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MINIREVIEW

Mechanisms of asymmetric cell divisions in *Drosophila melanogaster* neuroblasts

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Abstract

Stem cells possess the properties of self-renewal and differentiation, and mainly rely on two strategies for division, including symmetric and asymmetric cell divisions. In this review, we summarize the latest progress on asymmetric cell divisions in *Drosophila melanogaster* neuroblasts (NBs), which focus on the establishment of cell polarity, mitotic spindle orientation, the asymmetric segregation of cell fate determinants as well as cell-cycle control. Here we also introduce five major cell fate determinants, including Numb, Prospero, Brat, Miranda, and Pon, which are thought to be unequally segregated to the ganglion mother cells (GMCs) and play an important role in the formation of stem cell-derived tumors.

Key Words: asymmetric cell divisions; cell fate determinants; neuroblasts; *Drosophila melanogaster*

Introduction

Stem cells are defined as the cells which not only undergo self-renewal but also produce daughter cells devoting to differentiation for all ages (Sada and Tumbar, 2013). Moreover, they employ two main strategies for division including symmetric and asymmetric cell divisions. Both of them can regulate the stem cell and the differentiated cell population (Sousa-Nunes and Somers, 2013). Symmetric cell divisions produce two uniform daughter cells. However, asymmetric cell divisions lead to one daughter cell destined to differentiate and one stem cell, the latter is crucial for the development of multicellular organisms (Yamashita, 2009). Recently, comprehensive research on the mechanisms of asymmetric cell divisions has been done. Specifically, *Drosophila melanogaster* sensory organ precursor (SOP) cells and neuroblasts (NBs) provide excellent models for studying the cellular and molecular mechanisms of asymmetric cell divisions (Rusan and Rogers, 2009).

In contrast to SOP cells, most NBs are derived from the embryonic procephalic and ventral neuroectoderm (Wu *et al*., 2008). During embryonic neurogenesis, NBs delaminate from the epithelium and undergo repeated rounds of asymmetric division to self-renew and to produce one proliferating NB and one small ganglion mother cell (GMC) that terminally differentiates (Sawa, 2010). This process is strictly controlled, and disrupting it can lead to both uncontrolled proliferation and aberrant differentiation. NBs restricted self-renewal capacity also limits their usefulness as a true stem cell model. For this reason, we primarily focus on NBs.

During asymmetric cell divisions, it has been proposed that NBs depend on external environmental factors and internal regulations (Sada and Tumbar, 2013). Both of them could work together or independently (Yamashita, 2009).

For extrinsic asymmetric divisions, stem cells reside in microenvironment, which is called the niche (Sada and Tumbar, 2013). The niche is used to maintain the stem cells identity and proliferation. During stem cell divisions, the cell fate appears to be determined solely by their localization (Yamashita, 2009). In general, there is only one daughter cell maintaining the stem cell identity in the niche area.

For intrinsic asymmetric divisions, cell fate determinants are asymmetrically segregated into two daughter cells in mitosis. Thus, one cell undergoes differentiation and the other one keeps the stem cell identity (Roegiers and Jan, 2004; Knoblich, 2008). In most cases, there are four steps in the intrinsic asymmetric divisions (Fig. 1). Namely, the mother cell sets up an axis of asymmetry during the interphase of cell division, following by the cell polarized. After that, the cell fate determinants, such as Numb, Prospero, Brat, Miranda, or Pon, are segregated towards the regions of the polarized

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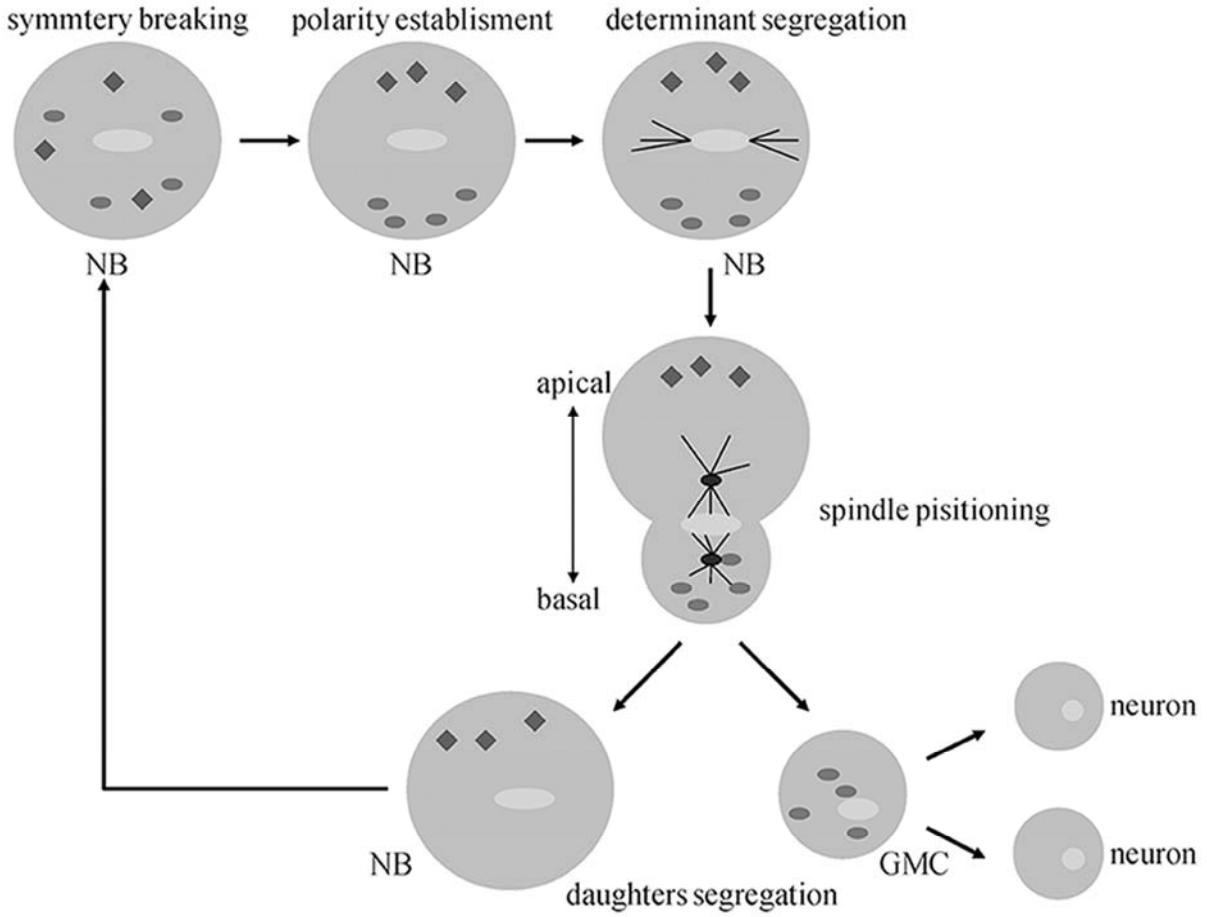

Fig. 1 Intrinsic asymmetric division in *D. melanogaster* NBs. There are four steps in the intrinsic asymmetric division. Firstly, the symmetry of NBs is broken during the interphase. Secondly, NBs becomes polarized. Thirdly, the major cell fate determinants (diamond), such as Numb, Prospero, Brat, Miranda and Pon are segregated to the basal cortex, and the apical proteins (oval) are located in the apical cortex. Fourthly, the mitotic spindle orientation along the cell polarity axis is establishment so that the cell fate determinants are divided into the newly forming GMC which can generate two neurons.

mother cell. Finally, the mitotic spindle orientation along the cell polarity axis is established, so that cell fate determinants can been separated into the daughter cells. Due to these four steps, one mother cell can generate two daughter cells which own different cell fates (Gonczy, 2008).

Here, we summarize the recent progress on asymmetric cell divisions in *D. melanogaster* NBs and describe the mechanism which regulates asymmetric NBs divisions during development. We also focus on the establishment of cell polarity, the regulation of mitotic spindle orientation, and the asymmetric segregation of cell fate determinants as well as cell-cycle control (Wu *et al.*, 2008).

Establishment of cell polarity

The central nervous system of *D. melanogaster* originates from the basal delamination of the surface neurectoderm, which inherits its epithelial apical-basal (A-B) polarity (Gonczy, 2008). While the cell polarity is established by the asymmetric accumulation of the Par complex, including Baz (*D. melanogaster* homologue of *C. Elegans* Par-3), Par-6, as well as the atypical protein kinase C (aPKC) to form a crescent at the apical cell cortex (Haenfler *et al.*, 2012; Chen and Zhang, 2013), which is evolutionarily conserved. The Par complex is originally expressed in the neuroectoderm, while keeping in the NBs after delamination (Wu *et al.*, 2008).

Baz is a potential component of the apical organizer containing three PDZ domains, which can also interact with aPKC. Following NBs delaminating, Baz is co-localized with Inscuteable (Insc) to the crescent at the apical cell cortex (Matsuzaki, 2000). However, not only Baz but also Insc is undetectable during late mitosis in every cell cycle. As a small protein, Par-6 contains one PDZ domain and a N-terminal PB1 domain, which binds to a similar domain on the aPKC (Knoblich, 2008). Furthermore, Baz recruits Cdc42, which in turn binds to the semi-CRIB domain of Par-6 (Goldstein and Macara, 2007). The Cdc42 is a small GTPase which is vital for Par-6 localization (Atwood *et al.*, 2007). The

three compotents of Par complex also recruit Insc, Pin (the adapter protein partner of Insc), and Gαi (a subunit of heterotrimeric G protein) to the neuroectoderm, which are required for A-B polarity. Their apical localization is maintained during NBs delamination, and the complex is preferred to divide into the daughter cell which localizes in the apical cortex. While Baz, Par-6, and aPKC are closely related, any one mutanted can cause the other proteins delocalized. In addition, they also direct the cell fate determinants to the basal GMCs (Knoblich, 2008). Thus, the Par complex is crucial for asymmetric cell divisions since it provides essential positional information for cell division.

Spindle orientation

In general, NBs are arose from neuroectodermal cells and divided perpendicularly to the epithelial plane with a horizontal mitotic spindle axis (Wu *et al.*, 2008), so their mitotic spindle rotates 90 degree. Thus NBs may undergo repeated division along the A-B axis (Kaltschmidt and Brand, 2002).

Spindle orientation needs to be done with the involvement of the apically localized molecules as well as cell polarity establishment, any one of the molecules is indispensable, especially the protein Insc. Insc is undetectable in the neuroepithelial cell layer during each NBs cell cycle, and becomes detectable during NBs delamination and associates with the Par complex through Baz, Pin, and Gαi protein (Cai *et al.*, 2003). Moreover, Insc plays a sufficient role in spindle orientation, deletion of Insc or Baz leads to the randomization of the mitotic spindle and cell fate determinants delocalized in the basal crescent. Nevertheless, ectopic expression of Insc in the neuroepithelial cell layer can cause spindle move 90 degree (Siller and Doe, 2009).

As an adapter protein, Insc links the Par complex to a tripartite protein complex Gαi-Pins-Mud, which regulates NBs spindle orientation. It associates with the dynein-dynactin complex, directs the orientation of the mitotic spindle and coordinates with the A-B polarity axis, which plays an important role in recruiting and keeping one centrosome at the apical pole (Saini and Reichert, 2012). Moreover Pins-Dlg-Khc73 complex also regulates the spindle orientation. Losing a functional Par complex can active Dlg-Pins-Gαi complex formation in embryonic NBs, as a result of the interaction between astral microtubules and Khc73. Interestingly, down-regulation of Dlg or Khc73 induces partial spindle-orientation defects without affecting apical Pins-Gαi cortical polarity (Siegrist and Doe, 2005). However the regulation mechanism is not clear yet.

More data show that the accurate spindle alignment is required for normal NBs or GMCs fate. While *mud* mutant induces either spindle orientation defects or additional NBs numbers with normal cortical polarity. The transverse spindle causes symmetric divisions but not asymmetric divisions and generates two NBs but not GMCs any more (Homem and Knoblich, 2012).

Cell fate determinants

The cell fate determinants such as Numb, Prospero, Brat as well as their adapter proteins Miranda and Pon assemble in the basal cortical domain before the cytoplasm division, and then segregate into the basal GMCs (Fig. 2) (Paridaen and Huttner, 2014). Next we discuss those cell fate determinants in *D. melanogaster* NBs below.

Numb

Numb is the first defined asymmetrically partitioned determinant, which also belongs to a clathrin-associated sorting protein (CLASP). Recently it has been shown that Numb contains two interaction domain including phosphotyrosine binding (PTB) domain and C-terminus (Spana and Doe, 1996). Numb can be recruited and phosphorylated by the Par complex. The phosphorylation state of Numb determines that it forms a crescent on the basal cell cortex of the NBs, and finally it is excluded from the cortex (Egger *et al.*, 2007). Numb also works as a tissue-specific component of the Notch pathway. Therefore, Numb transfers intracellular signal to regulate cell divisions are related to different levels of Notch activity (Couturier *et al.*, 2013).

In general, Numb reduces or blocks the activity of Notch pathway through binding the endocytic protein α-adaptin, which is a component of the adapter protein-2 (AP2) complex that functions in clathrin-mediated endocytosis of transmembrane proteins (Tajbakhsh *et al.*, 2009). Some studies reveal that Numb interacts with the ear domain of α-adaptin, and they locate identically. Furthermore, α-adaptin acts as an upstream factor or in parallel with Notch pathway. In *α-adaptin* mutant, it blocks the interaction with Numb and no longer locates asymmetrically, which is similar to the *numb* mutant that NBs overproliferate and form a tumor-like phenotype (Ntelios *et al.*, 2012).

Prospero

Homeodomain transcription factor Prospero is another pivotal regulator in asymmetrically divisions (Choksi *et al.*, 2006). Prospero is detected in all embryonic and larval NBs, which congregates in the cytoplasm. It translocates to the basal cell cortex and forms a cresent pattern at one end of the cell during cell divisions, rather than distributes evenly (Matsuzaki, 2000). It migrates from the cortex into the nucleus, where it establishes the neural fate of the cell. In the end, Prospero asymmetrically divides into one of the two progeny called GMCs. Therefore, the GMCs have a different fate from that of its sister called NBs.

Moreover, Prospero is an essential regulator of the GMCs development, which binds more than 700 genes. Prospero binds to a majority of the temporal cascade genes, including Kruppel (Kr), Nubbin (Nub/pdm1), or Grainyhead (Grh), which regulate the timing of cell fate specification in NBs progeny. Interestingly, Prospero regulates Baz, Miranda, Insc, as well as aPKC, which directly regulate asymmetric NBs divisions. Prospero also suppresses those genes which are required for stem cell self-renewal, as well as cell cycle related genes including *Cyclins A* or *E* or *String* (the *D. melanogaster* homologue of *Cdc25*) (Choksi *et al.*, 2006). When *prospero* is mutated in NBs, the cell cycle related genes are up-regulated, NBs undergo multiple rounds of division, fail to differentiate and induce stem cell-derived tumors (Knoblich, 2008).

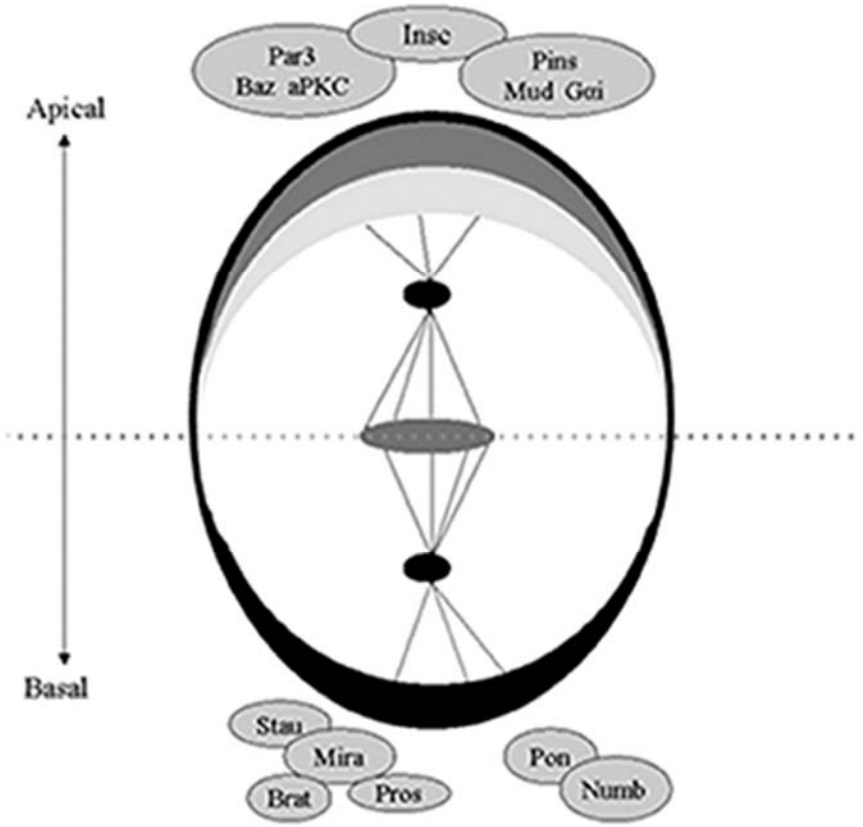

Fig. 2 Asymmetric cell divisions in *D. melanogaster* NBs. During the NBs mitosis, the apical aPKC-Par3-Par6 complex (dark gray) is linked to the Gαi-Pins-Mud complex (pale gray) through Inscuteable. All of the proteins are involved in the asymmetric partitioning of cell fate determinants, establishment of cell polarity and the spindle orientation. While, the determinants (black), including Miranda (Mira), Prospero (Pros), Staufen (Stau), Brat, Numb, and Pon are concentrated at the basal cortex. Finally, most of the basal determinants are segregated into the basal daughter cell called the GMC.

Brat

Brain tumor (Brat) encodes a member of the conserved C-terminal NHL family of proteins, including NCL-1, HT2A, and LIN-41 (Saini and Reichert, 2012). Apart from the NHL domain, Brat is also characterized by a coiled-coil region and an N-terminal Zinc binding B-box, which mediates its recruitment to the 3'UTR of posterior identity gene *Hunchback* (*HB*) through *Pumilio* (*Pum*) and *Nanos* (*Nos*) during early embryogenesis to repress translation of the *HB* (Slack *et al.*, 1998). Brat is also considered as a transcriptional activator of Prospero, and *pros/brat* mutants show complete loss of all GMCs by reason of that they corporately regulate GMC fate (Betschinger *et al.*, 2006). *Brat* mutants have reduced Prospero expression and show uncontrolled proliferation in the larval central brain which may induce tumor development (Bello *et al.*, 2006).

Miranda and Pon

Except the three crucial regulators of NBs self-renewal, there are two important adapter proteins including Miranda and Pon. Miranda is identified as a double-headed, double-tailed homodimer with a long central coiled-coil region (residues 150 - 700) flanked by non-coiled-coil N and C termini. The double-heads is essential for increasing avidity for specific binding partners (Yousef *et al.*, 2008).

Some reports suggest that Miranda may interact with not only Brat but also the RNA binding protein Staufen which acts as the Prospero-mRNA carrier. During mitosis, Miranda localizes asymmetrically and colocalizes with Prospero to the basal cell cortical crescent. At the end of telophase, both of them divide into GMCs. Unlike Prospero, Miranda fades away shortly after cytokinesis in GMCs. For that reason, it is possible that Miranda is degraded in a cell-cycle-dependent manner and its degradation is a consequence of cargo proteins release (Shen *et al.*, 1997). In addition, the extreme C-terminal 103 amino acids (residues 727 - 830) of Miranda, which also contains multiple consensus aPKC phosphorylation sites, are found to be crucial for those processes (Yousef *et al.*, 2008).

The asymmetric localization of Miranda depends on polarized Baz activity and has no related with Pins-Gαi function. Moreover, Baz regulates localization of aPKC to make Miranda

migrate from the apical cell cortical crescent to the basal through Lethal (2) phosphorylation (Izumi *et al.*, 2004). Anaphase-promoting complex/cyclosome (APC/C) also can regulate the asymmetric localization of Miranda, as well as its interrelated cargo proteins including Prospero, Brat, and Staufen. Several APC/C core subunits mutants displayed that Miranda is transferred from cortex to cytoplasm (Slack *et al.*, 2007).

Pon is a coiled-coil protein, which is not essential for its cargo protein, but Pon assists to establish the A-B polarity. As an important component of a multimolecular machinery, Pon colocalizes with Numb (Lu *et al.*, 1998), knockdown of Pon postpones Numb's crescent formation in metaphase, which may lead to a defect in NBs self-renewal (Wang *et al.*, 2008).

Cell-cycle control

Recent published data provide an evidence that cell cycle regulators can affect the asymmetric NBs divisions, Cdc2/CDK1, Aurora A, Polo, Cyclin E, and APC/C core components take action in pro-metaphase and metaphase. Those regulators mutants directly affect asymmetric cell fate determinants' localization (Reichert, 2011). Cdc2/CDK1 is essential for driving cells enter into mitosis, and cells lacking of CDK1 activity cause cell cycle arrest in the G2 phase (Reichert, 2011).

Aurora A is a centrosomal kinase which can cause Par6 phosphorylated and aPKC auto-phosphorylated, and which reaches a peak in the early mitosis (Barr and Gergely, 2007). As another centrosomal kinase, Polo also regulates cell cycle which is similar to Aurora A. Both of them are required for a subset of mitotic events, including centrosome maturation, spindle formation and orientation, as well as cytokinesis (Knoblich, 2008). Moreover, they also can act as tumor suppressors to prevent excess self-renewal (Reichert, 2011).

Cyclin E regulates the G1 to S-phase transition (Chia *et al.*, 2008). It is necessary for converting symmetric NBs divisions into asymmetric divisions through downstream of *Hox* genes (Berger *et al.*, 2005). During asymmetric divisions, Cyclin E may inhibit Prospero and facilitate its cortical localization in the NBs, which is closely related with its self-renewal during asymmetric divisions (Berger *et al.*, 2010).

APC/C, in transient association with the activating subunits Cdc20 and Cdh1, can promote cell cycle transitions through several key processes including regulation of DNA replication, centrosome duplication and mitotic spindle assembly as well as the mitotic cyclins disfunction and inhibition of chromosome separation (Leismann and Lehner, 2003). APC/C also can control axon growth and regulate synaptic size and transmission (Juo and Kaplan, 2004).

Concluding remarks

D. melanogaster NBs provide a well-studied model system for illustrating the cellular and molecular mechanisms of asymmetric cell divisions. In this review, some viewpoints have been made to describe the establishment of cell polarity, the orientation of mitotic spindle, the asymmetric segregation of cell fate determinants, as well as cell-cycle control.

Here we focused on five major cell fate determinants, including Numb, Prospero, Brat, Miranda, and Pon, which are thought to be unequally segregated to one of the two daughter cells. All or most of them relate with the formation of stem cell-derived tumors, however, the regulatory mechanism of this process is not clear yet.

Understanding of asymmetric cell divisions in *D. melanogaster* NBs provides some clues for stem cell behaviors, stem cell therapy, as well as cancer research.

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