Asymmetric stem cell division: Lessons from *Drosophila*

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Abstract

Asymmetric cell division is an important and conserved strategy in the generation of cellular diversity during animal development. Many of our insights into the underlying mechanisms of asymmetric cell division have been gained from *Drosophila*, including the establishment of polarity, orientation of mitotic spindles and segregation of cell fate determinants. Recent studies are also beginning to reveal the connection between the misregulation of asymmetric cell division and cancer. What we are learning from *Drosophila* as a model system has implication both for stem cell biology and also cancer research.

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Keywords: Asymmetric cell division; Neuroblast; Cell size; Spindle orientation; Cancer

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1. Introduction

Early studies on ascidian and leech cell lineages more than one century ago first postulated that the asymmetric distribution of cell substances in cell division might affect cell fate [1,2].

In *Drosophila* the molecular basis of asymmetric cell division began to be unravelled with the discovery of the cell fate determinant Numb in sensory organ precursors (SOP) and its role in cellular identity specification [3]. Since then this topic has been intensively studied and considerable insights have been gained from many laboratories using different model systems including the *Caenorhabditis elegans* zygote, *Drosophila* neuroblasts, sensory organ precursors, and more recently, germline stem cells (GSCs), as well as mammalian neuroepithelial cells.
[4–13]. Given the cancer stem cell hypothesis [14–16], the study of asymmetric cell division is likely to contribute to our understanding of stem cell biology and cancer biology.

Stem cells are unique in their ability to self-renew and to produce daughter cells committed to differentiation. In addition, they use a balance of symmetric and asymmetric cell divisions to regulate the number of stem cells and differentiated cells. Symmetric divisions lead to two identical daughter cells, whereas following an asymmetric cell division the smaller of the two daughter cells differentiates. It is crucial for stem cells to tightly control this choice between symmetric proliferative divisions and asymmetric differentiative divisions during development and tissue repair, because if cellular homeostasis is not maintained, premature differentiation may lead to incomplete tissue or organ development, whereas uncontrolled proliferation can lead to tumour formation [16].

In this review, we summarise recent key discoveries in this field mostly from Drosophila neural stem cells, or neuroblasts (NB). Our main goal is to describe the machinery that orchestrates asymmetric NB division during development. We focus on the establishment of cell polarity, the regulation of mitotic spindle orientation, centrosome asymmetry and the asymmetric segregation of cell fate determinants. And we explore how this affects the proliferation and differentiation of the generated daughter cells. We also briefly discuss how asymmetrically dividing NBs arise from symmetrically dividing neuroepithelial cells and what mechanisms might be involved in the switch from symmetric to asymmetric division. In the last part of the review, we discuss the link between asymmetric cell division and tumour formation, as recent evidence from Drosophila shows that the impairment of asymmetric cell division can lead to tumourigenesis [17–28].

2. Asymmetric cell division of Drosophila neuroblasts

Drosophila neural stem cells provide an excellent model system in which to study asymmetric cell division, due to their physical and genetic accessibility. Most NBs derive from the embryonic procephalic and ventral neuroectoderm. During embryonic neurogenesis, about 30 NBs in each hemisegment delaminate from the ventral neuroectoderm and undergo stem cell-like, asymmetric divisions, to self-renew and to generate smaller daughter cells, called ganglion mother cells (GMCs). Each GMC divides only once to generate two neurons and/or glial cells (Fig. 1). Each NB produces a stereotyped lineage of motorneurons, interneurons and glial cells and can be identified by its unique position within the neuroectoderm and its characteristic gene expression profile [29–33]. Embryonic NBs divide up to about 18 times, decreasing in size at each division until the end of embryogenesis, when they stop dividing. A subset of NBs becomes quiescent until the larval stage, when they enlarge and divide to generate the adult nervous system.

Larval NBs undergo classical asymmetric divisions but, unlike embryonic NBs, enlarge between each cell division. Interestingly, a novel mode of division has recently been discovered in a dorsomedial region of each larval brain hemisphere.

Fig. 1. Classical division modes of Drosophila neural progenitors. Most Drosophila NBs arise from neuroepithelial cells embedded in the embryonic neuroectoderm. Neuroectodermal cells divide symmetrically within the plane of the epithelium generating equal daughter cells. NBs rotate their spindle axis 90° and undergo asymmetric divisions along the apico-basal axis. Each asymmetric NB division generates a NB (self-renewal) and a smaller GMC that divides once more to give rise to two postmitotic neurons.
These specific NBs divide asymmetrically to self-renew and generate an intermediate progenitor. Intermediate progenitors can then again undergo multiple asymmetric divisions, self-renewing and budding off GMCs [34]. A similar intermediate neural progenitor has also been found in mammalian brain development [35,36], indicating that the molecular mechanisms of neurogenesis in insect and vertebrates are evolutionarily conserved.

2.1. Establishment of neuroblast polarity and coordination of mitotic spindle orientation

NBs arise from neuroectodermal cells, polarized epithelial cells that divide symmetrically in the plane of the epithelium with a horizontal mitotic spindle axis. NBs delaminate from the neuroectoderm and rotate their mitotic spindle 90° perpendicular to the epithelial plane. Subsequent NB divisions are thus oriented along the apico-basal axis [37–39] (Fig. 1). The polarity in neuroectodermal cells and NBs is established by the Par complex, an evolutionarily conserved protein complex consisting of three proteins: Par-6, Bazooka (Drosophila homologue of C. elegans Par-3) and atypical kinase C (aPKC). The Par complex is first expressed in the neuroectoderm and is maintained in the NB after delamination, where it localises in a crescent at the apical cell cortex [40–43]. During NB delamination the protein Inscuteable (Insc) becomes expressed. Insc binds the Par/Baz/aPKC complex, through Baz, and recruits Partner of Inscuteable (Pins, also called Rapsynoid, a GoLoco domain protein) and the heterotrimeric G protein subunit Goi [44–46]. It is this Insc/Pins/Goi complex that regulates spindle orientation and spindle geometry (Fig. 2) [47–50].

How is NB polarity and spindle orientation coordinated during NB division? Siegrist and Doe suggest that the link between spindle orientation and polarity is achieved by a microtubule induced mechanism and mediated through interaction between Pins/Goi, Discs large (Dlg) and Kinesin heavy chain 73 (Khc-73) [51]. Their experiments showed that Khc-73 is localised at the plus ends of astral microtubules where it binds and clusters cortical Dlg at one end of the spindle. Dlg in turn binds and induces Pins/Goi clustering. They suggest that this sequence of events eventually leads to spindle-induced “cortical” polarity. In a last step, Pins/Goi binds to the Insc/Par complex to align the spindle with the apico-basal tissue axis. In insc/par mutants, cortical polarity is still induced, but fails to align along the apico-basal axis, resulting in randomised spindle orientation. In dlg/khc-73 mutants, Pins/Goi can still bind the apically localised Insc/Par complex, but the spindle pole fails to connect to cortical polarity cues, similarly leading to misorientated mitotic spindles [51].

What then maintains the apical localisation of the Insc/Par complex and the orientation of the apico-basal spindle axis through subsequent mitotic cell cycles? It has been shown that the apical localisation of the Insc/Par complex requires contact between NBs and neuroectodermal cells [52]. This suggests that extrinsic cues from the overlying epithelium provide positional information allowing the realignment of the mitotic spindle with tissue polarity throughout multiple NB divisions. Isolated and cultured NBs fail to maintain the position of the Insc/Par complex and spindle orientation. The identity of extrinsic cues is still unclear but possible candidates are signalling molecules such as extracellular matrix proteins expressed in neuroectodermal cells [52].

![Diagram of Apical and Basal Protein Complexes](image_url)

Fig. 2. Apical and basal complexes involved in asymmetric NBs divisions. Apical and basal protein complexes are illustrated together with their main functions in NB division. The apical Par complex (Par6/Baz/aPKC) is mainly involved in establishing polarity and in directing cell fate determinants to the basal NB cortex. Insc/Pins/Goi is crucial for coordinating the spindle axis with tissue polarity, whereas free Goi affects spindle geometry. At the basal side, cell fate determinants Mira/Pros/Brat and Pon/Numb control proliferation and differentiation in the presumptive GMC.
2.2. Machinery of spindle geometry and cell size control

The correct orientation and geometry of the mitotic spindle is essential to ensure the asymmetric segregation of cell fate determinants and to generate daughter cells of unequal size (NBs are 10–12 μm in diameter versus 4–6 μm for GMCs). In NB divisions, the mitotic spindle is initially symmetric, with the metaphase plate placed at the centre of the cell. At anaphase, the apical aster enlarges and the basal aster shrinks as spindle microtubules elongate at the apical side and shorten at the basal side, generating an asymmetric spindle. This results in a shift of the cleavage plane and the generation of two daughter cells of different size [38,53].

The Par and Pins/Goi complexes act in a redundant manner to regulate spindle asymmetry and cell size. Single mutations in either complex do not cause substantial cell size or spindle defects. However, double mutants affecting both complexes result in symmetric NB divisions [47–50]. The heterotrimeric G-proteins Gβ13F and Gγ1 also play a crucial role in the regulation of spindle geometry and cell size asymmetry [48–50]. In Gβ13F null mutants, NB spindle asymmetry is lost, leading to equal-sized daughter cells with high penetrance. Although in these cells Pins/Goi is mislocalised, the Par complex remains at the apical cortex at reduced level and the segregation of cell fate determinants is not affected. In contrast overexpression of Gβ13F leads to a reduction in spindle size [48]. Gγ1 mutants reveal a phenotype that is almost identical to Gβ13F mutants [49].

The classical heterotrimeric G-protein cycle requires the binding of a ligand to a seven-transmembrane G-protein-coupled-receptor (GPCR). The ligand-bound GPCR activates the G-protein by catalysing the exchange of GDP to GTP resulting in the dissociation of Goi and Gβγ subunits which activate downstream signalling pathways. In Drosophila NBs, spindle geometry is regulated by a receptor-independent heterotrimeric G-protein cycle (Fig. 3) [54,55]. GDP dissociation inhibitors (GDI), Pins and Locomotion defective (Loco), promote the dissociation of heterotrimeric G-proteins and maintain Goi in a GDP-bound state [55]. Pins and Loco both encode a conserved GoLoco motif that interacts with Goi at the apical cortex leading to the release of Gβγ [45,56]. Thus, double mutants of Loco and Pins exhibit similar phenotypes to Gβ13F and Gγ1 mutants: NBs divide symmetrically and produce two similarly size daughter cells [56]. Dissociated Gβγ acts on unknown downstream effectors to regulate daughter cell size [55,56]. Interestingly, in addition to the function as a GDI, Loco also has GAP (GTPase activating protein) activity, which converts Goi-GTP to Goi-GDP through its RGS domain [56].

Recently a new component of the receptor-independent heterotrimeric G-protein cycle was discovered in NBs, Ric-8. Studies in C. elegans and mammalian cells demonstrated that Ric-8 acts as a non-receptor guanine nucleotide-exchange factor (GEF) of Goi, which catalyses GDP-Goi to GTP-Goi [57–59]. Subsequent studies of Drosophila NBs and sensory organ precursors (SOP) showed that Ric-8 is also required for the cortical localisation of heterotrimeric G protein subunits [54,60–62]. It has been proposed that Ric-8 may act as a chaperone that enhances the assembly of heterotrimeric G-proteins [61,63]. The studies suggest that it is the free, unbound Gβγ that affects spindle geometry. The future challenge will be to identify the targets of heterotrimeric G-protein signalling that execute spindle asymmetry.

2.3. Segregation of cell fate determinants

 Establishment of apico-basal polarity and alignment of the mitotic spindle along the apico-basal axis enables the segregation of cell fate determinants into the GMC. Basally segregated markers include Miranda (Mira), Staufen (Stau), Prospero (Pros) protein and pros mRNA, Brain tumour (Brat), Partner of Numb (Pon) and Numb (Fig. 2). Mira is a coiled coil protein that acts as an adaptor carrying the cargo proteins Pros, Stau and Brat [18,25,64–66]. Pros, a homeodomain transcription factor, is released from Mira after segregation into the GMC. It then translocates to the nucleus to regulate GMC transcription and differentiation [21,67,68]. pros mRNA is bound by the RNA binding protein Stau, which is also asymmetrically partitioned by Mira [69]. This ensures the robust segregation of Pros to specify the GMC fate [64]. Recently Brat was identified as a further binding partner of Mira, which segregates asymmetrically together with Pros [18,25]. Brat is a translational repressor and belongs to an evolutionarily conserved protein family with a C-terminal NHL (NCL-1, HT2A and LIN-41) domain [70,71].

The segregation of cell fate determinants involves the interaction of the Par complex with the cortically localised tumour suppressor proteins lethal(2) giant larvae (Lgl), Discs large (Dlg) and Scribble (Scrib) [72–75]. Mutations in these proteins lead to defects in basal determinant targeting and spindle geometry [76]. Lgl has been shown to bind to non-muscle myosin II (NMY-2) [77] and to repress its function at the basal cortex. At the apical cortex, aPKC phosphorylates Lgl [73] and inactivates it preventing binding to NMY-2. Apically localised NMY-2 is now able to modify the actin cytoskeleton in order to exclude Mira (the Pros adapter) from the apical cortex. Live imaging
during mitosis reveals that NMY-2 moves toward the cleavage plane, driving Mira and its cargo basally in preparation for their asymmetric segregation upon cytokinesis [72].

Another actin binding motor protein, myosin VI or Jaguar (Jar), is also involved in spindle orientation and Mira localisation. Jar has been shown to bind Mira directly [78]. In jar mutants, Mira/Pros are delocalised and the mitotic spindle is misoriented. Interestingly the Insc/Par crescent is unaffected by loss of Jar and remains apical. Vesicle transport and exocytosis may also be involved in basal protein targeting since the yeast homologues of Lgl, Sro7p and Sro77p, bind t-SNARE proteins, which function in docking and fusion of vesicles to the plasma membrane [79]. The mammalian homologue of Lgl can also bind to the basolateral exocytosis machinery [80] and mammalian myosin VI regulates vesicle transport [81].

2.4. Centrosome asymmetry

Several studies indicate that centrosome asymmetry correlates with asymmetric cell division in Drosophila larval NBs [82–84], similar to what has been shown for the male germline stem cell (GSCs) [12]. In male GSCs, Yamashita et al. used green fluorescence protein (GFP)-tagged PACT (pericentrin/AKAP450), a centrosome protein required for centriolar localisation, to differentially label mother and daughter centrosomes using a “pulse-chase” strategy. The study showed that in GSC asymmetric division, the mother centrosome is always positioned at the interface of the GSC and the Hub cells. Hub cells are the “niche” in the germline. Upon division the mother centrosome is retained in the GSC while the newly formed centrosome is inherited by the gonialblast (differentiated cell) [12]. The mother centrosome has more pericentriolar material (PCM) than the daughter centrosome [12]. In mutants for Centrosomin (Cnn), which is required for microtubule nucleation and centrosome anchoring to astral microtubules, the position of mother and daughter centrosomes is randomised in male GSCs. The phenotype is accompanied by an increase in the number of GSCs. This may be the consequence of spindle misorientation leading to the loss of the stereotyped asymmetric division observed in the wildtype [12,85]. Alternatively mother and daughter centrosomes may carry fate determinants themselves and so influence different cell fates in a more direct way.

Recently centrosome asymmetry has also been reported in larval NBs (Fig. 4) [83,84]. In both studies centrosome dynamics were examined by time-lapse live cell recordings, in either larval brain explants or cultured NBs. Immediately after centriole duplication, only the apical centrosome acquires pericentriolar material (PCM), which organises an aster that is positioned at the apical cortex. The second aster moves towards the basal cortex. It only becomes visible shortly before the onset of prophase and nuclear envelope breakdown. Interestingly, in pins mutants the apical centrosome stays on the apical side initially but then begins to move to apparently random positions, similar to the basal centrosome [83]. In asterless (asl) mutants, which lack functional centrosomes and astral microtubules, mitotic spindles are still formed and orient along the apical-basal polarity axis. In very few cases (10%) are symmetric divisions observed. This suggests that there is an alternative centrosome/astral microtubule-independent mechanism for correct spindle orientation and asymmetric division [82,84]. It is not known whether the mother centrosome is consistently retained in larval NBs, as is the case for male GSCs. The future challenge is to explore the molecular mechanisms regulating centrosome asymmetry and to determine how centrosome asymmetry affects the fates of daughter cells.

Fig. 4. Spindle and centrosome asymmetry in larval NBs. The sequence of events during an asymmetric division is shown for a single larval NBs (from top left to bottom right). The asymmetric segregation of determinants and the asymmetric aster/spindle geometry lead to daughter cells of different fates and sizes. Note also the difference between apical and basal centrosome behaviour during the asymmetric division cycle.
3. Asymmetric cell division and cancer

Asymmetric cell division is one strategy that stem cells have adopted to control self-renewal and differentiation. Several recent studies in *Drosophila* indicate that disruption of the molecular machinery regulating asymmetric cell division (including polarity establishment, mitotic spindle orientation and cell fate determinant segregation) may lead to uncontrolled cell proliferation and malignant tumour formation [28,86,87]. There is also evidence emerging that some human cancers originate from a small group of neoplastic cells, or cancer stem cells (CSCs), that are able to sustain tumour growth [15,16,88–90]. The cellular and molecular basis of these cancer stem cells in humans remains elusive. Therefore, *Drosophila* NBs serve as an excellent model system to explore the cellular and molecular mechanisms that lead to the transformation from normal stem cells to cancer stem cells. Many genes originally identified as tumour suppressors in *Drosophila* have been shown to regulate asymmetric cell division, such as *dlg* and *lgl*. In addition, genes that were first associated with a role in asymmetric NB division have recently been discovered to be tumour suppressors. Here we summarize recent findings in *Drosophila* that link asymmetric cell division and tumour formation.

In elegant transplantation experiments, Caussinus and Gonzalez demonstrated that the brains of polarity mutants can develop neoplastic tumours [20]. They implanted mutant larval brain tissue into the abdomens of otherwise healthy adult flies to assay whether these mutations would induce tumour growth. Strikingly, tissue from brains mutant for the polarity cues Pins and the cell fate determinants Mira, Pros, Brat, and Numb produce tumours in healthy flies. In some cases the tumour mass was found to enlarge to more than 100 times the size of the original transplanted tissue. These tumours invade other tissues, eventually killing the hosts within several weeks. These tumour cells could also be maintained for years through rounds of transplantation, demonstrating that they are effectively "immortal" [20]. Examination of the karyotypes of the tumours revealed genomic instability and, in some cases, an abnormal number of centrosomes [20]. Loss of cell polarity and genomic instability combined with tissue invasion and metastasis are hallmarks of malignant human tumours [91]. Therefore, the *Drosophila* model recapitulates many of the properties of human cancer. In the last two years a number of reports show that loss-of-function mutants for cell fate determinants such as Pros, Brat and Numb or gain-of-function mutants of apical polarity cues such as aPKC can also cause tumorous growth in the developing fly [17,18,21,23–25,27].

4. Role of cell fate determinants

4.1. Atypical protein kinase C (aPKC)

As mentioned above, aPKC is part of the Par complex and regulates NB polarity. Strikingly, aPKC is also involved in the control of NB self-renewal and differentiation. Lee et al. show that the tumour suppressor Lgl and the polarity cue Pins restrict aPKC to the apical cortex in dividing NBs. *lgl, pins* double mutants exhibit ectopic cortical localisation of aPKC and a significant increase in the number of larval brain NBs [24]. Similarly, overexpression of a membrane-tethered form of aPKC leads to ectopic NB proliferation [24], while aPKC loss-of-function reduces the number of NBs [24,92]. Therefore, a model has been proposed in which Pins anchors aPKC apically, while Lgl inhibits aPKC localisation basally, restricting aPKC to the apical side and promoting NB self-renewal [24]. It has been shown that aPKC phosphorylates Lgl, which restricts determinants to the basal NB cortex [73]. Whether aPKC also directly promotes NB self-renewal through phosphorylation of NB-specific proteins remains to be determined. Notably, during mammalian neocortex neurogenesis, loss of aPKC, one of the two aPKC members expressed in the mammalian CNS, results in the loss of neuroepithelial cell adherens junctions [93]. However, this does not change the ratio between proliferation and differentiation of neural progenitors. It is possible that the two aPKC homologues work in a redundant manner in this system. The determination of whether or not aPKC proteins function in mammalian neural stem cells as they do in NBs must await the examination of the double knock-out mouse.

4.2. Prospero (Pros)

Previous work suggested that Pros is a critical regulator of cell division in the embryonic *Drosophila* CNS. Loss of *pros* increases the expression of mitotic genes such as *cyclin E, cyclin A*, and *string* (the *Drosophila* homologue of *cdc25*) [94–96]. A recent study demonstrated that Pros regulates the choice between stem cell self-renewal and differentiation [21]. By identifying the *in vivo* targets of Pros throughout the genome, Choksi et al. showed that Pros represses genes required for self-renewal, such as stem cell fate genes and cell cycle genes. Unexpectedly, Pros also binds to, and is required to activate, genes controlling terminal differentiation. When the division pattern of single, Di labelled GMCs was followed in *pros* mutant embryos, it was found that in the absence of Pros GMCs revert to a stem cell-like fate, express markers of self-renewal (Mira, Worniu and Deadpan), exhibit increased proliferation, fail to differentiate and generate small tumours. Therefore, the Pros-mediated transcriptional repression of stem cell genes together with Pros-mediated activation of differentiation genes prevents tumorigenic growth [21].

Interestingly, Prox1 (Prospero-related homeobox), the vertebrate homologue of Pros, is expressed in the embryonic rat forebrain at a time when neural stem cells cease self-renewal and begin to differentiate [97]. Prox1 also regulates exit from the cell cycle in mouse embryonic retinal progenitors, so that cells lacking Prox1 fail to stop dividing, whereas Prox1 overexpression results in premature exit from the cell cycle [98]. These findings suggest Pros/Prox1 function as a regulator of differentiation is conserved from insect to mammals.

4.3. Brain tumour (Brat)

Brat was first identified by Gateff in a screen for tumour suppressor genes in *Drosophila* [99,100] and was shown to induce
larval brain overgrowth and neoplastic transformation [70,101]. Recently, it was found that Brat binds to Mira, and is segregated to the GMC upon asymmetric cell division [18,25]. Loss-of-function mutations in brat cause the exponential proliferation of NB-like cells. These cells fail to differentiate, leading to a high mitotic index. Based on a BrdU pulse-chase experiment, Lee et al. also suggested that some brat mutant “GMCs” enlarge in size and re-enter the cell cycle to become proliferative [25]. Although the mechanisms by which Brat regulates NB proliferation are not clear, brat mutant clones express high levels of cell cycle proteins such as Cyclin E and many NB markers [17,18,25]. Additionally Brat was shown to downregulate the expression of dMyc, a transcription factor crucial for cell cycle control and cell growth [18,102]. Interestingly, the expression of Pros is lost or highly reduced in brat mutant cells [17,18,25]. As observed in the embryonic CNS [21], pros mutant clones also overproliferate in the larval central brain [17,18,25] and the ectopic expression of Pros in brat mutant clones can suppress the brat overproliferation phenotype [17]. These data suggest that Brat and Pros might act in a common molecular pathway.

There are two other NHL domain family proteins in the Drosophila genome: Mei-P26 and Dappled. Mei-P26 controls germline cell differentiation in both sexes and mutations in Mei-P26 cause ovarian tumours [103]. Dappled mutations cause melanotic tumours in the fat body [104]. Therefore, NHL domain proteins seem to have a common function in cell proliferation and growth. It will be intriguing to investigate whether Brat, Mei-P26 and Dappled also use similar mechanisms to control cell growth and differentiation. The vertebrate NHL domain family has three members, TRIM-2, TRIM-3 and TRIM-32 [70]. TRIM-3 is located on human chromosome 11p15 and deletions in this region have been associated with human astrocytoma and glioma [105–107].

4.4. Numb

Numb was the first asymmetrically partitioned cell fate determinant discovered in Drosophila sensory organ precursor (SOP) and in NBs [3,67]. Numb antagonises Notch signalling and in embryonic NB lineages specifies the asymmetric fates of sibling neurons [108]. Although a role for Numb in asymmetric cell fate specification in embryonic NB division has yet to be demonstrated, loss-of-function mutants affecting Numb lead to aberrant GMC specification and cause tumours in the post-embryonic brain [23,27]. Notch is thought to promote and maintain the stem cell fate in mammals, and previous reports show that Notch signalling is active in larval NBs [109]. Therefore it is likely that Numb acts to repress Notch signalling in GMCs. Indeed, Wang et al. show that reducing Notch levels post-embryonically leads to a decrease in NB number [27]. Interestingly, the basal localisation and asymmetric segregation of Numb requires the function of the Aurora-A kinase. Loss of Aurora-A results in the abnormal localisation of aPKC and Numb, misorientation of the mitotic spindle and the generation of brain tumours. Similarly, misexpression of a membrane-tethered form of aPKC leads to abnormal Numb localisation and to NB overproliferation [23,24]. A genetic interaction model has been proposed whereby Aurora-A acts in two pathways: one regulates spindle orientation (through Mud, see below) and the second alters Numb/Notch signalling by repressing aPKC [23,27].

Very recently a second key cell cycle regulator, Polo kinase, was shown to inhibit NB self-renewal and tumorous overgrowth [110]. As seen for aurora-A mutants, strong hypomorphic polo mutants disrupt the asymmetric localisation of Numb and aPKC as well as spindle orientation. Wang et al. show that Polo phosphorylates the Numb adaptor protein, Partner of Numb (Pon), and that this phosphorylation is important for Numb localisation. Since the segregation of Pros and Brat is unaffected in polo and aurora-A mutants, it seems that the misregulated Notch activity or aPKC can override their ability to inhibit proliferation. How Aurora-A and Polo regulate the localisation of aPKC, the activity of Numb, and the orientation of the mitotic spindle requires further investigation [110].

5. Role of spindle orientation

Most NBs are generated in the Drosophila embryo and derive from the neuroectoderm. However, optic lobe NBs, which form the visual system of the adult fly, are generated during larval stages. Like embryonic NBs, optic lobe NBs arise from a superficial neuroepithelium [111]. Optic lobe neuroepithelial cells undergo planar symmetric divisions to produce equivalent daughter cells that remain in the epithelium, whereas optic lobe NBs reorient their spindles along the apico-basal axis perpendicular to the epithelium. They then divide asymmetrically to generate an apical NB (self-renewal) and a basally positioned cell that is destined to differentiate. Egger et al., used Insc misexpression to test whether reorientation of the mitotic spindle in this system is sufficient to induce a change in cell fate, from neuroepithelium to NB. Although Insc localised apically and was sufficient to alter spindle orientation, from horizontal to apico-basal, it did not induce the formation of ectopic NBs, nor was there any evidence of excessive neuron production. Therefore, spindle reorientation is a consequence of, rather than instructive for, a change in cell fate [111]. A number of recent studies of vertebrate neuroepithelial cells have reached similar conclusions [112–115]. Neuroepithelial cells in the chicken neural tube, or in the mammalian forebrain, reveal a range of spindle orientations during neurogenesis. Most neuroepithelial cells exhibit a spindle orientation that is horizontal to the plane of the epithelium whether or not the outcome of the division is symmetric (progenitor/progenitor) or asymmetric (progenitor/differentiating cell). Two recent reports show that mitotic spindle orientation becomes randomised when the activity of the vertebrate Pins homologue, LGN, is reduced or lost [112,113]. Komno et al. also showed that overexpression of mammalian INSC induces apico-basal divisions [112]. Both studies investigated whether the increase in the percentage of apico-basal-oriented divisions promotes asymmetric divisions and/or increases rates of neurogenesis. Similar to the Drosophila optic lobe, neuronal production rate is not affected by increased apico-basal divisions. However, divisions are seen where one daughter cell exits the neuroepithelium and proliferates aberrantly in the
subventricular or mantle zone [112,113]. These ectopic, non-surface, progenitors still express neuroepithelial markers, such as Pax6 [112]. These findings suggest that spindle reorientation in *Drosophila* and vertebrate neuroepithelial cells cannot by itself convert symmetric to asymmetric divisions. However, at least in vertebrates, spindle orientation is important for the maintenance of epithelial integrity.

Is it possible to induce symmetric divisions by simply misorienting the mitotic spindle in the NB in such a manner that equal apical and basal cell fate determinants are inherited by both daughter cells? So far this question has been difficult to address for two reasons: first, most loss-of-function mutations that affect spindle orientation, such as *insc* or *pins*, also affect the asymmetric localisation of basal cell fate determinants. Second, in loss-of-function mutants for *baz*, *insc* and *pins*, basal cell fate determinants often mislocalise at metaphase, but are rescued at late anaphase and telophase as a consequence of a compensatory mechanism that aligns the basal crescent with the spindle pole inherited by the GMC, a phenomenon called ‘telophase rescue’ [116]. Recently, mutations in mushroom body defect (Mud), another microtubule binding protein, have been shown to affect spindle orientation [19,22,26]. Mud is the *Drosophila* homologue of mammalian NuMA (nuclear mitotic apparatus protein) and *C. elegans* LIN-5. In *C. elegans* and mammals, LIN-5 and NuMA bind to the Pins homologues AGS3 and LGN, respectively [117,118]. NuMA binds microtubules and stimulates polymerisation and assembly [117,119,120]. *Drosophila* Mud also co-localises with and directly binds to Pins, mud loss-of-function NBs fail to align their mitotic spindles with the axis of cortical polarity but, in contrast to *pins* mutants, telophase rescue does not occur. Therefore, apical and basal determinants are segregated symmetrically to both daughter cells, which continue to proliferate [19,22,26]. Interestingly, in the mammalian retina, the INSC homologue is expressed in both vertically and horizontally dividing neuroepithelial cells. Reduced levels of INSC lead to a decrease in vertical divisions and a concomitant increase in horizontal divisions, which appear to increase the progenitor pool at the expense of early-generated neurons [121]. Therefore, spindle orientation dictates whether cell division is symmetric or asymmetric in both NBs and vertebrate retinal progenitors. These experiments show that aberrant spindle orientation can affect self-renewal and proliferation, raising the possibility that spindle misalignment can induce tumours too.

Many human homologues of genes characterised in *Drosophila* have been reported to be associated with various forms of cancer. For example, the mammalian Mud homologue, NuMA is associated with breast cancer susceptibility and acute promyelocytic leukaemia [122–124]. Expression of the human homologue of Lgl, is significantly reduced in human colorectal cancer and malignant melanoma [125,126]. Loss of mouse Lgl results in severe brain malformations similar to human neuroectodermal tumours [127]. An aPKC homolog in human has been identified as an oncogene and is highly expressed in lung cancer patients [128,129]. Alterations in Aurora-A expression are associated with many human cancers including prostate cancer, pancreatic cancer, ovarian tumours and colorectal cancer [130–133]. Due to its potential as an anti-cancer target, research is now underway to identify Aurora-A inhibitors [134].

6. Conclusion

In the past decade, many advances have been made in uncovering the molecular mechanisms controlling asymmetric cell division. This includes the establishment of polarity, mitotic spindle orientation and the segregation of cell fate determinants. Furthering this understanding of symmetric and asymmetric divisions is important for elucidating stem cell behaviour and has tremendous implications for stem cell biology, stem cell therapy and cancer research.

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