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Cell Division Orientation in Animals

Review

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Cell division orientation during animal development can serve to correctly organize and shape tissues, create cellular diversity or both. The underlying cellular mechanism is regulated spindle orientation. Depending on the developmental context, extrinsic signals or intrinsic cues control the correct orientation of the mitotic spindle. Cell geometry has been known to be another determinant of spindle orientation and recent results have shed new light on the link between cellular shape and cell division orientation. The importance of controlling spindle orientation is manifested in neurodevelopmental defects such as microcephaly, tumor initiation as well as defects in tissue architecture and cell fate misspecification. Here, we summarize the role of oriented cell division during animal development and also outline the cellular and molecular mechanisms in selected invertebrate and vertebrate systems.

Introduction

The orientation of the division axis is a basic regulator of metazoan development. Oriented cell division serves two purposes: first, to elongate cell sheets and shape tissues and, second, to generate cellular diversity. In order to achieve these two mutually non-exclusive functions, the orientation of the mitotic spindle has to be controlled. Over 120 years ago, Oscar Hertwig [1] recognized that cell shape is a determinant of spindle orientation and cell division orientation. He was among the first to discover that cells divide along their long cell axis, an observation known as the 'long axis rule'. Cell shape has been considered to be a default mechanism of spindle orientation [2,3]. However, several cell types override the cell shape pathway and control spindle orientation through external or internal polarity cues [4,5].

In this review, we first describe oriented cell division in different developmental contexts by outlining some of the classic and emerging model systems. We further summarize the underlying molecular and cellular mechanisms and highlight recent reports showing how external cues affect cellular shape (and thus oriented cell division) and how external or internal cues are linked to the mitotic spindle.

Shaping Tissues and Organs

Studies performed in zebrafish (*Danio rerio*) have made significant contributions to our understanding of oriented cell division during animal development. Analyzing the patterns of cell division within the surface layer of the epiblast during zebrafish gastrulation revealed that in the dorsal region of the midline, and later in the ventral region, cell divisions are highly oriented along the animal-vegetal axis [6–8]. Similarly, the development of the immature epithelium of the zebrafish neural keel, which forms a lumenized neuroepithelium, depends on stereotyped oriented cell divisions, generating two bilaterally distributed neural progenitors. As these progenitors divide, one cell is placed on the ipsilateral side of the neural keel and its daughter intercalates across the midline integrating into the contralateral neuroepithelium (Figure 1A) [6,7,9–13]. Cell division is required for neural keel development, and blocking cell division will prevent the majority of cells from crossing the midline [12]. The correct cell division orientation is achieved through a 90 degree rotation of the mitotic spindle (Figure 1A) [9,11]. Disruption of division orientation in the neuroepithelium results in mostly unilateral placement of progenitors in the neural tube [11].

Similarly, in embryos of the frog *Xenopus laevis* it was shown that cells divide in three different manners in relation to the embryonic surface: parallel, oblique and perpendicular. The majority of divisions take place parallel to the surface. However, from the 32-cell stage onward, perpendicular cell divisions occur, resulting in the generation of deeper cells. An isolated 64-cell stage blastomere dividing in culture can generate a superficial cell that will express the bHLH gene *ESR6e* and a sibling cell that does not. Although the distribution of perpendicular divisions appears to differ between embryos and the division angle is not fixed between successive divisions, there is a strong correlation between cell division orientation and the generation of molecularly distinct deep and superficial cell layers [14].

Oriented cell division has also been observed in invertebrate species. During early *Drosophila melanogaster* embryogenesis the germband extends and elongates. During the fast phase of the elongation process cells divide preferentially along the anterior-posterior axis, corresponding to the long axis of the extending tissue. Blocking cell division does not completely prevent tissue elongation but reduces the amount of extension. Furthermore, mutant embryos lacking segmental patterning show randomized spindle orientation and isotropic increase in tissue size [15]. Although the above experiments suggest that oriented cell division is involved in germband extension, the overlap between cell division and morphogenetic movements makes it difficult to discern the individual contributions of these two processes to *Drosophila* embryogenesis.

Recently, it was shown that organ development can also be controlled, at least in part, through oriented cell division. *Drosophila* imaginal discs are epithelial structures originating from the embryonic ectoderm developing into adult organs such as the wing, legs and compound eyes. In wing and eye imaginal discs, a striking correlation between the shape of labeled clones of cells and the orientation of cell division has been described [16,17] (Figure 1B). Measuring spindle orientation in the wing blade revealed that the majority of divisions are oriented along the proximal-distal axis [16]. The correlation between oriented cell divisions and the shape of clones is maintained throughout wing development. Analysis of two- and four-cell clone clusters revealed that cell relocation plays a minor role in defining clonal shape. Measurements of division orientation outside the wing blade or in the eye disc showed similar results. Thus, oriented cell division appears to be a general mechanism to shape organs during fly organogenesis [16,17].

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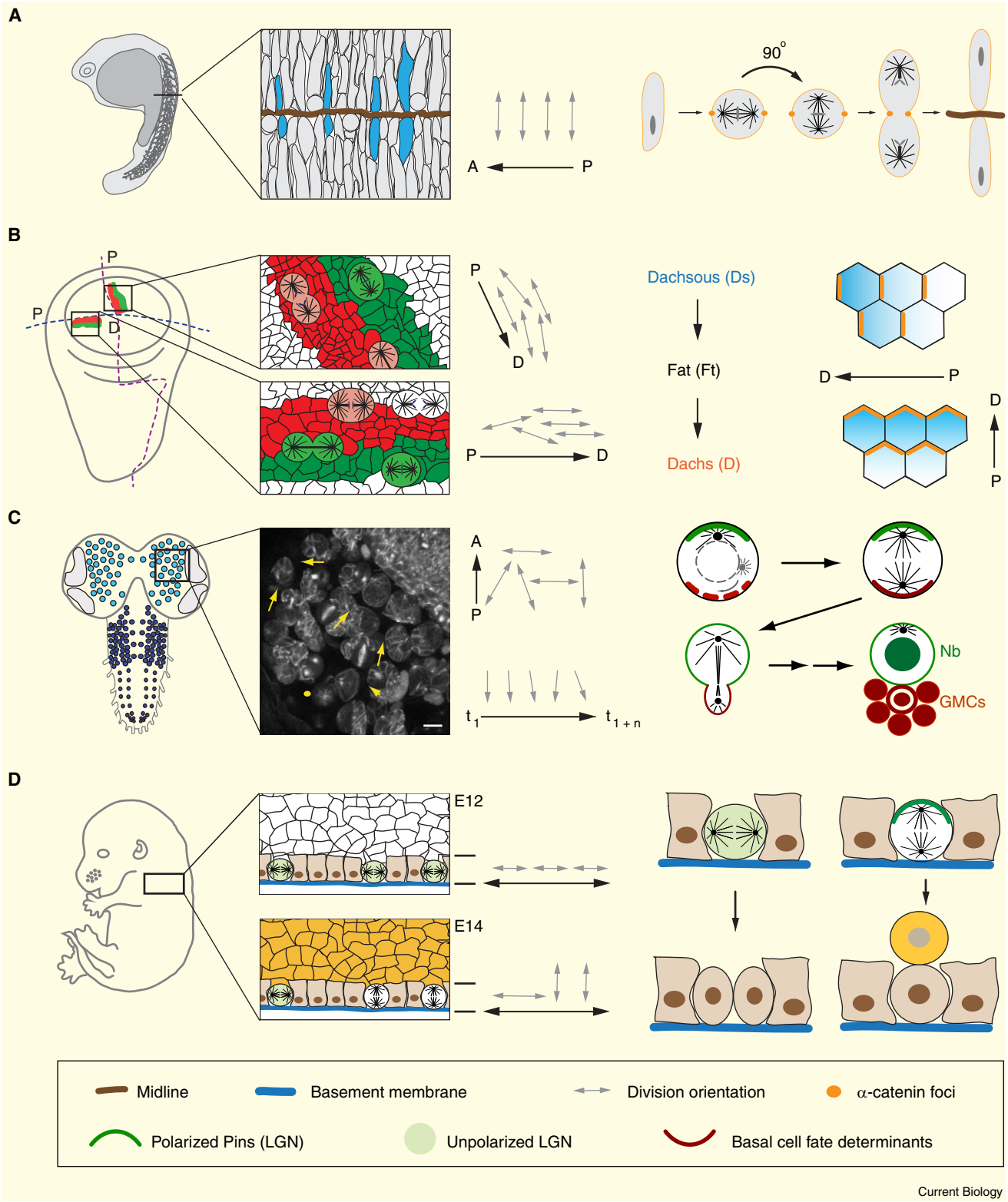

Figure 1. Cell division orientation in model systems.

(A) Zebrafish neural keel development. Neural progenitors divide perpendicular to the midline (brown line) and position sibling cells on both sides of the midline (four representative cell pairs are highlighted in blue). The stereotypic division axis is indicated by grey arrows. The mitotic spindle undergoes a 90 degree rotation, positioning the cell division orientation perpendicularly to the midline. (A, anterior; P, posterior). (B) *Drosophila* wing imaginal disc. Mitotic twin spot clones close to the anterior-posterior (dashed purple line) and dorsal-ventral (D-V) axis (dashed blue line) are indicated in green and red, respectively. Clones show elongated shape based on cell division orientation respecting the proximal-distal (P-D) axis. The stereotypic division axis in boxed regions is indicated with grey arrows. Dachsous (Ds) is expressed in a gradient along the P-D axis. Dachs

These examples illustrate how oriented cell division acts as a nearly ubiquitous morphogenetic force in multiple species. As we will see in the next paragraph, a different form of oriented cell division, asymmetric cell division, is iteratively used during development and across species.

Generating Cellular Diversity

Asymmetric cell division generates cellular diversity [18]. This is achieved through the asymmetric partitioning of cell fate determinants, resulting in the generation of molecularly distinct sibling cells. An important aspect of asymmetric cell division is the correct alignment of the mitotic spindle in relation to an axis of internal or external polarity, ensuring asymmetric segregation of cell fate determinants. Asymmetric cell division has been studied in great detail and to great effect in model organisms such as the early *Caenorhabditis elegans* embryo [19–21], *Drosophila* neuroblasts [18] and *Drosophila* sensory organ precursor (SOP) cells [22].

Shortly after fertilization, the early *C. elegans* embryo becomes polarized — a prerequisite for the correct orientation of the mitotic spindle. Spindle orientation consists of two phases: first, during prophase, the nucleus–centrosome complex moves to the cell center and undergoes a 90 degree rotation; second, during metaphase and anaphase, the spindle is pulled towards the posterior of the cell. Spindle rotation and displacement require interactions between the mitotic spindle and the cortex and are dependent on intrinsic polarity cues [5,23]. Proper spindle orientation also ensures the correct segregation of cell fate determinants. While the anterior cell inherits cell fate determinants, the zinc-finger proteins MEX-5/MEX-6, another set of zinc-finger proteins, MEX-1, PIE-1 and POS-1, are partitioned asymmetrically into the posterior cell [20,24].

Similarly, *Drosophila* neural stem cell-like cells, called neuroblasts, orient their mitotic spindle along an established internal polarity axis. Stereotypic spindle orientation ensures that cell fate determinants, such as the coiled-coil protein Miranda (Mira), the transcriptional repressor Prospero (Pros; Prox1 in vertebrates), Numb (Numbl in vertebrates), Partner of Numb (Pon) and Brain tumor (Brat), are asymmetrically segregated into a small differentiating ganglion mother cell (GMC), while retaining a self-renewed apical neuroblast [18,25] (Figure 1C). Controlling the orientation of the mitotic spindle is required for the correct segregation of cell fate determinants [26]. As in *C. elegans*, delaminated embryonic neuroblasts rotate their mitotic spindle by 90 degrees and divide perpendicular to the neuroectoderm, which places the newly born GMCs into deeper tissue layers [27,28]. However, spindle rotation occurs only during the first neuroblast cell cycle after delamination [28]. From the second cell cycle onwards, one centrosome remains attached to the apical cortex, predetermining the future orientation of the mitotic spindle [28–30]. Elegant cell dissociation experiments showed that embryonic neuroblasts associated with neuroepithelial cells maintain their division axis over successive rounds of divisions; however, unassociated neuroblasts divide along random division axes [31]. Thus, in addition to neuroblast intrinsic polarity cues, some unknown extrinsic factors are required to maintain neuroblast division orientation in the fly embryo [31]. Surprisingly, live imaging experiments performed in intact larval brains or dissociated larval neuroblasts did not suggest a requirement for an extracellular signal to orient the mitotic spindle along a 'global' tissue axis [29]. Nevertheless, individual larval neuroblasts repetitively divide along the same axis with minor deviations, ensuring that sibling cells are positioned on the basal side of the neuroblast exclusively [26,32] (Figure 1C).

Switching the Division Axis to Generate Cellular Diversity

Cell division orientation can be switched during development. The switch from symmetric, proliferative divisions towards asymmetric, diversifying ones occurs in several different cell types.

Mammalian skin epidermis is a stratified epithelium. Stratification occurs through a change in the division axis. Before that, proliferative basal cells predominantly divide within the plane of the epithelium. In mice, around embryonic day 15 basal cells change their division orientation and start dividing perpendicular to the underlying basement membrane. This orientation will place one sibling, the suprabasal cell, into deeper layers of the epithelium and away from the underlying basement membrane (Figure 1D). Perpendicular divisions are also associated with a change in cell fate as the suprabasal cells express differentiation markers not seen in proliferative basal cells. Thus, the stratification of the mammalian epidermis consists of two phases: a proliferative, amplification phase in which symmetric divisions increase the surface area of the epithelium, followed by an asymmetric division phase generating distinct molecular identities [33–35]. Elegant lineage tracing experiments in mice further revealed that epidermal cells are not committed to one type of division but can change between symmetric and asymmetric divisions [35] (Figure 1D).

A similar situation is found in the vertebrate neuroepithelium. These columnar epithelial cells function as neural progenitors, extending from the apical to the basal surface of the cortex. In metaphase, they round up and divide at the apical side. Neuroepithelial cells initially expand their population during cortical development through symmetric divisions before they switch to an asymmetric division mode, giving rise to a self-renewing neural progenitor and a differentiating neuron [36–38]. Recent live imaging studies in mice revealed that the majority of divisions occur in the plane of the epithelium. However, randomization of the division plane in LGN mutant mice results in the loss of apically located neural progenitors [39]. In the chick neuroepithelium, it was found that randomizing spindle orientation does not affect cell fate but leads to a premature loss of neuroepithelial cells from the apical cortex; they become localized to the mantle zone where they proliferate aberrantly [40].

(D) localization depends on the orientation of the Ds gradient. (C) Neuroblasts in the larval *Drosophila* CNS (dark and light blue dots). Snapshot showing dividing neuroblasts in the larval brain. Several division axes are highlighted with a yellow arrow. Note that the division axis is random in relation to the A-P axis (yellow arrows and grey arrows on top), but is fixed with respect to the time axis (grey arrows below). Schematic neuroblast (Nb) showing the apical (green) and basal (red) polarity complexes. Neuroblasts divide asymmetrically, resulting in an apical self-renewed neuroblast and a basal ganglion mother cell (GMC). (D) Mouse epidermis. At embryonic day 12, basal cells divide preferentially within the plane of the epithelium. From embryonic day 15.5 onwards, perpendicular asymmetric cell divisions occur, producing differentiating siblings (yellow). LGN protein (green) is diffusely localized (light green throughout the cell) in symmetrically dividing basal cells but becomes asymmetrically localized (green crescent) from embryonic day 15.5 onwards, inducing asymmetric cell division.

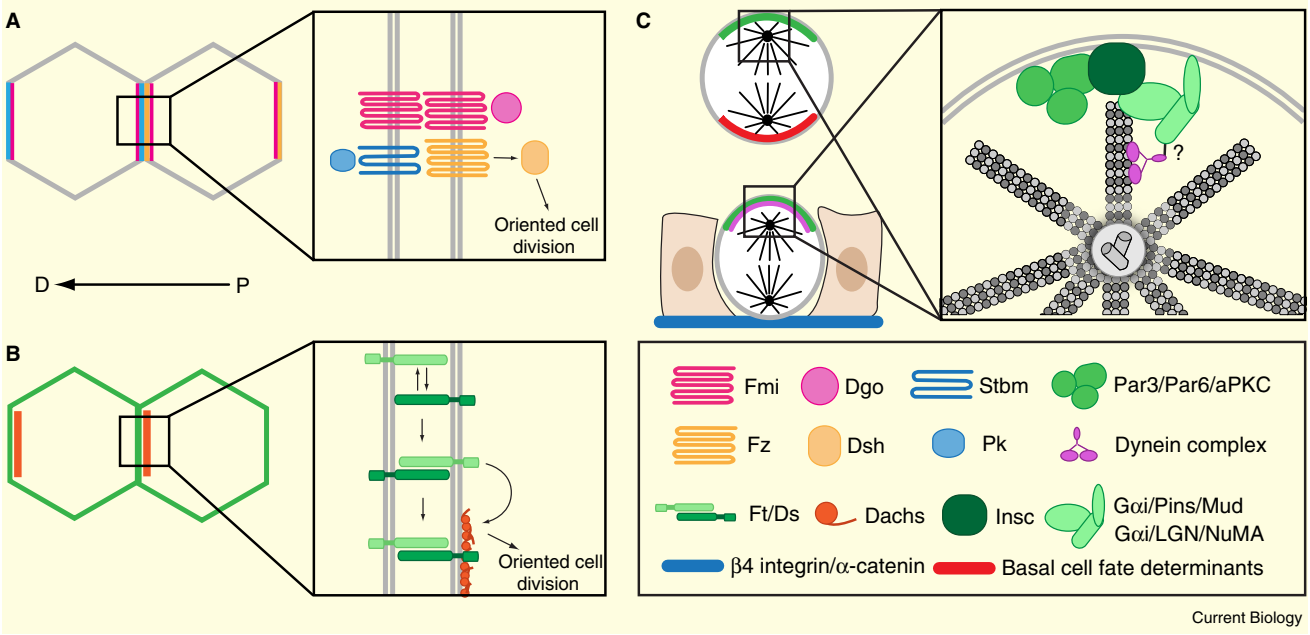

Figure 2. Planar cell polarity pathways and cell intrinsic polarity pathway in oriented cell division.

(A) The core planar cell polarity (PCP) pathway. The seven-pass transmembrane protein Frizzled (Fz) and the cytoplasmic proteins Dishevelled (Dsh) and Diego (Dgo) are localized to distal cell junctions. Strabismus (Stbm), another transmembrane protein, and the cytoplasmic protein Prickle (Pk) are localized to proximal cell junctions, while Flamingo (Fmi) is localized distally and proximally. The core PCP signaling pathway can be used for oriented cell division. (B) Fat/Dachsous system. Fat (Ft) and Dachsous (Ds) are large, atypical cadherins that interact heterophilically at cell junctions. A downstream effector of the Ft/Ds pathway, the atypical myosin Dachs, localizes to distal cell junctions and directs oriented cell division by influencing cell shape. (C) Pins/Mud/Gαi (in *Drosophila*), or LGN/NuMA/Gαi (in vertebrates) pathway. Asymmetric localization of Pins/LGN, Mud/NuMA, and Gαi directs spindle orientation in *Drosophila* neuroblasts and mammalian basal cells. The dynein complex, a minus-end directed microtubule motor protein complex, has been proposed to act as a force generator in both basal cells and neuroblasts. The Pins–Mud–Gαi, and LGN–NuMA–Gαi complexes, respectively, are connected to the Par3–Par6–aPKC apical polarity complex via Insc.

Interestingly, studies in mice further revealed that Lissencepaly1 (Lis1), its upstream regulator Magoh (a component of the exon junction complex (EJC)) and abnormal spindle-like microcephaly associated protein (ASPM) are all required to control spindle orientation in neuroepithelial stem cells [41–43].

The consequence of disrupted spindle orientation on cellular fate is controversial, as experimentally randomizing spindle orientation in neural progenitors yielded varying results. However, it is clear that neural progenitors tightly control the switch from proliferative to differentiating divisions.

Mechanism of Cell Division Orientation: Extracellular Cues

Based on the diversity of cell types undergoing oriented cell division, the following immediate questions arise: what are the cellular and molecular determinants regulating correct cell division orientation, and how do these determinants differ between cell types and species?

Oriented cell division relies on the orientation of the mitotic spindle. Spindle orientation can be controlled through extrinsic factors, cell intrinsic molecules or physical constraints such as cell shape and cellular environment. During zebrafish gastrulation, cells of the epiblast give rise to the neural ectoderm on the dorsal side of the epidermis and the epidermis on the ventral side [10,44]. Blocking of the DEP-domain protein Dishevelled (Dsh) results in randomization of cell division orientation in all layers of the epiblast. In addition, the polarity of elongation of these cells is also affected [8]. The effect of Dsh knock-down is independent of convergent extension and acts cell-autonomously based on mosaic experiments. Dsh functions in multiple Wnt pathways such as the canonical Wnt/β-catenin pathway and the core planar cell polarity (PCP) pathway. Blocking canonical Wnt signaling did not affect oriented cell division. Conversely, inhibiting the two PCP ligands Wnt11 or Wnt5 partially affected oriented cell division, suggesting that they both act in parallel [8]. Furthermore, zebrafish lacking Fz7, the receptor for Wnt11 [45], and Stbm show disrupted oriented cell division [6,8]. Although these experiments demonstrated that PCP signaling is instrumental for oriented cell division, PCP-controlled oriented cell division has neither an instructive nor permissive role in body-axis elongation [6].

PCP signaling seems to be a general pathway to orient cell divisions within tissues and there are two separate but interconnected PCP pathways: the ‘core PCP’ pathway and the Fat/Dachsous (Ft/Ds) system, an overlapping local alignment pathway [46] (Figure 2A,B). The Ft/Ds system has been shown to be required for oriented cell division in the developing fly wing [16] (Figure 1B). Clones of cells lacking Dachsous or Fat protein show a rounded morphology as opposed to elongated wild-type clones; similar results have also been obtained if Dachsous or Fat are expressed ectopically. The cellular basis for this phenotype is a loss of oriented cell division [16,47]. Dachsous is expressed in a gradient along the proximal-distal axis in response to cues emanating from the compartment boundaries [48,49]. Dachsous is the ligand for Fat [50] and transduces the signal to the atypical myosin Dachs (D) (Figure 2B). Dachs is localized to the distal

side of each cell's apical surface axis in response to the Dachsous gradient, corresponding to division orientation in the developing fly wing [17,48] (Figures 1 and 2). Mutant *dachs* clones showed rounded morphology and randomized spindle orientation similar to *fat* or *dachsous* mutant clones. Furthermore, Dachsous is sufficient to change oriented cell division: reorienting Dachsous localization through genetically altering the direction of the Dachsous gradient changed Dachsous localization within cells and, as a consequence, altered the direction of oriented cell division [17]. These experiments strongly suggest that *Drosophila* organ shape is dependent on oriented cell division, regulated through the Ft/Ds PCP signaling system.

How does PCP signaling control the orientation of the mitotic spindle? Before we review literature addressing this question, we will summarize how cell intrinsic polarity is linked to spindle orientation and oriented cell division.

Mechanism of Cell Division Orientation: Intrinsic Cues

Cell intrinsic polarity is a prominent feature of asymmetrically dividing cells (Figure 2C). The link between cell intrinsic polarity and spindle orientation, and thus oriented cell division, is best understood in invertebrate model systems. As this topic was the subject of a recent review [5], we will just provide a brief synopsis and then highlight similarities to vertebrate systems.

Genetic and molecular analyses in *C. elegans* zygotes revealed that cortical polarity is required for spindle positioning. In addition, laser severing experiments demonstrated that pulling forces on the posterior pole are larger than on the anterior, resulting in a displacement of the spindle towards the posterior pole [51–53]. This anaphase spindle positioning, as well as centration and rotation of the nucleus–centrosome complex, is controlled by the minus-end microtubule motor dynein (together with its components dynactin/Lis1). This molecular motor provides the pulling force and binds to cortically localized Lin-5 (nuclear mitotic apparatus (NuMA) in vertebrates). Lin-5 is found in a protein complex with the cortical proteins GPR1/2 (LGN/AGS3) and G-protein α-subunit (Gα) [54–57]. As the GPR1/2/Lin-5/Gα complex is asymmetrically localized, pulling forces differ between the anterior and posterior ends of the worm embryo, resulting in a displacement of the mitotic spindle [5,58,59].

Similarly, in *Drosophila* neuroblasts, lack of the NuMA orthologue *mushroom body defect* (*mud*) results in misaligned spindles in relation to the internal polarity axis; *mud* mutant neuroblasts are properly polarized. It was further shown that Mud is localized to the apical and basal cortex and to both centrosomes [60–62]. However, as Mud only co-localizes with Pins (LGN/AGS3 in vertebrates) on the apical cortex, the prevailing models propose that the apically localized Pins/Mud/Gαi complex is involved in neuroblast spindle orientation through interactions with dynein [60–62]. A physical connection between dynein and Mud has not been reported in *Drosophila* neuroblasts but genetic analysis revealed that mutations in the two dynein-complex proteins Lis1 and Glued result in spindle orientation defects similar to *mud* mutants [63]. Furthermore, dynein has been shown to physically interact with NuMA in *Xenopus* [64]. These results suggest that the *Drosophila* and *C. elegans* NuMA orthologues (Mud and Lin-5, respectively) are key effector proteins controlling the alignment of the mitotic spindle in relation to cell intrinsic polarity cues (Figure 2C) [5].

Recently, it was shown that a similar pathway is also used for spindle orientation in mammalian cells. In mitotic basal cells, NuMA has been found to be localized to spindle poles but also forms a polarized cortical crescent co-localizing with LGN, Inscuteable and Par3. This complex is localized opposite of integrins and the basement membrane. Furthermore, NuMA co-localizes with the p150glued subunit of the dynactin complex [34]. Neither NuMA nor LGN require microtubules for their localization to the cortex, since depolymerizing microtubules does not alter NuMA's cortical localization. Instead, the basement membrane component β1 integrin and α-catenin are required for LGN and NuMA localization [34]. LGN is also required for cortical NuMA localization but LGN does not depend on NuMA; the same relationship has been reported for the *Drosophila* orthologues Mud and Pins in neuroblasts (Figure 2C) [5,33,60,61,65].

How is the switch between symmetric and asymmetric spindle orientation controlled? Basal epidermal cells do not display predetermined spindle orientation before metaphase. During prometaphase, spindle orientation is still random but by metaphase the mitotic spindle aligns either perpendicularly or in parallel to the basement membrane, indicating that the spindle rotates into its final position [35]. Furthermore, in mice, LGN protein shows diffuse localization in interphase or symmetrically dividing basal cells but changes towards apical localization from embryonic day 15.5 onwards. Metaphase spindles are further aligned perpendicular to the LGN crescent [34]. Knock-down of NuMA, LGN or dynactin randomizes spindle orientation in basal cells and shifts the balance from asymmetric towards symmetric divisions [33,34]. These results suggest that the controlled asymmetric localization of LGN (together with NuMA and the dynein complex) determine spindle orientation and thus the switch from proliferative symmetric towards asymmetric basal cell division in the developing skin epidermis (Figures 1D and 2C). This interpretation is validated by findings from *Drosophila* neuroblasts, where lack of the NuMA orthologue Mud results in symmetric neuroblast divisions generating two neuroblasts as opposed to one neuroblast and a differentiating GMC [26]. However, single-cell analysis would be required in mammalian skin to truly demonstrate the causal relationship between spindle orientation and cell fate changes.

Vertebrate neuroepithelial cells also switch from symmetric to asymmetric division modes in order to create differentiating neurons while maintaining a self-renewed neural progenitor [37,66]. How is spindle orientation controlled in the neuroepithelium? Live imaging of chicken neuroepithelial cells revealed that mitotic spindles display a dynamic but stereotypic behavior: during early metaphase, spindles orient themselves parallel to the apical surface. This planar orientation is maintained during late metaphase and anaphase while the spindle is free to revolve randomly around the apical-basal axis [65]. Orienting the mitotic spindle into the plane parallel to the apical surface requires NuMA, LGN and Gαi. Both NuMA and LGN are excluded from the apical and basal cortex but form a cortical lateral belt in the plane parallel to the apical surface. This localization is independent of aPKC but depends on Gαi [40,65]. Randomization of spindle orientation furthermore results in an increase of ectopic progenitors [65]. Thus, an evolutionarily conserved pathway composed of LGN (Pins, GPR1/2), NuMA (Mud, Lin-5) and Gαi seems to play an important role in orienting the mitotic spindle in *Drosophila* neuroblasts,

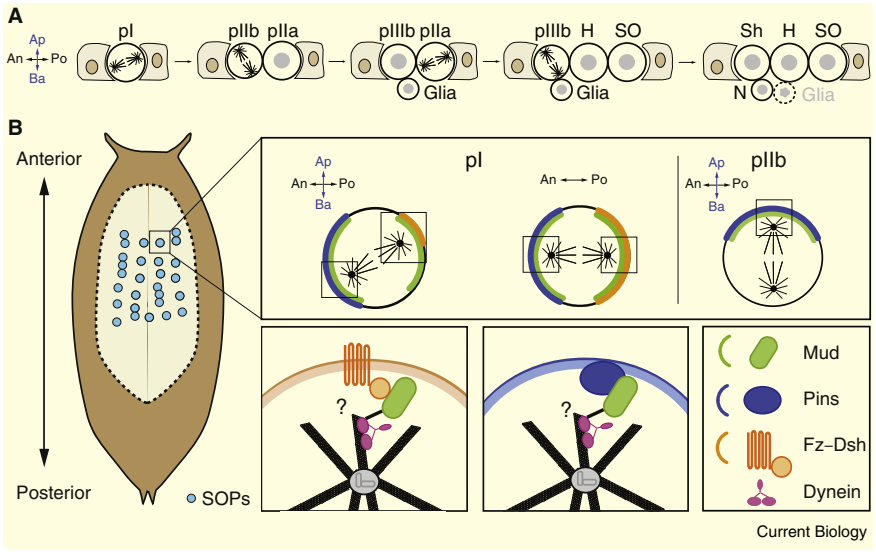

Figure 3. Interplay between PCP and Mud/Pins pathway in the sensory organ precursor lineage in *Drosophila.*

(A) In *Drosophila*, the multiple divisions of the sensory organ precursor (SOP) lineage gives rise to the hair (H), socket (SO), sheath (Sh), neuron (N) and glia which comprise an external sensory organ. Divisions occur along the anterior (An)-posterior (Po) axis (pI and pIIa) and the apical (Ap)-basal (Ba) axis (pIIb and pIIIb). (B) SOP cells are located on the pupal notum and are organized in a relatively regular pattern. In the pI cell the Pins–Mud complex is localized to the anterior cortex, whereas Fz–Dsh-Mud is bound to the posterior cortex. Note that Fz–Dsh is localized apically on the anterior cortex, co-localizing only partially with Mud. Both molecular complexes exert pulling forces on the mitotic spindle, resulting in a slightly tilted spindle. The Mud–Pins complex is localized apically in the pIIb cell. In the dividing pI cell Fz binds Dsh, which interacts with Mud at the posterior cortex. Mud presumably interacts directly with dynein to establish proper spindle orientation. In both the pI and pIIb cell, Mud interacts with anteriorly localized Pins, orienting the spindle along the apical-basal axis.

C. elegans early zygote, as well as vertebrate neuroepithelial cells and mammalian basal epidermal cells.

This is, however, not the only spindle orientation pathway connecting cell intrinsic polarity with the mitotic spindle. In *Drosophila* neuroblasts, it has been shown earlier that a parallel pathway, consisting of the PDZ protein Discs large (Dlg) and the Gakin orthologue kinesin heavy chain 73 (Khc73), is also involved in spindle orientation [67]. The current model suggests that phosphorylation of Pins through Aurora-A, together with the activation of Pins via Gαi [68], enables recruitment of Dlg to the cortex, where it could anchor microtubules via Khc73 to the apical cortex [69]. Mud, previously shown to bind to the TPR motif of Pins [60–62], could provide a force-generating complex through binding of dynein/dynactin [69]. It would be interesting to see whether these two pathways are also working together in other systems.

Connecting Extrinsic and Intrinsic Polarity Cues to Control Oriented Cell Division

A central question in oriented cell division concerns the relationship between the PCP and LGN (Pins)/NuMA (Mud)/Gαi spindle orientation pathways. Is there a molecular interaction between these two pathways, and if so, is it universal? An intuitive solution would be to link the PCP pathways (either the core PCP pathway and/or the Ft/Ds system) and the cell intrinsic LGN (Pins)/NuMA (Mud)/Gαi pathway through effector proteins, which could tether the mitotic spindle to the cortex. Alternatively, PCP effector proteins could directly affect the shape of cells, which is instrumental in orienting the mitotic spindle based on Hertwig's 'long axis rule'. Evidence for the former possibility was recently shown in zebrafish epiblast cells and *Drosophila* SOP cells [70]. However, data supporting the latter hypothesis have recently been provided in epithelial cells of the wing imaginal disc (see below) [17].

The SOP lineage in the peripheral *Drosophila* nervous system provides a beautiful example of how asymmetric cell division and oriented cell division cooperate during development (reviewed in [22,71,72]). The approximately 100 SOPs in the fly notum divide multiple times, each producing five cells which give rise to a mechanosensory organ (Figure 3A). The first two divisions in the SOP lineage, the division of the pI cell and one of its daughters, the pIIa cell, occurs strictly along the anterior-posterior axis within the plane of the epithelium [73,74]. The two daughter cells produced by pIIa become the hair and socket of the sensory organ. The pIIb cell (the sibling of pIIa) undergoes two asymmetric divisions along the apical-basal axis, much like the division of the *Drosophila* neuroblast. These divisions result in a basal glia cell and an apical cell, known as pIIIb, followed by the production of an apical sheath cell and a basal neuron [74] (Figure 3A).

Division orientation of the pI cell is mediated by the core PCP pathway. In the pI cell, Fz is localized to the posterior pole, and *fz* mutant pupa lose their anterior-posterior planar polarity and divide with random orientation [75]. Downstream of Fz, Flamingo (Fmi; Celsr1-3 in mouse) is necessary for proper anterior localization of the Numb crescent and positioning of the division axis. Another downstream target of Fz is the Partner of Inscuteable–Discs Large (Pins–Dlg) complex. This complex localizes at the anterior cortex of the pI cell and works together with Fz to localize Bazooka (Baz; Par-3 in vertebrates) to the posterior pole [76]. As in neuroblasts, Pins has been shown to be required for the anterior localization of Mud in SOPs [70]. Interestingly, Mud also localizes to the posterior cortex, where it co-localizes with the key PCP component Dsh. Mud directly interacts with Dsh [70], which is recruited to the membrane via direct interaction with Fz [77]. Proper localization of Mud to both cell poles is necessary for proper anterior-posterior polarity and cell fate specification of the pIIa and pIIb cells [70]. Thus, in SOPs, the core PCP pathway is linked to the cell intrinsic Pins–Dlg–Mud pathway through Dsh, which thus provides a direct molecular connection between extracellular signaling and the mitotic spindle, presumably via dynein (Figure 3). The connection of the Fz–Dsh and Pins–Dlg–Mud pathways is evident in the slight tilt of the spindle of the pI cell. The apical posterior localization of Fz–Dsh pulls the posterior centrosome, and thus the spindle, away from

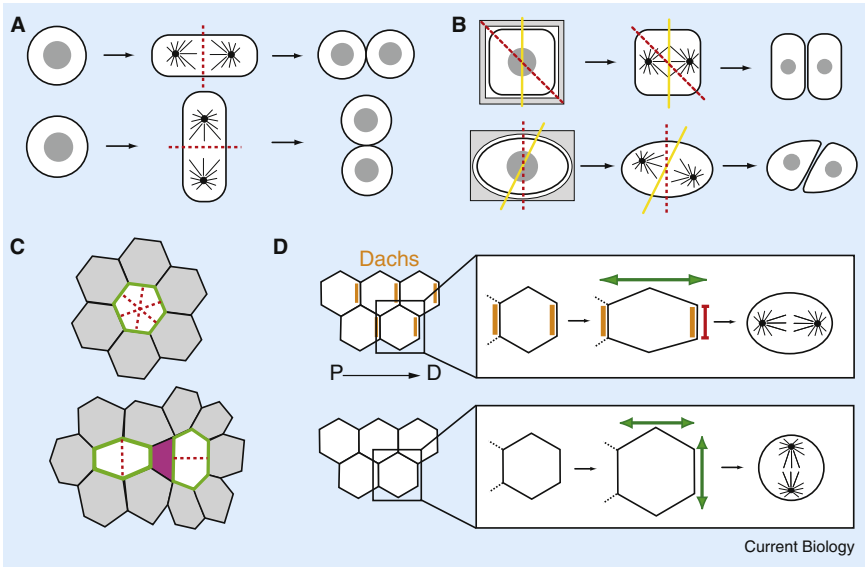

Figure 4. Cell shape and division orientation are linked.

(A) The cleavage plane (red dashed line) is oriented perpendicular to the cellular long axis, in accordance with Hertwig's long axis rule. (B) Sea urchin eggs forced into squares or elliptical chambers do not divide according to Hertwig's rule. The position of the actual cleavage plane (yellow line) is shifted from the predicted position (red dashed line). (C) Cells in a hexagonal cell sheet may divide along a number of long axes present in each cell. Addition of a non-hexagonal cell in the cell sheet induces a single long axis in neighboring cells, dictating their division orientation. (D) Planar polarized localization of Dachs to the apical distal membrane in the developing *Drosophila* wing disc constricts the proximal and distal cell membranes, forcing the cell to grow along the proximal-distal (P-D) axis. This P-D elongation dictates the position of the cell's cleavage plane and the direction of tissue growth. In *dachs* mutants cell division orientation is randomized; cell expansion is not confined to the P-D axis.

a planar orientation. These data demonstrate how in the pI cell these two pathways work together to orient the spindle along the anterior-posterior and apical-basal axis, respectively (Figure 3B) [70].

Interestingly, the interaction between Dsh and Mud seems to be evolutionarily conserved, as it was shown that NuMA physically interacts with the DEP domain of Dsh in epiblast cells in zebrafish. This interaction is physiologically relevant: knockdown experiments revealed that NuMA affects the orientation of the mitotic spindle in epiblast cells [70].

PCP Control of Cell Shape and Cell Division Orientation

In addition to the two PCP pathways and the LGN–NuMA–Gαi pathway, physical constraints can affect spindle orientation and oriented cell division. The existence of a connection between cellular shape and division axis has been evident to cell biologists since the 1880s. Cells may take on a wide variety of shapes during development, and in many systems cell shape determines the orientation of the division plane. This correlation is described by Hertwig's 'long axis rule': "*The two poles of the division figure come to lie in the direction of the greatest protoplasmic mass*" [1] (Figure 4A). In the developing *Xenopus* embryo, cells follow the long axis rule until the late blastula and cells isolated from a blastomere will divide along an experimentally induced long axis [3,14]. Similarly, experimental manipulation of cell shape in early mouse embryos influences the division axis [78] (Figure 4A).

In a recent experiment by Minc and colleagues [79], microfabricated wells were used to manipulate sea urchin eggs into several defined shapes, such as stars, ovals, squares and rectangles (Figure 4B). The cells were found to divide with normal timing, indicating that general physiology was relatively unaffected. Many cell shapes were found to undergo divisions that followed Hertwig's rule, but there were exceptions. The division axis did not follow the long diagonal axis in rectangular cells but instead formed along the largest axis of symmetry. The long axis rule also did not hold for ellipses of small aspect ratio or squares [79] (Figure 4B). When the urchin cells were forced into their new shapes, the nucleus repositioned to the new center of mass and elongated in respect to the future spindle axis. Nuclear centering and elongation are dependent on microtubules but not filamentous actin, and the cell most likely determines the position and orientation of the division axis by 'measuring' the length of microtubules.

In animals, cells are usually embedded in tissues and the shape of a cell is influenced by that of its neighbors. Monolayer cell sheets tend to be composed of three main cell shapes, hexagons, pentagons, and heptagons [80]. Mathematical modeling revealed that manipulating the shape of a cell's neighbor can influence the positioning of its division plane. For example, inserting a non-hexagonal neighbor into a sheet of hexagonal cells will cause the induction of a long axis in a neighboring hexagonal cell. Live imaging studies and mathematical modeling suggested that the presence of a long axis during interphase can influence spindle orientation during mitosis in *Drosophila* wing discs [81] (Figure 4C).

Exceptions to the long axis rule can also be found under normal physiological conditions [8,82]. Nevertheless, cell shape seems to have a strong influence on cell division orientation in certain cell types. Recently, an interesting study connected PCP signaling with the control of cell shape [17]. As mentioned earlier, the *Drosophila* wing epithelium elongates based on oriented cell division orientation along the proximal-distal axis during development (Figure 1B). This elongation is accomplished through the Ft/Ds system and its effector protein Dachs. How is Dachs transducing the positional information provided by Dachsous? Dachs might tether the mitotic spindle to the cortex. Alternatively, Dachs, an atypical myosin, could be controlling cell shape. Analysis of *dachs* mutant cells showed that Dachs exerts a contractile force on apical cell junctions, controlling the cell's shape. Planar polarized Dachs increases tension in the distal cell junction and the proximal junction of that cell's neighbor, forcing the cells to grow along to the proximal-distal axis. This elongation influences the positioning of the division axis, since these cells follow the long axis rule [17]. These data provide a conceptual framework of how positional information provided by PCP signaling is translated into cell shape changes, which influence the orientation of the mitotic

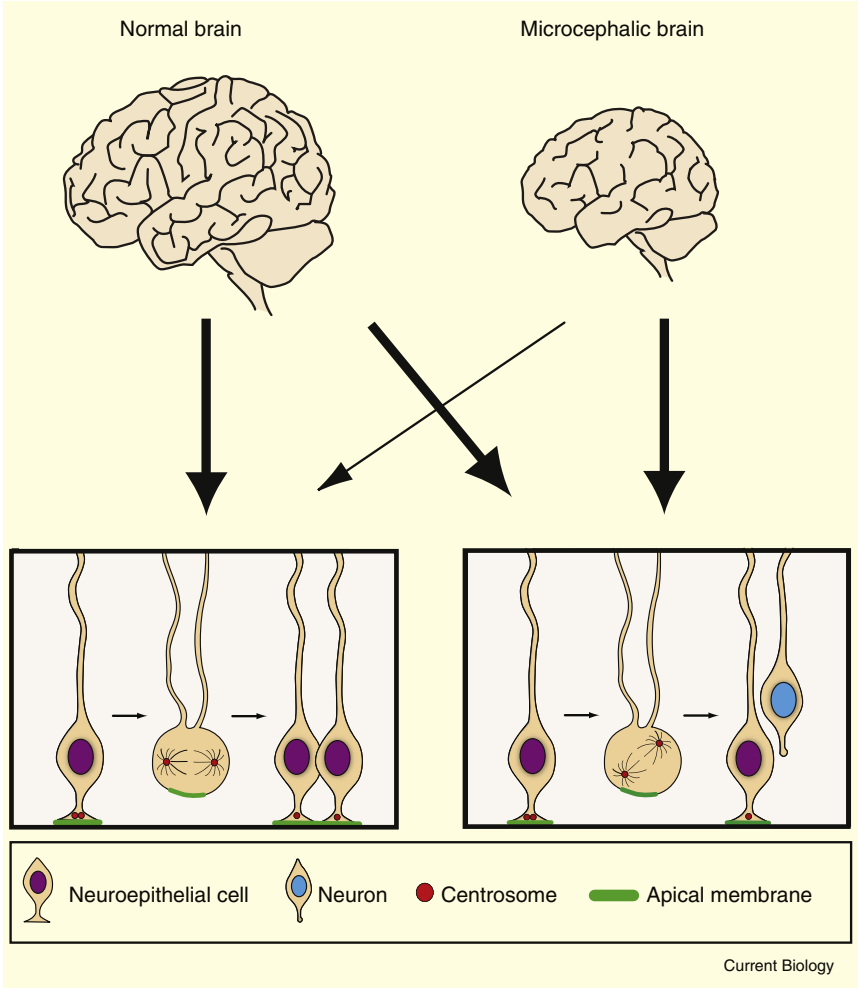

Figure 5. Improper cell division orientation contributes to the development of microcephaly.

Normal brains contain symmetrically (left panel) and asymmetrically (right panel) dividing progenitors, resulting in the amplification of the progenitor pool but also the production of differentiating siblings. Asymmetric division of these cells produces one neuroepithelial progenitor and one cell which detaches from the apical membrane and becomes a basal progenitor or a neuron. In the microcephalic brain, randomized spindle rotation could result in a premature shift towards asymmetric divisions resulting in a decrease of the neuroepithelial progenitor pool, and limited production of neurons. This leads to a decrease in overall cell number and thus a reduction in brain size.

spindle, resulting in oriented cell division (Figure 4D). However, mechanistic insight into how cell shape influences spindle orientation is still lacking.

NuMA- and PCP-Independent Spindle Orientation Pathways

The evolutionary conserved LGN–NuMA–Gαi and core PCP pathways are utilized repeatedly to orient the mitotic spindle throughout metazoan development. However, recent results in zebrafish revealed that other pathways might also be important for oriented cell division and spindle orientation [11]. As described earlier, in the developing zebrafish neural keel, the mitotic spindle rotates 90 degrees and aligns itself perpendicular to the midline (Figure 1A) [9,11]. Knocking down Vangl2, Wnt11, Wnt5 or Dsh2 does not affect the correct orientation of the mitotic spindle, suggesting that the core PCP pathway is not required for spindle orientation in the neural keel [6,11]. However, removal of the Wnt receptor Fz7 affected the stereotypical division orientation of neural progenitors [6]. Furthermore, *scrib* mutant zebrafish embryos display a randomization of spindle orientation. The Scrib phenotype is not a consequence of disrupted polarity, as the general organization of the apical cell cortex seemed to be unperturbed and knockdown of Par-6 or aPKC does not compromise spindle orientation [11]. Thus, Scribble-dependent spindle orientation does not act through the core PCP pathway and is distinct form Scribble's role in establishing and maintaining apical-basal polarity [11].

Could Fz7 and Scrib constitute a new cell division orientation pathway working independently of the core PCP pathway? More experiments are needed to resolve this issue. In *Drosophila* SOP cells [83], *Drosophila* male germline stem cells [84] and basal cells of the developing epidermis in mice [34], cadherin-based cell–cell contacts play important roles in spindle orientation. Could a similar mechanism be used in the zebrafish neural keel? Zigman and colleagues [11] studied the localization of α-catenin in wild-type fish and found that foci are localized on the cortex and enriched at the presumptive cleavage plane (Figure 1A). Furthermore, knockdown of Scribble resulted in a decrease of cortical α-catenin levels. *N-cadherin* mutants also display a decrease in cortical α-catenin levels and showed a *scribble*-like spindle orientation phenotype [11]. Thus, Scribble-dependent assembly of cell–cell adhesion complexes plays an important role in division orientation in the developing neural keel.

Developmental Consequences of Altered Cell Division Orientation

Defective cell division orientation can result in tissue architecture defects, cell fate misspecification and cancer. In the colon and small intestine of mice and humans, stem cells orient their spindles preferentially perpendicularly to the apical surface [85]. In order to see whether oriented cell division is changed in cancer, spindle orientation was measured in the stem cell compartment of *Apc* mutant mice. Mutations in *Apc* are responsible for familial adenomatous polyposis (FAP) and are the most prevalent initiating mutations in colorectal cancers [86]. In contrast to wild-type tissue, there was no spindle orientation bias in *Apc* mutant mice or FAP human intestine; spindle orientation was significantly different between mutant and wild-type tissue [85]. Genetic analysis of one of the two *Drosophila* Apc orthologues, Apc2, suggested that Apc2 could anchor astral microtubules to the cortex [87]. However, other possible mechanisms could

account for the *Apc* spindle orientation phenotype. In the mammalian gut, it was shown that *Apc* mutant cells still contain astral microtubules, although they do not fully attach to the cortex [88]. *Apc* mutant stem cells also displayed a widening of the basal region, which altered the shape of these long columnar epithelial cells [85,88]. Thus, changes in cell shape and loss of cortical contact of astral MTs could contribute to a change in spindle orientation. Clearly, more mechanistic data are required to fully understand the role of Apc in spindle orientation and its role in cancer. Furthermore, although spindle misorientation may contribute to tumor formation, not all tumors display spindle orientation defects [86].

Autosomal recessive primary microcephaly is another disease that is, at least in part, associated with defective spindle orientation and cell division orientation. Microcephaly is manifested in the occurrence of small, but structurally normal, brains and mild-to-moderate mental retardation [89,90] (Figure 5). There are at least eight microcephaly loci and five of the affected genes have been cloned [90]. Interestingly, at least three of these genes have proposed roles in spindle orientation and oriented cell division based on molecular genetic analysis of homologues in model systems [90]. CDK5RAP2 (Centrosomin (Cnn) in *Drosophila*), Abnormal spindle-like microcephaly associated protein (ASPM; abnormal spindle (Asp) in *Drosophila*; ASPM-1 in *C. elegans*) and CenpJ (Sas4 in *Drosophila*) are localized to centrosomes and mutations in *cnn*, *Sas4* and *aspm-1* have been associated with spindle orientation defects *in Drosophila*, *C. elegans* and mouse. Loss of Cnn and Sas4 in flies has been directly shown to affect centrioles and centrosomes and also manifests in a lack of astral microtubules [43,63,87,91]. Mutations in these genes uncouple the mitotic spindle from the cortex, resulting in an increase in the stem cell pool, as was shown with both neuroblasts and male germline stem cells [26,87]. Loss of ASPM in *C. elegans* resulted in meiotic spindle orientation defects [92]. In mice, ASPM has been shown to regulate cell division orientation; lack of ASPM resulted in an increase of asymmetric divisions and a loss of neuroepithelial cells abutting the ventricular zone [43]. Thus, a larger proportion of neuroepithelial cell progeny is found in the neuronal layer, associated with a concomitant loss of neuroepithelial cells in the ventricular zone [43]. Microcephaly could thus be caused through spindle orientation defects resulting in a premature shift from symmetric, amplifying divisions towards premature, asymmetric, neurogenic divisions. As a consequence, the neural stem cell pool is reduced. As neurons exit the cell cycle and proliferating neural progenitors are successively lost, microcephalic brains do not contain the same amount of cells as their wild-type counterparts manifested in the small brain phenotype (Figure 5) [90].

Misregulated spindle orientation and oriented cell division can also lead to cell differentiation defects in the mammalian epidermis [33]. Knockdown of LGN, NuMA or p150glued resulted in a thinner epidermis with impaired barrier function leading to dehydration and the death of genetically manipulated mice. Careful analysis revealed that spindle orientation defects resulted in an increase in symmetric basal cell divisions, preferentially generating basal progenitors as opposed to wild-type asymmetric divisions, which give rise to one basal progenitor and a suprabasal differentiating cell. In agreement with this observation, fewer suprabasal cells and a reduction in differentiation marker expression were observed [33]. Thus, morphogenesis of the mammalian skin depends on controlled oriented cell division to generate enough differentiating suprabasal cells while maintaining a pool of basal progenitors.

Taken together, these observations suggest that spindle orientation defects can result in altered cell division orientation and cell fate misspecification. Depending on the cellular context, this can lead to tumor initiation and tissue organization defects with impaired function.

Conclusion

Oriented cell division in animals has been most intensely studied in systems allowing powerful genetic analyses combined with live imaging. Lately, molecular connections between extracellular signaling pathways and spindle orientation have emerged. Over the next years, it will be interesting to gain more mechanistic insight into how the core PCP signaling pathway and the Ft/Ds system are connected with intrinsic cell polarity but also how this extracellular signaling pathway is translated into cell shape changes. Furthermore, how exactly cell shape controls spindle orientation remains to be seen. As oriented cell division is such a recurring theme during animal development, it will be interesting to see how diverse the mechanism and molecular players are in animals.

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