Stem cell tumors in *Drosophila melanogaster*

Dividing the defects in asymmetric cell division

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Contents

Abstract

Stem cells are the precursors of various tissues in multicellular organisms. Their unique ability to divide both symmetrically and asymmetrically enable them to expand cell populations and to generate various cell types. In the initial stages of tissue formation, stem cells divide symmetrically into two daughter stem cells to increase the progenitor pool. The stem cells are required to switch their division mode to asymmetry to provide differentiating daughter cells upon modeling of the tissues. In an asymmetric stem cell division, the differentiating daughter cell is budded off from a self-renewing stem cell. The balance between symmetric and asymmetric divisions is tightly regulated to control the levels of proliferating cells versus differentiating cells. Defects in the ability to generate a differentiating daughter results in an overpopulation of proliferating stem cell daughters, a phenomenon described as stem cell cancer. A lot of research was dedicated to understand the mechanisms of asymmetric division and the distribution of the different daughter fates. In this review, I will give an overview of the mechanisms involved in controlling asymmetry of a cell division, and the defects that have been unraveled up till now leading to stem cell tumor formation.

1. Introduction - stem cells in development

During development of a multicellular organism billions of cells have to arise from just a few progenitor cells, the stem cells. Stem cells are unique in their ability to divide both symmetrically and asymmetrically, which allows them to eventually generate an entire organism. Symmetric cell divisions generate two identical daughter cells, whereas an asymmetric division generates daughter cells with different developmental potential. During fetal development stem cells divide in a symmetric pattern to increase the proliferative stem cell population. After gastrulation the division mode of these cells switches to asymmetry. In one round of asymmetric division stem cells self-renew and bud off a daughter cell that will differentiate, to form the various tissues [Hartenstein and Campos-Ortega, 1984, Pardal et al., 2003]. There is a tight balance between symmetric and asymmetric division modes to produce appropriate amounts of proliferating stem cells versus differentiated cells. Developmental and environmental signals control this balance. Defects in this balance that unable the stem cell to generate differentiating daughter cells was shown to result in an overpopulation of proliferative stem cell daughters, a phenomenon described as stem cell cancer [Neumuller and Knoblich, 2009].

1.1 Model systems to study asymmetric cell division: fruit fly and nematode

Asymmetric cell division has been shown to be regulated by both intrinsic and extrinsic cues. In order to capture the overall effect of disruption of asymmetric division during developmental stages, model organisms are used to study the regulation mechanisms of asymmetry. Two of the current leading model organisms to study the mechanisms of asymmetric division are the fruit fly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans* [Doe and Bowerman, 2001].

The *C. elegans* zygote, also referred to as P0, is the classic example of an intrinsic asymmetric cell division system. The oocyte has a symmetric distribution of cell fates; upon fertilization an anterior-posterior polarization of now P0 is induced and cell fates are distributed according to this anterior-posterior polarization [Hyenne et al., 2010]. P0 undergoes five rounds of asymmetric division after fertilization to pattern the embryo. Within these five divisions the germline is established, as are the three body axes [Hyenne et al., 2010]. The first division of P0 is asymmetric along the anterior-posterior axis and generates a large anterior daughter blastomere (AB), and a smaller posterior blastomere (P1) (figure 1). The AB and P1 cells differ in cell size, cell cycle progression, and have distinct developmental potential. AB is the precursor for the ectoderm lineages, where P1 generates the mesoderm, endoderm, and germline lineages [reviewed in Doe and Bowerman, 2001]. The mechanisms that result in patterning of the embryo and asymmetric cell division were shown to be applied by many other stem cells and progenitor cells [Morrison and Kimble], making the *C. elegans* one-cell embryo an excellent model system to study intrinsic asymmetric cell division.

A second class of stem cells that rely on intrinsic asymmetric division are the *Drosophila* neural stem cells [Wu et al., 2008]. The asymmetric division of these cells was shown to depend on the segregation of cell fate determinants. Many of these fate determinants are conserved in vertebrates, where they also regulate daughter cell potential, suggesting a common mechanism that a subset of stem cells apply to regulate asymmetric outcome. Most current knowledge about asymmetric division in *Drosophila* was derived from experiments performed on the central nervous system. The central nervous system contains the precursor cells for the neurons: the neuroblasts. Depending on their position in the brain, *Drosophila* larval neuroblasts are subdivided into central brain (CB), optic lobe (OL), or ventral nerve chord (VNC) neuroblasts [Neumuller and Knoblich, 2009]. The CB and VNC neuroblasts are descendants from embryonic neuroblasts. During embryogenesis these become quiescent, but reactivate proliferation during larval development [Truman and Bate, 1988]. The OL neuroblasts are only generated during larval development. During larval development the OL consists of three compartments of cells. It starts out as a neuroepithelium of stem cells that divide symmetrically to solely expand the stem cell population. After gastrulation a region of these neuroepithelial cells start to express the neuroblast identity genes asense (*ase*) and deadpan (*dpn*), which facilitate the transition of neuroepithelium into neuroblasts. Neuroblasts divide asymmetrically to self-renew and bud off a second smaller daughter cell that is primed to divide into two differentiating

neurons, called the Ganglion Mother Cell (GMC) [Egger et al., 2010]. The VNC and CB neuroblasts also start out as a neuroepithelium that divides symmetrically to expand the stem cell population. During stages 8-11 of embryonic development about one third of the neuroblasts delaminate from the epithelium due to a loss of epithelial junctions [Egger et al, 2007, Yasugi et al., 2008, Campos-Ortega and Hartenstein, 1997, Rebollo et al., 2009]. After delamination the neuroblasts switch to an asymmetric division mode to regenerate themselves and to generate a GMC [Egger et al., 2007] (figure 1).

Drosophila contains a second class of neural progenitors that are used to study intrinsic asymmetric division. Sensory organ precursor (SOP) cells are precursors of the external sensory organs of the fly. These organs are not essential for viability, making them very suitable to study mutations in cell fate specification. Cell fate transformations can be easily read-out since these cells are at the surface of the fly [Mummery-Widmer et al., 2009, Neumuller and Knoblich, 2009]. SOP cells undergo three rounds of asymmetric divisions before generating a sensory organ. The first asymmetric division results in an anterior pIIb and a posterior pIIa cell. These cells will both divide once more to generate differentiating cells. pIIb daughter cells will form the inner neuron and its sheat, whereas the pIIa daughter cells will generate the hair and its socket [Bardin et al., 2004] (figure 1).

Drosophila is also an excellent model system to study extrinsic asymmetric divisions. The germline stem cells (GSC) do not depend on segregation determinants, but merely on their position relative to an extrinsic signal source [Morrison and Kimble, 2006]. GSC reside in a specialized microenvironment that provides extracellular signals to the stem cell that maintain its stem cell fate. This specialized microenvironment is called the stem cell niche. Niches are important as they were shown to negatively regulate the expansion of the stem cell pool [Morrison and Spradling, 2008]. GSC in the testis reside in niches comprised of hub cells, whereas ovarian GSC niches comprise cap cells. Both hub and cap cells anchor the GSC to the niche via E-cadherin based cell-cell junctions. Division of the GSC always results in a daughter GSC that remains anchored to the niche via these E-cadherin junctions, and a daughter cell that dissociates and is set to differentiate, the gonialblast [Yamashita et al., 2003] (figure 1). The GSC are required to stay in close proximity to the niche cells in order to receive stem cell maintenance signals [Kiger et al., 2001, Yamashita et al., 2003]. Gonialblast GSC daughter cells were shown to start differentiation as soon as they dissociate from the niche, supporting the suggestion that the extracellular signals determine daughter cell fate in these cells [Burness and Sipkins 2010].

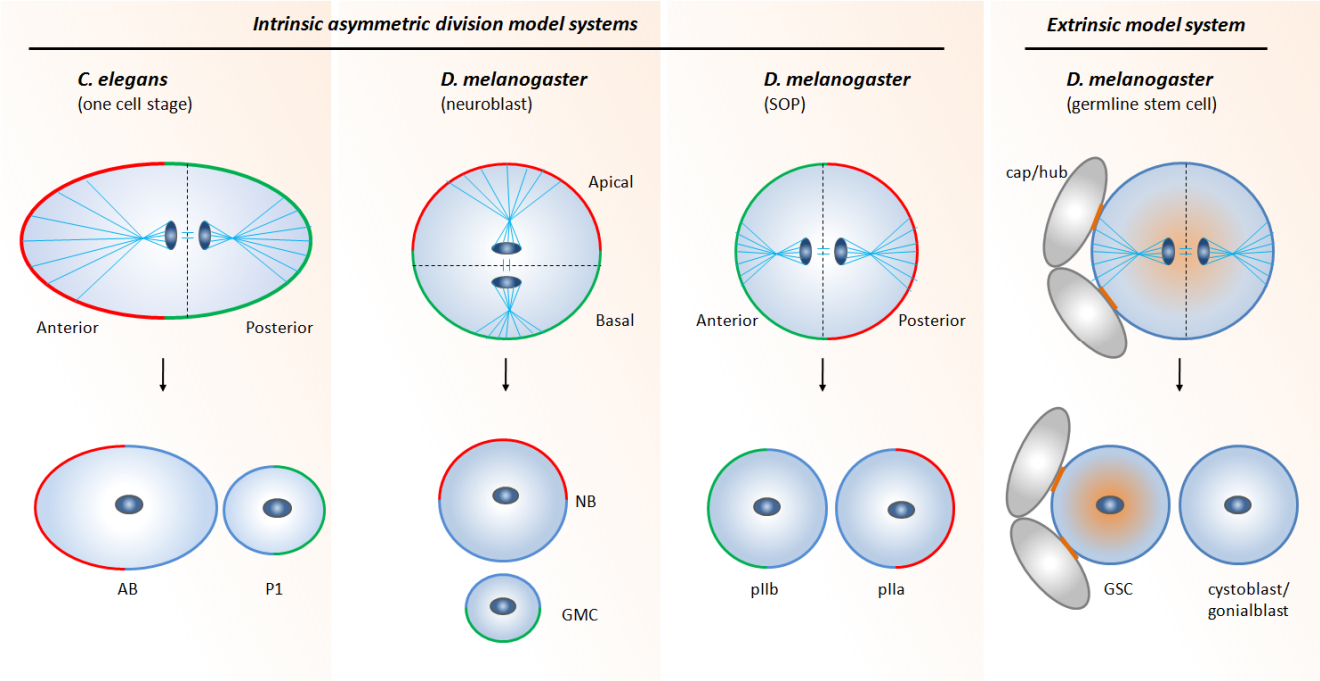


Figure 1. Current model systems used to study intrinsic and extrinsic asymmetric cell division. The *C.elegans* one cell embryo and the *D. melanogaster* neuroblast and SOP cell are the main models used to study intrinsic asymmetric division. The intrinsic models use cellular polarity to compartmentalize the cell into two distinct cell fate zones, red versus green in the image. The *C. elegans* embryo is divided in an anterior half (red) and a posterior half (green). The anterior daughter cell, the AB cell, is the precursor of ectoderm lineages. Its smaller daughter sibling inherited the posterior compartment primed to form the mesodermal, endodermal, and germline lineages. The *Drosophila* neuroblast divides asymmetrically to separate the apical compartment (red) from the basal compartment (green). The apical larger daughter cell is a self-renewed neuroblast (NB) having a stem cell identity, whereas its smaller basal daughter sibling is a precursor for the neuron lineage. This cell was named the Ganglion Mother Cell (GMC). The *Drosophila* SOP cell divides asymmetrically to result in two equally sized daughter cells. The pIIb daughter cell inherited the anterior domain and is primed to generate the inner cells of the sensory organ, where its daughter sibling pIIa, which inherited the posterior domain, is the precursor of the outer cells of the sensory organ. The *Drosophila* germline stem cell is used as a model to study extrinsic asymmetric cell divisions. The GSC is anchored to the niche cells (cap in female, hub in male) that provide it with stem cell maintenance factors (orange patches at niche-stem cell contact). GMCs are not polarized, and upon division the daughter siblings initially have identical potential. However, the stem cell fate is retained in the daughter anchored to the niche (orange glow), whereas its sibling that is not attached anymore lost these signals and starts to differentiate into a cystoblast (female) or a gonialblast (male). Adapted from Gonzcy et al., 2008.

1.2 Stem cell tumors result from defects in the asymmetric segregation of cell fate determinants

Up to date stem cell tumors have only been identified in *Drosophila* neuroblasts. Overproliferation of neuroblasts at the expense of differentiating neurons is observed in *Drosophila* mutants for asymmetric cell fate distribution. This suggests that the loss of intrinsic cell fate in this system cannot be rescued by extrinsic signals, and that this loss of cell fate is the tumor-initiating event [Neumuller and Knoblich]. The observation that single mutations could initiate overproliferation makes the *Drosophila* neuroblasts an interesting model to study stem cell tumor formation.

Transition from embryonic stem cells into adult stem cells also induces the transition from an active proliferating state into quiescence [Li and Clevers, 2010]. These are thought to be only activated upon tissue damage signals to regenerate the damaged cells [Arai et al., 2004]. Adult stem cells that are in a quiescent state should therefore not be affected by mutations that disrupt the asymmetric distribution of cell fate determinants, since they are not provided with external proliferation cues and will therefore not divide. Stem cells that have been transformed into proliferating cancer cells were shown to no longer respond to external cues. These continuously proliferate, even during adult stages, an observation that made Neumuller and Knoblich propose that loss of asymmetry might induce immortalization of the daughter cells [Bello et al., 2006, Neumuller and Knoblich, 2009].

Therefore, it is important to study the progression of these transformed stem cells to reveal the (epi)genetic changes underlying this immortalization.

A problem that many model systems encounter is that upon tumor progression, and thus increasing tumor sizes, the host organism dies. The development of transplantation assays overcomes these problems. Cassinus and Gonzales described in a paper how transplantation of tumor samples into *Drosophila's* host abdomen can be used to study tumor progression over long periods of time. These tumors were not affected by the transplantation; they continued proliferation [Cassinus and Gonzales, 2005]. This makes *Drosophila* a very suitable model organism to study stem cell tumor formation and progression.

Stem cell tumors are proposed to arise from mutations affecting asymmetric cell division that result in a loss of differentiation potential. As these overproliferating cells have only been observed in *Drosophila* neuroblasts, which depend on the asymmetric distribution of cell fate determinants, it is suggested that disruption of the asymmetric distribution of the determinants is what sets off tumor formation. Therefore, close observation of the different mechanisms involved in the asymmetric distribution of cell fates might provide insight on these tumors. I will give an overview of the regulatory steps in asymmetric cell division based on research performed on *C.elegans* and *Drosophila* in the next chapter. Furthermore, I will give an overview of the cell fate determinants implicated in stem cell tumor formation that have been identified up to date.

2. Daughter cell asymmetry – dividing the mechanisms

A stem cell is defined by the ability to both self-renew and generate a differentiating daughter cell to repopulate the tissue within one cell division. This can only be achieved when both daughter cells inherit different cell fate determinants from the mother cell. There has to be a tight regulation of cell fate distribution mechanisms to accurately divide the different factors. Research has revealed that a cell uses both extrinsic and intrinsic regulation mechanisms to control daughter cell fates, which can become asymmetric both before and after mitosis. Despite of an equal distribution of cell fates, daughter siblings can still adopt different fates due to different environmental cues they are exposed to, as was described for the germline stem cell daughters. These extrinsic cues trigger an intracellular cascade that alters cell fate.

Unequal distribution of cell fate determinants before mitosis automatically results in daughter cells with different properties, as was described for the *Drosophila* neuroblasts. Stem cells are the only cell type that can induce different daughter cell fates via asymmetric mitosis. Daughter cells of asymmetric mitosis differ in size, contain different levels of cell fate determinants and have different differentiation potential [Horvitz and Herskowitz, 1992]. However, cellular components are sometimes unequally divided in symmetric somatic divisions as well. Gromley and colleagues described that the midbody is passed to one daughter cell [Gromley et al., 2005]. Chromatin was also shown to be distributed unevenly in some cases [Rando 2007, Neumuller and Knoblich 2009]. This points out that unequal distribution of cellular content alone does not characterize asymmetry. It is the combination of difference in size, content, and differentiation potential that makes a division asymmetric.

2.1 E-cadherin mediates spindle orientation by locking the mother centrosome to the niche

An intrinsic asymmetric division relies on the unequal distribution of two different cell fates to the daughter cells. In order to pass on the two different cell fates to its daughters the cell must divide itself into two compartments; one for the proliferative stem cell fate and one for the differentiation fate. By orienting the mitotic spindle along the different fate compartments, the cell ensures separation of these compartments upon division. The cell adhesion protein E-cadherin was recently shown to be involved in spindle orientation. Evidence for this came from the Yap lab, who suggested in 2009 that cell-cell adhesions are involved in spindle orientation in epithelial cells. Spindle orientation ensures the asymmetric distribution of cell fate determinants by setting up the two future daughter poles along the polarity axis. Yap and coworkers observed that 96% of the epithelial MDCK cells that contained cell-cell contacts divided in a symmetric, planar way, where monolayers of the same cells lacking cell-cell

contacts only contained 17% planar dividing cells [den Elzen et al., 2009]. Non-polarized CHO cells were seeded on coverslips coated with the extracellular domain of E-cadherin, which resulted in E-cadherin clustering at the coverslip interface and increased levels of radial oriented spindles. These results suggest that spindle orientation depends on E-cadherin localization, but not directly on cell polarity.

The suggestion that E-cadherin regulates spindle orientation was confirmed in a recent follow-up study by Inaba and colleagues, who studied spindle orientation in the *Drosophila* male GSC. Expression of a dominant negative E-cadherin protein that contains a truncated extracellular domain unable to form homodimers, and thus cannot form a connection with the niche hub cell, resulted in misoriented centrosomes in 35% of the stem cells, and 39% misoriented spindles (misoriented meaning loss of the centrosome binding to the hub cell interface). These observations imply that E-cadherin sequesters the GSC mitotic spindle to a specific site at the cortex As GSC are not polarized, these cells solely depend on E-cadherin for spindle orientation [Inaba et al., 2010]. Both den Elzen and Inaba describe a possible mechanism how E-cadherin determines orientation. They suggest a role for APC2 (adenomous polyposis coli 2) as a scaffold protein that anchors the microtubules emerging from the spindle to the E-cadherins at the cortex, possibly via interaction with Armadillo (homolog of mammalian β-catenin) [den Elzen et al., 2009, Inaba et al., 2010].

What is very interesting about these observations is that the presence of E-cadherin within a polarized epithelium preserved symmetric division, while within the non-polarized germline stem cell niche E-cadherin is required to ensure asymmetric division. Apparently, localization of E-cadherin determines localization of the spindle. Both cells apply the extrinsic cell-cell junction formation induced via their niche to localize E-cadherin and thus position the centrosomes. It was recently shown that in non-polarized germline stem cells (GSC) E-cadherin determines spindle orientation by specifically binding the mother centrosome to the niche. By this specific binding, the niches ensures that he mother centrosome is always inherited by the daughter stem cell [Yamashita et al., 2003]. Centrosomes consist of a pair of centrioles surrounded by pericentriolar material (PCM). At telophase the mother centriole pair splits and both single centrioles are replicated. Cells were shown to be able to distinguish between original and newly synthesized centrioles, suggesting that the retention of the original centriole could determine daughter cell fate [Neumuller and Knoblich, 2009]. Indeed, recent experimental data supports the suggestion that the stem cell daughter is the original centriole receiving daughter cell. Pulse-chase experiments in *Drosophila* male germline stem cells (GSC) using a GFP-tagged centrosome protein revealed that asymmetric localization of the centrosomes is a landmark for asymmetric division [Yamashita et al., 2003, Wu et al., 2008].

The fact that the differentiating daughter cell always obtains the newly formed centrosome in GSC divisions [Yamashita et al., 2007], and the self-renewed daughter stem cell always retains the centrosome containing the original centriole, triggered Spradling and Zheng to propose that permanent inheritance of the original centriole enables stem cells to retain their proliferation status indefinitely [Spradling and Zheng, 2007]. This theory was challenged in 2008 by a study of Stevens and colleagues in which they show that the asymmetric segregation of centrioles is not an essential feature of stem cells. *Drosophila* ovaries of a *dsas4* mutant fly which are unable to replicate centrioles revealed that the few centrioles that were left in the female GSCs were randomly distributed among the daughter cells. There was no stringency in anchoring these to the mother cell cortex. The stem cells lacking centrosomes still displayed mitotic spindles that localized in such a way that asymmetric division was achieved. These cells also displayed normal polarity. Wildtype and *dsas4* mutants were stained for the posterior oocyte marker Orb, which displayed similar localization patterns. Microtubule association in these mutant flies was also not affected by loss of centrioles as was read-out by nuclear migration during oogenesis. These data all suggest that centrosomes might not be important at all for cell fate determination. However, following these cells after fertilization revealed that these flies die of mitotic errors in early stages of embryonic development [Stevens et al., 2007]. These data suggest that asymmetric distribution of centrioles is not a common feature for stem cells, but it does affect a subpopulation of stem cells during embryonic development. Many researchers attempt to unravel what the exact function of the asymmetric distribution of centrioles during development could be. [Castellanos et al., 2008, Neumuller and Knoblich, 2009].

These results describe an important role for E-cadherin based cell-cell junctions in determining spindle orientation. In asymmetrically dividing, non-polarized stem cells, E-cadherin was shown to have a higher affinity for the mother centrosome, thereby specifically locking the original centrosome to the stem cell daughter. These observations uncouple spindle orientation from cellular polarity, which is remarkable as polarized stem cells require a spindle orientation according to their polarity to separate the two cell fate compartments. Polarized epithelial cells ensure symmetric divisions using E-cadherin at their lateral membranes as an anchoring site for the spindle poles, a mechanism that is also applied by the *Drosophila* neuroepithelium that initially divides symmetrically to expand the stem cell population during embryonic development. As described earlier, neuroepithelial cells switch to an asymmetric division mode once they delaminate from the epithelium. Interestingly, delamination is partially the result of E-cadherin downregulation [Neumuller and Knoblich, 2009]. The loss of E-cadherin and the resulting asymmetric division imply that E-cadherin is indeed required for symmetric epithelial divisions by anchoring of the spindle to the cortex. But this also implies that there is a second mechanism that locks the spindle into the apical-basal orientation of asymmetrically dividing neuroblast cells.

2.2 Polarity proteins pattern the cell to establish different daughter cell compartments

Spindle orientation in the asymmetrically dividing polarized cell was shown to be dependent on the proteins involved in polarization of the cell, the polarity proteins (box1). Polarity proteins are involved in the set-up of an anterior-posterior axis in the *C. elegans* zygote. In unfertilized *C. elegans* oocytes Par3, Par-6 and PKC-3 are equally distributed along the cell cortex. Upon sperm entry Par-3/Par-6 dissociate from the cortical area that overlies the sperm entry point as a result of a local loss of tension on the actin cortex. This local loss of tension is thought to be induced by the Rho GTPase GAP protein CYK-4, which was introduced by the sperm. CYK-4 suppresses myosin-2 activity and induces a relocation of the actomyosin cytoskeleton to the opposite pole of the cell [Munro et al., 2004]. The cortex has now been subdivided into two domains: an anterior contractile domain and a posterior non-contractile domain [Hyenne et al., 2010]. Par3 and Par6 migrated along with the actomyosin cytoskeleton in anterior direction, enabling Par-2 to localize to the posterior non-contractile domain via microtubules. Par-2 expands its cortical localization region to the posterior half of the cell, where Par-1 can also localize [Munro et al., 2004]. The cortex is divided in an anterior domain containing Par-3/Par-6 and a posterior domain containing Par-1/Par-2.

Drosophila uses the homologues of these polarity proteins to set-up an apical-basal polarity in neuroblasts and an anterior-posterior polarity in SOP cells. Par-3 homolog Bazooka and Par-6 both localize to the apical cortex in neuroblasts, and to the posterior cortex in SOP cells [reviewed in Neumuller and Knoblich, 2009]. The basal/anterior cortex however is marked by a different polarity protein. As described in box 1, *par-2* is not conserved from *C. elegans* to *Drosophila*. Drosophila neuroblasts and SOP cells use the lethal (2) giant larvae (lgl) protein to mark their basal/anterior cortexes. *lgl* does have a homolog in C. elegans, *lgl-1,* which was shown to be redundant to *par-2* [Boyd et al., 1996, Atwood and Prehoda, 2009].

The two polarity domains mutually inhibit each other. Anterior Par-2 localization in *C. elegans* is inhibited by Par-3 that phosphorylates Par-2, leading to its dissociation from the cortex. Par-2 at its turn inhibits posterior Par-3 localization together with Par-1 and Par-5 proteins [Munro et al., 2004]. This mutual inhibition is conserved to *Drosophila*. The *Drosophila* Par-3 homolog Bazooka was shown to be directly phosphorylated by Par-1, which enables 14-3-3e (Par5 in *C. elegans*) to bind and sequester Bazooka from the cortex [Munro et al., 2004, Labbe et al., 2006]. The mutual inhibition of the polarity complexes underlines the intrinsic nature of cell polarity.

Cellular polarity is induced during the early stages of mitosis, when the polarity proteins are activated. However, research has shown that in *Drosophila* larval neuroblasts asymmetric localization of aPKC is already set up during interphase by the surrounding tissue. Marthiens and ffrench showed that aPKC is asymmetrically inherited in *Drosophila* neuroblasts, suggesting that it needs to be locked to the apical cortex to ensure inheritance by the apical stem cell daughter [Marthiens and ffrench-

Constant, 2009]. Par-3 and Par-6 also tend to localize apically before mitosis, suggesting a ready primed polarity [reviewed in Knoblich, 2008]. These observations indicate that polarity proteins are asymmetrically distributed during interphase upon extrinsic cues, but seem to depend on mitotic factors to be activated.

Box. 1 Polarity genes

The polarity genes were originally discovered in *C. elegans* zygotes in a screen for partitioning defect mutants. Six mutants were identified that share a similar phenotype: a switch from asymmetric division into abnormal symmetric division that results equal sizes of the AB and P1 cells. The genes were named after their mutation: *par*-1 to *par*-6 [Kemphues et al, 1988]. Except for *par-2*, these genes are conserved in *Drosophila*.

Despite their shared mutant phenotype, the Par proteins have different functions in the cell. Par-1 is a serine/threonine kinase that localizes to the posterior cell cortex [Guo and Kemphues, 1995]. Par-2 also localizes at the posterior cortex. It is a RING finger protein that is only found in *C. elegans*. The PDZ domain proteins Par-3 (Bazooka in *Drosophila*) and Par-6 both localize to the anterior cortex. Par-4 is a serine-threonine kinase that is evenly distributed in the cell [Watts et al., 2000], as is the 14-3-3 protein Par-5 (14-3-3e in *Drosophila*) [Morton et al., 2002]. The atypical *pkc3* gene (*apkc* in *Drosophila*) encodes a serine/threonin kinase that was later added to the *par* family as its depletion resulted in a similar phenotype as *par3* or *par6* deletion. The PKC3 protein was shown to form a complex with Par-3 and Par-6 at the anterior cell cortex referred to as the anterior PAR complex [Kemphues et al., 1988, Hyett et al., 2010, Tabuse et al., 1998].

2.3 Activation of polarity proteins is cell-cycle dependent

If aPKC is already localized to the apical membrane during interphase, then why is the apical-basal polarity only established during mitosis? During interphase aPKC forms a complex with Par-6 and Lgl (lethal (2) giant larvae) which is uniformly distributed along the cell cortex in *Drosophila* [Betschinger et al., 2005]. At the early stages of mitosis AuroraA is activated by Bora in a Cdc-2 dependent manner [Hutterer et al., 2006]. Activated AurA phosphorylates Par-6 in the Lgl/aPKC complex. Unphosphorylated Par-6 suppresses aPKC activity, indicating that upon Par-6 phosphorylation aPKC is activated. aPKC subsequently phosphorylates Lgl, which can no longer bind to the complex and will translocate to the basal cortex. Dissociated Lgl enables Par-3 to bind aPKC. Par-3 together with phosphorylated Par-6 and aPKC forms an active polarity complex (PAR complex). These results imply that aPKC is only activated during mitosis because its activator AurA is only active in this stage. Besides AurA there is another aPKC activator, described by Lin and colleagues who showed that the Rho GTPase Cdc42 can also activate aPKC via binding to Par-6 [Lin et al., 2000]. Once activated, the apical and basal polarity complexes can restrict each other's localization.

The cell has adopted additional mechanisms to restrict polarity complex localization. A recent study by Chabu and Doe describes how aPKC phosphorylation is regulated in an autonomous manner. Biochemical assays have revealed that aPKC interacts with a subunit of the B-type protein phosphatase-2A (PP2A) called Twins. Together with a catalytic and a variable subunit, Twins forms an active serine/threonin phosphatase complex, that is required to retain aPKC at the apical cortex, possibly by directly dephosphorylating it at the basal cortex. Besides acting on aPKC directly, the PP2A complex also antagonizes aPKC function basally by dephosphorylating Par-1, thereby restoring its cortical localization. *twins* mutant neuroblasts display basal aPKC localization and an overproliferation of neuroblasts at the expense of GMC, implying that retention of aPKC is related to retention of stem cell fate [Chabu and Doe, 2009]. Furthermore, the basal daughter cell was described to lose neuroblast stem cell fate upon the loss of cortical aPKC and the gain of cortical Lgl, supporting the suggestion that the polarity proteins are essential for the distribution of cell fates. Mutation in the aPKC activator *aura* in *Drosophila* neuroblasts was also shown to induce stem cell-derived tumors.

Tumor formation is likely an effect of missegregation of unphosphorylated aPKC into the differentiating daughter cell, as aPKC mutations suppress the phenotype [Wang et al., 2006, Wirtz-Peitz et al., 2008]. These results underline the importance of controlled cellular polarity on cell fate distribution.

2.4 The apical PAR complex cooperates with Inscuteable to rotate the spindle along the apical-basal axis

Local activation of the Par proteins was shown to be indispensable for correct spindle orientation in polarized stem cells by acting on the spindle - cell cortex interaction. The aster microtubules are anchored to the cortex via a subset of proteins. The NuMa-related protein mushroom body defect (Mud) binds to the spindle microtubule plus-ends, likely via association with the minus-end tracking motor protein dynein that localizes to these plus-ends [Bowman et al., 2006, Siller et al., 2006]. Mud forms a docking site for a GoLoco-domain protein, either Partner of Inscuteable (Pins) or Loco, which is a scaffold protein that connects it to a membrane-anchored protein [Schaefer et al., 2000, Yu et al., 2005]. The heterotrimeric G-protein alpha subunit Gαi is the proposed membrane-anchor protein. Gαi is ubiquitously expressed at the cell cortex and was shown to recruit Pins to the cell cortex. Upon Gαi binding Pins conformation changes, exposing a binding site for Mud [Schaefer et al., 2001]. Pins and Gαi together mediate the linkage of the mitotic spindle to the cell cortex and thus determine spindle orientation. However, they depend on positioning cues on where to anchor the spindle in the cortex, like E-cadherin that sequesters the poles and determines the sites of spindle anchoring to the cortex.
In case of the neuroepithelium, symmetric divisions are regulated by E-cadherin that sequesters the spindles to the lateral membranes. This is facilitated by the apical Par complex; aPKC phosphorylates Pins, thereby inhibiting its connection to the apical cortex. As Pins is repelled from the apical cortex, the default spindle orientation is planar.

In order to switch to asymmetrically dividing neuroblasts, the spindle needs to rotate 90 degrees to orient along the apical-basal axis, despite of aPKC presence. The expression of Inscuteable (Insc) during neuroblast delamination is the key to the switch to asymmetric division [Schober et al., 1999]. Insc cooperates with the apical Par complex in spindle rotation. *par* complex expression starts in the *Drosophila* neuroepithelium and retains in the delaminated neuroblasts, meaning that upon induction of Insc expression these cells already have an apical-basal polarity. Bazooka/Par3 in the polarity complex was shown to recruit Insc to the apical cortex. Apical bound Insc recruits Pins to the cortex [Yu et al., 2000]. In a yet unknown way, Insc prevents aPKC from phosphorylating Pins. The result is that both the PAR complex and Pins are localized at the apical membrane where Insc anchors the spindle via Pins to the Par complex [Hao et al., 2010]. The PAR complex and Pins/Gαi do not seem to depend on each other. Single mutations in either complexes resulted in wildtype daughter cell sizes and revealed no defects in spindle formation. It required the double mutant to disturb the asymmetry, suggesting the Par complex is just an anchoring site for Pins and not a regulatory component [Izumi et al., 2004, Wu et al., 2008]. These results indicate why in the neuroepithelial cells the polarity proteins have no effect on spindle orientation; they require Insc presence to couple them to the spindle anchoring complex. The Par/Insc orientation is dominant, as in asymmetrically dividing neuroblasts E-cadherin is downregulated [Neumuller and Knoblich] (figure 2).

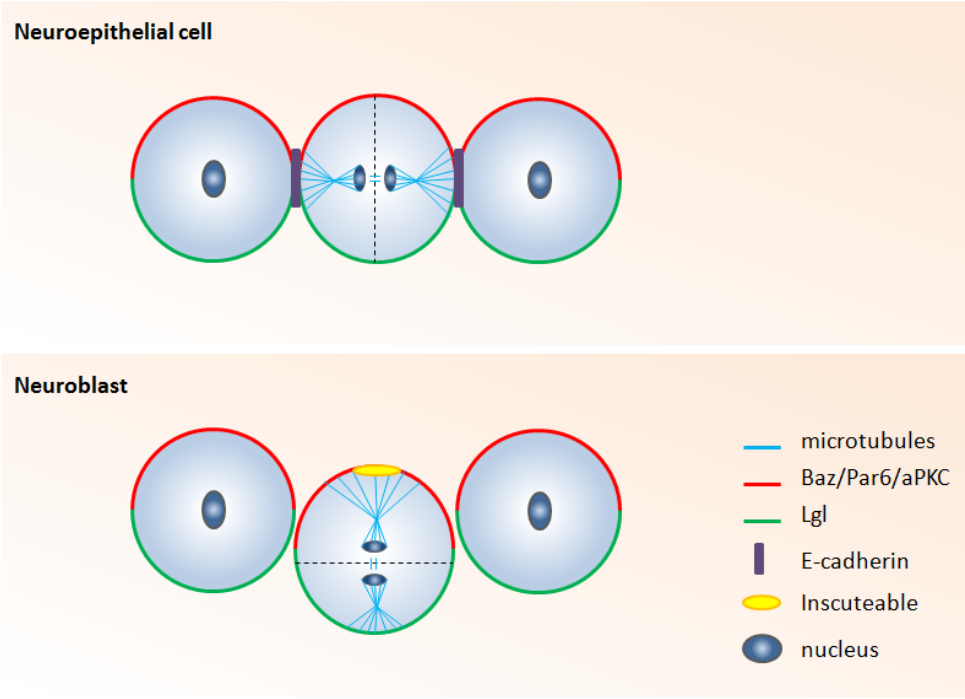


Figure 2. Spindle orientation in neuroepithelial cells versus neuroblasts. Neuroepithelial cells divide symmetrically to expand the stem cell pool. They apply the lateral E-cadherin complexes (purple) as spindle anchoring sites, and in this way ensure planar division. Neuroblasts that delaminated from the neuroepithelium have downregulated E-cadherin, which cannot anchor the spindle anymore. These cells have upregulated the Inscuteable protein (yellow) which is a scaffold protein coupling the spindle anchoring complex to the apical Par complex.

Several mutants of this anchoring complex have been described. *pins* mutant neuroblasts are not able to keep the spindle associated to the membrane at later stages of mitosis; resulting in a random migration of the apical spindle. These mutants lose their ability to self-renew [Cai et al., 2003, Lee et al., 2009]. Mutations in *mud* have been shown to lead to misoriented spindles without affecting the overall apical-basal polarity [Bowman et al., 2006]. These observations again uncouple cellular polarity from spindle orientation. *mud* mutants have slightly increased neuroblast numbers, suggesting a shift in asymmetric division into two neuroblast daughter cells. As a loss of spindle anchoring proteins does not affect cellular polarity, overproliferation of neuroblasts cells is more likely an effect of disturbed separation of the daughter cell compartments containing different cell fate determinants. These divisions appear to resemble an epithelial symmetric division that generates two daughter stem cells. Cabernard and Doe indeed observed a shift to the generation of neuroblasts instead of GMC, supporting the suggestion that more symmetric divisions resulting in two neuroblast daughters occur in *mud* mutants. But they suggest that the increase in neuroblast number could also be due to an effect on the cell cycle, in which the neuroblasts have a shorter cycle length than the GMC which also results in overpopulation of neuroblasts [Cabernard and Doe, 2009]. Nevertheless, these results reveal that correct spindle orientation depends on its stable anchoring to the cell cortex, and can influence asymmetric distribution of daughter cell fates.

C. elegans uses a similar mechanism to connect the spindles to the cell cortex. The microtubule plus ends are bound by a complex composed of the minus-end directed motor protein Dynein and its binding partners Dynactin and LIS-1 [Gonczy et al., 2008]. This complex generates a pulling force on microtubules, and thus on the asters, via anchoring to the cell cortex. The pulling force is facilitated by local depolymerization of the microtubule plus ends et the cortex contact points [Kozlowski et al., 2007, Nguyen-Ngoc et al., 2007]. The Dynein/LIS-1 complex is coupled to membrane-associated G-protein alpha subunits GOA-1 or GPA-16, the homologues of the just described Gαi G protein subunit, via a set of adaptor proteins [Gotta and Ahringer, 2001, Nguyen-Ngoc et al., 2007]. The NuMa homolog LIN-5 interacts with Dynein in a direct way [Gotta et al., 2003, Srinivasan et al., 2003]. Contrary to *Drosophila* Mud, LIN-5 cannot directly bind to the Gα subunit. It

needs a scaffold, which is either of the adaptor proteins GPR1 or GPR2 [Srinivasan et al., 2003]. These GPR proteins create a stable interaction with the Gα protein subunit via GoLoco domains (figure 3). These data reveal that the spindle anchoring mechanism is largely conserved between *C. elegans* and *Drosophila*.

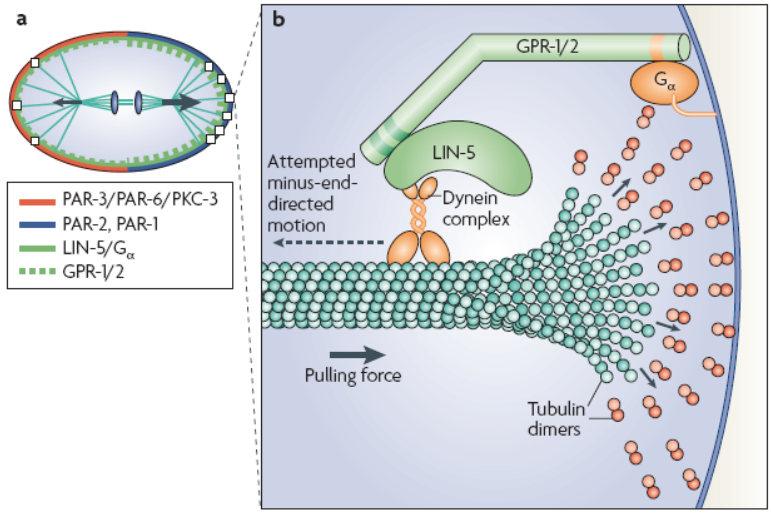


Figure 3. Spindle anchoring complex in *C. elegans*. The C. elegans one cell embryo is polarized in an anterior domain, marked by Par-3/Par-6/PKC-3, and a posterior domain defined by Par-1/Par-2. The mitotic spindle in oriented in an apical-basal plane, and attached to the cortex via a complex of LIN-5/Gα/GPR-1/-2. The GPR-1/-2 proteins are more abundant on the posterior half, as can be seen by the dashed line. The posterior pulling forces on the spindle are stronger as shown by the larger arrow (a). The spindle association complex. The aster microtubule plus ends are bound by the Dynein complex composed of the minus-end tracking motor protein Dynein and its binding partners Dynactin and LIS-1 (not shown). The Dynein complex is bound by the LIN-5 protein, which is connected to the membrane anchoring G protein alpha subunit Gα via the GPR1/2 adaptor proteins (b) [Gonzcy et al., 2008].

2.5 Mitotic spindle positioning along the polarity axis determines daughter cell size

Once the centrosomes are separated and anchored to the cortex in the correct orientation, the spindle needs to be positioned along the pole-pole axis. Spindle positioning directly determines the site where the cell is cleaved, as this is always at the center of the spindle. Thus, a posterior shift of the poles results in a smaller posterior daughter due to a posterior shift of the cleavage furrow. By accurate positioning of the spindle the cell ensures separation of the daughter cell compartments.

Spindle position along the polarity axis is determined by the extent of pulling forces that are exerted on the opposite poles [Hyman and White, 1987]. This suggests an important function for the centrosome anchoring complexes in inducing tension on the spindles, as the connection to the cortex enables tension formation. Several research lines in *C.elegans* revealed that tension is stronger on the posterior aster than on the anterior, and indeed point to the anchoring complexes as the mediators of tension (figure 3). Dynein was shown to generate a cortex-directed pulling force on the microtubules [Gonczy, 2008]. At the cortex microtubule ends depolymerize, which is important to generate these forces [Kozlowski et al., 2007]. But apparently, something causes the posterior cortex to generate more pulling forces than the anterior cortex. Experiments have shown that the levels of membrane-anchor protein Gα are similar anterior and posterior. The levels of Dynein and LIS-1 also do not differ. The only protein levels that differ are those of GPR1 and GPR2, which are increased posteriorly (figure 3) [Colombo et al., 2003, Gotta et al., 2003]. How would these increased amounts of adaptor proteins influence tension on the spindle when the actual motor protein and the membrane-linker have unchanged levels? Results point to the cycling between active GTP bound Gα and inactive GDP bound Gα, as was shown that the adaptor proteins GPR1/2 GoLoco domains specifically bind GDP-bound Gα [Cismowski et al., 2001].

A study by Tall and Gilman suggests that the originally identified guanine exchange factor Ric-8 might act directly on GDP-bound Gαi that is part of a stable complex with the mammalian Pins homolog LGN and the mammalian Mud homolog NuMa. Ric-8A (the mammalian homolog of Ric-8) catalyzes the dissociation of GDP-Gαi from the complex, releasing GTP-Gαi *in vitro* [Tall and Gilman, 2005]. Dissociation of GDP-Gαi results in NuMa releasing from LGN and local loss of spindle attachment. This dynamic release of NuMa from LGN might regulate aster microtubule pulling during mitosis. Effective regulation of spindle pulling forces were shown to require the Gα GAP RSG-7 besides Ric-8. Loss of RSG-7 resulted in increased posterior tension, suggesting stable interactions with the microtubules [Hess et al., 2004]. Ric-8 mutants displayed a phenotype similar like GPR1/2 mutants or Gα mutants, suggesting a loss of spindle anchoring. A recent study by Woodard and colleagues shows that this spindle anchoring regulation by Ric-8A is also required for effective spindle positioning; loss of either Ric-8A or Gαi resulted in misoriented spindles, a phenotype that was to be expected since these proteins connect the spindle to the cortex [Woodard et al., 2010].

This mechanism is largely conserved in *Drosophila*. Upon GTP hydrolysis of Gα subunits, a complex of two heterotrimeric G protein subunits Gβ13F and Gγ1 associates with Gα-GDP [Yu et al., 2003]. The GoLoco domain proteins Pins and Loco associate with GDP-Gα and inhibit the release of GDP. Upon Pins/Loco binding, Gβγ is released from Gα [Yu et al., 2000]. This release is thought to be involved in spindle positioning, as upon Gβγ release Ric-8A can substitute the GDP for GTP [Hampoelz et al., 2005]. At this point it has not been uncovered what establishes the asymmetric tension on the spindle poles, but it is thought to involve free Gβγ complexes [Wu et al., 2008]. These observations suggest there is a need for a balance between release and binding of the spindle microtubules to generate a cortical-directed tension and correct positioning of the spindle.

Spindle positioning is thought to be essential for the distribution of cell fate determinants by separating the two cell fate compartments. Neuroepithelial planar division ensures that one daughter cell receives both apical and basal cell fate determinants, despite of the position of the spindle along the planar axis. But in neuroblasts that divide along the apical-basal axis, the basal determinants need to be separated from the apical determinants in order to lose stem cell fate in the basal daughter cell. Accurate positioning of the spindle along the apical-basal axis is essential here to exclude any apical factors from the basal compartment. Spindle positioning therefore seems to not directly determine cell fate, but it does induce the separation of cell fate determinants which are the key to asymmetry [Kosodo et al.,2004, Konno et al., 2008].

Interestingly, *Drosophila* contains an additional spindle anchoring complex that is active during late stages of mitosis. This complex consists of Pins which binds to the cell cortex via tumor suppressor protein disc large (Dlg). The complex is coupled to microtubule plus-ends via the kinesin motor protein Khc-73 [Siegrist and Doe, 2005]. Dlg subsequently clusters the polarity complexes of both poles according to spindle orientation, thereby restoring the polarity axis. *Drosophila* uses this complex as a back-up system, to restore polarity when the asymmetric segregation of cell fate determinants has failed during the first spindle anchoring by Mud/Pins/Gαi. It is suggested that because of this complex *mud* mutants only display mild phenotypes compared to segregation mutants like *brat* (see paragraph 2.12), as a loss of *mud* is partially restored by this downstream mechanism [Knoblich, 2008]. This additional step of spindle anchoring is therefore also referred to as telophase rescue [Siegrist and Doe, 2005]. The mechanism is active in many mutants of cell fate determinants in *Drosophila* during late stages of mitosis, attempting to restore their asymmetric distribution [Knoblich, 2008]. The development of an additional backup spindle orientation mechanism to direct cortical polarity underlines the importance of spindle orientation in stem cell maintenance and tissue homeostasis. It shows that contrary to symmetrically dividing cells, spindle orientation and cellular polarity are both essential to obtain a controlled distribution of cell fates in asymmetrically dividing cells.

2.6 Centrosomes might carry cell fate determinants in their pericentriolar material

Rebollo and colleagues recently provided insights on why and how E-cadherin and Insc have a higher affinity for the mother centrosome using *Drosophila* neuroblasts. Following centrosome duplication, one of the centriole pairs was shown to shed off its pericentriolar material (PCM) and migrate towards the opposite pole rapidly. Here it accumulates new PCM and forms the second centrosome [Rebollo et al., 2007, 2009]. They again suggest that the centrosome that remains anchored to the cell cortex contains the original centriole, a similar mechanism as the GSCs apply. It might also explain why centrioles can be randomly distributed in a cell, it is the PCM that requires to stay in the future daughter stem cell.

The suggestion that the mother centrosome is required to stay in the self-renewing stem cell daughter is based on a timelapse experiment performed by Rebollo and colleagues on the differentiating daughter cell of a neuroblast division, the GMC. After asymmetric neuroblast division, they observed the GMC daughter cell during another round of now symmetric division into two neurons. This experiment revealed that the two centrosomes of the dividing GMC are functionally similar. These data suggest that only in an asymmetric division centrosomes seem to have different potential. Furthermore the centrosome that was not anchored to the stem cell cortex and set to migrate to the opposite cell pole, was shown to shed off its PCM before starting migration. This observation suggests that the PCM in stem cells contains proliferative factors that need to be excluded from the differentiating GMC [Rebollo et al., 2009].

The observation that one centriole, presumably the newly synthesized, sheds off its PCM before migration to the GMC pole made Rebollo and colleagues question whether the apically anchored centrosome in neuroblasts contains important factors that play a role in setting up new apical-basal polarity in the daughter stem cell after mitosis [Rebollo et al., 2009]. Taking into account the fact that in embryonic, symmetrically dividing neuroepithelial cells both centrosomes still have equal potential in becoming the apical centrosome, and that the specific inheritance of the mother centrosome is observed not earlier than larval neuroblast stage, when the division mode is switched to asymmetry, supports the suggestion that the PCM is somehow correlated to stem cell fate [Kaltschmidt et al., 2000, Knoblich, 2008]. Additional data supporting this suggestion were provided by Yamashita and colleagues using a different stem cell of *Drosophila,* the germline stem cell GSC. They showed that the mother centrosome contains more PCM than its gonialblast daughter cell centrosome in a GSC division [Yamashita et al., 2007]. The extent of PCM could contain stem cell fate determinants which are required to remain in the daughter stem cell [Wu et al., 2008]. These results imply that besides marking the future daughter cell compartments, spindle rotation is also involved in distributing cell fate determinants via direct binding to the PCM.

2.7 Asymmetric segregation of cell fate determinants determines daughter potential

The mechanisms of polarization, spindle orientation and spindle positioning are all suggested to pattern the cell into two daughter cell compartment to which different cell fates can be distributed. The distribution of the fate determinants to these compartments was shown to be regulated by polarity proteins. Apical aPKC and basal Lgl were shown act on some determinants in a direct manner by mediating the apical localization of the PAR complex and the basal localization of differentiation proteins Miranda, Staufen, Prospero (both protein and mRNA), Brain tumour, Neuralized, Partner of Numb, and Numb in *Drosophila* neuroblasts (figure 3). Some of these determinants were implicated in stem cell tumor formation, as described below [Wu et al., 2008].

2.8 Notch inhibitor Numb stimulates differentiation in the basal daughter cell

One of the cell fate determinants that is directly regulated by aPKC is Numb. Numb is a membrane-associated repressor of Notch signaling that was originally identified as a tumor suppressor protein in vertebrates [Pece at al., 2004, Colaluca et al., 2008, Neumuller and Knoblich, 2009]. During interphase Numb is uniformly distributed at the cell cortex. Upon activation of the PAR complex at the anterior cell cortex during mitosis, Numb is recruited to bind Bazooka/Par-3. Bazooka introduces Numb to aPKC in the complex which phosphorylates it. Phosphorylation of Numb causes it to dissociate from the cell cortex. Numb is asymmetrically segregated into the posterior daughter cell of the sensory organ precursor (SOP) cell [Hutterer and Knoblich, 2005] and basal differentiating daughter cell of dividing neuroblasts where it negatively regulates the Delta/Notch pathway [Neumuller and Knoblich, 2009] (figure 4). *numb* mutant larval neuroblasts result in the formation of a stem cell-derived tumor which overproliferates neuroblasts at the expense of differentiating GMC, implying the importance of Numb localization to the basal daughter cell [Lee et al., 2006].

Asymmetric distribution of Numb is facilitated by its adaptor protein Partner of Numb (Pon). Pon is activated upon phosphorylation by Polo (the *Drosophila* homolog of mammalian Polo-like kinase 1 Plk-1) during early stages of mitosis, which increases its affinity for Numb. The Pon/Numb complex is recruited to the anterior cell cortex by Lgl in SOP cells [Langevin et al., 2005], and it is suggested that a similar mechanism is applied in neuroblasts, where Lgl marks the basal cortex. Numb is only cortical in the basal half of the neuroblast and localizes specifically to the membrane region overlying the spindle pole. In the basal daughter GMC Numb negatively regulates the Notch/Delta signal transduction pathway. The Delta/Notch signaling pathway was shown to be essential to remain stem cell fate in the neuroblast, which clarifies why the receptor needs to be removed from the differentiating daughter cell. Numb phosphorylation diminishes its activity as a Notch repressor [Nishimura and Kaibuchi, 2007]. There should be a tight balance on Numb phosphorylation, as ectopic Numb phosphorylation leads to a transformation of the basal daughter cell into a neuroblast [Wirtz-Peitz et al., 2008]. It also implies that proteins regulating Numb activity, like dephosphatases in the basal daughter, cell are equally important in the distribution of daughter cell fate via Numb.

Indeed, mutations in the Numb transport machinery can also lead to stem cell tumors. Pon activation by Polo was shown to be required for efficient Numb distribution; *polo* mutant flies display increased levels of neuroblasts at the expense of neurons, similar to *numb* mutants. Overproliferation is likely the result of the mislocalization of Pon, Numb and aPKC in these mutants, as overexpression of Numb in *polo* mutants rescues the phenotype [Wang et al., 2007]. *pon* mutant neuroblasts displayed a delay in Numb localization during metaphase, but eventually had an asymmetric distribution of Numb, implying Pon is not strictly required for Numb localization [Wang et al., 2007]. Asymmetric distribution of Numb further depends on actin, but not on microtubules [Knoblich et al., 1997, Berdnik and Knoblich 2002]. The different mechanisms to ensure Numb localization reveal its importance as a cell fate determinant.

It was long thought that Numb represses Notch signaling by regulating endocytosis of the Notch mediator Sanpodo [Hutterer and Knoblich 2005]. However, a recent study by Tong and colleagues revealed that Numb indeed negatively regulates Notch signaling, but not via Sanpodo internalization. Mutation of the NPAF motif in the Numb binding region of Sanpodo resulted in increased plasma membrane localization of Sanpodo, suggesting that Numb indeed cannot bind and internalize it anymore. However, the expected increase in Notch signaling was not detected. These results imply that Numb represses Notch via a different mechanism [Tong et al 2010]. Numb also interacts with the Notch receptor via its N-terminus, and Notch was shown to colocalize with Delta, Sanpodo and Numb in endocytic vesicles marked by Rab5 and Rab7 proteins [Hutterer and Knoblich 2005]. The direct interaction of Numb and Notch might be the regulatory step in Notch internalization.

2.9 Neuralized mediates Delta/Notch signaling between the daughter siblings

Another Notch mediator referred to as Neuralized, is asymmetrically distributed by the polarity proteins. Neuralized is a E3 ubiquitin ligase for the Delta ligand, that is implicated in the endocytosis and activation of Delta. Only activated Delta can act as a ligand to activate the Notch pathway in neighboring cells. Neuralized is therefore suggested to be a positive regulator of Notch signaling, contrary to Numb. Neuralized accumulates at the cell cortex near one of the spindle poles and is asymmetrically segregated into the anterior pIIb daughter cell of the dividing SOP cell, similar to Numb [Le Borgne and Schweisguth, 2003] (figure 4). Neu localization was suggested to be regulated by Lgl [Langevin et al., 2005]. Neu mutant SOP cells divide into two pIIb cells, suggesting that loss of Neu within the original pIIb inhibits the original pIIa cell from adopting the pIIa fate [Neumuller and Knoblich, 2003]. These results imply an important role for Neu in cell fate determination.

A recent study in epithelial MDCK cells suggests a regulatory role for Neu in Delta endocytosis. Neu was shown to promote the internalization of Delta from the basolateral membrane. Subsequently it regulates the transport of these endocytic vesicles through the cell from the basal membrane to the apical membrane, where Notch is normally expressed in epithelial cells These cells might use Neu to localize Delta specifically to the apical surface to activate Notch signaling on an apical neighboring membrane [Benhra et al., 2010]. At this point, it is not known yet whether Neu also functions in neuroblasts, but it is tempting to speculate that Neu could be required to maintain stem cell fate of the neuroblast daughter sibling by providing the Notch receptor ligand Delta.

2.10 Bratstimulates differentiation in the basal daughter via translational repression

Numb and Neu together mediate Delta/Notch signaling between the daughter siblings. The Delta/Notch pathway ensures two different cell fates via lateral inhibition, which is another mechanism to control asymmetric outcome of the daughter siblings. Now that Delta/Notch regulate lateral inhibition of the siblings, the actual cell fate determinants can induce proliferation in the self-renewing daughter and differentiation in the other daughter cell. The direct localization of cell fate determinants is required for this with aPKC as an essential factor for the unequal distribution.

Brain tumour (Brat) was identified in 2006 as being a segregating determinant [Bello et al., 2006]. Brat is a translational repressor. It belongs to a protein family that share the C-terminal NHL domain (NCL-1, HT2A, LIN-41) [Sonoda and Wharton, 2001]. Brat requires the adaptor protein Miranda (Mira) to carry it to its final destination within the cell during mitosis [Lee et al., 2006]. Mira is an adaptor protein whose localization was shown to be dependent on aPKC phosphorylation during mitosis [Atwood and Prehoda 2009]. Mira phosphorylation results in a loss of Mira binding to the apical cortex. As a result Brat co-segregates with Mira into the differentiating basal GMC daughter in *Drosophila* (figure 4). Brat is suggested to inhibit the growth stimulating transcription factor Myc in the GMC [Betschinger et al., 2006]. In *Drosophila brat* mutants, an overproliferation of neuroblasts is observed at the expense of differentiating GMCs, a phenotype that would support the suggestion that Brat is a growth inhibitor [Neumuller and Knoblich, 2009].

Besides playing a role in stimulating differentiation in neuroblast daughter cells, Brat also functions in the establishment of the anterior-posterior polarity in the one cell embryo of *Drosophila*. Here, Brat forms an RNA-binding complex with Nanos and Pumilio during early embryogenesis, which inhibits the translation of maternally provided hunchback mRNA in the posterior half of the embryo. This leads to an anterior-posterior gradient of Hunchback protein [Tautz, 1988, Wharton et al., 1998, Curtis et al., 1997]. Nanos, Pumilio and Brat are all conserved in *C. elegans*, but their homologues function differently [Sonoda and Wharton, 2001]. The homologues do regulate anterior-posterior polarity, but modulate different proteins to do so. Embryonic lethality due to a loss of *par-2* was restored by mutating either the Nanos homolog *nos-3* or the Pumilio homolog *fbf1/2*, suggesting that these proteins either positively regulate the anterior Par3/Par6/PKC3 complex or suppress Par2 activity in wildtype conditions [Labbé et al., 2006, Hwang and Rose, 2010]. Comparison of these data to the *Drosophila* homologues made Hyenne and colleagues question whether *nos3* and *fbf1/2*

regulate polarity in *C. elegans* via forming a complex with the brat homolog *ncl-1*. *ncl-1* was indeed shown to be involved in establishing polarity by modulating cortical Par localization. *ncl-1* mutants were able to restore polarity in *par-2* mutant embryos, just as *nos-3* and *fbf1/2*. However, *nlc-1* mutants displayed a different phenotype than *nos-3* mutants, suggesting these are not part of the same complex. *nos-3* mutants displayed decreased levels of Par-6 protein, suggesting that *nos3* is required to maintain Par-6 protein levels by inhibiting its polyubiquitination for degradation. It was shown to do this by inhibiting the translation of the *fem-3* adaptor protein of the ubiquitin ligase complex [Pacquelet et al., 2008]. *ncl-1* mutants did not show decreased Par-6 levels, suggesting these restore polarity in a different way. Hwang and Rose suggest that *ncl-1* positively regulates Par-2 ubiquitination and breakdown in wildtype conditions independently of *nos-3* of *fbf1/2* [Hyenne et al., 2008, Hwang and Rose, 2010]. These results suggest that Brat, and its partners Pumilio and Numb have a conserved protein function in inhibiting translation and regulating cell polarity, but have different target mRNAs in *C. elegans* and *Drosophila*.

2.11 Prospero is a transcriptional regulator that induces differentiation in the basal daughter

Brat acts redundantly with Prospero in determining differentiation fate in the basal daughter cell of embryonic neuroblasts [Betschinger et al., 2006]. Prospero (Pros) is a homeodomain transcription factor that can act both as a transcriptional repressor of cell cycle genes [Li and Vaessin, 2000] and as a transcriptional activator of differentiation genes [Choksi et al., 2006]. Pros also binds to Mira during mitosis in a transient manner [Shen et al., 1997]. Like Brat, Pros and Mira are segregated into the basal GMC daughter cell as a complex [Spana and Doe, 1995] (figure 4). After cytokinesis, Mira is degraded in the GMC and Pros translocates to the nucleus [Knoblich et al, 1995]. Pros was suggested to be a positive regulator of differentiation of the GMC by repressing the transcription of cell cycle genes. Larval *pros* mutant neuroblasts developed into neuroblast tumors that were unable to generate differentiating GMCs [Betschinger et al., 2006], while over expression of Pros resulted in a depletion of the neuroblast population, since all daughter cells develop into GMCs [Cabernard and Doe 2009]. To ensure neuroblast fate in the self-renewed daughter cell, all *pros* mRNA is also exported to the GMC daughter cell. *pros* mRNA is bound by the RNA binding protein Staufen (Stau). Stau also uses the adaptor protein Mira to translocate to the GMC daughter cell [Li et al., 1997].

Both *pros* and *brat* single mutants display only mild phenotypes. *pros* mutants affect a small subset of GMCs, whereas the *pros/brat* double mutant results in an almost complete loss of GMC [Betschinger et al., 2006]. Brat was suggested to be the transcriptional activator of Pros, since *brat* mutant cells are Pros negative [Bello et al., 2006]. A transcriptional activator that is part of the same linear pathway should not enhance the phenotype when mutated as well. Since the combined *brat*/*pros* mutant does show an enhanced phenotype compared to the *pros* mutant, it is unlikely that Brat is the actual activator of Pros [Knoblich, 2008]. Prospero was shown to be required to ensure differentiation fate in the basal GMC daughter, and seems to regulate this independently of Brat.

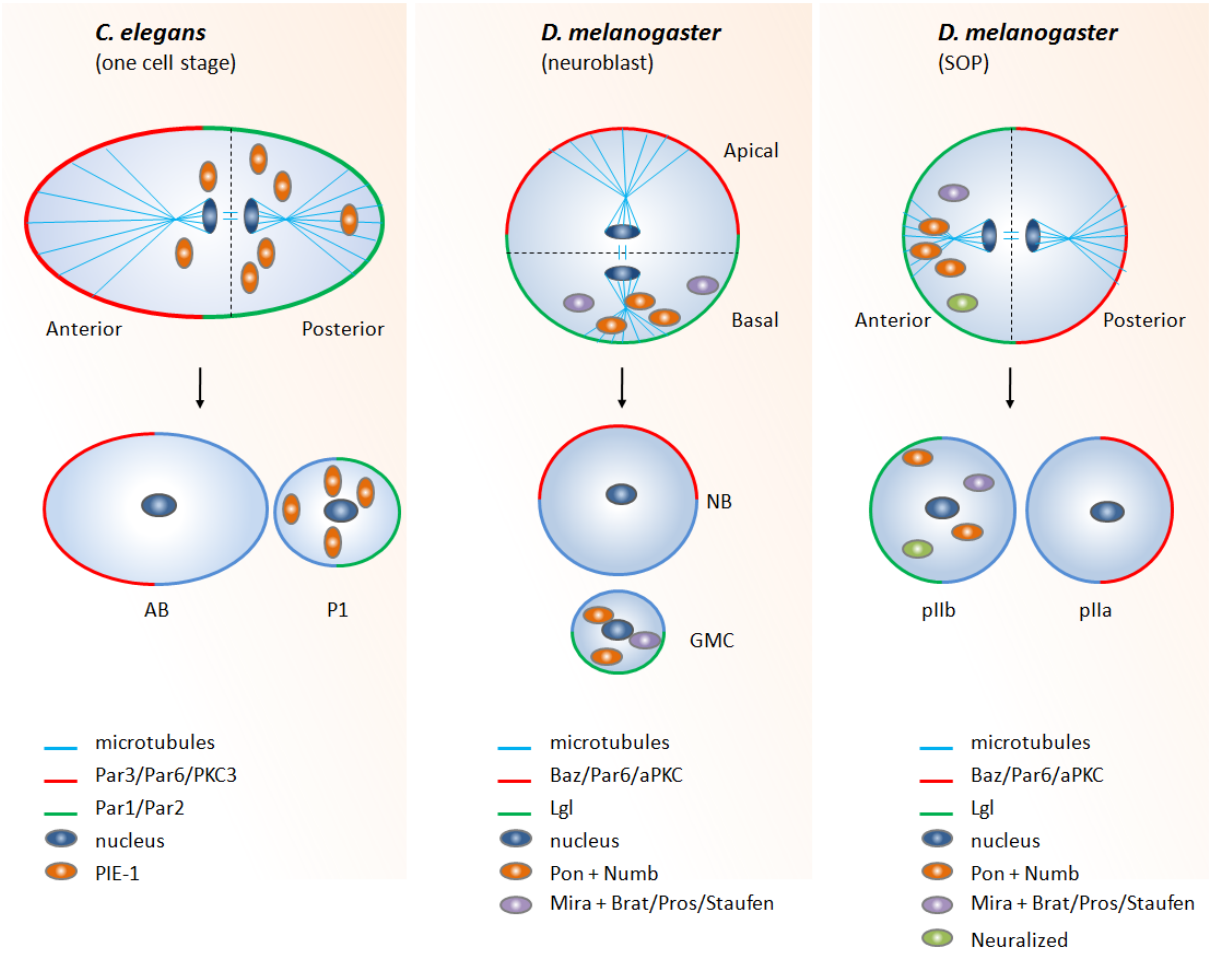


Figure 4. Distribution of cell fate determinants in the *C. elegans* one cell embryo and the *Drosophila* neuroblast and SOP cell. The *C. elegans* embryo has an anterior-posterior polarity defined by an anterior Par-3/Par-6/PKC3 complex and a posterior Par1/Par2 complex. The germline determinant PIE-1 segregates asymmetrically into the posterior P1 daughter cell. The *Drosophila* neuroblast has an apical-basal polarity, defined by the apical Baz/Par-6/aPKC complex and the basal Lgl marker. The differentiation determinants Numb, Brat and Pros are asymmetrically distributed into the basal GMC daughter cell via their cargo proteins Pon and Mira. The Drosophila SOP cell has an anterior-posterior polarity defined by anterior Lgl and a posterior Par-3/Par-6/aPKC complex. The cell fate determinants Numb, Brat, Pros, and Neuralized are asymmetrically segregated in the anterior pIIb daughter. Adapted from Gonczy et al., 2008.

2.12 aPKC is a cell fate determinant itself inducing the expression of neuroblast genes

aPKC itself was recently shown to be a cell fate determinant as well. aPKC does not solely localize in the polarity complex at the cell cortex. Immunostainings on *Xenopus* embryos revealed that aPKC shuttles between the nucleus and the cortex. Furthermore these experiments revealed that in order to suppress neurogenesis in the nucleus, aPKC needs to be activated in the cytoplasm near the cortex. Constitutively active aPKC fused to a nuclear localization signal (NLS) strongly reduced neurogenesis, as was read-out by N-tubulin expression levels. The wildtype aPKC-NLS fusion protein did not affect N-tubulin levels, suggesting it was inactive as a nuclear determinant. Nuclear entrapment of wildtype aPKC apparently prevents its activation. These results imply that aPKC obtains an activation cue in the cytoplasm before it can act as a determinant in the nucleus [Sabherwal et al., 2009]. To investigate what aPKC's function in the nucleus might be, Sabherwal and colleagues overexpressed the constitutive active aPKC-NLS in *Xenopus*. Active nuclear aPKC suppressed neural differentiation of daughter neuroblasts by induction of neuroblast gene expression and proliferation. The introduction of a nuclear dominant negative mutant of aPKC resulted in the opposite phenotype: neurogenesis was induced. Even the membrane-tethered dominant negative aPKC induced a loss of apical-basal cell

fate distribution, suggesting that both membrane and nuclear localizations of aPKC affect cell fate. Altogether these results suggest a regulatory role for aPKC in maintaining the expression of stem cell genes to prevent differentiation of precursor cells [Sabherwal et al., 2009]. An involvement of aPKC in promoting proliferation has been reported for *Drosophila* [Lee et al., 2006] and chicken [Ghosh et al., 2008], suggesting it is a conserved function. Whether *Drosophila* neuroblast aPKC promotes proliferation via a similar mechanism remains to be experimentally tested.

aPKC is suggested to be antagonized in the differentiating daughter cell by Lgl. *lgl* over expression was shown to phenocopy the knockout of aPKC, suggesting these antagonize each other. Chalmers and colleagues further showed that overexpression of *lgl2* in the frog resulted in loss of apical aPKC and the depolarization of the epithelium [Chalmers et al., 2005]. But this loss of polarization was not sufficient to cause neurogenesis in these cells. They need additional proneural factors to fully differentiate [Sabherwal et al., 2009]. This is probably disturbed in these cells since aPKC can no longer direct the differentiation-inducing fate determinants to the basal cortex. These results all imply a functional role for aPKC as a direct suppressor of differentiation.

2.13 Asymmetric localization of Mira cargo proteins is also regulated by the actin cytoskeleton

Polarity proteins were shown provide the cell positional cues on where to distribute the different daughter fates, and regulate each others' potential to act as a cell fate determinant directly. Where Lgl was shown to antagonize aPKC in its function as suppressor of differentiation, it also antagonizes aPKC as a polarity cue. Lgl forms the basolateral polarity complex together with the cortical tumor suppressors Discs large (Dlg), and Scribble (Scrib) [Betschinger et al., 2003, Peng et al., 2000, Albertson and Doe, 2003, Wu et al., 2008]. Knockdown studies revealed that loss of either Lgl, Dlg or Scrib resulted in mislocalization of the cell fate determinants and disrupted spindle orientation, since the apical domain is extended in these mutants [Albertson and Doe, 2003]. Furthermore, *scrib* mutants displayed mislocalized apical marker proteins which resulted in a partial loss of polarization, suggesting that the basal complex regulates the apical polarity markers [Bilder and Perrimon, 2000].

The basal polarity complex has an additional function in the distribution of cell fate determinants. By acting on the actin cell cortex, the basal polarity complex indirectly regulates distribution of fate determinants that depend on the Mira cargo protein. Biochemical assays have shown that Lgl directly binds to and suppresses non-muscle myosin II (NMY-2), which is involved in PAR-1 distribution to the basal membrane [Strand et al., 1994, Guo and Kemphues, 1996]. Since Lgl is phosphorylated and suppressed apically due to aPKC phosphorylation [Betschinger et al., 2003], this implies that Lgl can only inhibit NMY-2 function in the basal half of the cell. Active apical NMY-2 acts on the actin cytoskeleton and modifies it in such a way that Mira cannot localize to the cortex anymore. Mira dissociates from the apical cortex and migrates towards the mitotic spindle during mitosis [Barros et al., 2003]. Thus, besides direct phosphorylation of Mira that repels it from the apical cortex, Mira localization is also inhibited by modulation of its anchor sites on actin filaments. NMY-2 is not the only actin effector that influences Mira localization. The Jaguar myosin IV protein (Jar) binds directly to Mira and is implicated in (vesicle) transport along the actin filaments. Jar mutants displayed defect Mira/Pros localization and a disrupted spindle orientation. The actin-associated Par complex however is not affected by loss of Jar, which excludes a global effect in actin dynamics [Petritsch et al., 2003, Buss et al., 2002]. These results suggest that the cell has different mechanisms that together mediate Mira –and its cargo proteins Brat, Pros, and Staufen- translocation to the basal cortex by locally acting on the Mira protein itself and on the actin filaments to which Mira binds.

2.14 Local degradation of cell fate determinants – another route to asymmetry

Several other mechanisms to ensure asymmetric distribution of fate determinants were discovered in *C. elegans*. *C. elegans* body axes are already setup in the P0 zygote. At this point little to no *de novo* transcription takes place, meaning that the patterning process is regulated by maternally provided

mRNA and proteins. Controlling the presence of these maternal factors at a local level is a way to regulate their asymmetric distribution. One of the mechanisms to ensure asymmetric protein distribution is the selective binding to posterior structures such as P granules. These are ribonucleoprotein particles that are asymmetrically inherited by the posterior P1 daughter cell. The germline determinant PIE-1 is suggested to cosegregate with P granules in the P1 daughter cell [Neumuller and Knoblich, 2009].

Another mechanism to ensure asymmetric localization of protein cell fate determinants is by local degradation. Besides being guided by P-granules to the posterior side, the cell eliminates the posterior germline determinant PIE-1 from the anterior half using the anterior muscle lineage determinants MEX-5 and MEX-6 [Cowan and Hyman, 2004]. PAR-1 phosphorylates MEX-5 at its C-terminus which stimulates MEX-5 movement in anterior direction [Tenlen et al., 2008]. PAR-1 is distributed at the posterior half of the cell, implying that MEX-5 is only phosphorylated in the posterior half. Anteriorly, MEX-5 negatively regulates PIE-1 presence by inducing its polyubiquitination for proteasomal breakdown [Kile et al., 2002]. Anterior breakdown of PIE-1 results in a posterior-anterior gradient of PIE-1 (figure 4). PIE-1 suppresses the transcription of somatic genes in the posterior daughter cell, thereby maintaining the posterior germline cells [Tenenhaus et al., 2001]. Upon division of the one-cell embryos, MEX-5 and MEX-6 will specifically be inherited by the anterior AB daughter cell, and PIE-1 by the posterior P1 cell. These observations implicate that besides anchoring a protein to cargo structures, local protein breakdown is also applied to facilitate the asymmetric distribution of fate determinants.

Where proteasomal breakdown of proteins plays a role in asymmetric distribution of cell fate proteins, the microRNA pathway regulates asymmetry via the controlled degradation of mRNA transcripts. In *Drosophila* the *brat* paralog Mei-P26 was shown to be active in the differentiating daughter cell of an asymmetric division where it regulates the size of the nucleolus. Mei-P26 induced shrinking of the nucleolus is suggested to inhibit overall protein synthesis. The mouse homolog TRIM32 is implied in the same mechanism [Schwamborn et al., 2009] as is one of the five *C. elegans brat* homologues NCL-1 [Frank and Roth, 1998, Neumuller and Knoblich 2009]. Mei-P26 and TRIM32 were both shown to implicated in the suppression of the Myc transcription factor by targeting it for proteasomal degradation. Their relative *brat* lacks this ubiquitinating domain, but shares a binding site for the RNase Argonaut (Ago1) with TRIM32 [Schwamborn et al., 2009] and a second *C.elegans brat* homolog NHL-2 [Hammell et al,. 2009., Neumuller and Knoblich., 2009]. Ago1 functions as member of the microRNA (miRNA) pathway. The mouse Let-7a microRNA involved in neuronal differentiation was shown to be a target of TRIM32, which activated it via Ago1 [Schwamborn et al 2009]. These results imply a functional, conserved role for Brat in suppressing mRNA translation by generating specific microRNAs. The directed mRNA breakdown via the microRNA pathway, is just like protein degradation by the proteasome, another way of the cell to control asymmetric segregation of cell determinants.

3. Stem cell tumors arise from a loss of differentiation inducers

The results describing the different steps of asymmetric cell division and their mutant phenotypes have revealed that it is not so much the mechanism of asymmetric division that leads to tumor formation, but the cell fate determinants that were incorrectly distributed. The loss of differentiation factors Brat, Prospero, and Numb was shown to induce overproliferation of neuroblasts in *Drosophila* at the expense of neurons. In these mutants there is a loss of neuron identity, and a gain of a neuroblast-like identity. *Drosophila* neuroblasts mutant for the Pon activator *polo*, the mitotic kinase *aura,* and the protein phosphatase *twins* also result in overpopulation of neuroblasts, likely because Numb distribution is disrupted in these mutants. Last, spindle anchorer *mud* was shown to have a mild overproliferation phenotype which is less severe than that of the segregating determinants, possibly due to partial missegregation of determinants (summarized in figure 5). Apparently, the mutant cells switch their fate upon loss of differentiation cues to generate a neuroblast-like cell type. All these different factors induce a similar phenotype upon mutation, but the cellular mechanisms underlying this common phenotype have not been uncovered yet.

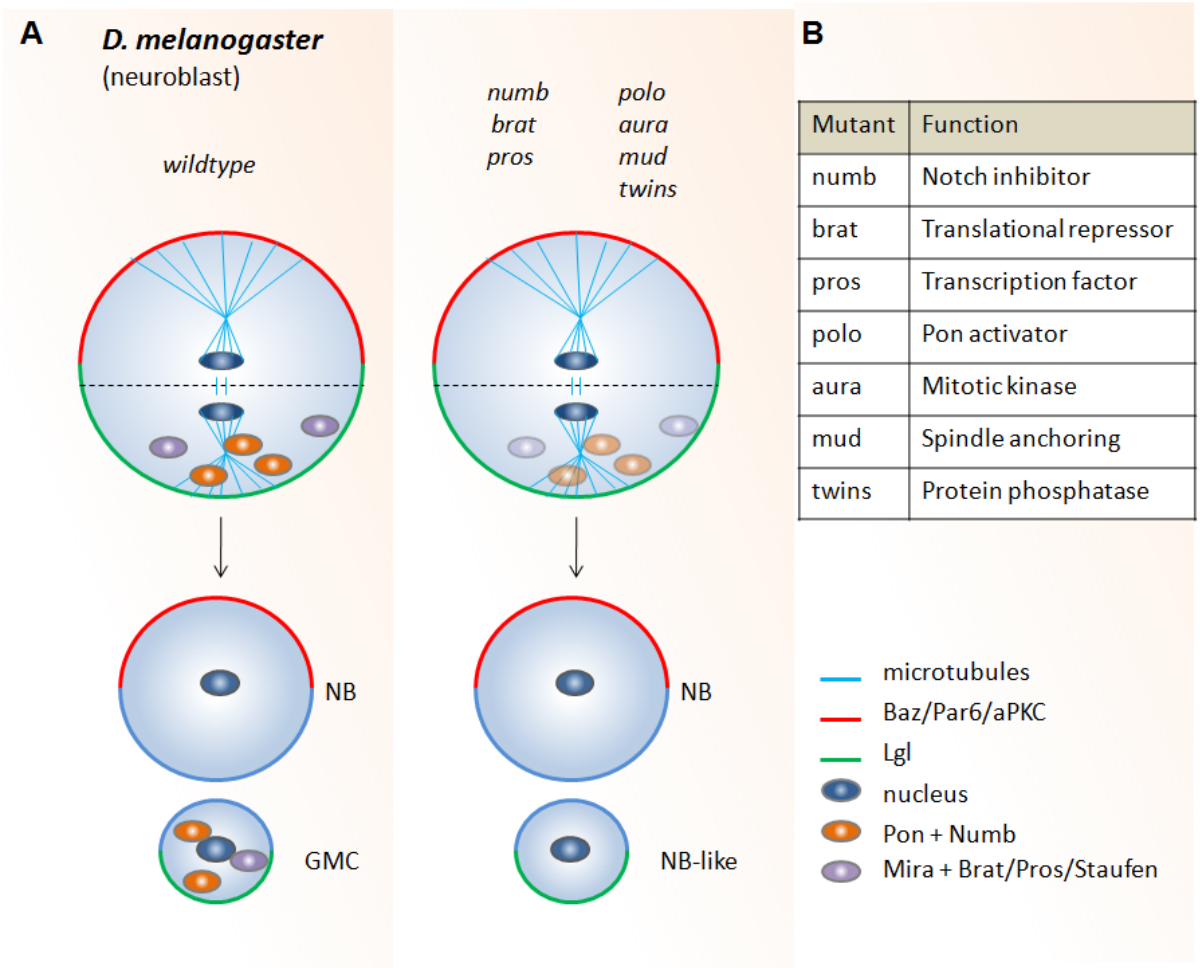


Figure 5. *Drosophila* neuroblast mutants that cause an overproliferation of neuroblasts at the expense of differentiating GMCs. Wildtype neuroblasts regulate a basal distribution of Pros, Brat and Numb. Upon loss of these proteins the GMC cell can no longer differentiate and is suggested to become a neuroblast-like cell. Also, mutations in *aura*, *mud*, *polo,* and *twins* were shown to result in neuroblast overproliferation (A). The functions of these mutant proteins are presented in a table (B) Adapted from Gonzcy et al., 2008..

3.1 Type II neuroblasts are the origin of stem cell tumors in *Drosophila*

Closer observation of *brat* mutant flies has revealed several interesting aspects about neural stem cell tumors. The overproliferating cells arise in a specific area of the *Drosophila* central brain, which made the Hirth and Knoblich labs suggest that a subpopulation of neuroblasts is particularly sensitive to *brat* mutation [Bello et al., 2006, Betschinger et al., 2006]. This subclass of neuroblasts, which was a previous uncharacterized population of only eight neuroblasts per brain lobe, has been investigated in follow-up studies. Contrary to the bulk of neuroblasts, these stem cells generate transit-amplifying intermediate progenitors (IP) that bud off GMCs upon self-renewal. The IP cells each generate up to ten neurons, implying that this subclass of neuroblasts is responsible for the bulk of neurons in the central brain [Boone and Doe, 2008]. Due to their posterior localization in the central brain this subclass of neuroblasts was termed Posterior Asense Negative (PAN) neuroblasts. The PAN neuroblasts are often also referred to as type II neuroblasts (type II NB) [Bowman et al., 2008]. The more predominant neuroblasts are now referred to as type I neuroblasts (type I NB).

As described in the introduction, type I NBs divide to self-renew and to bud off a GMC. The GMC will divide once more to generate two differentiating neurons. This means that the type I NB contains both cell fates during mitosis, and passes on all required differentiation factors to the GMC. Type II NBs will ultimately also generate a GMC, but do this via an intermediate step. Upon type II NB division, the neuroblast self-renews and buds off an IP (figure 6A). This IP cell differs from type I NBs in that it does not contain cytoplasmic Prospero during interphase. Eventually it will generate Pros during mitosis to pass on to its GMC daughter cell, but Pros is not present yet during interphase [Boone and Doe, 2008, Bayraktar et al., 2010].

The absence of Pros is not the only difference between the IP cell and type I NBs. The type II NB and itsIP daughter cell repress the asense (*ase*) neuroblast identity gene [Bowman et al., 2008]. As described in the introduction, *ase* facilitates the transition from neuroepithelial cell into neuroblast. Ase is a transcription factor that is a member of the achaete-scute complex (AS-C), a combination of four genes that cooperate in specifying neural precursor fate [Brand et al., 1993]. This observation makes it striking that the type II NBs suppress this gene; it suggests that they have not acquired the neural precursor fate yet, and that the IP is a transition step in the maturation process to form fully functional neuroblasts.

The IP cells differ from type I NBs in the absence of nuclear Ase and cytosolic Pros, suggesting that these factors protect type I NBs from tumor formation upon *brat* mutation. Careful observation of the IP after a type II NB cell division has revealed that at the point of budding off, the IP does not contain proliferation status nor differentiation status. It seems to be waiting for a cue. Upon expression of Ase, that is induced via a yet unknown mechanism, the IP starts to proliferate [Bowman et al., 2008]. Subsequently, the expression of a second neuroblast identity transcription factor, Deadpan (Dpn), is induced. At this point, the IP has become a mature neuroblast and starts to divide asymmetrically to self-renew and to bud-off a GMC [Bayraktar et al., 2010] (figure 6A). The mature IP closely resembles the type I NB, but lacks cytoplasmic Pros during interphase. To investigate whether Asense indeed protects neuroblasts from overproliferation upon loss of a cell fate determinant, Ase was ectopically overexpressed in type II NB. Subsequently, *brat* was knocked down in control type II NBs versus the Ase overexpressing type II NBs. Indeed, where control cells developed over-proliferating neuroblasts, the type II NBs overexpressing Ase were not affected by the loss of Brat [Bowman et al., 2008]. This supports the suggestion that Ase protects neuroblasts from a loss of Brat, and might explain why type I NBs, that do express Ase, are unaffected by *brat* mutations.

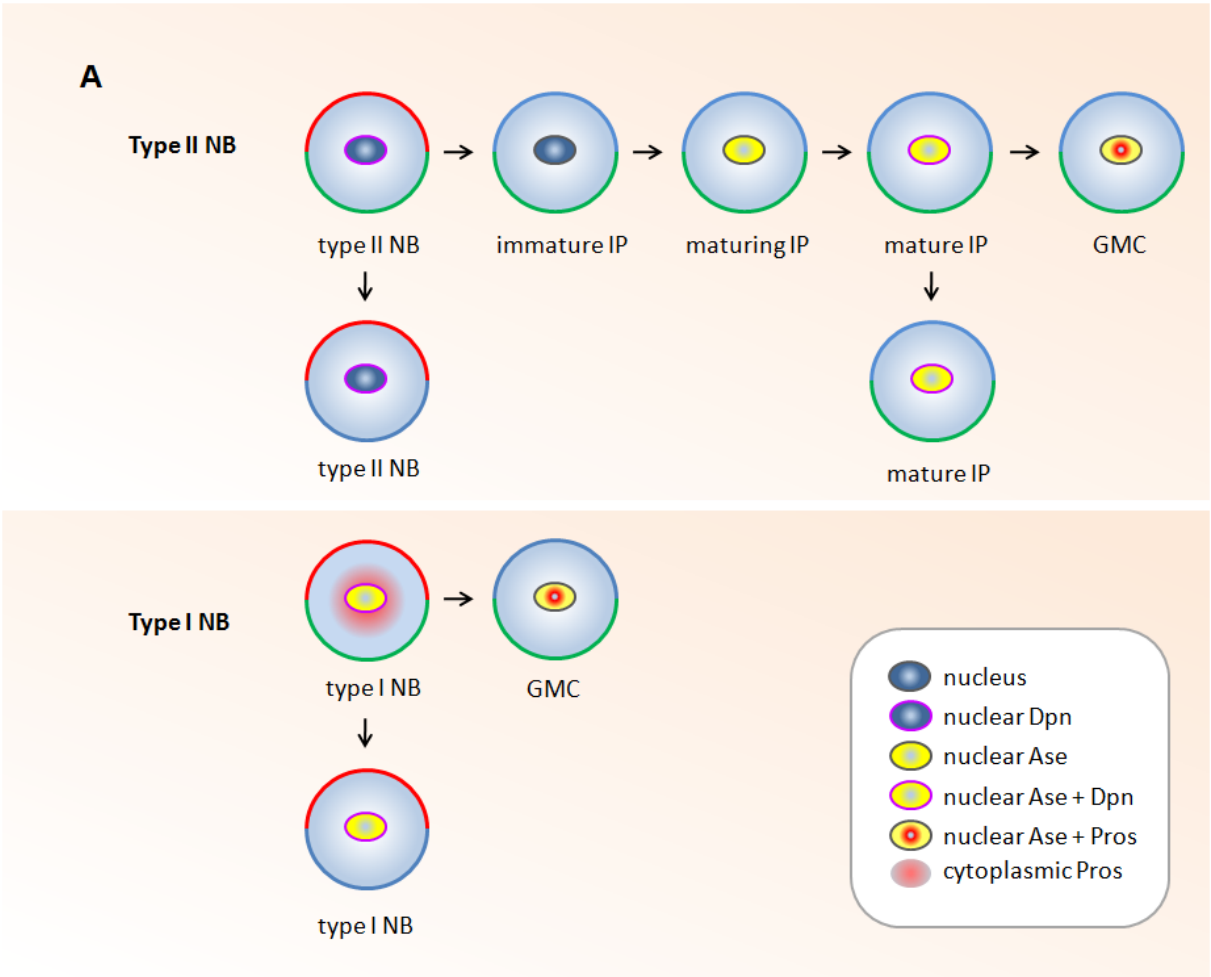
Interestingly, *brat* mutant type II NBs do bud off an Ase-negative IP cell, but this IP is not able to induce Ase expression. These IPs fail to progress beyond the immature Ase-negative state [Bowman et al., 2008] (figure 6B), implying that Brat is indispensable for IP neuroblast maturation. Brat being required for Ase expression explains why a loss of Brat does not affect IPs that have already upregulated Ase. The Ase-negative IPs were shown to halt their cell cycle in G2 phase, likely to wait for Ase expression. Eventually, these IPs will enter mitosis despite the absence of Ase to generate two Ase-negative IPs. It is suggested that this no longer controlled division of immature Ase-negative IPs is the tumor initiating event [Bowman et al., 2008].

An identical result was obtained using *numb* mutant type II NBs. IPs that had just budded off and lack nuclear Ase, block their cell cycle in G2, whereas the maturing IPs that do express nuclear Ase were not affected by the loss of *numb*. As in *brat* mutants, the Ase-negative *numb* IPs will eventually proceed mitosis to generate two daughter IPs [Bowman et al., 2008] (figure 6B). The absence of Numb correlates with the presence of Notch receptor on these cells. Some mammalian transit-amplifying stem cell lineages were shown to require Notch signaling to control proliferation and differentiation [Wilson and Radtke, 2006, Bowman et al., 2008], which suggests that the *numb* mutant IPs start proliferation too early in development as a result of disturbed Notch signaling. To investigate whether the loss of *numb* induces overproliferation by disturbing Notch signaling, the cytosolic fragment of the Notch receptor was ectopically expressed in Ase-negative IPs. Notch overactivation also led to uncontrolled expansion of these IPs, supporting the suggestion that Numb acts via Notch to induce the overproliferation phenotype [Bowman et al., 2008] It seems that Brat and Numb affect IP maturation via their function as cell fate determinants controlling differentiation mechanisms and suppressing proliferation. As Brat and Pros were shown to function redundantly in embryonic neuroblasts, this observation raises the question whether the other identified basal determinant *pros*, which is absent from type II IPs, is involved in the overproliferation of IPs upon loss of *brat* or *numb*.

Ase-positive clones mutant for *brat* or *numb* were shown to produce functional GMCs and neurons, suggesting these determinants function upstream of the differentiation onset. Ase-positive *pros* mutant clones to the contrary, were not able to produce neurons and generated more Ase-positive IPs [Bowman et al., 2008]. These observations indicate that Pros is required for GMC differentiation, uncoupling it from the upstream Brat and Numb that play a role in the maturation of the GMC. Interestingly, ectopic expression of Pros was shown to rescue *brat* or *numb* mutations, supporting the suggestion that Pros acts downstream of Brat/Numb. It was suggested that Pros rescues *brat* and *numb* mutants by transforming type II NBs into type I NBs. However, ectopic expression of Pros in type II NB lineages mutant for *brat* was not able to induce Ase expression in immature IPs [Bayraktar et al., 2010]. The absence of Pros in type II NB and immature IPs is suggested to sensitize these cells for *brat* mutations, but does not seem to cooperate with these factors directly [Betschinger et al., 2006, Neumuller and Knoblich, 2009]. But *pros* mutations do result in tumor formation of type II NB lineages as well. As *pros* expression is only induced in mature IPs, the effect should be on these cells, inhibiting their transition into a mature GMC (figure 6B)

It is interesting that ectopic Pros can rescue a loss of *brat*, without the expression of Ase. If Ase is the upstream inducer of Pros, this would explain the rescue mechanism. Overexpression of Pros in type I NBs was shown to result in loss of neuroblasts due to differentiation. Ectopic expression of Pros in type II lineages resulted in a decrease in IP number and total cell number without affecting the NB pool. Increasing the Pros expression levels even affected the type II NBs by inducing their differentiation. These results suggest that the ability of Pros to rescue *brat* or *numb* mutants from tumor formation is merely an effect of Pros' ability to inhibit proliferation of the IPs by suppressing cell cycle genes, rather than changing cell fate [Bayraktar et al., 2010].

These results show that fate determinants Brat and Numb affect a different cell type within the type II NB lineage than Pros. By retaining immature IPs in an Ase-negative state, *brat* and *numb* mutants induce an uncontrolled expansion of these non-differentiated, type II NB-like cells. These tumors can be suppressed by Prospero, which acts downstream of the Ase-switch. Prospero itself is also a tumor suppressor. Its expression is only induced after Ase activation in the mature IP in order to transit into a GMC. Upon mutation it cannot exert its transcription function anymore in the GMC, leading to a loss of differentiation induction and retention of a mature IP-like state. The observation that different cell types are affected by these mutations uncouples the *brat*/*numb* tumors from the *pros* tumors, and indicates that we should be observing the downstream mechanisms of these factors to better understand the different tumors.


A
Type II NB
type II NB
immature IP
maturing IP
mature IP
GMC
type II NB
mature IP
Type I NB
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GMC
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nucleus
nuclear Dpn
nuclear Ase
nuclear Ase + Dpn
nuclear Ase + Pros
cytoplasmic Pros

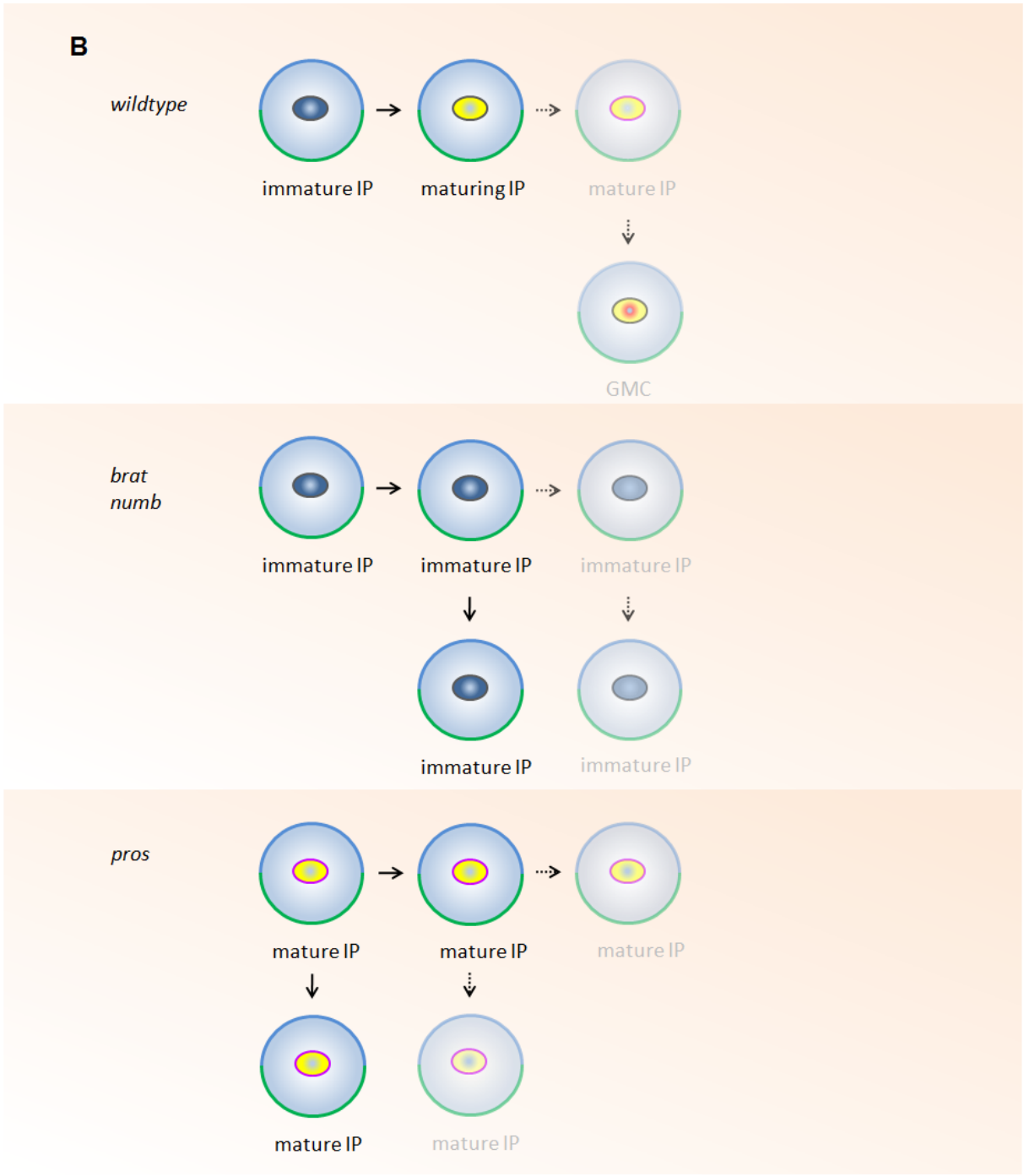

Figure 6. *Drosophila* type II versus type I neuroblasts and segregation mutant phenotypes. Type II neuroblasts (NB) divide to self-renew and bud off an intermediate progenitor (IP) cell. This IP cell is an immature neuroblast. Upon unknown triggers the transcription factor Asense (Ase) is expressed, and subsequently the Deadpan (Dpn) transcription factor. Nuclear Ase and Dpn define a mature IP cell that starts proliferation. The mature induces Prospero transcription factor expression during mitosis. It divides asymmetrically to self-renew and to bud off a GMC. Pros is exclusively inherited by the GMC where it localizes to the nucleus to suppress Dpn and Ase, and to induce differentiation factors. Type I neuroblasts contain nuclear Ase and Dpn, and already induce Pros expression during interphase. In the mother cell Pros is cytosolic. Upon asymmetric division the NB self-renews and buds off a smaller GMC that inherits the Pros. Pros localizes to the GMC nucleus where it inhibits Ase and Dpn, and it induces differentiation factors (A, adapted from Bayraktar et al., 2010). Type II NB that are mutant for *brat* or *numb* were shown to unable to progress from an immature IP into a Ase-expressing IP. As a result, the Ase-negative IPs divide symmetrically to generate two Ase-negative IPs, which leads to an expansion of the IP population. *pros* mutant type II NB lineages are unable to induce differentiation in the GMC, resulting in an overproliferation of mature IPs (B).

Future perspectives

The neuroblast mutants described in this thesis all involve cell fate determination, and specifically the decision to differentiate into neurons is lost in these tumors. The tumor-inducing factors involved regulators of proliferation cascades, and the mechanisms involved in the specific distribution of these factors to the differentiating daughter. The *numb* and *brat* mutants displayed a similar phenotype; they halted the development of neural stem cells at a stage of which the neural identity transcription factor Ase is not expressed. In wildtype *Drosophila*, this cell type does not occur but is just a transient state of a developing cell. The mutant cells mimic the developmental stage in which symmetric division of stem cell occurs, in order to expand the stem cell population. However, these are not type II neuroblasts anymore, nor will they ever mature to form a GMC. They are not supposed to divide; only after transition into a mature IP their proliferation routes are induced.
The observation that *brat* and *numb* mutant IPs block their cell cycle in G2 phase to wait for Ase expression, but ultimately proceed mitosis anyway is an interesting observation. It raises the question whether Brat and Numb directly regulate Ase expression, or whether the absence of differentiation factors indirectly affects IPs maturation and Ase upregulation. What makes these cells proceed into mitosis despite the absence of the presumed inducer of proliferation? The initial delay in cell cycle in these mutants implies that a cell cycle checkpoint is bypassed upon initiation of mitosis. The next level will be identifying the factors that stimulate the cell cycle to complete despite the absence of Ase. What epigenetic changes are involved in the process of Ase induction in wildtype IPs that are absent in *brat* or *numb* mutants. And do *brat* and *numb* induce this common phenotype using similar pathways or do they have different targets?

Ectopic activation of Notch in type II NBs, a condition that mimics the loss of Numb, was shown to result in overproliferation of the Ase-negative precursors, phenocopying *numb* mutants. This observation implies that Numb facilitates the maturation of the IPs via acting on the Notch pathway. This also implies that the second mutant *brat* might induce the phenotype as a result of the release of translational repression by Brat. The proliferation inducing transcription factor Myc was shown to be a target for Brat in differentiating cells [Schwamborn et al., 2009]. It might be that the Myc proliferation cues facilitate the continuation of the cell cycle despite the absence of Ase. Nevertheless, there should be more factors involved, as type I NBs that lack *brat* should also develop overproliferating GMCs if Myc were the only inducer. This also applies for *numb* mutants. If an overactivation of Notch would be sufficient to induce overproliferation, type I NB mutants should also develop tumors. Apparently, the presence of proliferation inducing factors is not sufficient to induce overproliferation, but it might facilitate tumor progression. It will be challenging to unravel the factors that release the cell cycle block.

Up to date stem cell tumors as a result of a loss if differentiation have only been described for *Drosophila* neuroblasts. However, mammals contain homologues for Numb [Pece et al., 2004] and Brat/TRIM32 [Schwamborn et al., 2009] as well, where they are suggested to have a similar function. Besides, the mechanisms in asymmetric division of a stem cells have also been largely conserved between *Drosophila* and mammals. Is a loss of differentiation fate a specific phenomenon in *Drosophila* type II neuroblasts, or are we looking at a common mechanism? And what can we learn from other model systems? Interestingly, a study on *C. elegans* neuroblast lineages has revealed that the loss of cell fate determinants can also lead to overproliferation in the nematode. A study by Frank and colleagues has uncovered two genes involved in the asymmetric distribution of cell fates in the neural HSN/PHB lineage. Mutations in the *ham-1* and *pig-1* genes were shown to revert daughter cells of the neuroblast lineage HSN/PHB that are primed to undergo apoptosis back into neurons, resulting in an expansion of the neuron population. It is suggested that these genes are involved in the distribution of cell fate determinants as in wildtype conditions these are anchored to the surviving neurons and are absent in the daughter cells primed to undergo apoptosis. A loss of these factors would facilitate equal distribution of survival factors resulting in retention of the neurons [Frank et al., 2005, Singhvi et al., 2008]. Despite that these mutations do not lead to tumor formation, an uncontrolled maintenance of a pool of mutant cells is observed. It will be interesting to further study this lineage for other mutants leading to overpopulation of neurons.

Understanding the mechanisms that lead to uncontrolled proliferation of stem cells in *Drosophila* might provide us insights on how defects in asymmetric division can induce tumor formation. In this review I described the importance of the correct distribution of cell fate determinants to daughter cells. Mutation of these factors was shown to result in a loss of differentiation fate. But mutations in the mechanisms distributing these factors to the correct cell compartment were shown to induce a similar phenotype. Third, the mechanisms dividing the two daughter cells were shown to be equally important, as these can also disrupt daughter fate separation. These tumors start out as cells that lost their differentiation status, but did not necessarily gain a proliferation fate. Somehow, these cells acquire the proliferation state after an initial pause. It is thought that this is the actual disease-initiating event. Therefore, stem cell tumors are a result of defects in asymmetric cell division, but not so much directly caused by. It will be interesting to study the progression of stem cell tumors to see whether the tumor cells will remain immature progenitors or revert back into a type II neuroblast. Furthermore, it will be interesting to study a different stem cell type with the corresponding differentiation factors. Is a loss of differentiation fate a common inducer for a stem cell tumor, or is this a neuroblast specific disorder? We are now at the point of identifying tumor-initiation for the identified cell fate determinants *numb*, *brat*, and *pros* at the molecular level. Unraveling their modes of action will not only gain information on tumorigenesis, but might also provide new insights on how these factors function during embryonic development of the neuroblasts.

List of abbreviations

14-3-3e	Par-5 homolog in *Drosophila*
AB	anterior daughter cell of *C. elegans* zygote
Ago1	argonaut, RNase, involved in microRNA pathway
APC2	adenomatosis polyposis coli 2, spindle anchoring protein
aPKC	atypical protein kinase C, part of anterior PAR complex
Armadillo	β-catenin homolog. E-cadherin complex member
Ase	asense, transcription factor
Aura	auroraA, mitotic kinase, activator of aPKC
Baz	Bazooka, Drosophila homolog of Par-3
Brat	brain tumour, cell fate determinant in *Drosophila*
CB	central brain, brain region
Cdc42	cell division control protein 42, activator of aPKC
CYK-4	Rho GTPase GAP protein
Dlg	Disc large, basal cortex marker in *Drosophila*
Dpn	deadpan, transcription factor
Dynein	microtubule minus-end tracking protein, spindle anchoring protein
E-cadherin	epithelial cell adhesion protein
Fbf-1/2	*C. elegans* homolog of Pumilio
Gα	G protein alpha subunit, membrane anchor protein
GMC	ganglion mother cell, differentiating daughter cell of *Drosophila* neuroblast
GPR1/2	G-protein coupled receptor 1/2, spindle anchoring protein
GSC	germline stem cell, non-polarized stem cell in *Drosophila*
Insc	Inscuteable, couples spindle to apical Par complex
IP	intermediate progenitor, daughter of type II neuroblast
Jar	Jaguar, myosin IV protein, binds Mira
Khc-73	kinesin motor protein, spindle anchoring protein of telophase rescue complex
Lgl	lethal (2) giant larvae, Drosophila basal cortex marker
LGN	mammalian Pins homolog, spindle anchoring protein
LIN-5	spindle anchoring protein
LIS-1	member of Dynein complex
MDCK	Madin-Darby Canine Kidney, epithelial cell type
MEX5/6	anterior muscle lineage determinants in *C. elegans*
Mei-P26	brat paralog in *Drosophila*
Mira	Miranda, cargo protein distributing cell fate determinants to basal daughter cell
Mud	mushroom defect, spindle anchoring protein in *Drosophila*
Myc	transcription factor
NB	neuroblast, neural precursor cell
Ncl-1	*C. elegans* brat homolog
Neu	neuralized, cell fate determinant in *Drosophila*
NLS	nuclear localization signal
NMY-2	non-muscle myosin, motor protein for actin filaments
Nos-3	C. elegans homolog of Nanos
NuMa	spindle anchoring protein
Numb	cell fate determinant basal daughter cell, Notch inhibitor
OL	optic lobe, brain region
P1	posterior daughter cell of *C. elegans* zygote
Par	partitioning defect, family of genes that polarize the cell cortex
PCM	pericentriolar material
PIE-1	germline determinant in *C. elegans*
Pins	partner of inscuteable, spindle anchoring protein
Polo	mitotic kinase, activates Pon

Pon	partner of numb, transports Numb to basal daughter cell
PP2A	protein phosphatase 2A, dephosphorylates aPKC
Pros	prospero, cell fate determinant
Ric-8A	guanine exchange factor for Gα
Scrib	scribble, basal cortex marker in *Drosopila*
SOP	sensory organ precursor, neural precursor in *Drosophila*
Staufen	RNA-binding protein, binds prospero mRNA
TRIM32	brat homolog in mouse
Twins	subunit of PP2A
VNC	ventral nerve cord, brain region

Literature

Adams, J.M., and Strasser, A. (2008). Is tumor growth sustained by rare cancer stem cells or dominant clones?, Cancer Res. 68:4018–21.

Albertson, R., Doe, C.Q., (2003), Dlg, Scrib and Lgl regulate neuroblast cell size and mitotic spindle asymmetry, Nat. Cell Biol. 5:166–70

Arai, F., Hirao, A., Ohmura, M., Sato, H., Matsuoka, S., Takubo, K., Ito, K., Koh, G.Y., Suda, T., (2004), Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche, Cell 118:149-61

Arata, Y., Lee, J.Y., Goldstein, B., Sawa, H., (2010), Extracellular control of PAR protein localization during asymmetric cell division in the C.elegans embryo, Development 137:3337-45

Asselin Labat, M.L., Vaillant, F., Sheridan, J.M., Pal, B., Wu, D., Simpson, E.R., Yasuda, H., Smyth, G.K., Martin, T.J., Lindeman, G.J., Visvader, J.E., (2010), Control of mammary stem cell function by steroid hormone signaling, Nature 465:798-802

Atwood, S.X., Prehoda, K.E., (2009), aPKC phosphorylates Miranda to polarize fate determinants during neuroblast asymmetric cell division, Curr. Biol. 12:723-9

Bardin, A.J., Le Borgne, R., Schweisguth, F., (2004), Asymmetric localization and function of cell-fate determinants: A fly's view. Curr. Opin. Neurobiol. 14:6–14

Barker, N., van Es, J.H., Kuipers, J., Kujala, P., van den Born, M., Cozijnsen, M., Haegebarth, A., Korving, J., Begthel, H., Peters, P.J., Clevers, H., (2007) Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 449:1003–7

Barros, C.S., Phelps, C.B., Brand, A.H, (2003), *Drosophila* nonmuscle myosin II promotes the asymmetric segregation of cell fate determinants by cortical exclusion rather than active transport. Dev Cell 5:829–40.

Bass, A.J., Watanabe, H., Mermel, C.H., Yu, S., Perner, S., Verhaak, R.G., et al. (2009), SOX2 is an amplified lineage-survival oncogene in lung and esophageal squamous cell carcinomas. Nat. Genet. 41:1238–42

Bayraktar, O.A., Boone, J.Q., Drummond, M.L., Doe, C.Q., (2010), Drosophila type II neuroblast lineages keep Prospero levels low to generate large clones that contribute to the adult brain central complex, Neural Dev. 5:26

Beatty, A., Morton, D., Kemphues, K., (2010), The C. elegans homolog of Drosophila Lethal giant larvae functions redundantly with PAR-2 to maintain polarity in the early embryo, Development 137:3995-4004

Bello, B., Reichert, H., Hirth, F., (2006), The brain tumor gene negatively regulates neural progenitor cell proliferation in the larval central brain of Drosophila. Development 133:2639–48

Benhra, N., Vignaux, F., Dussert, A., Schweisguth, F., le Borgne, R., (2010), Neuralized promotes basal to apical transcytosis of Delta in epithelial cells, Mol. Biol. Cell 21:2078-86

Berdnik, D., Knoblich, J.A., (2002), Drosophila Aurora-A is required for centrosome maturation and actin-dependent asymmetric protein localization during mitosis, Curr. Biol. 12:640-7

Berdnik, D., Torok, T., Gonzalez-Gaitan, M., Knoblich, J.A., (2002), The endocytic protein a-adaptin is required for numb-mediated asymmetric cell division in Drosophila. Dev Cell 3:221–231

Betschinger J, Eisenhaber F, Knoblich JA. (2005), Phosphorylationinduced autoinhibition regulates the cytoskeletal protein Lethal (2) giant larvae. Curr. Biol. 15:276–82

Betschinger, J., Mechtler, K., Knoblich, J.A., (2003), The Par complex directs asymmetric cell division by phosphorylating the cytoskeletal protein Lgl, Nature 422:326–30.

Betschinger, J., Mechtler, K., Knoblich, J.A., (2006), Asymmetric segregation of the tumor suppressor brat regulates self-renewal in Drosophila neural stem cells. Cell. 124:1241–53

Bilder, D., Perrimon, N., (2000), Localization of apical epithelial determinants by the basolateral PDZ protein Scribble, Nature 403:676–80

Blagosklonny, M.V., (2005). Why therapeutic response may not prolong the life of a cancer patient: selection for oncogenic resistance. Cell Cycle 4:1693–98.

Bonaccorsi, S., Giansanti, M.G., Gatti, M., (2000), Spindle assembly in *Drosophila* neuroblasts and ganglion mother cells. Nat. Cell Biol. 2:54–6

Bonnet, D., Dick, J.E., (1997), Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat. Med. 3:730–7.

Boone, J.Q., Doe, C.Q., (2008), Identification of Drosophila type II neuroblast lineages containing transit amplifying ganglion mother cells, Dev. Neurobiol. 68:1185-95

Bowman, S.K., Neumuller, R.A., Novatchkova, M., Du, Q., Knoblich, J.A., (2006), The Drosophila NuMA homolog mud regulates spindle orientation in asymmetric cell division. Dev. Cell 10:731–42

Bowman, S.K., Rolland, V., Betschinger, J., Kinsey, K.A., Emery, G., Knoblich, J.A., (2008), The tumor suppressors Brat and Numb regulate transit-amplifying neuroblast lineages in Drosophila, Dev. Cell 14:535–546

Boyd, L., Guo, S., Levitan, D., Stinchcomb, D.T., Kemphues, K.J., (1996), PAR-2 is asymmetrically distributed and promotes association of P granules and PAR-1 with the cortex in C. elegans embryos. Development 122:3075–84

Brand, M., Jarman, A.P., Jan, L.Y., Jan, Y.N. (1993), asense is a Drosophila neural precursor gene and is capable of initiating sense organ formation. Development 119:1–17

Brinster, R.L., Zimmermann, J.W., (1994), Spermatogenesis following male germ-cell transplantation. Proc. Natl. Acad. Sci. USA 91:11298–302

Brou, C., Logeat, F., Gupta, N., Bessia, C., LeBail, O., Doedens, J.R., Cumano, A., Roux, P., Black, R., Israel, A., (2000), A novel proteolytic cleavage involved in Notch signaling: the role of the disintegrin–metalloprotease TACE. Mol. Cell. 5:207–216

Brunet, S., Verlhac, M.H., (2010), Positioning to get out of meiosis: the asymmetry of division, Human Reprod. Update 0:1-9

Burness, M.L., Sipkins, D.A., (2010), The stem cell niche in health and malignancy, Semin. Cancer Biol. 20:107-15

Buss, F., Luzio, J.P., Kendrick-Jones, J., (2002), Myosin VI, an actin motor for membrane traffic and cell migration. Traffic 3:851–8

Cabernard, C., Doe, C.Q., (2009), Apical/Basal Spindle Orientation Is Required for Neuroblast Homeostasis and Neuronal Differentiation in Drosophila, Dev. Cell 17:134-41

Cai, Y., Yu, F., Lin, S., Chia, W., Yang, X., (2003), Apical complex genes control mitotic spindle geometry and relative size of daughter cells in *Drosophila* neuroblast and p*I* asymmetric divisions. Cell 112:51–62

Cao, J., Crest, J., Fasulo, B., Sullivan, W., (2010), Cortical actin dynamics facilitate early-stage centrosome separation, Curr. Biol. 20:770-6

Carmena, M., Earnshaw, W.C., (2003), The cellular geography of aurora kinases. Nat. Rev. Mol. Cell Biol. 4, 842–854

Caudron, M., Bunt, G., Bastiaens, P., Karsenti, E., (2005), Spatial coordination of spindle assembly by chromosome-mediated signaling gradients, Science 309:1373-6

Caussinus, E., Gonzalez, C., (2005) Induction of tumor growth by altered stem-cell asymmetric division in Drosophila melanogaster. Nat. Genet. 37:1125–9

Castellanos, E., Dominguez, P., Gonzalez, C., (2008), Centrosome dysfunction in Drosophila neural stem cells causes tumors that are not due to genome instability. Curr. Biol. 18:1209–14

Cheng, J., Turkel, N., Hemati, N., Fuller, M.T., Hunt, A.J., Yamashita, Y.M., (2008), Centrosome misorientation reduces stem cell division during ageing, Nature 456:599-604

Chenn, A., McConnell, S.K., (1995), Cleavage orientation and the asymmetric inheritance of Notch1 immunoreactivity in mammalian neurogenesis, Cell 82:631-41

Choksi, S.P., Southall, T.D., Bossing, T., Edoff, K., de Wit, E., Fischer, B.E., van Steensel, B., Micklem, G., Brand, A.H. (2006), Prospero acts as a binary switch between self-renewal and differentiation in Drosophila neural stem cells. Dev. Cell 11:775–89

Cismowski, M.J., Takesono, A., Bernard, M.L., Duzic, E., Lanier, S.M., (2001), Receptor-independent activators of heterotrimeric G-proteins. Life Sci. 68:2301–8

Clarke, M.F., Fuller, M., (2006), Stem cells and cancer: two faces of eve, Cell 124:1111–5.

Colombo, K., Grill, S.W., Kimple, R.J., Willard, F.S., Siderovski, D.P., Gonczy, P., (2003), Translation of polarity cues into asymmetric spindle positioning in Caenorhabditis elegans embryos. Science 300:1957–61

Cowan, C.R., Hyman, A.A., (2004), Asymmetric cell division in C. elegans: Cortical polarity and spindle positioning. Annu. Rev. Cell Dev. Biol. 20:427–53

Curtis, D., Treiber, D.K., Tao, F., Zamore, P.D., Williamson, J.R., and Lehmann, R. (1997), A CCHC metal-binding domain in Nanos is essential for translational regulation. EMBO J.16:834–43

Den Elzen, N., Buttery, C.V., Maddugoda, M.P., Ren, G., Yap, A.S., (2009), Cadherin adhesion receptors orient the mitotic spindle during symmetric cell division in mammalian epithelia, Mol. Cell Biol. 20:3740-50

Deng, M., Suraneni, P., Schultz, R.M., Li, R., (2007), The Ran GTPase mediates chromatin signaling to control cortical polarity during polar body extrusion in mouse oocytes, Dev. Cell 12:301-8

Ebnet, K., Suzuki, A., Horikoshi, Y., Hirose, T., Meyer zu Brickwedde, M.K., Ohno, S., Vestweber D, (2001), The cell polarity protein ASIP/PAR-3 directly associates with junctional adhesion molecule (JAM), EMBO J. 20:3738-48

Egger, B., Boone, J.Q., Stevens, N.R., Brand, A.H., Doe, C.Q., (2007), Regulation of spindle orientation and neural stem cell fate in the Drosophila optic lobe. Neural Develop 2:1

Egger, B., Gold, K.S., Brand, A., (2010), Notch regulates the switch from symmetric to asymmetric neural stem cell division in the Drosophila optic lobe, Dev. 137:2981-7

Fortini, M.E., (2001), Notch and presenilin: a proteolytic mechanism emerges, Curr. Opin. Cell Biol. 13:627–634.

Frank, C. A., Hawkins, N.C., Guenther, C., Horvitz, H.R., Garriga, G., (2005), C. elegans HAM-1 positions the cleavage plane and regulates apoptosis in asymmetric neuroblast divisions. Dev. Biol. 284:301–10

Frank, D.J., Roth, M.B., (1998), ncl-1 is required for the regulation of cell size and ribosomal RNA synthesis in Caenorhabditis elegans. J. Cell Biol 140:1321–29

Ghosh, S., Marquardt, T., Thaler, J. P., Carter, N., Andrews, S. E., Pfaff, S. L., Hunter, T., (2008), Instructive role of aPKCzeta subcellular localization in the assembly of adherens junctions in neural progenitors. Proc. Natl. Acad. Sci. USA 105:335-40

Glover, D.M., Leibowitz, M.H., McLean, D.A., Parry, H., (1995), Mutations in aurora prevent centrosome separation leading to the formation of monopolar spindles. Cell 81:95–105.

Gonczy, P., (2008), Mechanisms of asymmetric cell division: Flies and worms pave the way. Nat. Rev. Mol. Cell Biol. 9:355–66

Gotta, M., Ahringer, J., (2001), Distinct roles for Ga and Gbg in regulating spindle position and orientation in Caenorhabditis elegans embryos. Nat. Cell Biol. 3:297–300.

Gotta, M., Dong, Y., Peterson, Y.K., Lanier, S.M., Ahringer, J., (2003), Asymmetrically distributed C. elegans homologs of AGS3/PINS control spindle position in the early embryo. Curr. Biol. 13:1029–37

Gotz, M., Huttner, W.B., (2005) The cell biology of neurogenesis, Nat. Rev. Mol. Cell Biol. 6:777–88

Gromley, A., Yeaman, C., Rosa, J., Redick, S., Chen, C.T., Mirabelle, S., Guha, M., Sillibourne, J., Doxsey, S.J., (2005) Centriolin anchoring of exocyst and SNARE complexes at the midbody is required for secretory-vesicle-mediated abscission. Cell 123:75–87

Guo, S., Kemphues, K.J., (1995) Par-1, a gene required for establishing polarity in C. elegans embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. Cell 81: 611–20

Guo, S., Kemphues, K., (1996), A non-muscle myosin required for embryonic polarity in Caenorhabditis elegans, Nature 382:455-8

Gupta, P.B., Chaffer, C.L., Weinberg, R.A., (2009), Cancer stem cells: mirage or reality? Nat Med 15: 1010–2

Hammell, C.M., Lubin, I., Boag, P.R., Blackwell, T.K., Ambros, V., (2009), nhl-2 Modulates microRNA activity in Caenorhabditis elegans. Cell 136:926–38

Hampoelz, B., Hoeller, O., Bowman, S.K., Dunican, D., Knoblich, J.A., (2005), *Drosophila* Ric-8 is essential for plasma-membrane localization of heterotrimeric G proteins. Nat Cell Biol. 7:1099–105

Hartenstein, V., Campos-Ortega, J. A., (1984), Early neurogenesis in wild-type Drosophila melanogaster, Wilhelm Roux Arch. Dev. Biol. 193:308–26

Hess, HA. , Roper, J.C., Grill, S.W., Koelle, M.R, (2004). RGS-7 completes a receptor-independent heterotrimeric G protein cycle to asymmetrically regulate mitotic spindle positioning in C.elegans, Cell 119:209–18

Hird, S.N., White, J.G., (1993), Cortical and cytoplasmic flow polarity in early embryonic cells of Caenorhabditis elegans. J. Cell Biol. 121:1343–55

Horvitz, H.R., Herskowitz, I., (1992), Mechanisms of asymmetric cell division: Two Bs or not two Bs, that is the question. Cell 68:237–55

Hutterer, A., Berdnik, D, Wirtz-Peitz, F., Zigman, M., Schleiffer, A., Knoblich, J. A., (2006), Mitotic activation of the kinase Aurora-A requires its binding Partner Bora, Dev. Cell 11:147-57

Hutterer, A., Knoblich, J. A., (2005), Numb and alpha-Adaptin regulate Sanpodo endocytosis to specify cell fate in *Drosophila* external sensory organs. EMBO Rep. 6:836–842.

Huttner, W.B., Brand, M., (1997), Asymmetric division and polarity of neuroepithelial cells. Curr Opin. Neurobiol. 7: 29–39

Hwang, S.Y., Rose, L.S. (2010), , Control of asymmetric cell division in early C. elegans embryogenesis: teaming-up translational repression and protein degradation, BMB Rep. 43:69-78

Hyenne, V., Chartier, N.T., Labbe, J.C., (2010), Understanding the role of asymmetric cell division in cancer using C. elegans, Dev. Dyn. 239:1378-87

Hyenne, V., Desrosiers, M., Labbe, J.L., (2008), C. elegans Brat homologs regulate PAR protein-dependent polarity and asymmetric cell division, Dev. Biol. 321:368-78

Hyman, A.A., White, J.G., (1987), Determination of cell division axes in the early embryogenesis of Caenorhabditis elegans. J. Cell Biol. 105:2123–35

Inaba, M., Yuan, H., Salzmann, V., Fuller, M.T., Yamashita, Y.M., (2010), E-cadherin is required for centrosome and spindle orientation in Drosophila male germline stem cells, Plos One 5:1-7

Izumi, Y., Ohta, N., Itoh-Furuya, A., Fuse, N., Matsuzaki, F., (2004), Differential functions of G protein and Baz-aPKC signaling pathways in *Drosophila* neuroblast asymmetric division. J Cell Biol. 164:729–38

Johnstone, R. W., Cretney, E. & Smyth, M. J. P-glycoprotein protects leukemia cells against caspase-dependent, but not caspase-independent, cell death. *Blood* **93**, 1075–1085 (1999).

Kaltschmidt, J.A., Davidson, C.M., Brown, N.H., Brand, A.H. (2000). Rotation and asymmetry of the mitotic spindle direct asymmetric cell division in the developing central nervous system. Nat. Cell Biol. 2:7–12

Kemphues, K.J., Priess, J.R., Morton, D.G., Cheng, N.S., (1988) Identification of genes required for cytoplasmic localization in early C. elegans embryos. Cell 52:311–20.

Kiger, A.A., Jones, D.L., Schulz, C., Rogers, M.B., Fuller, M.T., (2001), Stem cell self-renewal specified by JAK-STAT activation in response to a support cell cue, Science 294:2542-5

Kile, B.T., Schulman, B.A., Alexander, W.S., Nicola, N.A., Martin, H.M., Hilton, D J., (2002), The SOCS box: A tale of destruction and degradation. Trends Biochem. Sci. 27:235–41

Knoblich, J.A., (2001) Asymmetric cell division during animal development, Nat. Rev. Mol. Cell Biol 2: 11–20

Knoblich, J.A., (2008), Mechanisms of asymmetric stem cell division, Cell 132:583-97

Knoblich, J.A., Jan, L.Y., Jan, Y.N., (1995), Asymmetric segregation of Numb and Prospero during cell division, Nature 377:624–627

Knoblich, J.A., Jan, L.Y., Jan, Y.N., (1997), The N terminus of the Drosophila Numb protein directs membrane association and actin-dependent asymmetric localization. Proc. Natl. Acad. Sci. 94:13005-10

Konno, D., Shioi, G., Shitamukai, A., Mori, A., Kiyonari, H., Miyata, T., Matsuzaki, F. (2008), Neuroepithelial progenitors undergo LGN-dependent planar divisions to maintain self-renewability during mammalian neurogenesis. Nat. Cell Biol. 10:93–101

Kosodo, Y., Roeper, K., Haubensak, W., Marzesco, A.M., Corbeil, D., Huttner, W.B., (2004), Asymmetric distribution of the apical plasma membrane during neurogenic divisions of mammalian neuroepithelial cells, EMBO J. 23:2314-24

Kosodo, Y., Toida, K., Dubreuil, V., Alexandre, P., Schenk, J., Kiyokage, E., Attardo, A., Mora-Bermudez, F., Arii, T., Clarke, J.D., Huttner, W.B., (2008), Cytokinesis of neuroepithelial cells can divide their basal process before anaphase. EMBO J. 27:3151–3163

Kozlowski, C., Srayko, M., Nedelec, F., (2007), Cortical microtubule contacts position the spindle in C. elegans embryos. Cell 129:499–510

Kraut, R., Chia, W., Jan, L.Y., Jan, Y.N., Knoblich, J.A., (1996), Role of inscuteable in orienting asymmetric cell divisions in Drosophila. Nature 383:50–5

Labbé, J.C., Pacquelet, A., Marty, T., Gotta, M., (2006) A genomewide screen for suppressors of Par-2 uncovers potential regulators of PAR protein-dependent cell polarity in Caenorhabditis elegans. Genetics 174:285–95

Langevin, J., le Borgne, R., Rosenfeld, F., Gho, M., Schweisguth, F., Ballaiche, Y., (2005), Lethal giant larvae controls the localization of Notch-signaling regulators Numb, Neuralized, and Sanpodo in Drosophila Sensory-Organ Precursor cells, Current Biol. 15:955-62

Lapidot, T., Sirard, C., Vormoor, J., Murdoch, B., Hoang, T., Caceres-Cortes, J., Minden, M., Paterson, B., Caligiuri, M.A., Dick, J.E., (1994). A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 367:645–648

Le Borgne, R., Schweisguth, F., (2003), Unequal segregation of neuralized biases Notch activation during asymmetric cell division. Dev. Cell 5:139–48

Lee, C. Y., Robinson, K. J. and Doe, C. Q., (2006), Lgl, Pins and aPKC regulate neuroblast self-renewal versus differentiation. Nature 439:594-8

Lee, C.Y., Wilkinson, B.D., Siegrist, S.E., Wharton, R.P., Doe, C.Q., (2006), Brat is a Miranda cargo protein that promotes neuronal differentiation and inhibits neuroblast self-renewal. Dev Cell 10:441–9.

Li, L., Clevers, H., (2010), Coexistence of Quiescent and Active Adult Stem Cells in Mammals, Science 327:542-5

Li, L., Vaessin, H., (2000). Pan-neural Prospero terminates cell proliferation during Drosophila neurogenesis. Genes Dev. 14:147–51

Li, P., Yang, X., Wasser, M., Cai, Y., Chia, W., (1997), Inscuteable and Staufen mediate asymmetric localization and segregation of prospero RNA during *Drosophila* neuroblast cell divisions. Cell 90:437–47

Lin, D., Edwards, A. S., Fawcett, J. P., Mbamalu, G., Scott, J. D., Pawson, T., (2000). A mammalian PAR-3-PAR-6 complex implicated in Cdc42/Rac1 and aPKC signalling and cell polarity. Nat. Cell Biol. 2:540-7

Maeda, K., Takemura, M., Umemori, M., Adachi-Yamada, T., (2008), E-cadherin prolongs the moment for interaction between intestinal stem cell and its progenitor cell to ensure Notch signaling in adult Drosophila midgut, Genes Cells 13:1219-27

Mani, S.A., Guo, W., Liao, M.J., Eaton, E.N., Ayyanan, A., Zhou, A.Y., Brooks, M., Reinhard, F., Zhang, C.C., Shipitsin, M., et al. (2008). The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 133, 704–715

Marthiens, V., ffrench-Constant, C., (2009), Adherens junction domains are split by asymmetric division of embryonic neural stem cells, EMBO reports 10:515-20

Morton, D.G. , Shakes, D.C. , Nugent, S., Dichoso, D , Wang, W., Golden, A., Kemphues, K.J., (2002), The Caenorhabditis elegans Par-5 gene encodes a 14–3–3 protein required for cellular asymmetry in the early embryo. Dev Biol. 241:47–58.

Mummery-Widmer, J.L., Yamazaki, M., Stoeger, T., Novatchkova, M., Bhalerao, S., Chen, D., Dietzl, G., Dickson, B.J., Knoblich, J.A., (2009), Genome-wide analysis of Notch signalling in Drosophila by transgenic RNAi. Nature 458:987–92

Munro, E., Nance, J., Priess, J.R., (2004), Cortical flows powered by asymmetrical contraction transport PAR proteins to establish and maintain anterior-posterior polarity in the early C. elegans embryo. Dev. Cell 7:413–24

Neumuller, R., Knoblich, J.A., (2009), Dividing cellular asymmetry: asymmetric cell division and its implications for stem cells and cancer, Genes Dev. 23:2675-99

Nguyen-Ngoc, T., Afshar, K., Gonczy, P., (2007), Coupling of cortical dynein and Ga proteins mediates spindle positioning in Caenorhabditis elegans. Nat. Cell Biol. 9:1294–1302

Noatynska, A., Panbianco, C., Gotta, M., (2010), SPAT-1/Bora acts with Polo-like kinase 1 to regulate PAR polarity and cell cycle progression, Dev. 137:3315-25

O'Brien, C.A., Pollett, A., Gallinger, S., Dick, J.E., (2007), A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature 445:106–110

Ohlstein, B., Spradling, A., (2007), Multipotent Drosophila intestinal stem cells specific daughter cell fates by differential notch signaling, Science 315:988-92

Ooshio, T., Fujita, N., Yamada, A., Sato, T., Kitagawa, Y., Okamoto, R., Nakata, S., Miki, A., Irie, K., Takai, Y., (2007), Cooperative roles of Par-3 and afadin in the formation of adherens and tight junctions, J. Cell Sci. 120:2352-65

Ossipova, O., Ezan, J., Sokol, S.Y., (2009), PAR-1 phosphorylates Mind Bomb to promote vertebrate neurogenesis,Dev. Cell 17:222-233

Pacquelet, A., Zanin, E., Ashiono, C. and Gotta, M., (2008), PAR-6 levels are regulated by NOS-3 in a CUL 2 dependent manner in Caenorhabditis elegans. Dev. Biol. 319:267-72

Pallis, M., Russell, N., (2000), P-glycoprotein plays a drug-efflux independent role in augmenting cell survival in acute myeloblastic leukemia and is associated with modulation of a sphingomyelin-ceramide apoptotic pathway. Blood 95:2897–2904

Pardal, R., Clarke, M.F., Morrison, S.J., (2003), Applying the principles of stem-cell biology to cancer, Nature Rev. 3:895-902

Pece, S., Serresi, M., Santolini, E., Capra, M., Hulleman, E., Galimberti, V., Zurrida, S., Maisonneuve, P., Viale, G., Di Fiore, P.P., (2004), Loss of negative regulation by Numb over Notch is relevant to human breast carcinogenesis, J. Cell Biol. 167:215–221

Peng, C.Y., Manning, L., Albertson, R., Doe, C.Q., (2000), The tumour-suppressor genes *lgl* and dlg regulate basal protein targeting in *Drosophila* neuroblasts, Nature 408:596–600.

Pereira, G., Tanaka, T.U., Nasmyth, K., Schiebel, E., (2001), Modes of spindle pole body inheritance and segregation of the Bfa1p-Bub2p checkpoint protein complex, EMBO J. 20:6359-70

Petritsch, C., Tavosanis, G., Turck, C.W., Jan, L.Y., Jan, Y.N., (2003), The *Drosophila* myosin VI Jaguar is required for basal protein targeting and correct spindle orientation in mitotic neuroblasts. Dev. Cell 4:273–81.

Quintana, E., Shackleton, M., Sabel, M.S., Fullen, D.R., Johnson, T.M., Morrison, S.J., (2008), Efficient tumour formation by single human melanoma cells. Nature 456:593–98

Rando, T.A., (2007), The immortal strand hypothesis: Segregation and reconstruction. Cell 129:1239–43

Rebollo, E., Sampaio,, P., Januschke, J., Llamazares, S., Varmark,, H., Gonzalez, C., (2007), Functionally Unequal Centrosomes Drive Spindle Orientation in Asymmetrically Dividing Drosophila Neural Stem Cells, Dev. Cell 12:467-74

Rebollo, E., Roldan, M., Gonzalez, C., (2009), Spindle alignment is achieved without rotation after the first cell cycle in Drosophila embryonic neuroblasts. Development 136: 3393–7

Reya, T., Morrison, S.J., Clarke, M.F., Weissman, I.L., (2001), Stem cells, cancer, and cancer stem cells. Nature 414:105–11

Roesch, A., Fukunaga-Kalabis, M., Schmidt, E.C., Zabierowski, S.E., Brafford, P.A., Vultur, A., Basu, D., Gimotty, P., Vogt, T., Herlyn, M., (2010), A temporarily distinct subpopulation of slow-cycling melanoma cells is required for continuous tumor growth, Cell 141:583-94

Rosenblatt, J., Cramer, L.P., Baum, B., McGee, K.M., (2004), Myosin II-dependent cortical movement is required for centrosome separation and positioning during mitotic spindle assembly, Cell 117:361-72

Sabherwal, N., Tsutsui, A., Hodge, S., Wei, J., Chalmers, A.D., Papalopulu, N., (2009), The apicobasal polarity kinase aPKC functions as a nuclear determinant and regulates cell proliferation and fate during Xenopus primary neurogenesis, Development 136:2767-77

Schaefer, M., Petronczki, M., Dorner, D., Forte, M., Knoblich, J.A., (2001), Heterotrimeric G proteins direct two modes of asymmetric cell division in the *Drosophila* nervous system. Cell 107:183–94

Schaefer, M., Shevchenko, A., Shevchenko, A., Knoblich, J.A., (2000), A protein complex containing Inscuteable and the Gabinding protein Pins orients asymmetric cell divisions in Drosophila. Curr Biol 10:353–62

Schober, M., Schaefer, M., Knoblich, J.A., (1999), Bazooka recruits Inscuteable to orient asymmetric cell divisions in *Drosophila* neuroblasts. Nature 402:548–51

Schwamborn, J.C., Berezikov, E., Knoblich, J.A., (2009), The TRIMNHL protein TRIM32 activates microRNAs and prevents self-renewal in mouse neural progenitors. Cell 136:913–25

Seki, A., Coppinger, J.A., Jang, C.Y., Yates, J.R., Fang, G., (2008), Bora and the kinase Aurora a cooperatively activate the kinase Plk1 and control mitotic entry, Science 320:1655-8

Sell, S., Pierce, G.B., (1994), Maturation arrest of stem cell differentiation is a common pathway for the cellular origin of teratocarcinomas and epithelial cancers. Lab. Invest. 70:6–22

Sharma, S.V., Lee, D.Y., Li, B., Quinlan, M.P., Takahashi, F., Maheswaran, S., McDermott, U., Azizian, N., Zou, L., Fischbach, M.A., Wong, K.K., Brandstetter, K., Wittner, B., Ramaswamy, S., Classon, M., Settleman, J., (2010), A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations, Cell 141:69-80

Siegrist, S.E., Doe, C.Q., (2005), Microtubule-induced Pins/Gαi cortical polarity in *Drosophila* neuroblasts, Cell 123:1323–35

Siller, K.H., Cabernard, C., Doe, C.Q., (2006), The NuMA-related Mud protein binds Pins and regulates spindle orientation in Drosophila neuroblasts. Nat. Cell Biol. 8:594–600

Singh, A., Settleman, J., (2010), EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer, Oncogene 29:4741-51

Singhvi, A., Frank, C.A., Garriga, G., (2008), The T-box gene tbx-2, the homeobox gene egl-5 and the asymmetric cell division gene ham-1 specify neural fate in the HSN/PHB lineage, Genetics 179:887-98

Sharma, S.V., Lee, D.Y., Li, B., Quinlan, M.P., Takahashi, F., Maheswaran, S., McDermott, U., Azizian, N., Zou, L., Fischbach, M.A., Wong, K.K., Brandstetter, K., Wittner, B., Ramaswamy, S., Classon, M., Settleman, J., (2010), A Chromatin-Mediated Reversible Drug-Tolerant State in Cancer Cell Subpopulations, Cell 141:69-80

Shen, C.P., Jan, L.Y., Jan, Y.N, (1997), Miranda is required for the asymmetric localization of Prospero during mitosis in Drosophila.Cell 90:449–458

Smith, G.H., Boulanger, C.A., (2003), Mammary epithelial stem cells: transplantation and self-renewal analysis, Cell Prolif. 36:3–15.

Sonoda, J., Wharton, R.P., (2001), *Drosophila* brain tumor is a translational repressor. Genes Dev.15:762–73

Spana, E.P., Doe, C.Q., (1995), The prospero transcription factor is asymmetrically localized to the cell cortex during neuroblast mitosis in *Drosophila*, Development 121:3187–95.

Spradling, A.C., Zheng, Y., (2007), Developmental biology. The mother of all stem cells?, Science 315:469 70

Srinivasan, D.G., Fisk, R.M., Xu, H., Van Den Heuvel, S., (2003), A complex of LIN-5 and GPR proteins regulates G protein signaling and spindle function in C. elegans. Genes & Dev 17:1225–39

Stevens, N.R., Raposo, A.A.S.F., Basto, R., St Johnston, D., Raff, J.W., (2007), From Stem Cell to Embryo without Centrioles, Curr. Biol. 17:1498-1503

Strand, D., Jakobs, R., Merdes, G., Neumann, B., Kalmes, A., Heid, H.W., Husmann, I., Mechler, B.M., (1994), The *Drosophila* lethal(2) giant larvae tumor suppressor protein forms homo-oligomers and is associated with nonmuscle myosin II heavy chain, J. Cell Biol. 127:1361–73.

Struhl, G., Adachi, A., (1998), Nuclear access and action of notch in vivo. Cell 93:649–60

Tabuse, Y., Izumi, Y., Piano, F., Kemphues, K.J., Miwa, J., Ohno, S., (1998) Atypical protein kinase C cooperates with PAR-3 to establish embryonic polarity in Caenorhabditis elegans. Development 125: 3607–14

Takahashi, K., Yamanaka, S., (2006), Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors, Cell 126:663-76

Takekuni, K., Ikeda, W., Fujito, T., Morimoto, K., Takeuchi, M., Monden, M., Takai, Y., (2003). Direct binding of cell polarity protein PAR-3 to cell-cell adhesion molecule nectin at neuroepithelial cells of developing mouse. J. Biol. Chem. 278, 5497-5500

Tall, G. G., Gilman, A. G., (2005). Resistance to inhibitors of cholinesterase 8A catalyzes release of Gαi-GTP and nuclear mitotic apParatus protein (NuMA) from NuMA/LGN/Gαi-GDP complexes. Proc. Natl. Acad. Sci. U. S. A. 102:16584–9

Tautz, D., (1988), Regulation of the *Drosophila* segmentation gene hunchback by two maternal morphogenetic centres. Nature 332**:**281–4

Tenenhaus, C., Subramaniam, K., Dunn, M.A., Seydoux, G., (2001), PIE-1 is a bifunctional protein that regulates maternal and zygotic gene expression in the embryonic germ line of Caenorhabditis elegans. Genes and Dev. 15:1031–40.

Tenlen, J.R., Molk, J.N., London, N , Page, B.D., Priess, J.R, (2008), MEX-5 asymmetry in one-cell C. elegans embryos requires PAR-4- and PAR-1-dependent phosphorylation. Development 135:3665–75

Truman, J.W., Bate, M., (1988), Spatial and temporal patterns of neurogenesis in the central nervous system of Drosophila melanogaster. Dev. Biol. 125:145–157

Trumpp, A., and Wiestler, O.D. (2008). Mechanisms of disease: cancer stem cells–targeting the evil twin. Nat. Clin. Pract. Oncol. 5, 337–347

Udolph,, G., Rath, P., Tio, M., Toh, J., Fang, W., Pandey, R., Technau, G., Chia, W., (2009), On the roles of Notch, Delta, kuzbanian, and inscuteable during the development of Drosophila embryonic neuroblast lineages, Dev. Biol. 336:156-68

Wang, H., Ouyang, Y., Somers, W.G., Chia, W., Lu, B. (2007). Polo inhibits progenitor self-renewal and regulates Numb asymmetry by phosphorylating Pon. Nature 449:96–100

Wang, H., Somers, G.W., Bashirullah, A., Heberlein, U., Yu, F., Chia, W. (2006). Aurora-A acts as a tumor suppressor and regulates self-renewal of Drosophila neuroblasts. Genes Dev. 20:3453–63

Watts, J.L., Morton, D.G. , Bestman, J., Kemphues, K.J., (2000), The C. elegans Par-4 gene encodes a putative serine-threonine kinase required for establishing embryonic asymmetry. Development 127: 1467–75

Wharton, R.P., Sonoda, J., Lee, T., Patterson, M., and Murata, Y. (1998) The Pumilio RNA-binding domain is also a translational regulator. Mol. Cell 1:863–72

Wilson, A., Radtke, F., (2006). Multiple functions of Notch signaling in self-renewing organs and cancer. FEBS Lett. 580:2860–8

Wirtz Peitz, F., Nishimura, T., Knoblich, J.A., (2008), Linking cell cycle to asymmetric division: Aurora-A phosphorylates the Par complex to regulate Numb localization, Cell 135:161-73

Woodard, G.E., Huang, N.N., Cho, H., Miki, T., Tall, G.G., Kehrl, J.H., (2010), Ric-8A and Gia recruit LGN, NuMa, and Dynein to the cell cortex to help orient the mitotic spindle, Mol. Cell Biol. 30:3519-30

Wu., P.S., Egger, B., Brand, A., (2008), Asymmetric stem cell division: lessons from Drosophila, Sem. Cell Dev. Biol. 19:283-93

Yamashita, Y.M., (2010), Cell adhesion in regulation of asymmetric stem cell division, Curr. Opin. Cell Biol. 22:605-10

Yamashita, Y.M., Jones, D.L., Fuller, M.T., (2003), Orientation of asymmetric stem cell division by the APC tumor suppressor and centrosome, Science 301:1547–50.

Yamashita, Y.M., Mahowald, A.P., Perlin, J.R., Fuller, M.T., (2007), Asymmetric inheritance of mother versus daughter centrosome in stem cell division, Science 315:518–21

Yasugi, T., Umetsu, D., Murakami, S., Sato, M., Tabata, T., (2008), Drosophila optic lobe neuroblasts triggered by a wave of proneural gene expression that is negatively regulated by JAK/STAT. Development 135:1471–80

Yu, F., Cai, Y., Kaushik, R , Yang, X., Chia, W., (2003), Distinct roles of Galphai and Gbeta13F subunits of the heterotrimeric G protein complex in the mediation of *Drosophila* neuroblast asymmetric divisions. J. Cell Biol.162:623–33

Yu, F., Morin, X., Cai, Y., Yang, X., Chia, W., (2000), Analysis of Partner of inscuteable, a novel player of *Drosophila* asymmetric divisions, reveals two distinct steps in inscuteable apical localization. Cell 100:399–409

Yu, F., Wang, H , Qian, H , Kaushik, R , Bownes, M., Yang, X., Chia, W., (2005) Locomotion defects, together with Pins, regulates heterotrimeric G-protein signaling during Drosophila neuroblast asymmetric divisions. Genes & Dev. 19:1341–53

Zhou, S. *et al.* The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. *Nature Med.* **7**, 1028–1034 (2001).

Zieba, P., Strojny, P., Lamprecht, J., (1986), Positioning and stability of mitotic spindle orientation in the neuroepithelial cell. Cell Biol. Int. Rep. 10:91–100

Zigman, M., Cayouette, M., Charalambous, C., Schleiffer, A., Hoeller, O., Dunican, D., McCudden, C.R., Firnberg, N., Barres, B.A., Siderovski, D.P., Knoblich, J.A., (2005), Mammalian inscuteable regulates spindle orientation and cell fate in the developing retina. Neuron 48:539–45.