric-8 possesses a GEF activity for $G\alpha_i$. One plausible model for receptor-independent cycling of heterotrimeric G proteins is illustrated in Figure 2B.

In the canonical mode, GTP- $G\alpha_i$ is generally considered the active protein that activates various downstream effectors. It remains to be determined whether the GDP bound or GTP bound $G\alpha_i$ is active in the receptor-independent model. In *Drosophila*, embryos overexpressing GTP- $G\alpha_i$ exhibit mild defects, whereas overexpression of GDP- $G\alpha_i$ produces much stronger defects (Schaefer et al., 2001; Yu et al., 2005), suggesting that GDP- $G\alpha_i$, together with Pins and Loco, might play important roles in *Drosophila* NBs. Furthermore, it would be important to identify the downstream effectors of activated $G\alpha_i$ and $G\beta\gamma$ during NB asymmetric cell divisions.

Heterotrimeric G Proteins and Asymmetric Spindle Geometry

One important aspect of NB asymmetric cell divisions is the correlation between asymmetric spindle geometry and daughter cell size. The mitotic NB forms an apically-biased spindle during anaphase/telophase with the following characteristics (Figure 1): first, the apical centrosome is larger in size than the basal one; second, astral microtubules emanating from the apical centrosome grow more robustly; third, the apical centrosome is located far from the proximal cortex whereas the basal centrosome is almost attached to the basal cortex (so called “basal displacement of mitotic spindle”); fourth, the apical half of spindle that extends from the apical centrosome to the cleavage plane is longer than the basal one (Bonaccorsi et al., 2000; Kaltschmidt et al., 2000; Fuse et al., 2003). Asymmetric spindle geometry, in general, correlates well with daughter cell size differences during NB asymmetric cell divisions (Cai et al., 2003; Fuse et al., 2003; Izumi et al., 2004; Yu et al., 2003). It results in dramatic size disparity between the NB daughter and GMC daughter: the diameter of a NB is three times larger than that of a GMC (Cai et al., 2003; Fuse et al., 2003; Izumi et al., 2004). One exception to this correlation, however, is the *asterless* (*asl*) mutant, whose NBs divide asymmetrically in the absence of centrosomes and astral microtubules (Bonaccorsi et al., 2000). It remains possible that *asl* mutant NBs retain a residual amount of functional astral microtubules and centrosomes.

Both the spindle geometry and daughter cell size difference are controlled by two redundant pathways mediated by the Insc/Baz/Par-6/aPKC proteins and the Pins/ $G\alpha_i$ /Loco proteins (Cai et al., 2003; Yu et al., 2003, 2005). The majority of mutant NBs that lose a single component of either complex still divide asymmetrically to produce daughter cells of unequal size. However, the spindle asymmetry and daughter cell size difference are entirely disrupted when an additional component of the Insc/Baz/Par-6/aPKC complex is removed in *pins*, *loco*, or $G\alpha_i$ mutants. In $G\beta 13F$ or $G\gamma 1$ mutant, the majority of NBs exhibit symmetric spindle structure and divide into

two equally sized daughter cells (Fuse et al., 2003; Izumi et al., 2004), suggesting that cortically localized $G\beta\gamma$ proteins play a more prominent role than other apical proteins in this process.

There are currently several models for how cortically localized $G\beta 13F$ can affect daughter cell size asymmetry. The first model proposes that active $G\beta 13F$ functions to suppress the length of basal spindle arm while its activity at the apical side is antagonized by $G\alpha_i$, resulting in the formation of an asymmetric spindle (Fuse et al., 2003). This is supported by the fact that $G\beta 13F$ mutant NBs form a large symmetric spindle whereas the elevated activity of $G\beta 13F$ results in a small symmetric spindle. The second model uses the data that Insc/aPKC and Pins/ $G\alpha_i$ are delocalized in $G\beta 13F$ mutant NBs, which suggests that $G\beta 13F$ may control both the Insc/Par and Pins/ $G\alpha_i$ pathways to regulate spindle geometry (Yu et al., 2003). The third model suggests that $G\beta 13F$ regulates spindle geometry by restricting $G\alpha_i$ localization to the apical side (Wang et al., 2005), which are supported by the following: first, in $G\beta 13F$ mutant embryos, $G\alpha_i$ is localized uniformly around the NB cortex, raising a possibility that cortically localized $G\alpha_i$ may be active to regulate spindle geometry from both apical and basal sides; second, a $G\beta 13F$ *ric-8* double mutant, in which $G\alpha_i$ and Pins are cytosolic, resembles a *ric-8* or $G\alpha_i$ single mutant but not $G\beta 13F$ mutant in terms of daughter cell size differences.

Thus, $G\alpha_i$ may be a key regulator in controlling spindle geometry and daughter cell size difference, such that the high frequency of symmetric division in $G\beta 13F$ or $G\gamma 1$ mutants is the indirect consequence of the cortical localization of $G\alpha_i$ /Pins, which may override polarity cues from Par proteins (Wang et al., 2005). It was also reported that a different *ric-8* mutant allele exhibits equal size division in the majority of NBs, similar to that seen in $G\beta 13F$ mutants. This evidence supports the view that Ric-8-mediated $G\beta 13F$ localization to the cortex may be a key pathway in controlling spindle geometry and daughter cell size difference (Hampoelz et al., 2005). In the future, the roles of $G\alpha_i$ and $G\beta 13F$ in spindle geometry and daughter cell size difference could be further assessed if double germline clone mutants of $G\beta 13F$ $G\alpha_i$ can be generated and compared to either $G\beta 13F$ or $G\alpha_i$ single mutants.

In mammalian cells, the $G\alpha$ subunit can bind microtubules (Willard et al., 2004). However, it is not known whether heterotrimeric G proteins can directly regulate spindle geometry by binding microtubules in *Drosophila*. Evidence from the *ric-8* mutant, in which cytosolic $G\alpha_i$ fails to associate with the mitotic spindle, suggests at least that GDP- $G\alpha_i$ (and Pins) are unlikely to possess an affinity for microtubules in *Drosophila*. Thus, one important remaining question is what mechanisms control the reorganization of microtubules that coordinates the formation of the cleavage furrow to generate daughter cell size differences during NB asymmetric cell division.

Asymmetric Protein Localization and Mitotic Spindle Orientation

The sequential recruitment model described earlier in this minireview explains how apical-basal polarity is initially established and subsequently maintained in order for NBs to orient their mitotic spindle along the apical-basal axis during the first round of asymmetric cell

division. However, it is not clear how the NB can “memorize” this apical-basal polarity to always divide perpendicular to the epithelial surface. During *in vitro* culture conditions, NBs maintain an intrinsic polarity and divide into two unequal-sized daughter cells in the absence of neighboring cell contacts (Siegrist and Doe, 2006). However, in these isolated NB cultures, NBs randomize the spindle axis orientation over a series of divisions, leading to the generation of daughter GMCs from random cortical positions. In contrast, in a culture of clustered cells, NBs that retain contact with epithelial cells orient their mitotic spindle perpendicular to the epithelial surface and consequently bud off GMC daughters at the same site away from epithelial cells. This suggests that cortical polarity and/or spindle orientation in NBs is regulated by either extrinsic signals emanating from epithelial cells or by cell-cell contact between the NB and neighboring epithelial cells (Figure 1; Siegrist and Doe, 2006).

Support for this idea comes from the *Drosophila* sensory organ precursors (SOPs), located in the developing PNS. SOPs divide asymmetrically along the anterior-posterior axis within the plane of the epithelium, and the orientation of their division depends on receptors such as Frizzled and Flamingo (Gho and Schweisguth, 1998; Lu et al. 1999). Although NBs divide under the epithelium plane unlike SOPs, it is still possible that some unidentified receptor(s) could be clustered at the site of contact between NBs and epithelial cells and thereby recruits the apical complex. Interestingly, the intercellular region of Echinoid, a transmembrane cell adhesion molecule, interacts with the PDZ domains of Baz to regulate cell adhesion in the wing disc epithelial cells (Wei et al., 2005), raising the possibility that this or some other similar interactions may be responsible for providing polarity cues to NBs.

One hallmark of asymmetric cell division in *Drosophila* is the coupling of asymmetrically localized proteins to each centrosome, leading to mitotic spindle alignment and subsequent asymmetric protein segregation. Given that apically localized proteins are required to orient the mitotic spindle during mitosis, one may expect that adaptor proteins that can interact simultaneously with apical cortical proteins, and microtubules could serve to align the spindle along the apical-basal axis. In *C. elegans*, Lin-5, a microtubule-associated protein, can interact with GPR-1/2, the nematode counterpart of *Drosophila* Pins, to displace the mitotic spindle toward the posterior cortex in the one-cell zygote (Srinivasan et al., 2003). In mammals, the microtubule binding protein, NuMA can interact with LGN, the mammalian homolog of *Drosophila* Pins, to control spindle dynamics (Du and Macara, 2004). The fly homolog of Lin-5/NuMA is Mushroom body defect (Mud), a large coiled-coil domain protein, which can interact with the N-terminal region of Pins containing seven TPR repeats, and is localized to both the apical cortex and centrosomes during mitosis. Loss of *mud* function leads to uncoupling of mitotic spindle with apical crescent in some mitotic NBs (Bowman et al., 2006; Izumi et al., 2006; Siller et al., 2006). Thus, it appears that Pins can align the mitotic spindle along the apical-basal axis through direct interactions with the microtubule-associated adaptor protein Mud (Bowman et al., 2006; Izumi et al., 2006; Siller et al., 2006).

Not only the apical proteins are able to orient the mitotic spindle, this interaction is not a one-way street, as recent study suggests that a microtubule-dependent pathway can also asymmetrically localize apical proteins such as Pins/G α i in metaphase NBs (Siegrist and Doe, 2005). How does the microtubule-dependent pathway exert its function at the cellular cortex during metaphase? Kinesin heavy chain 73 (Khc-73), a microtubule-based plus-end motor protein, plays an important role, as it can interact with cortical protein Dlg *in vivo*. Through this interaction, Khc-73 may act to change the conformation of Dlg: in the absence of Khc-73, the GK domain of Dlg can bind intramolecularly to its SH3 domain, whereas in the presence of Khc-73, the GK domain may preferentially associate with the Khc-73 MAGUK binding site (MBS), freeing the SH3 domain to bind Pins in an intermolecular interaction. How might Khc-73 function *in vivo*? One model is as follows: at metaphase, Khc-73 present at the plus end of astral microtubules can cluster Dlg to one side of the cortex. Then the Khc-73 bound Dlg proteins expose their SH3 domains and interact with Pins, thereby localizing Pins/G α i asymmetrically.

The Dlg/Khc-73/microtubule pathway is primarily active at metaphase, whereas the Insc/Par pathway used to localize Pins/G α i at the apical cortex is active prior to metaphase. When the Insc/Par pathway is inactivated, Pins/G α i are uniformly localized around the cell cortex prior to metaphase. However, the Dlg/Khc-73/microtubule pathway can act independently of Insc/Par to induce Pins/G α i crescent at metaphase. Although the Pins/G α i crescent is formed overlying one of the centrosomes, its position along the cortex is randomized and not restricted to the normal apical side, leading to formation of a misoriented spindles. When the Dlg/Khc-73/microtubule pathway is inactivated, Pins/G α i can be recruited to the apical cortex by the Insc/Par complex, as in wild-type NBs. Yet, the apical Pins/G α i crescent fails to overlie one of centrosomes, leading to formation of a misoriented spindle that is uncoupled from apical proteins. Only when the microtubule and the Insc/Par pathways are simultaneously activated can mitotic spindle oriented along the apical-basal axis be coupled to the asymmetric localization of proteins (Siegrist and Doe, 2005; Figure 3).

This model of the microtubule/Khc-73/Dlg pathway helps to explain how Pins/G α i forms a crescent at metaphase in *insc* mutants. Moreover, this model may also explain how spindle rotation occurs at metaphase in the wild-type situation. When Khc-73/Dlg and Dlg/Pins complexes form at the apical cortex, Khc-73 may depolymerize astral microtubules at their plus ends to pull one of the centrosomes toward the apical side. It's a mechanism similar to kinetochore-attached kinesin acting as a microtubule-destabilizing enzyme to disassemble tubulin monomers at the plus ends of kinetochore microtubules and thereby generate a pulling force leading to sister chromatid separation at anaphase (Rogers et al., 2004). The potential role of Khc-73 as a microtubule depolymerase remains to be determined in *Drosophila* NBs.

But how is a particular centrosome chosen to migrate toward the apical end? Previous studies showed that a basally positioned interphase centrosome can cause

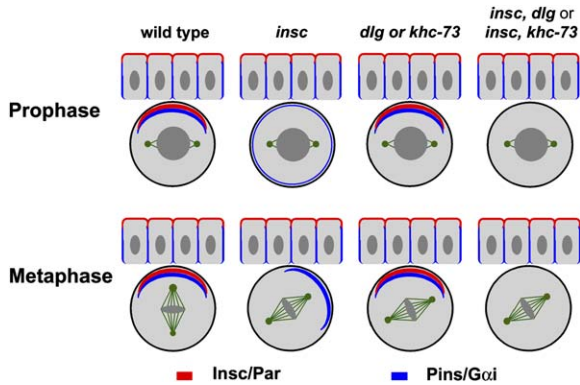


Figure 3. Both *Insc/Par* and *Dlg/Khc-73* Are Required for *Pins/Gαi* Crescent Formation and Spindle Alignment

In the wild-type NB, *Insc/Par* (red crescent) and *Pins/Gαi* (blue crescent) are colocalized at the apical cortex during prophase/metaphase and overlie the apical centrosome. In *insc* mutant NBs, during prophase *Pins/Gαi* (blue) is localized uniformly around the cortex and becomes a randomly placed crescent overlying one centrosome during metaphase. In *dlg* or *khc-73*-RNAi mutants, the *Pins/Gαi* crescent forms during prophase/metaphase, but the mitotic spindle becomes uncoupled during metaphase. In mutant NBs lacking *insc/dlg* or *insc/khc-73*, *Pins/Gαi* fails to form a crescent during metaphase, suggesting that *Pins* crescent formation and spindle alignment require both pathways. Centrosomes are represented as green dots.

an anticlockwise spindle rotation, whereas an apically positioned interphase centrosome frequently leads to a clockwise spindle rotation at metaphase (Kaltschmidt et al., 2000). The mechanisms underlying this phenomenon are currently unknown. One possibility is that the two centrosomes in a mitotic NB may be intrinsically different. For example, the mother centrosome may produce more astral microtubules than the daughter centrosome, reminiscent of budding yeast division (Wang et al., 2003). At metaphase, the mother centrosome may preferentially migrate to the apical side by actively emanating its astral microtubules to capture the apical cortex. It would be of great interest to determine whether in mitotic NB such a mechanism exists and whether the apical daughter NB consistently inherits the mother centrosome following each round of asymmetric cell division.

In summary, at least two mechanisms, mediated by *Khc-73/Dlg* and *Mud*, can contribute to spindle orientation during NB asymmetric cell division. In the case of *khc-73/dlg* or *mud* mutants, each exhibit low penetrance in spindle-orientation defects. It remains possible that double-stranded RNA interference of *khc-73* only reduces its protein level, and maternal components of *mud* may compensate for its zygotic loss. It is also possible that *Dlg/Khc-73* and *Mud* may synergistically align mitotic spindle, and each may compensate for defects in the other.

Asymmetric Cell Division Coordinates Proliferation and Differentiation

During *Drosophila* neurogenesis, a NB divides to produce a proliferating NB (regenerative) and a GMC daughter cell that terminally differentiates. This process is tightly controlled, and disrupting these asymmetric cell divisions can lead to both uncontrolled proliferation and aberrant terminal differentiation (Figure 4). Several

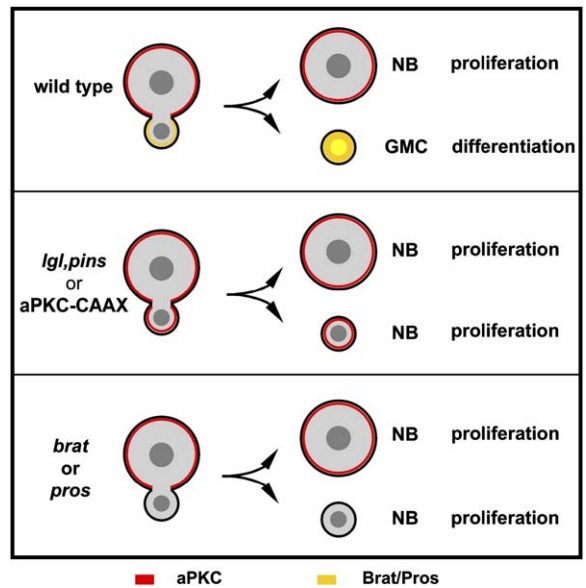


Figure 4. Asymmetrically Segregated Proteins aPKC and Brat/Pros Can Regulate Proliferation/Differentiation of Daughter Cells Following Larval NB Division

In the wild-type situation (top row), during cell division a larval NB segregates aPKC (red) to a proliferating NB daughter cell, and Brat/Pros (yellow) to a differentiating GMC daughter cell. Brat/Pros can suppress the expression of cell cycle genes and *dMyc* in the GMC daughter cell, thereby inducing differentiation. In the double mutants of *lgl/pins* or overexpression of a membrane-tethered aPKC (middle row), aPKC is segregated into both daughter cells, leading to a transformation of GMC into proliferating NB. In the *brat* or *pros* mutant larval NBs (bottom row), cell cycle progression and protein synthesis are not inhibited in the GMC, also leading to the transformation of GMC into proliferating NB.

recent studies provide insights into how this process is regulated during development and how its misregulation may contribute to the tumor formation (Betschinger et al., 2006; Caussinus and Gonzalez, 2005; Lee et al., 2006a, 2006b). For example, transplantation studies revealed that mutant larval brain tissue from *pins*, *mira*, *numb*, or *pros* when transplanted into wild-type adult abdomen grows to sizes more than 100-fold larger than control wild-type transplants (Caussinus and Gonzalez, 2005). NBs, which are highly enriched in the transplanted mutant tissue, exhibits genomic instability and altered centrosome, which likely contribute to the observed uncontrolled proliferation. Strikingly, the transplanted NBs can form malignant tumors that migrate away from the implantation site to invade neighboring tissues. These studies support the notion that at least some tumors may be derived from mutant stem/progenitor cells.

How are the proliferation/differentiation capacities of daughter cells coordinated following each round of asymmetric cell division? One possibility is that NBs use the asymmetric localization/segregation machinery to distribute “proliferation factors” to the NB daughter and/or “differentiation factors” to the GMC daughter during asymmetric cell division. Then what might these factors be? Recent work indicates that aPKC is sufficient to promote NB self-renewal and that the daughter cell destined to be a NB inherits aPKC and continues to proliferate, whereas the GMC daughter cell without

aPKC undergoes differentiation (Lee et al., 2006a). As described above, aPKC preferentially phosphorylates and inactivates Lgl on one side of the cell, while non-phosphorylated Lgl at the opposite side can positively direct asymmetric localization of Mira/Pros and Pon/Numb in both NBs and SOPs (Betschinger et al., 2003; Mayer et al., 2005). However, in larval NBs, Lgl appears to negatively regulate aPKC activity, which limits its self-renewal ability to the apical daughter (NB) by restricting aPKC kinase activity to the apical cortex, inherited by the NB daughter. When Lgl is absent, aPKC, although localized nonuniformly, is partially delocalized to the basal cortex in some metaphase larval NBs, resulting in the generation of supernumerary NBs at the expense of neurons. Uniform cortical localization of aPKC in the *pins lgl* double mutant leads to the generation of more self-renewing NBs. That is consistent with overexpression of a membrane-tethered aPKC, which localizes uniformly around the cortex, which is sufficient to induce NB self-renewal. All of these data point to aPKC kinase activity is sufficient for promoting NB self-renewal (Figure 4; Lee et al., 2006a).

Concurrently, inhibition of daughter cell proliferation may also be achieved by asymmetric segregation of key differentiation factors into the GMC. One such factor is the basally localized Brain Tumor (Brat) (Betschinger et al., 2006; Lee et al., 2006b). Brat was independently isolated in a genetic screen for mutations that increase the number of NBs in larval brain (Lee et al., 2006b) and in a biochemical assay analyzing the basal Mira protein complex (Betschinger et al., 2006). Brat is a translational repressor containing an evolutionarily conserved NHL (NCL-1, HT2A, and LIN-41) domain that recruits the Pumilio-Nanos-hunchback mRNA complex through an interaction between its NHL domain and Pumilio during early embryogenesis (Sonoda and Wharton, 2001). In embryonic NBs, Brat colocalizes with Mira at the basal cortex as a crescent from prometaphase to telophase. Upon cytokinesis, Brat is segregated to the basal GMC and released from the cellular cortex. In *brat* mutant larval brains, supernumerary NBs are produced at the expense of neurons. Like wild-type NBs, *brat* mutant NBs divide asymmetrically into two daughter cells of unequal size, but the GMC daughter fails to terminally differentiate. Instead, the mutant "GMC" continues to grow, enlarge, and express NB markers such as Deadpan and Mira. Moreover, MARCM clones generated from a single larval *brat* mutant NB form a large number of Mira, Worniu, and CycE-positive NBs, indicating that *brat* functions in a cell-autonomous manner (Bello et al., 2006; Betschinger et al., 2006; Lee et al., 2006b). Thus, these data suggest that asymmetric segregation of Brat into GMCs is intended to inhibit cellular proliferation.

So how does Brat inhibit GMC proliferation? One possibility is that in GMCs Brat acts to inhibit the translation of dMyc protein, an important regulator of cell cycle progression and cell growth. dMyc is specifically expressed in NBs but not GMCs. Although the role of dMyc in NB proliferation is not yet clear, loss of *brat* function elevates dMyc levels in all daughter cells, suggesting that Brat may act as a posttranscriptional inhibitor of dMyc in GMCs (Betschinger et al., 2006).

Similarly to Brat, Pros also functions as a tumor suppressor in larval brain (Bello et al., 2006; Betschinger

et al., 2006; Lee et al., 2006b; Figure 4). In fact, there may be some functional relationships between Brat and Pros. Brat is required for the asymmetric localization of Pros in larval NBs, and in *pros* mutant clones, Brat expression is downregulated (Betschinger et al., 2006; Lee et al., 2006b). Tumorigenesis phenotypes in *brat* and *pros* larva brain may thus be due to mutual dependency on the expression/localization of these two proteins (Betschinger et al., 2006; Lee et al., 2006b). Ectopic expression of Pros in *brat* mutant clones can rescue the NB overproliferation phenotype, also suggesting that Brat may function through Pros (Bello et al., 2006). Given that mutations in *lgl* perturb the asymmetric segregation of Pros and Brat and can cause tumor formation in larval brains, Lgl may function at least in part by ensuring that Brat and Pros segregate specifically into GMCs (Betschinger et al., 2006). However, since neither the removal of aPKC nor the overexpression of Lgl3A (a suppressor of aPKC-induced NB overproliferation) in *brat* mutants can rescue the overproliferation defect, these results suggest that aPKC may not be required for NB self-renewal in *brat* mutants.

Several interesting questions are raised by these findings. For example, what are the NB-specific substrates that are phosphorylated and activated by aPKC during NB self-renewal? It is important to determine how the aPKC-dependent and Brat/Pros-dependent pathways coordinate to regulate proliferation versus differentiation during larval NB cell division.

Implications for Mammalian Stem Cell Biology

These studies in *Drosophila* NB have provided some insights to our general understanding of how stem and progenitor cells divide, proliferate, and differentiate. With more mammalian homologs of fly asymmetric cell division machinery being identified, it is now clear that considerable mechanistic conservation exists across species. For example, the mammalian G $\beta\gamma$ subunit of the heterotrimeric G protein complex and activator of G protein signaling-3 (AGS3) have been shown to control mitotic spindle orientation and cell fate during mouse cortical neurogenesis (Sanada and Tsai, 2005). Likewise, mammalian aPKC, Par-3, and LGN are thought to be involved in asymmetric division of basal epidermal progenitor cells in the skin (Lechler and Fuchs, 2005). The once elusive mammalian homolog of the fly *insc* has also been cloned (Kato and Katoh, 2003; Lechler and Fuchs, 2005; Zigman et al., 2005). Although its role in mammalian asymmetric cell division is not yet clear, it is reasonable to suspect that mammalian *insc* plays similarly important roles during progenitor cell division. These results make the point that asymmetric cell division is a fundamental process that regulates stem/progenitor cell division and fate determination, and it is likely that more examples of mechanistic conservation will come out of future studies of such mammalian homologs.

Beyond developmental biology, there has been considerable recent interest in stem cells and cancer. Not only is it hypothesized that oncogenic mutations in adult stem cells can give rise to cancer, but that some tumors may actually be propagated at the core by slowly dividing "tumor stem cells" (Brabletz et al., 2005; Feinberg et al., 2006). Although these ideas make attractive models, there remains one universal property of stem

cells, which is the ability for self-renewal. Therefore, oncogenic mutations in stem cells that limit self-renewal are unlikely to generate cancer stem cells. Similarly, oncogenic mutations in differentiated cells which gained the ability to self renew may give rise to cancer stem cells. As we have discussed, since asymmetric cell division governs self-renewal and differentiation of stem cells, it is conceivable that mutations in part of this machinery maybe linked to tumor formation.

By showing that Brat and Prospero asymmetrically segregate during fly neuroblast division to inhibit the self-renewal capacity of one daughter cell (Betschinger et al., 2006; Lee et al., 2006b), these investigators established a mechanism of action between asymmetric cell division and cell proliferation. Betschinger et al. (2006), then went on to demonstrate Brat's inhibitory action on dMyc (the fly homolog of the mammalian oncogene MYC), thereby showing a possible link between asymmetric cell division and oncogenesis. Although these results are very exciting, the upregulation of dMyc in the absence of Brat leaves several unanswered questions. It is not clear if Brat directly inhibits posttranslational inhibition of dMyc or if the observed dMyc upregulation results from other processes, such as extracellular signals, that drive cellular proliferation. The MYC transcription factor responds to many cellular signals that govern proliferation; thus, loss of Brat inhibition may represent only part of the cascade that leads to dMyc activation in Brat mutant daughter cells. Future experiments will be needed to fully elucidate this interaction.

If mutations in the asymmetric cell division pathway that downregulate proliferative capacity can indeed lead to stem cell hyperproliferation and cancer, one might expect to find genetic mutations in the mammalian homologs of Brat and Prospero in mammalian tumors. Very little is known about the putative Brat mammalian homolog Tripartite motif proteins (Trim2, 3, and 32) in cellular proliferation (van Diepen et al., 2005), but the Prospero homolog Prospero-related homeobox 1 (Prox1) has conserved functions in regulating retinal progenitor cell proliferation (Dyer et al., 2003). Prox1 expression can force retinal progenitor cells to exit the cell cycle and differentiate (Dyer et al., 2003). In addition, mutations in and aberrant DNA methylation of *prox1* have been observed in some liver tumors and lymphomas (Nagai et al., 2003; Schneider et al., 2006). Although the number of such reported cases are few and despite the fact that Prox1 has so far not been implicated in mammalian asymmetric cell division, more investigators looking in the right places may eventually shed light on the links between asymmetric cell division, stem cell proliferation, and cancer.

Selected Reading

- Afshar, K., Willard, F.S., Colombo, K., Johnston, C.A., McCudden, C.R., Siderovski, D.P., and Goczy, P. (2004). *Cell* 119, 219–230.
- Afshar, K., Willard, F.S., Colombo, K., Siderovski, D.P., and Goczy, P. (2005). *Development* 132, 4449–4459.
- Barros, C.S., Phelps, C.B., and Brand, A.H. (2003). *Dev. Cell* 5, 829–840.
- Bellaiche, Y., Radovic, A., Woods, D.F., Hough, C.D., Parmentier, M.L., O'Kane, C.J., Bryant, P.J., and Schweisguth, F. (2001). *Cell* 106, 355–366.
- Bello, B., Reichert, H., and Hirth, F. (2006). *Development* 133, 2639–2648.
- Betschinger, J., and Knoblich, J.A. (2004). *Curr. Biol.* 14, R674–R685.
- Betschinger, J., Mechtler, K., and Knoblich, J.A. (2003). *Nature* 422, 326–330.
- Betschinger, J., Mechtler, K., and Knoblich, J.A. (2006). *Cell* 124, 1241–1253.
- Bonaccorsi, S., Giansanti, M.G., and Gatti, M. (2000). *Nat. Cell Biol.* 2, 54–56.
- Bowman, S.K., Neumüller, R.A., Novatchkova, M., Du, Q., and Knoblich, J.A. (2006). *Dev. Cell* 10, 731–742.
- Brabletz, T., Jung, A., Spaderna, S., Hlubek, F., and Kirchner, T. (2005). *Nat. Rev. Cancer* 5, 744–749.
- Cai, Y., Yu, F., Lin, S., Chia, W., and Yang, X. (2003). *Cell* 112, 51–62.
- Caussinus, E., and Gonzalez, C. (2005). *Nat. Genet.* 37, 1125–1129.
- Chia, W., and Yang, X. (2002). *Curr. Opin. Genet. Dev.* 12, 459–464.
- David, N.B., Martin, C.A., Segalen, M., Rosenfeld, F., Schweisguth, F., and Bellaiche, Y. (2005). *Nat. Cell Biol.* 7, 1083–1090.
- Du, Q., and Macara, I.G. (2004). *Cell* 119, 503–516.
- Dyer, M.A., Livesey, F.J., Cepko, C.L., and Oliver, G. (2003). *Nat. Genet.* 34, 53–58.
- Feinberg, A.P., Ohlsson, R., and Henikoff, S. (2006). *Nat. Rev. Genet.* 7, 21–33.
- Fuse, N., Hisata, K., Katzen, A.L., and Matsuzaki, F. (2003). *Curr. Biol.* 13, 947–954.
- Gho, M., and Schweisguth, F. (1998). *Nature* 393, 178–181.
- Hampelz, B., Hoeller, O., Bowman, S.K., Dunican, D., and Knoblich, J.A. (2005). *Nat. Cell Biol.* 7, 1099–1105.
- Izumi, Y., Ohta, N., Itoh-Furuya, A., Fuse, N., and Matsuzaki, F. (2004). *J. Cell Biol.* 164, 729–738.
- Izumi, Y., Ohta, N., Hisata, K., Raabe, T., and Matsuzaki, F. (2006). *Nat. Cell Biol.* 8, 586–593.
- Jan, Y.N., and Jan, L.Y. (2001). *Nat. Rev. Neurosci.* 2, 772–779.
- Kaltschmidt, J.A., Davidson, C.M., Brown, N.H., and Brand, A.H. (2000). *Nat. Cell Biol.* 2, 7–12.
- Katoh, M., and Katoh, M. (2003). *Int. J. Mol. Med.* 11, 111–116.
- Lechler, T., and Fuchs, E. (2005). *Nature* 437, 275–280.
- Lee, C.Y., Robinson, K.J., and Doe, C.Q. (2006a). *Nature* 439, 594–598.
- Lee, C.Y., Wilkinson, B.D., Siegrist, S.E., Wharton, R.P., and Doe, C.Q. (2006b). *Dev. Cell* 10, 441–449.
- Lu, B., Usui, T., Uemura, T., Jan, L., and Jan, Y.N. (1999). *Curr. Biol.* 9, 1247–1250.
- Mayer, B., Emery, G., Berdnik, D., Wirtz-Peitz, F., and Knoblich, J.A. (2005). *Curr. Biol.* 15, 1847–1854.
- Nagai, H., Li, Y., Hatano, S., Toshihito, O., Yuge, M., Ito, E., Utsumi, M., Saito, H., and Kinoshita, T. (2003). *Genes Chromosomes Cancer* 38, 13–21.
- Ohshiro, T., Yagami, T., Zhang, C., and Matsuzaki, F. (2000). *Nature* 408, 593–596.
- Parmentier, M.L., Woods, D., Greig, S., Phan, P.G., Radovic, A., Bryant, P., and O'Kane, C.J. (2000). *J. Neurosci.* 20, RC84.
- Peng, C.Y., Manning, L., Albertson, R., and Doe, C.Q. (2000). *Nature* 408, 596–600.
- Petritsch, C., Tavosanis, G., Turck, C.W., Jan, L.Y., and Jan, Y.N. (2003). *Dev. Cell* 4, 273–281.
- Rhyu, M.S., Jan, L.Y., and Jan, Y.N. (1994). *Cell* 76, 477–491.
- Rogers, G.C., Rogers, S.L., Schwimmer, T.A., Ems-McClung, S.C., Walczak, C.E., Vale, R.D., Scholey, J.M., and Sharp, D.J. (2004). *Nature* 427, 364–370.
- Sanada, K., and Tsai, L.H. (2005). *Cell* 122, 119–131.
- Schaefer, M., Shevchenko, A., Shevchenko, A., and Knoblich, J.A. (2000). *Curr. Biol.* 10, 353–362.
- Schaefer, M., Petronczki, M., Dorner, D., Forte, M., and Knoblich, J.A. (2001). *Cell* 107, 183–194.
- Schneider, M., Buchler, P., Giese, N., Giese, T., Wilting, J., Buchler, M.W., and Friess, H. (2006). *Int. J. Oncol.* 28, 883–890.

- Schober, M., Schaefer, M., and Knoblich, J.A. (1999). *Nature* **402**, 548–551.
- Siegrist, S.E., and Doe, C.Q. (2005). *Cell* **123**, 1323–1335.
- Siegrist, S.E., and Doe, C.Q. (2006). *Development* **133**, 529–536.
- Siller, K.H., Cabernard, C., and Doe, C.Q. (2006). *Nat. Cell Biol.* **8**, 594–600.
- Somers, W.G., and Chia, W. (2005). *Dev. Cell* **9**, 312–313.
- Sonoda, J., and Wharton, R.P. (2001). *Genes Dev.* **15**, 762–773.
- Srinivasan, D.G., Fisk, R.M., Xu, H., and van den Heuvel, S. (2003). *Genes Dev.* **17**, 1225–1239.
- Tall, G.G., and Gilman, A.G. (2005). *Proc. Natl. Acad. Sci. USA* **102**, 16584–16589.
- van Diepen, M.T., Spencer, G.E., van Minnen, J., Gouwenberg, Y., Bouwman, J., Smit, A.B., and van Kesteren, R.E. (2005). *Mol. Cell. Neurosci.* **29**, 74–81.
- Wang, H., Oliferenko, S., and Balasubramanian, M.K. (2003). *Curr. Opin. Cell Biol.* **15**, 82–87.
- Wang, H., Ng, K.H., Qian, H., Siderovski, D.P., Chia, W., and Yu, F. (2005). *Nat. Cell Biol.* **7**, 1091–1098.
- Wei, S.Y., Escudero, L.M., Yu, F., Chang, L.H., Chen, L.Y., Ho, Y.H., Lin, C.M., Chou, C.S., Chia, W., Modolell, J., and Hsu, J.C. (2005). *Dev. Cell* **8**, 493–504.
- Willard, F.S., Kimple, R.J., and Siderovski, D.P. (2004). *Annu. Rev. Biochem.* **73**, 925–951.
- Wodarz, A., Ramrath, A., Kuchinke, U., and Knust, E. (1999). *Nature* **402**, 544–547.
- Yu, F., Morin, X., Cai, Y., Yang, X., and Chia, W. (2000). *Cell* **100**, 399–409.
- Yu, F., Cai, Y., Kaushik, R., Yang, X., and Chia, W. (2003). *J. Cell Biol.* **162**, 623–633.
- Yu, F., Wang, H., Qian, H., Kaushik, R., Bownes, M., Yang, X., and Chia, W. (2005). *Genes Dev.* **19**, 1341–1353.
- Zigman, M., Cayouette, M., Charalambous, C., Schleiffer, A., Hoeller, O., Dunican, D., McCudden, C.R., Fimberg, N., Barres, B.A., Siderovski, D.P., and Knoblich, J.A. (2005). *Neuron* **48**, 539–545.