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Asymmetric cell division during neurogenesis in *Drosophila* and vertebrates

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Abstract

The majority of cells that build the nervous system of animals are generated early in embryonic development in a process called neurogenesis. Although the vertebrate nervous system is much more complex than that of insects, the underlying principles of neurogenesis are intriguingly similar. In both cases, neuronal cells are derived from polarized progenitor cells that divide asymmetrically. One daughter cell will continue to divide and the other daughter cell leaves the cell cycle and starts to differentiate as a neuron or a glia cell. In *Drosophila*, this process has been analyzed in great detail and several of the key players that control asymmetric cell division in the developing nervous system have been identified over the past years. Asymmetric cell division in vertebrate neurogenesis has been studied mostly at a descriptive level and so far little is known about the molecular mechanisms that control this process. In this review we will focus on recent findings dealing with asymmetric cell division during neurogenesis in *Drosophila* and vertebrates and will discuss common principles and apparent differences between both systems.

Keywords: *Drosophila*; Neurogenesis; Vertebrates; Asymmetric cell division; Stem cells

1. Asymmetric division of neuroblasts in the *Drosophila* central nervous system

The central nervous system (CNS) in *Drosophila* develops from precursor cells with stem cell-like properties, so called neuroblasts (NBs). The NBs of the ventral neurogenic region (VNR), which give rise to the ventral nerve cord, delaminate as individual cells from the neuroectodermal epithelium into the interior of the embryo, where they are positioned between the epidermis and the mesoderm. Shortly after delamination, NBs start to divide asymmetrically, generating another NB and a ganglion mother cell (GMC) in each division. While the larger NB continues to divide in a stem cell-like fashion, the smaller GMC divides only once more to generate a pair of neurons or glia cells (Campos-Ortega, 1993; Goodman and Doe, 1993). During NB division the mitotic spindle rotates by 90°, leading to a stereotypic spindle orientation perpendicular to the plane of the overlying neuroectodermal epithelium (Kaltschmidt et al., 2000). Consequently, the GMC is always pinched off at the basal pole of the NB. Several proteins and mRNAs that serve as cell fate determinants are localized to the basal pole of the NB during mitosis and are segregated exclusively to the GMC before cytokinesis. One of these factors, the homeobox transcription factor Prospero (Pros), is required for the transcription of GMC specific genes. In addition, Pros suppresses transcription of multiple cell cycle regulators, leading to exit from the mitotic cycle and allowing terminal differentiation of neurons and glia cells after one final cell division (Doe et al., 1991; Vaessin et al., 1991; Li and Vaessin, 2000).

In contrast to the NBs of the VNR, a subset of procephalic NBs that give rise to the brain are formed by horizontal cleavage of neuroectodermal epithelial cells rather than by delamination (Campos-Ortega and Hartenstein, 1997; Urbach et al., 2003). Subsequently, these NBs may divide in a manner similar to ventral NBs, but their precise lineage remains to be determined.

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In the first section of our review we will focus on novel findings related to the following questions: (i) How does the NB aquire its apical–basal polarity? (ii) How is the orientation of the mitotic spindle coordinated with the polarized localization of cell fate determinants? (iii) What is the mechanism to generate the difference in cell size between NB and GMC?

1.1. Establishment of apical–basal NB polarity

NBs are large spherical cells that do not possess elaborate contacts to adjacent cells but nonetheless they are highly polarized along the apical–basal axis. NB polarity is the prerequisite for proper spindle orientation and asymmetric localization of cell fate determinants, but what do we know about the establishment of polarity? Prior to delamination and its first division, each NB of the VNR is integrated into the neuroectodermal epithelium and is connected to adjacent cells by the zonula adherens (ZA), a belt like adherens junction (AJ) encircling the apex of the cells (Fig. 1). When the NB delaminates, these cell contacts are broken up, the NB changes its shape and moves into the interior of the embryo. Transiently, an apical stalk is left behind in between the epithelial cells (Fig. 1). Proteins of the PAR/aPKC complex (Ohno, 2001; Wodarz, 2002) that are localized to the apical cortex of the cells in the neuroectodermal epithelium remain localized in this apical stalk and form a crescent in the apical cortex after the NB has fully delaminated and the stalk has been retracted (Fig. 1) (Schober et al., 1999; Wodarz et al., 1999, 2000; Petronczki and Knoblich, 2001). Mutations in the genes encoding components of the PAR/aPKC complex (*bazooka, baz; atypical protein kinase C, DaPKC; DmPAR-6*) lead to loss of apical–basal polarity in epithelia and in NBs, indicating that the polarity of these two cell types is controlled by a related mechanism (Müller and Wieschaus, 1996; Schober et al., 1999; Wodarz et al., 1999, 2000; Petronczki and Knoblich, 2001). However, NB polarity is not absolutely dependent on an intact neuroectodermal epithelium. In *crumbs* (*crb*) and *stardust* (*sdt*) mutants that show a loss of epithelial polarity, NB polarity is unaffected (Bachmann et al., 2001; Hong et al., 2001). Apparently, *crb* and *sdt* act in concert with the PAR/aPKC complex to control epithelial polarity (Müller and Wieschaus, 1996; Bilder et al., 2003; Johnson and Wodarz, 2003; Tanentzapf and Tepass, 2003), but only the PAR/aPKC complex is also required in NBs.

This finding raises the questions of how the apical plasma membrane domain of epithelia and NBs differs in its protein and lipid composition and how the apical localization of the PAR/aPKC complex is established and maintained in these two cell types. Localization to the apical cortex could be achieved either by binding of a component of the complex

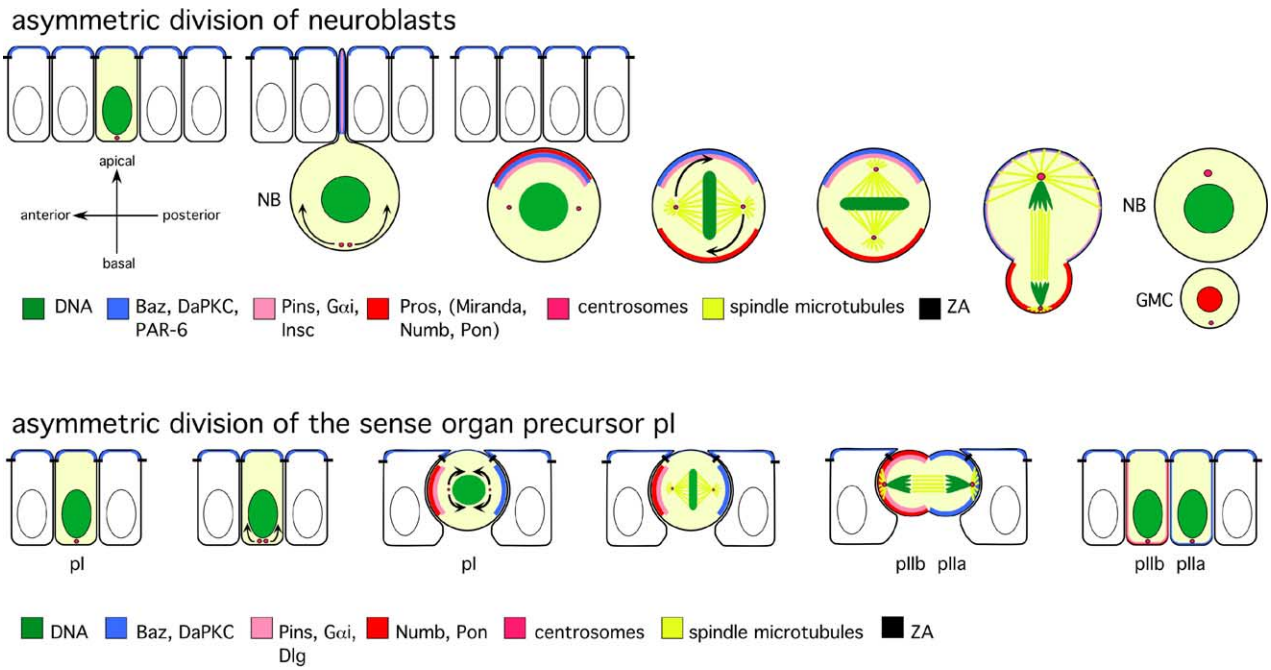

Fig. 1. Asymmetric division of NBs and sense organ precursor cells in *Drosophila*. The top panel shows a simulated time course of delamination and division of a single NB in the ventral neurogenic region of the *Drosophila* embryo. The subcellular localization of several polarity regulators, cell fate determinants and their adaptor proteins is indicated in different colors (see legend). For simplicity, in the epithelium and in the delaminating NB only the subcellular localization of the PAR/aPKC complex is indicated. The red color represents the localization of Pros. In meta- through anaphase, Miranda, Numb and Pon are localized in a very similar fashion, but there are differences in the localization of these proteins in pro- and late telophase. The bottom panel shows the asymmetric division of the sense organ precursor pI. Color coding is the same as for the top panel. Insc and Pros are not expressed in pI, and no published information is available on expression of PAR-6 and Miranda. Arrows show the movement of centrosomes. For abbreviations, see text.

to a transmembrane protein or by interaction with lipids on the inner face of the plasma membrane. A candidate transmembrane protein in the epithelium is Crb, which binds directly to the MAGUK protein Sdt (Bachmann et al., 2001; Hong et al., 2001; Roh et al., 2002). The mammalian Sdt homolog, Pals1, binds directly to PAR-6 and recruits it to the membrane by simultaneously binding to the Crb homolog Crb3 (Hurd et al., 2003). However, in NBs Crb and Sdt are not expressed and no other candidate transmembrane protein that might bind to the PAR/aPKC complex in *Drosophila* has been identified yet.

The recruitment of the PAR/aPKC complex by membrane lipids is a particularly intriguing possibility because the phosphatidyl-inositol-3-kinase (PI-3-kinase) pathway is required for the polarized localization of the PAR/aPKC complex to the tip of the axon in cultured hippocampal neurons of rats (Shi et al., 2003). Moreover, in *Drosophila*, the PAR-3 homolog Baz binds directly to the lipid phosphatase PTEN which antagonizes PI-3-kinase activity by removing the phosphate at position 3 of the inositol ring of phosphatidyl inositol (3,4,5) trisphosphate (PIP_3) to generate phosphatidyl inositol (4,5) bisphosphate (PIP_2) (W. von Stein, A. Ramrath, A. Grimm and A.W., unpublished). It is important to note here that PIP_2 can directly bind to proteins with PDZ domains (Zimmermann et al., 2002). Whether this is also true for the PDZ domains of Baz or PAR-6 remains to be shown. Alternatively, a component of the PAR/aPKC complex could bind to another protein with a lipid binding domain, e.g. a pleckstrin homology (PH) or a FYVE domain that could localize the complex to the membrane.

1.2. Asymmetric localization of cell fate determinants and the control of spindle orientation in NBs

In mutants for components of the PAR/aPKC complex the asymmetric localization of the cell fate determinants Pros and Numb and their adaptor proteins Miranda and Partner of Numb is lost. Moreover, the orientation of the mitotic spindle is randomized in these mutants (Kuchinke et al., 1998; Schober et al., 1999; Wodarz et al., 1999, 2000; Petronczki and Knoblich, 2001). Similar phenotypes have been observed in mutants for the gene *inscuteable* (*insc*) (Kraut et al., 1996; Kaltschmidt et al., 2000). Starting at delamination, Insc is recruited to the apical cortex of NBs due to its physical association with the PAR/aPKC complex and becomes undetectable in ana- and telophase, most likely by cell cycle dependent degradation (Kraut et al., 1996; Schober et al., 1999; Wodarz et al., 1999, 2000; Petronczki and Knoblich, 2001; D. Egger and A.W., unpublished). Interestingly, the maintenance of apical Insc localization and the correct localization of cell fate determinants depend on the activity of the mitotic kinase Cdc2 (Tio et al., 2001), revealing a link between cell polarity and cell cycle regulation. In contrast to the PAR/aPKC complex, Insc is not expressed in the ventral neuroectodermal epithelium. However, when ectopically expressed there, Insc induces rotation of the mitotic spindle by 90°, leading to a horizontal rather than vertical plane of cell division (Kraut et al., 1996). Interestingly, Insc is expressed in epithelial cells of the PNR which give rise to the brain, and these cells divide with a horizontal cleavage plane (Kraut et al., 1996). Together, these results indicate that Insc acts as a switch that controls the orientation of the mitotic spindle. The mechanism of how Insc induces spindle rotation is unclear. Insc is asymmetrically localized in the apical NB cortex where it may interact with astral microtubules from one spindle pole, causing rotation of the spindle. The involvement of aster microtubules in spindle rotation has indeed been suggested by the analysis of NB divisions in mutants lacking asters (Giansanti et al., 2001). However, no direct interaction of Insc with microtubules has been reported so far, nor are there any binding partners of Insc known that bind to microtubules.

The identification of Partner of Inscuteable (Pins) as a binding partner of Insc revealed an intriguing connection between Insc and heterotrimeric G-proteins (Schaefer et al., 2000; Yu et al., 2000). Pins binds simultaneously to both Insc and the Gαi subunit of heterotrimeric G-proteins (Parmentier et al., 2000; Schaefer et al., 2000, 2001). All three proteins colocalize in the apical NB cortex in a mutually dependent way. Loss of Pins function and overexpression of Gαi both lead to mislocalization of Insc, aberrant spindle orientation and mislocalization of basal cell fate determinants, supporting the functional relevance of the observed protein interactions (Schaefer et al., 2000, 2001; Yu et al., 2000).

So far it is not known which downstream effectors of G-protein signaling are involved in the control of NB polarity. 'Classical' G-protein signaling cascades in mammalian cells are triggered by ligand binding to a G-protein coupled seven-transmembrane receptor, followed by dissociation of the G-protein trimer into the free α and βγ subunits. Overexpression experiments with wild type and constitutively GTP bound forms of Gαi suggest that the βγ dimer rather than the free, GTP-bound Gαi is the relevant signaling molecule in NBs (Schaefer et al., 2001). In many cell types, the free βγ dimer activates phospholipase C, which cleaves PIP_2 to generate the second messengers diacylglycerol and inositol (1,4,5) trisphosphate (IP_3). Although we have no direct evidence for the activation of this pathway during NB division, this scenario points to another potential connection between phosphoinositide lipids and cell polarity.

An important question regarding NB division is how the proteins localized in the apical NB cortex (the 'apical complex') can control the localization of cell fate determinants to the opposite, basal pole. One possibility is that the apical complex affects the organization of the cortical cytoskeleton. Indeed, an intact actin cytoskeleton is required for the asymmetric localization of cell fate determinants and their adaptors, whereas microtubules are

dispensable (Broadus and Doe, 1997; Knoblich et al., 1997; Lu et al., 1999a). These results indicate that actin-based motors may be important for the basal transport of cell fate determinants. Consistent with this hypothesis, the adaptor protein Miranda binds directly to the unconventional myosin VI Jaguar (Jar) and is mislocalized in *jar* mutant neuroblasts (Petritsch et al., 2003). Cdc42, a small GTPase that acts as a key regulator of actin dynamics, is a component of the PAR/aPKC complex in vertebrates and binds directly to the CRIB domain of PAR-6 (Joberty et al., 2000; Johansson et al., 2000; Lin et al., 2000; Qiu et al., 2000). This protein interaction is conserved in *Drosophila* (D. Egger and A.W., unpublished), providing additional evidence for a close connection between the apical complex and the actin cytoskeleton.

In addition to the proteins of the apical complex, the gene products of the tumor suppressor genes *lethal giant larvae* (*lgl*), *discs large* (*dlg*) and *scribble* (*scrib*) are also required for the basal localization of cell fate determinants without affecting localization of the apical complex (Fig. 2)

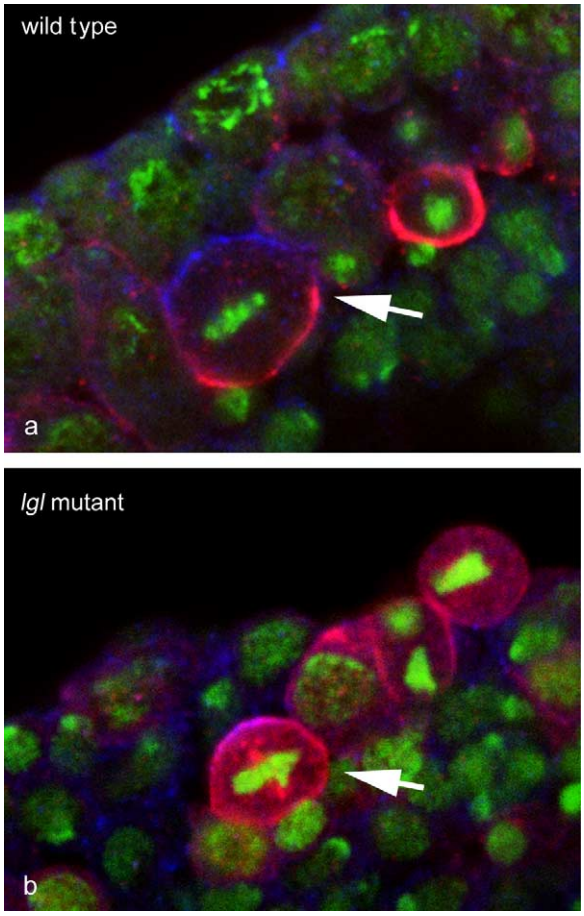

Fig. 2. Subcellular localization of Baz and Miranda in metaphase NBs of wild type and *lgl* mutant embryos. (a) In a wild type NB, Baz (blue) is localized to the apical cortex and Miranda (red) to the basal cortex in a mutually exclusive fashion. (b) In NBs of *lgl* mutant embryos, Baz is localized apically as in wild type, but Miranda is distributed all around the cortex and is also found on the mitotic spindle and in the cytoplasm. Apical is in the upper left corner in both panels. NBs are marked by an arrow.

(Ohshiro et al., 2000; Peng et al., 2000; Albertson and Doe, 2003). All three tumor suppressor proteins are present in the whole NB cortex and could thus be more directly involved in the targeting or tethering of cell fate determinants to the basal cortex. Interestingly, Lgl binds to the non-muscle myosin 2 Zipper (Strand et al., 1994) which might be involved in the basal transport of cell fate determinants along actin filaments. Homologs of Lgl in yeast and mammals are required for fusion of secretory vesicles with the plasma membrane (Lehman et al., 1999; Musch et al., 2002), providing another hint on the function of Lgl in basal localization of cell fate determinants. Like the PAR/aPKC complex, Lgl, Dlg and Scrib are also involved in the control of apical–basal polarity in epithelia (Bilder et al., 2000; Bilder and Perrimon, 2000; Wodarz, 2000). Recent findings suggest that Lgl, Dlg and Scrib antagonize the activity of the apical PAR/aPKC complex, and that this antagonism is important for the proper ratio of apical to basolateral plasma membrane domains in epithelia (Bilder et al., 2003; Johnson and Wodarz, 2003; Tanentzapf and Tepass, 2003). A similar antagonism appears to be at work in NBs. It has recently been shown that DaPKC binds directly to Lgl and phosphorylates Lgl at several highly conserved serine residues (Betschinger et al., 2003). Phosphorylation by apically localized DaPKC inactivates Lgl and allows recruitment of Miranda to the cortex only basally, where Lgl is active (Betschinger et al., 2003).

In all the mutants discussed above, cell fate determinants fail to form basal crescents in metaphase. Surprisingly, a considerable recovery of basal determinant localization takes place in late ana- and telophase, resulting in preferential segregation of cell fate determinants into the GMC upon cytokinesis (Lu et al., 1998; Schober et al., 1999; Wodarz et al., 1999; Peng et al., 2000; Petronczki and Knoblich, 2001). This phenomenon has been termed ‘telophase rescue’ (Peng et al., 2000) and points to the existence of a localization mechanism that acts late in mitosis and is independent of the localization machinery responsible for basal crescent formation in metaphase. Mutants homozygous for a deletion that removes the genes *snail*, *escargot* and *worniu*, which encode snail family transcription factors, show defects in localization of cell fate determinants without telophase rescue (Ashraf and Ip, 2001; Cai et al., 2001). These three transcription factors act redundantly and are required for expression of *insc* in NBs. However, lack of *insc* alone cannot be the cause for the severe mislocalization of cell fate determinants throughout mitosis in the triple mutants, as *insc* mutants do show telophase rescue. The data indicate that there must be at least one additional target gene of the snail family transcription factors that is responsible for telophase rescue in the absence of *insc*. Again, G-protein signaling may be involved in this phenomenon, as overexpression of delocalized, truncated versions of Pins and overexpression of Gαi leads to mislocalization of cell fate determinants

without telophase rescue (Schaefer et al., 2001; Yu et al., 2002).

1.3. Cell size regulation during NB divisions

Another important aspect of NB divisions is the different size of the daughter cells, the NB being always larger than the GMC. This size asymmetry is the consequence of two unusual features of the mitotic spindle in NBs: (i) beginning in anaphase, the mitotic spindle moves closer to the basal cortex of the NB where the GMC will bud off, and (ii) the spindle itself becomes asymmetric during anaphase, meaning that the cleavage plane is off center with respect to the two spindle poles (Fig. 1) (Kaltschmidt et al., 2000). The two spindle poles also differ in size and position in ana- and telophase NBs. The basal (GMC) spindle pole is always smaller and closer to the cortex than the apical (NB) spindle pole (Spana and Doe, 1995). Also, aster microtubules nucleated from the apical spindle pole are always longer and more elaborate than those of the basal spindle pole (Albertson and Doe, 2003; Fuse et al., 2003). However, centrosomes and aster microtubules seem to be dispensable for the generation of spindle asymmetry and unequal NB division, as NBs of *asterless* mutants that completely lack centrosomes and aster microtubules show normal, asymmetric spindles similar to wild type (Giansanti et al., 2001). The authors of the latter study speculate that an interaction between a factor associated with the GMC chromatin and the central spindle may be responsible for the asymmetric positioning of the cleavage plane in NBs (Giansanti et al., 2001).

This hypothesis still does not answer the question of how the GMC chromatin becomes different from the NB chromatin. In a genetic model organism like *Drosophila*, the solution to this problem may again be provided by the analysis of mutant phenotypes. Mutants for the tumor suppressors *lgl*, *dlg* and *scrib* show symmetric divisions in 20–30% of the neuroblasts (Albertson and Doe, 2003). Comparable frequencies of symmetric divisions are observed in NBs of homozygous mutant *pins* animals (Parmentier et al., 2000; Cai et al., 2003). Overexpression of Pins deletion mutants that still bind to Gαi but not to Insc leads to symmetric NB divisions (Yu et al., 2002). These mutant forms of Pins are not localized apically and may therefore activate G-protein signaling all around the cortex. Consistent with this hypothesis, overexpression of Gαi also results in symmetric division of NBs (Schaefer et al., 2001). Recent work by Cai et al. showed very convincingly that there are apparently two redundant activities that control spindle positioning and asymmetry in NBs. One activity is provided by the complex of Gαi and Pins, and the other activity is the PAR/aPKC complex together with Insc (Cai et al., 2003). In double mutants for components of both complexes, e.g. *baz* and *pins*, symmetric NB divisions are observed with 100% penetrance. Mutations in the Gβ13F gene affect the localization of both apical complexes, which results in a high percentage of NB divisions with equally sized daughter cells (Fuse et al., 2003).

2. Asymmetric division of sense organ precursor cells in the *Drosophila* peripheral nervous system

In the peripheral nervous system of *Drosophila*, sense organs are generated by a series of asymmetric cell divisions from a precursor cell, pI (Jan and Jan, 1993; Gho et al., 1999). Similar to NBs, the cell fate determinant Numb is asymmetrically localized in pI and segregates into only one of the two daughter cells (Fig. 1) (Rhyu et al., 1994). In contrast to NB divisions, the pI cell divides in the plane of the epidermal epithelium rather than perpendicular to it and the two daughter cells have nearly the same size (Fig. 1). The mitotic spindle aligns with the anterior–posterior axis of the animal with one spindle pole being centered over the anterior Numb crescent (Gho and Schweisguth, 1998; Bellaiche et al., 2001a; Roegiers et al., 2001b). Despite these differences, many of the genes that control the asymmetric division of NBs are also involved in the division of pI. Baz and DaPKC form a posterior crescent in mitotic pI cells, opposite to the anterior Numb crescent and are required for the asymmetric localization of Numb, similar to NBs (Bellaiche et al., 2001b; Roegiers et al., 2001a; Wodarz, 2001). However, spindle orientation is normal in pI cells mutant for *baz* and is instead controlled by planar polarity genes including *frizzled*, *dishevelled* and *flamingo* (Gho and Schweisguth, 1998; Lu et al., 1999b; Bellaiche et al., 2001a; Roegiers et al., 2001a,b). Another important difference between NBs and pI is the spatial relationship between the PAR/aPKC complex and the Pins/Gαi complex. While both complexes are colocalized in the apical cortex of NBs, the PAR/aPKC complex is at the posterior cortex and the Pins/Gαi complex at the anterior cortex in the pI cell (Bellaiche et al., 2001b; Wodarz, 2001). Interestingly, Pins binds directly to Dlg and both proteins mutually depend on each other for correct localization in the anterior cortex of pI (Bellaiche et al., 2001b). The reason for the localization of the two complexes to opposite poles of the pI cell is apparently the absence of Insc expression. When Insc is ectopically expressed in pI, the PAR/aPKC complex moves to the anterior cortex and colocalizes there with the Pins/Gαi/Dlg complex. Interestingly, Numb localization shifts to the posterior cortex in this situation, leading to a cell fate reversal of the two daughter cells (Bellaiche et al., 2001b). Furthermore, the mitotic spindle becomes asymmetric with an anterior bias when Insc is ectopically expressed in pI (Cai et al., 2003). This result is consistent with the idea that both the PAR/aPKC complex and the Pins/Gαi/Dlg complex can induce spindle asymmetry. In wild type pI, these two activities are localized to opposite poles of the cell, leading to symmetric division. Upon ectopic expression of Insc, however, both activities

are localized to the same pole of the cell, resulting in asymmetric division. Asymmetric divisions are also observed in *baz* or *pins* mutant pI cells. As expected according to the model, in *baz* mutants the anterior pIIb cell is larger, whereas in *pins* mutants the posterior pIIa cell is larger (Cai et al., 2003).

3. Asymmetric divisions during neurogenesis in the developing vertebrate central nervous system

3.1. Definitions and scope

When discussing asymmetric cell division in the vertebrate nervous system, the following subject categories should first be considered: (i) central vs. peripheral nervous system, (ii) embryonic development vs. adult, and (iii) generation of neurons vs. glial cells. Here, we shall confine our discussion to asymmetric cell divisions generating neurons of the CNS during embryonic development, with an emphasis on the mammalian CNS.

All neurons of the mammalian CNS are derived from neuroepithelial cells (NE cells), the cells constituting the innermost layer of the neural tube. NE cells exhibit an apical–basal polarity that will be discussed further below, extending from the lumen of the neural tube to the basal lamina at the pial boundary, at least prior to the onset of neurogenesis. After the onset of neurogenesis, spanning the entire neural tube wall from its lumen to the basal lamina is a characteristic feature of the so-called radial glial cells, which can be considered as a subpopulation of NE cells (Huttner and Brand, 1997; Parnavelas and Nadarajah, 2001; Fishell and Kriegstein, 2003). For simplicity, the term 'NE cells' as used here will therefore include radial glial cells.

Two definitions of asymmetric divisions of mammalian CNS neuronal progenitors have been used. Traditionally, cell divisions have been defined as asymmetric if the fate of the two daughter cells turned out to be different, without an analysis of the distribution of cell fate determinants upon mitosis of the progenitor as in the case of *Drosophila* NBs. We shall refer to these as *fate-defined* asymmetric divisions. More recently, with the progress in light microscopic methods, divisions of neuronal progenitors have been classified as asymmetric if they were (or could be anticipated to be) associated with an unequal distribution of cellular components between the resulting two daughter cells, even if the neuronal fate of one of the daughter cells was not always demonstrated. We shall refer to these as *distribution-defined* asymmetric divisions.

Fate-defined asymmetric cell divisions were first deduced from lineage studies which analyzed the progeny of single retrovirally labeled NE cells in vivo, which in some studies has been combined with quantitating the dilution of S-phase labeled DNA (see Kornack and Rakic, 1995; Mione et al., 1997; Reid et al., 1997 and refs. therein). Long-term time-lapse microscopy of isolated single neuronal progenitors in vitro has provided direct evidence for fate-defined asymmetric divisions (Qian et al., 1998, 2000). Discussing these important studies here would be beyond the scope of this review. Rather, we shall focus on distribution-defined asymmetric divisions of NE cells that have been studied within the neuroepithelial tissue.

Evidence for distribution-defined asymmetric divisions of NE cells (including radial glial cells) has been obtained by monitoring their divisions in intact tissue using time-lapse microscopy, as well as by the detailed analysis of mitotic NE cells in vivo (Chenn and McConnell, 1995; Fishell and Kriegstein, 2003). Before discussing these studies, we shall address the polarity of NE cells which, as in the case of *Drosophila* NBs, is a key aspect in this context. We shall concentrate here on the polarity in the cell-intrinsic, apical–basal axis. We would like to point out, however, that, extrapolating from the role of planar polarity in the *Drosophila* peripheral nervous system (see above), NE cell polarity in the other two dimensions, reflecting the tissue patterning along the principal body axes (anterior–posterior and dorso-ventral/medio-lateral), may well turn out to be very relevant for symmetric vs. asymmetric divisions of NE cells, as briefly addressed further below.

3.2. NE cell polarity

Like other epithelial cells, NE cells (including radial glial cells) are characterized by an apical–basal polarity. Besides the interkinetic nuclear migration along the apical–basal axis (Sauer, 1935; Murciano et al., 2002) and the localization of centrosomes beneath the apical surface (Chenn et al., 1998), this polarity is most obvious in the organization of the NE cell plasma membrane (Fig. 3A). Thus, consistent with the basal plasma membrane contacting the basal lamina, integrin α6 is concentrated in the basal plasma membrane (J. Koch and W.B.H., in preparation). Conversely, the apical plasma membrane (Fig. 3A, blue) selectively contains transmembrane proteins not found in the basolateral plasma membrane (Fig. 3A, red), such as prominin-1 (Weigmann et al., 1997). Even within the lateral plasma membrane, gradients of transmembrane proteins in the apical–basal direction can be detected, such as those of N-cadherin (Aaku-Saraste et al., 1996) or ephrin B1 (Stuckmann et al., 2001), which are more concentrated towards the apical end, and that of integrins, which are more concentrated towards the basal end (J. Koch and W.B.H., in preparation). Junctional complexes are found at the apical-most end of the lateral plasma membrane (Fig. 3A, black), and these recruit cytoplasmic proteins such as ZO-1, afadin/AF-6, ASIP/PAR-3/Bazooka, PAR-6 and aPKC (Aaku-Saraste et al., 1996; Zhadanov et al., 1999; Manabe et al., 2002). In other words, proteins concentrated at the apical cell cortex of *Drosophila* NBs

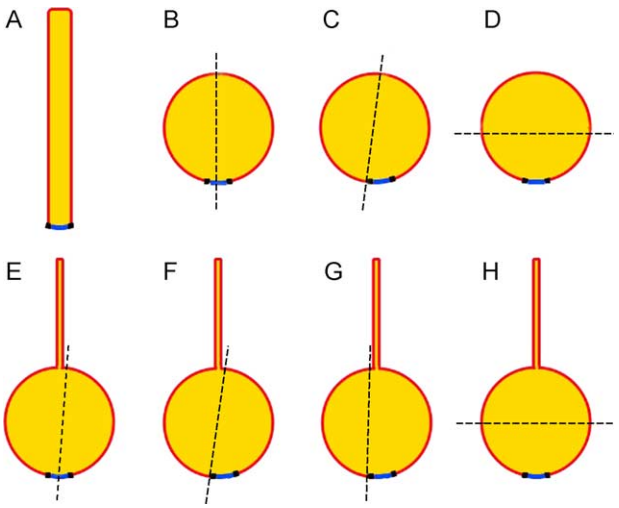

Fig. 3. Plasma membrane polarity, cleavage plane orientation and symmetric vs. asymmetric divisions of mammalian NE cells. (A) NE cell/radial glial cell in interphase; blue, apical plasma membrane; black, junctional complexes; red, basolateral plasma membrane. (B–H) Mitotic NE cells/radial glial cells; dashed lines, cleavage plane. (B–D) Mitotic NE cells without basal process, (E–H) mitotic NE cells/radial glial cells retaining a basal process. (B) Symmetric division, vertical cleavage plane. (C) Asymmetric division, vertical cleavage plane. (D) Asymmetric division, horizontal cleavage plane. (E) Asymmetric division, vertical cleavage plane; equal distribution of apical plasma membrane and junctional complexes but inheritance of basal process by only one of the daughter cells. (F) Asymmetric division, vertical cleavage plane; inheritance of basal process by one daughter cell and inheritance of apical plasma membrane and junctional complexes by the other daughter cell. (G) Asymmetric division, vertical cleavage plane; inheritance of apical plasma membrane, junctional complexes and basal process by only one of the daughter cells. (H) Asymmetric division, horizontal cleavage plane; inheritance of basal process by one daughter cell and inheritance of apical plasma membrane and junctional complexes by the other daughter cell.

(see Fig. 1) are also localized apically in mammalian NE cells.

In contrast to *Drosophila* NBs, however, mammalian NE cells do not delaminate when CNS neurons are being generated. Interestingly, however, prior to the onset of neurogenesis, NE cells loose tight junctions (TJ) (Aaku-Saraste et al., 1996) and down-regulate the apical vs. basal polarity of delivery of certain plasma membrane proteins (Aaku-Saraste et al., 1997). One may speculate that this down-regulation of certain epithelial features of NE cells may in some sense be equivalent to the *Drosophila* NB delamination. Remarkably, despite the loss of TJs, which are known to serve as a fence preventing the intermixing of integral membrane constituents between the apical and basolateral plasma membrane domains, NE cells, like delaminated *Drosophila* NBs, retain key features of polarity such as an apical-specific localization of centrosomes (Chenn et al., 1998) and prominin-1 (Weigmann et al., 1997); the retention of the latter in apical plasma membrane protrusions reflects its interaction with a cholesterol-based lipid microdomain (Röper et al., 2000; Corbeil et al., 2001). Moreover, the down-regulation of TJs is accompanied by an up-regulation of ZO-1, which appears to be associated with AJs (Aaku-Saraste et al., 1996). In contrast to the loss of TJs, interference with AJs by knocking-out afadin/AF-6, which is associated with these junctions in NE cells (Zhadanov et al., 1999; Manabe et al., 2002), perturbs the polarized organization of these cells, as evidenced by the mislocalization of prominin-1 to the basolateral plasma membrane (Zhadanov et al., 1999).

3.3. NE cell cleavage planes

Given the apical–basal polarity of mammalian NE cells (including radial glial cells), it has been proposed that, in analogy to *Drosophila* NBs, cleavage planes parallel to the apical–basal axis (vertical cleavages) will result in symmetric divisions of NE cells because critical apical and basal cell constituents (including cell fate determinants) would be distributed equally to the daughter cells (Fig. 3B), whereas cleavage planes perpendicular to the apical–basal axis (horizontal cleavages) will result in asymmetric divisions because the apical and basal constituents would be inherited by one or the other daughter cell (Chenn and McConnell, 1995) (Fig. 3D). Indeed, time-lapse microscopy revealed that the daughter cells arising from horizontally cleaving NE cells exhibit distinct behavior, i.e. an asymmetric fate, with the apical daughter likely remaining an NE cell and the basal daughter likely becoming a neuron, although this fate remained to be firmly established (Chenn and McConnell, 1995). Consistent with this concept, the proportion of horizontally cleaving NE cells has been reported to increase as more and more NE cells switch to generate neurons (Chenn and McConnell, 1995; Haydar et al., 2003).

However, in contrast to the delaminated *Drosophila* NB, where the plane of cell division is always perpendicular to the apical–basal axis, such an orientation of the cleavage plane (horizontal cleavage) (Fig. 3D) is observed only in a fraction of mammalian NE cells, which in most studies constitutes the minority of NE cells, even when neurogenesis is massively ongoing (Langman et al., 1966; Smart, 1973; Landrieu and Goffinet, 1979; Chenn and McConnell, 1995; Heins et al., 2001; Estivill-Torrus et al., 2002; Haydar et al., 2003). On the other hand, in the mammalian neuroepithelium, also only a certain fraction of NE cells undergoes neuron-generating divisions, with this fraction increasing as neurogenesis proceeds, whereas the other NE cells undergo proliferative divisions, generating more NE cells (including radial glial cells). Hence, an important issue has been to analyze cleavage plane orientation selectively in neuron-generating NE cells. The first molecular marker reported to be selectively expressed in neuron-generating, but not proliferating, NE cells is an antiproliferative gene called TIS21 in the mouse, PC3 in the rat and BTG2 in humans (Iacopetti et al., 1999). A recent analysis of mitotic TIS21-expressing vs. TIS21-non-expressing mouse NE cells during early neurogenesis has revealed that while horizontal cleavages are only found in neuron-generating, but not in proliferating, NE cells, they still constitute

the minority (<15%) of cleavage plane orientations even when only neuron-generating NE cells are being analyzed (Kosodo et al., 2002).

Given the scarcity of horizontal cleavages in mammalian NE cells, it has previously been postulated that vertical cleavage planes, which constitute 93% of all, and 86% of the neuron-generating, NE cell cleavages in the mouse embryo midbrain at E11.5 (Kosodo et al., 2002), can give rise to either (distribution-defined) symmetric (Fig. 3B) or asymmetric (Fig. 3C) divisions (Huttner and Brand, 1997). This hypothesis attributes particular significance to the apical–basal polarity of mammalian NE cells in the context of symmetric vs. asymmetric division. Specifically, the highly elongated shape of NE cells implies that the apical plasma membrane represents only a minute fraction (2–3%) of the total plasma membrane (Fig. 3A, blue). Hence not only horizontal, but even vertical cleavage planes may by-pass the apical plasma membrane and the junctional complexes at the apical-most end of the lateral plasma membrane, resulting in their asymmetric inheritance by only one of the daughter cells (Huttner and Brand, 1997) (Fig. 3C).

That vertical cleavages of NE cells can be neuron-generating has recently been shown in two time-lapse studies on the zebrafish retina (Das et al., 2003) and neural tube (Geldmacher-Voss et al., 2003), where essentially all cleavage planes were found to be parallel to the apical–basal axis of NE cells. Evidence in support for the above hypothesis has recently been provided by the observation that vertical cleavage planes may indeed bisect, or by-pass, the apical plasma membrane of mitotic mouse NE cells (Kosodo et al., 2002). Remarkably, ≈80% of the mitotic NE cells predicted to distribute apical plasma membrane to both daughter cells do not yet express the neurogenesis marker TIS21 and hence undergo proliferative divisions, whereas 90% of the mitotic NE cells predicted to distribute the apical plasma membrane to only one daughter cell do, indicative of neuron-generating divisions (Kosodo et al., 2002). These observations strongly suggest that there is some link between an equal vs. unequal distribution of apical plasma membrane (and the apical-most junctional complexes), i.e. a (distribution-defined) symmetric vs. asymmetric, vertical cleavage, and proliferative vs. neuron-generating divisions of mammalian NE cells, respectively.

Much remains to be learned about the mechanisms that determine cleavage plane orientation in mammalian NE cells. A primary factor determining the overall orientation such as vertical vs. horizontal will, of course, be the position of the mitotic spindle, but how this is controlled in mammalian NE cells is unknown. Consistent with observations made in the *C. elegans* embryo on the positioning of the spindle poles (Gonczy et al., 2001), the spindle poles in mitotic NE cells appear to oscillate around their final positions prior to anaphase, and this oscillation is greater for horizontal than vertical cleavages (Haydar et al., 2003).

A gene that deserves comment in this context is *LIS1*. Mutations in the human *LIS1* gene are responsible for a certain form (type I) of lissencephaly, a severe malformation of the brain ('smooth brain') (Olson and Walsh, 2002). Interestingly, the LIS1 protein can exist in a complex with cytoplasmic dynein and dynactin, and its overexpression leads to misorientation of the mitotic spindle (Faulkner et al., 2000). LIS1 has therefore been implicated in the interaction of mitotic microtubules with kinetochores and the cell cortex, and mutations in the *LIS1* gene may affect NE cell cleavage plane orientation, with consequences for the balance between NE cell proliferation vs. differentiation (Faulkner et al., 2000).

Transcription factors have been reported to affect cleavage plane orientation. Thus, NE cells of mice lacking the transcription factor Emx2 show a greater incidence of horizontal cleavage planes (Heins et al., 2001). The same is true for NE cells of mice lacking functional Pax6, a homeodomain transcription factor (Estivill-Torrus et al., 2002). These data indicate that Emx2 and Pax6 contribute to maintaining the normally predominantly vertical cleavage plane orientation, but the underlying mechanisms remain to be elucidated.

3.4. Asymmetrically distributed cellular components, including cell fate determinants

What are the cell constituents of the apical plasma membrane and the junctional complexes whose distribution appears to be so critical for determining whether both, or only one, of the NE cell daughters remain(s) an NE cell?

Apical Plasma Membrane (Fig. 3B–H, blue). Although no membrane protein of the apical plasma membrane of NE cells has yet been shown to affect NE cell fate, several scenarios can be envisaged, including the ones briefly outlined below. Common to all is the assumption that the presence of certain apical plasma membrane components somehow maintains the cell cycle, and hence the inheritance of these by both daughter cells would keep both of them in the cell cycle whereas lack of inheritance by one daughter would turn this cell into a post-mitotic neuron. First, prominin-1 itself, which in NE cells is selectively localized to the apical, but not basolateral, plasma membrane domain, may have a key role. Prominin-1 is expressed on various somatic stem cells, notably neural and hematopoietic stem cells (Corbeil et al., 2001), consistent with it being involved in maintaining the cell cycle of undifferentiated cells. Second, the lumen of the neural tube may contain a mitogenic factor and the apical plasma membrane the receptor for this factor. Third, the organization of the apical plasma membrane, for example in terms of lipid rafts (Simons and Ikonen, 1997; Röper et al., 2000), may be unique in promoting the maintenance of the cell cycle.

Junctional Complexes (Fig. 3B–H, black). Several of the components associated with the apical junctional complexes in the *Drosophila* neuroectoderm and the apical plasma

membrane of the delaminated neuroblast, i.e. ASIP/Bazooka/PAR-3, PAR-6 and aPKC, are concentrated at AJs of mammalian NE cells, together with ZO-1, afadin/AF-6, β-catenin and δ-catenin (Aaku-Saraste et al., 1996; Zhadanov et al., 1999; Ho et al., 2000; Manabe et al., 2002). To our knowledge, direct evidence showing that an unequal distribution of any of these components to only one of the daughter cells is associated with asymmetric cell fate has not yet been reported. It is worth pointing out, however, that the apparent increase in NE cell proliferation observed in transgenic mouse embryos expressing a constitutively stabilized β-catenin in these cells (Chenn and Walsh, 2002) would be consistent with a mitogenic signal originating from junctional complexes. These complexes are localized to the apical-most end of the lateral membrane of NE cells, and with regard to their distribution upon mitosis, similar considerations apply as for the apical plasma membrane (Fig. 3B–H, black). If a mitogenic signal were to originate from junctional complexes and mediated by β-catenin, their inheritance by both daughter cells would result in both of them continuing to divide, whereas inheritance by only one daughter cell would turn the other into a post-mitotic neuron; expression of constitutively stabilized β-catenin might override this distribution-based control mechanism.

The Basal Process (Fig. 3E–H). Given that the overwhelming majority of cleavage planes of mammalian NE cells show a vertical orientation, we have so far discussed the equal vs. unequal distribution of cellular components concentrated at the *apical* end of the mitotic cell. However, it appears equally important to consider the *basal* end of mitotic NE cells. Recent studies on the generation of neurons from radial glial cells, which (as stated above) can be regarded as the most epithelial type of mammalian NE cells (Huttner and Brand, 1997), as well as on neuroepithelial cells in the zebrafish retina, have revealed that these cells do not completely round up in mitosis but retain a very thin basal process throughout cytokinesis (Miyata et al., 2001; Noctor et al., 2001, 2002; Das et al., 2003; Fishell and Kriegstein, 2003). The available evidence indicates that this thin basal process is inherited by only one daughter cell (Miyata et al., 2001; Noctor et al., 2001, 2002; Das et al., 2003; Fishell and Kriegstein, 2003) and hence divisions of NE cells retaining a basal process are by definition distribution-wise asymmetric (Fig. 3E–H). Given the contact of the basal-most plasma membrane of NE cells (including radial glial cells) with the basal lamina, critical cellular components concentrated at the end of the basal process which would be distributed unequally upon mitosis include, for example, integrins and their downstream signal transduction machinery.

Does this imply that all divisions of NE cells are distribution-wise asymmetric? It should be noted that the observations showing that mitotic NE cells retain a basal process have all been made *after* the onset of neurogenesis. *Prior* to the onset of neurogenesis, NE cells proliferate, which eventually leads to their elongation along the apical–basal axis (Huttner and Brand, 1997). This raises the possibilities that NE cells prior to the onset of neurogenesis, being less elongated, either do not retain a basal process but truly round up in mitosis (Fig. 3B–D), or retain a relatively thicker process that is bisected by the cleavage furrow (not illustrated), and therefore are able to execute distribution-wise symmetric divisions.

Numb. One of the paradigms of a protein whose asymmetric distribution upon mitosis is intimately linked to the asymmetric division of *Drosophila* NBs is the intrinsic cell fate determinant Numb (see above). In vertebrate NE cells, however, the role of Numb and the related protein Numblike appear to be more complex. While it is clear that Numb in principle shows a polarized distribution in mitotic NE cells and determines neural cell fate, it has been reported to be concentrated either apically or basally depending on the species, and to promote progenitor fate or neuronal fate depending on the developmental stage and experimental manipulation (Wakamatsu et al., 1999; Zhong et al., 2000; Cayouette et al., 2001; Zilian et al., 2001; Petersen et al., 2002; Shen et al., 2002; Silva et al., 2002; Dooley et al., 2003). We shall not discuss the complexity of this issue in further detail, as this has been competently reviewed recently (Zhong, 2003). What should be added, however, is that the polarized (either apical or basal) distribution of Numb in mitotic NE cells as reported (Wakamatsu et al., 1999; Cayouette et al., 2001; Silva et al., 2002), i.e. with a crescent corresponding to a substantial portion of the entire cell cortex, is unlikely to lead to its inheritance by only one of the daughter cells, as is the case for *Drosophila* NBs (see above), because with the vast majority of mammalian NE cells showing an essentially vertical cleavage plane, both daughter cells should inherit Numb. Given the proposed role of Numb in the endocytosis, and thus down-regulation, of the Notch receptor (Shen and Temple, 2002), it may well be necessary to first dissect Notch internalization in the context of apical versus basolateral early endosomes of NE cells before the precise role of Numb in neurogenesis will be understood mechanistically.

Minibrain/DYRK1A. Another protein recently shown to be unequally distributed between the daughter cells arising from the mitosis of NE cells is Minibrain, also referred to as DYRK1A (*D*ual-specificity tyrosine (*Y*)-*R*egulated *K*inase) (Hämmerle et al., 2002). Interestingly, both the minibrain mRNA and protein show an unequal distribution upon mitosis of NE cells (Hämmerle et al., 2002). In *Drosophila*, Minibrain has been implicated in neurogenesis (hence its name) (Tejedor et al., 1995). In humans, Minibrain maps to the Down's syndrome critical region on chromosome 21 (Guimera et al., 1996; Shindoh et al., 1996; Song et al., 1996). Minibrain knock-out mouse embryos show an overall reduction in organ growth, including the developing brain where neurogenesis appears to be decreased or

delayed (Fotaki et al., 2002). How exactly the Minibrain kinase regulates neurogenesis, and what the significance of its asymmetric distribution upon mitosis of NE cells is, remains to be established.

PTEN. While the above date on Minibrain implicate *protein* phosphorylation–dephosphorylation, a major regulatory mechanism of cells, in neurogenesis, evidence is also emerging for a role of *lipid* phosphorylation–dephosphorylation. Specifically, the phosphoinositide 3′ phosphatase PTEN, which in *Drosophila* NBs has been shown to be part of the apical protein complex involved in asymmetric cell division (W. von Stein, A. Ramrath and A.W., unpublished), appears to negatively regulate mammalian NE cell proliferation. Mice lacking PTEN expression in NE cells and their progeny exhibit enlarged brains, which (besides larger cell size and decreased apoptosis) is due to a faster progression of neural progenitors through the cell cycle (Groszer et al., 2001). Extrapolating from *Drosophila* NBs, PTEN may well exhibit a polarized localization in mammalian NE cells and be unequally distributed upon their mitosis, but how this would be linked to cell cycle control is unclear at present.

Cell Cycle Regulators. Given that neurons are postmitotic whereas their progenitors are not, cell cycle regulators should be obvious candidates to exert key roles in cell fate determination and to be subject to unequal distribution upon asymmetric divisions of mammalian NE cells. Yet, we are not aware of a single study in which the distribution of cell cycle regulators in mitotic NE cells has been examined.

3.5. Planar polarity and asymmetric division of mammalian NE cells

All of the above discussion of symmetric vs. asymmetric division of mammalian NE cells has dealt with cell constituents showing a polarized intracellular distribution with regard to the apical–basal axis of NE cells. It is important to consider that NE cells may also exhibit polarity in the other two dimensions, which may reflect the patterning of the neural tube along the principal body axes, (i) the anterior–posterior axis and (ii) the dorsoventral or medio-lateral axis (depending on whether an NE cell has a lateral or dorsal/ventral location, respectively). In other words, in mitotic mammalian NE cells, intrinsic cell fate determinants may be differentially localized on the lateral cell cortex, being concentrated, for example, towards the anterior or dorsal pole of the embryo. We are not aware that such a differential lateral localization, as observed during development of the *Drosophila* peripheral nervous system (see above), has yet been reported for mammalian NE cells. However, the recent observation (Das et al., 2003) that during the development of the zebrafish retina, vertical cleavages of NE cells shift, by a 90° rotation within the plane of the neuroepithelium, from the daughters having central–peripheral positions to them having circumferential positions, indicates the existence of a machinery controlling cleavage plane orientation in a spatial dimension that would be consistent with such a scenario.

4. Conclusions

Comparison of the cell biology of asymmetric divisions of neural progenitors between *Drosophila* and mammals reveals both similarities and differences. Asymmetric division requires cell polarity, and foremost amongst the similarities between *Drosophila* and mammals is the conservation of the principle that a cleavage plane perpendicular to, or at least distinct from, the axis of a polarly localized cell fate determinant will result in an asymmetric division. Another notable commonality between *Drosophila* and mammals is the conservation of many key players involved in asymmetric division that contribute to the polarized organization of the progenitor cells and/or exhibit a polarized distribution within them. Striking differences between *Drosophila* NBs and mammalian NE cells include the lack, in mammals, of delamination and of the need of rotating the cleavage plane by 90° to switch from symmetric, proliferative to asymmetric, neuron-generating divisions. The main challenge for future research will be to understand, at the mechanistic cell biological level, how gene products with critical roles in the control of progenitor proliferation vs. differentiation, identified by genetic or genomic approaches, become differentially expressed between individual progenitor cells and execute their function.

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