Cell PRESS
Developmental Cell
Review

Mitotic Spindle Orientation in Asymmetric and Symmetric Cell Divisions during Animal Development

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DOI 10.1016/j.devcel.2011.06.012

The orientation of the mitotic spindle has been proposed to control cell fate choices, tissue architecture, and tissue morphogenesis. Here, we review the mechanisms regulating the orientation of the axis of division and cell fate choices in classical models of asymmetric cell division. We then discuss the mechanisms of mitotic spindle orientation in symmetric cell divisions and its possible implications in tissue morphogenesis. Many recent studies show that future advances in the field of mitotic spindle orientation will arise from combinations of physical perturbation and modeling with classical genetics and developmental biology approaches.

Introduction

During development, cell division rate is coordinated with cell growth to determine the number of cells and the size of multicellular organisms (Goranov and Amon, 2010). During adult life, tissue homeostasis and regeneration of damaged tissues demand a fine-tuned regulation of division and growth rate, and defects in their regulation lead to cancer. Cell division is controlled not only in time, but also in orientation. Over the last two decades, cell division plane orientation has emerged as one of the fundamental mechanisms to coordinate cell division rate with cell fate choices and cell position, hence specifying the repertoire of cell types as well as the structure and shape of tissues and organs. This coordination is carried out in part by an essential actor of cell division: the mitotic spindle. Here we review the mechanisms and the roles of mitotic spindle orientation in the context of both asymmetric and symmetric cell division during animal development.

During mitosis, the mitotic spindle ensures the separation of the two genomes and positions the cytokinesis furrow, therefore coordinating karyokinesis and cytokinesis. The mitotic spindle is an elongated dynamic structure consisting of three classes of microtubules (MTs) nucleated from the two spindle poles or centrosomes: (1) kinetochore MTs attach to the chromosomes to separate the two genomes at anaphase; (2) interpolar MTs form an antiparallel array between the spindle poles and are implicated in positioning the furrow at cytokinesis; and (3) astral MTs dynamically anchor the mitotic spindle to the cortex and also participate in furrow positioning (for review see Glotzer, 2009; Tanaka, 2010). The dynamic anchoring of the mitotic spindle to the cell cortex by astral MTs underlies most of the mechanisms that orient cell division relative to the shape of the cell or to cortical landmark deposits at the cell cortex (for review see Théry and Bornens, 2006). The structure of the mitotic spindle therefore coordinates cell division and the position of the daughter cells within the tissue. The role of astral MTs in the regulation of mitotic spindle orientation was proposed for some time in cultured cells, and the Dynein-Dynactin complex, a MT minus end-directed motor, appeared to be a major actor in the pathway (for review see Dujardin and Vallee, 2002). Yet, during animal development, understanding the mechanisms of mitotic spindle orientation began with the identification of cortical landmarks (Uemura et al., 1989; Etemad-Moghadam et al., 1995; Kraut et al., 1996), which were then connected to astral MTs via the NuMA (Nuclear Mitotic Apparatus) and Dynein-Dynactin motor complex (Srinivasan et al., 2003; Gotta et al., 2003; Bowman et al., 2006; Izumi et al., 2006; Siller et al., 2006; Couwenbergs et al., 2007; Nguyen-Ngoc et al., 2007; Siller and Doe, 2008, 2009).

More than 120 years ago, applying mechanical forces on sea urchin embryos, which trigger a cell shape deformation, revealed that cells tend to divide along their long axis (the so-called "Hertwig rule" [Hertwig, 1884]). This pushed forward the notion that mitotic spindle orientation originates from a mechanical regulation, whereby the cells are able to sense their shape or the applied stress. However, at the turn of last century, a correlation between oriented cell divisions (OCDs) and specific fate decisions or different daughter cell sizes was observed in the ascidian embryo (Conklin, 1905). This correlation suggested the existence of specific molecular signals, which finely control the positioning of the mitotic spindle to regulate developmental decisions. These two hypotheses were always at the heart of the mitotic spindle orientation field. Historically, the intense efforts in studying asymmetric cell divisions (ACDs) in invertebrate models have allowed the elucidation of several core molecular signaling mechanisms that control cell polarization, hence, mitotic spindle orientation.

Table 1. Summary of the Protein Names and Interactions in the Different Models Discussed in the Review

	C. elegans	*Drosophila*	Vertebrates	Interactors
NuMA	LIN-5	Mud	NuMA	Pins,
				Tubulin,
				Dynein-Dynactin complex,
				Dsh
Pins	GPR-1/2	Pins	mPins, LGN, GPSM2	Insc, NuMA, $G\alpha i_{GDP}$, Aurora A, aPKC, Dlg
Gα or Gαi	GOA-1	Gαi	Gαi1, Gαi2, Gαi3	Pins
	GPA-16			RGS proteins
				Ric8
Insc	–	Insc	mInsc	Par3, Pins
Par3	PAR-3	Bazooka (Baz)	Par-3	Par6, aPKC, Insc
Par6	PAR-6	DmPar6	Par-6	Cdc42, Par3, aPKC
aPKC	PKC-3	DaPKC	aPKC, PKCζ	Par3, Par6
Dsh	DSH-2, MIG-5	Dsh	Dvl	Frizzled, NuMA

Proteins involved in mitotic spindle orientation have distinct names in the different model systems. When possible, a single name has been used in the Main Text. The table gives the alternative names used in the literature and summarizes the relevant interactions (see Main Text for references).

ACD generates daughter cells of distinct identities and therefore couples cell division and cell fate specification. This process is often proposed to be composed of three steps: (1) a cell polarity axis is specified; (2) cell polarization is translated into the asymmetric localization of cell fate determinants; and (3) the mitotic spindle aligns with the cell polarity axis, thereby leading to the segregation of fate determinants in only one daughter cell. Examples of ACD abound, and polarizing cues have proved extremely diverse: mitotic spindle orientation can be controlled relative to an intrinsic cue such as the apical-basal (AB) axis of the epithelium, or to extrinsic cues, including the sperm entry point in a zygote, cell-cell contact, or a tissue polarity axis. ACD has mostly been studied in invertebrate systems showing a fixed lineage tree, which provide a diverse yet reproducible assay to study how mitotic spindle orientation is controlled relative to a cortical cue. Collectively, these studies and additional studies in vertebrates have defined a conserved framework whereby cues from the cell cortex most often converge on the NuMA family of proteins that regulates the activity of the Dynein-Dynactin motor complex to pull on astral MTs. Although its mechanisms are not yet fully understood, the framework permits an examination of the role of mitotic spindle positioning in fate specification and, therefore, its impact on tumorigenesis (for review see Knoblich, 2010).

Yet, most divisions in an organism are symmetric, and many have a stereotypical orientation. Understanding the relevance of OCD in tissue architecture and tissue morphogenesis is another major challenge in developmental biology. From a molecular standpoint, approaching this challenge by identifying the cortical cues regulating mitotic spindle orientation has proven to be relevant, in particular for understanding how planar tissue polarization pathways control OCD. In parallel, mechanical models allow us to predict the orientation of symmetric cell divisions and therefore provide an explanation for the century-old "Hertwig" rule. Collectively, this opens the path to integrate molecular signals and mechanical constraints in the regulation of tissue architecture and morphogenesis by mitotic spindle orientation.

The Lin5, Mud, and NuMA Orthologs Link Cortical Landmarks to the Dynein-Dynactin Motor Complex during ACD

The classical view of asymmetric division posits that upstream cortical cues are used to coordinate the polarized distribution of cell fate determinants and the orientation of the mitotic spindle. The control of cell fate determinant localization has been recently reviewed elsewhere (Knoblich, 2010) and will not be treated here. In this section, we focus on the mechanisms that translate cortical cues into spindle orientation. We first present the *C. elegans* zygote model to illustrate how cortical cues are translated in mechanical forces pulling on astral MTs. We then discuss two main modes of cell division orientation in *Drosophila* and vertebrate tissues by describing how the spindle can be aligned either perpendicular or parallel to the AB axis of the tissue. We illustrate the diversity of cortical cues, and describe how they all remarkably converge on the NuMA family of proteins and the Dynein-Dynactin motor complex. NuMA (*lin-5* in *C. elegans* and *mud* [mushroom body defect] in *Drosophila*, Table 1) encodes a large coiled-coil protein with multiple interaction partners, and was initially discovered and intensely studied for its role in mitotic spindle assembly in vertebrate cells in culture (Merdes et al., 1996; Radulescu and Cleveland, 2010). The first hint at a possible role for NuMA in spindle orientation came from the discovery of its association with the vertebrate Partner of Inscuteable (Pins, also known as mPins, LGN, and GPSM2) (Du et al., 2001; Du and Macara, 2004), whose homologs in *Drosophila* (Pins) and *C. elegans* (GPR-1 and GPR-2, thereafter referred to as GPR-1/2) are involved in ACD (Yu et al., 2000; Schaefer et al., 2000; Srinivasan et al., 2003; Gotta et al., 2003; Colombo et al., 2003).

The C. elegans *Zygote*

Owing to its large size, optical properties, and powerful genetics, the *C. elegans* zygote provides a wonderful model to dissect the molecular pathways and the dynamics of mitotic spindle positioning in metazoans (for review see Galli and van den Heuvel, 2008).

The fertilized *C. elegans* one-cell embryo is elongated along the anterior-posterior (a-p) axis. Upon fertilization, the two pronuclei and their associated centrosomes form the nucleus centrosome complex (NCC) in the posterior half of the zygote. The NCC moves to the center of the embryo and rotates to align the two centrosomes along the a-p axis (for review see Siller and Doe, 2009). Hence, the spindle forms in the center of the embryo and is already aligned with the a-p axis. Yet, as the spindle elongates during anaphase, it moves toward the embryo's posterior cortex, resulting in a large anterior blastomere and a smaller posterior one, which are both endowed with distinct cell fate determinants (see Gönczy, 2008 for review). Posterior spindle displacement is concomitant with posterior aster flattening and oscillation, which result from a larger net pulling force acting on the posterior spindle pole, as shown by laser severing of the spindle (Grill et al., 2001,

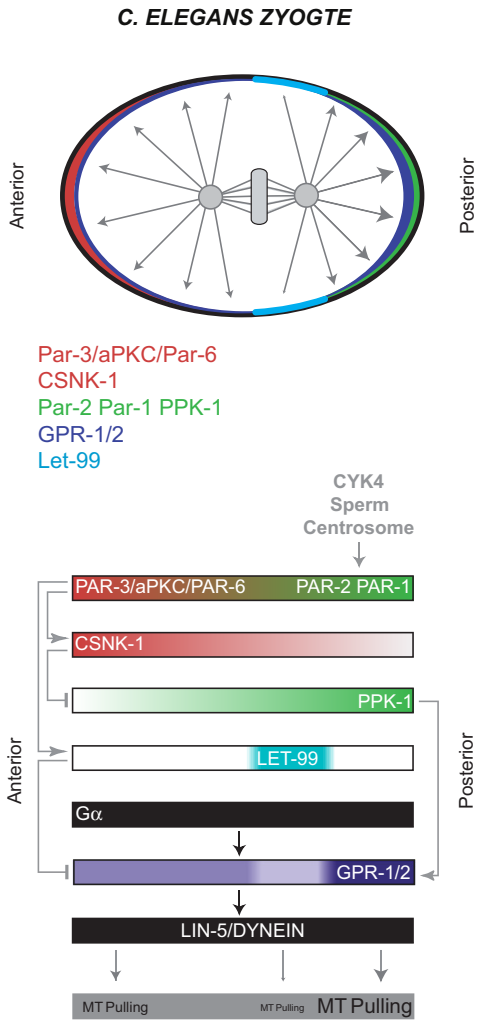

Figure 1. Mitotic Spindle Positioning in *C. elegans* zygote

Top view shows localization of relevant cortical cues in the *C. elegans* zygote leading to mitotic spindle positioning along the a-p axis during anaphase. Arrows emanating from the centrosomes schematize the astral MTs. The size of the arrowhead indicates the strength of the pulling forces as determined by the velocity of centrosome fragments upon centrosome laser ablation. Bottom view illustrates pathway leading to the differential localization of GPR-1/2 at the cell cortex and, hence, the repartition of FGs and MT pulling forces. Upon fertilization of the *C. elegans* embryo, the male centrosome and its aster lie in close contact with the cell cortex. There, in conjunction with the CYK-4 Rho GTPase brought by the sperm, they specify cortical polarization by regulating the distribution of Par proteins and the anterior accumulation of Myosin. The mechanisms by which Par proteins control GPR-1/2 localization and FG distribution are shown during anaphase. Gα is likely to cycle between its GDP and GTP-bound forms. The role of the Gα cycle is unknown, but it might permit the correct localization of Gα, the association of $G\alpha_{GDP}$ with GPR-1/2, or the production of distinct $G\alpha_{GDP}$ levels between the posterior and anterior cortex. The existence of such a cycle is suggested by the loss of the Gα GTPase RGS7 or the Gα Guanine Exchange Factor Ric8 function (not depicted), which result in opposite mitotic spindle defects; Ric8 is also required for GPA-16 membrane localization (Afshar et al., 2004; Hess et al., 2004; Couwenbergs et al., 2004). Gray arrows indicate genetic relationships, and black arrows indicate known direct or indirect molecular interactions. Molecules depicted in black are uniformly localized.

2005; Labbé et al., 2003; Pecreaux et al., 2006; Krueger et al., 2010); the higher posterior pulling force results from a 50% increase in the activity of so-called "force generators" (FGs) at the posterior cortex, as shown by laser ablation of the centrosome (Grill et al., 2003) (Figure 1).

The net higher posterior pulling force is controlled by a cascade of molecular interactions depicted in Figure 1. During the one-cell embryo division, the Par complex (Par3-Par6-aPKC) localizes at the anterior cortex, whereas Par1 and Par2 localize at the posterior cortex (for review see Gönczy, 2008). Par proteins regulate, in part via the Casein Kinase I (CSNK-1), the posterior enrichment of the PI(4)P5-kinase, PPK-1, which promotes the posterior cortical enrichment of GPR-1/2 during anaphase (Panbianco et al., 2008). In parallel, GPR-1/2 cortical localization is inhibited by LET-99 in a lateral-posterior domain (Park and Rose, 2008; Krueger et al., 2010). The combined activity of Par proteins and Let-99 therefore defines three distinct cortical domains at anaphase: an anterior domain, a lateral-posterior domain, and a posterior domain, where GPR-1/2 is weak, absent, and enriched, respectively (Krueger et al., 2010) (Figure 1). Through their GoLoco domain, GPR-1/2 bind to two partially redundant Gα proteins, GOA-1 and GPA-16 (collectively referred to as Gα), in their GDP-bound form. $G\alpha_{GDP}$ is anchored at the membrane by myristoylation and maintains GPR-1/2 there. The GPR-1/2-$G\alpha_{GDP}$ is the active form and is necessary for a net higher posterior pulling force, whereas the $G\alpha_{GDP}$-$G\beta\gamma$ heterotrimer acts as a negative regulator of Gα-dependent pulling forces (for review see Gönczy, 2008).

GPR-1/2, in association with $G\alpha_{GDP}$, interacts with LIN-5 (Srinivasan et al., 2003; Gotta et al., 2003). Coimmunoprecipitation experiments revealed interactions between the GPR-1/2-Gα-LIN-5 complex components and the Dynein-Dynactin complex components (Couwenbergs et al., 2007; Nguyen-Ngoc et al., 2007). Accordingly, GPR-1/2, Gα, and Lin-5 promote the cortical localization of Dynein-Dynactin complex components. Furthermore, the loss of function of Dynein Heavy Chain or of proteins associated with the Dynein-Dynactin complex results in the reduction of the pulling forces at the anterior and posterior cortex (Couwenbergs et al., 2007; Nguyen-Ngoc et al., 2007). While the Dynein-Dynactin complex is necessary to localize FGs at the cortex, its components are not enriched at the posterior cortex (Nguyen-Ngoc et al., 2007), and the mechanism by which the enrichment of GPR-1/2 triggers a higher activity of FGs at the posterior cortex remains to be elucidated.

Orientation of Cell Divisions along the AB Axis

Drosophila Neuroblasts. Embryonic and larval *Drosophila* NBs, the progenitors of the *Drosophila* central nervous system, have provided an excellent model to study the molecular mechanisms and the role of mitotic spindle orientation in stem cell-like progenitors. Embryonic NBs delaminate from the neuroepithelium, then divide asymmetrically along their AB axis to self-renew and generate the neurons of the larval nervous system. At the end of embryogenesis, they become quiescent, but reenter the cell cycle during larval life to generate the adult nervous system (Kaltschmidt et al., 2000; Rebollo et al., 2007; Rusan and Peifer, 2007; Chell and Brand, 2010; Sousa-Nunes et al., 2011). NBs divide in a stem-like manner to generate a large NB and a small daughter cell, which inherits the Brat, Numb, and Prospero cell fate determinants and becomes either a Ganglion Mother Cell (GMC) or an immature intermediate neural precursor (INP). The GMC and INP further divide to produce neurons and glial cells (for review see Sousa-Nunes et al., 2010).

In NBs, Par3 (also known as Bazooka), Par6, and aPKC proteins form an apical cortical complex from late interphase/early prophase onward (Figure 2A). Par3 interacts with Inscuteable (Insc) and recruits Insc to the apical cortex. Pins interacts with cortical $G\alpha i_{GDP}$ through its multiple C-terminal GoLoco domains, and both are recruited to the apical cortex via the interaction of Pins N-terminal TPR domains with Insc. Loss of Pins or Gαi affects Par3, aPKC, and Insc apical localization as well as mitotic spindle orientation (for review see Yu et al., 2006). As in *C. elegans*, the Pins-$G\alpha i_{GDP}$ complex is proposed to be the active form that orients the mitotic spindle.

At the apical pole, Pins/$G\alpha i_{GDP}$ acts as a platform to regulate two distinct signaling activities both necessary for mitotic spindle orientation. The first one, named the $Pins^{TPR}$ pathway, depends on the Pins TPR region and requires Mud activity. Pins interacts with Mud at the NB apical cortex via a direct interaction mediated by its TPR domain (Bowman et al., 2006; Izumi et al., 2006; Siller et al., 2006). Mud localization also requires the adherent junction PDZ protein Canoe, which associates with Pins (Speicher et al., 2008). Mud loss of function randomizes mitotic spindle orientation (Bowman et al., 2006; Izumi et al., 2006; Siller et al., 2006). Dynein-Dynactin complex components are not apically enriched during NB cell division; nevertheless, loss of Lissencephaly-1 (Lis-1) or Dynactin functions affects mitotic spindle rocking or orientation (Siller et al., 2005; Siller and Doe, 2008).

The second pathway is called the $Pins^{LINKER}$ pathway (Johnston et al., 2009). The Pins LINKER region is located between the TPR and GoLoco domains. Its activity in mitotic spindle orientation was discovered in *Drosophila* S2 cells using the "induced polarity" assay. By aggregating S2 cells via the extracellular domain of the adhesion molecule Echinoid, a polarized distribution of Pins fused to the intracellular region of Echinoid can be induced (Johnston et al., 2009). In this context, the Pins LINKER region is sufficient to orient the mitotic spindle. The $Pins^{LINKER}$ activity anchors the mitotic spindle to the edge of the Pins localization domain. Pins binds to Disc-Large (Dlg) (Bellaïche et al., 2001b), which binds to Kinesin-73 (Khc-73), a plus-end-directed motor located at the plus-end tips of taxol stabilized MTs (Siegrist and Doe, 2005). The $Pins^{LINKER}$ activity is independent of Mud function, but it requires both Dlg and Khc-73 activity in S2 cells (Johnston et al., 2009). Finally, the activity of the Pins LINKER domain is regulated by its phosphorylation by the mitotic kinase Aurora A (Johnston et al., 2009). The $Pins^{LINKER}$ pathway is likely to function in *Drosophila* NBs. Indeed, the loss of Dlg or Khc-73 activity perturbs the orientation of the mitotic spindle in embryonic NBs (Siegrist and Doe, 2005). Furthermore, the Pins Aurora A phosphorylation site is essential for Pins mitotic spindle activity in larval NBs (Johnston et al., 2009).

Collectively, these results demonstrate that spindle orientation in NBs depends on two Pins-dependent pathways: the $Pins^{LINKER}$ pathway provides astral MT anchoring activity via Dlg-Khc-73, and the $Pins^{TPR}$ pathway generates mitotic spindle pulling forces via Mud-Dynein-Dynactin, as shown also in *C. elegans*. Finally, the regulation of the $Pins^{LINKER}$ pathway by Aurora A provides a mechanism of integration between cell cycle progression and the regulation of mitotic spindle.

AB Division of Mouse Skin Basal Progenitors. Gαi, Pins, NuMA, and Dynein were shown to regulate AB spindle orientation in vertebrate cells in an Insc-dependent manner. The role of Inscuteable (mInsc) in the regulation of perpendicular division in vertebrates was first demonstrated in progenitor cells in the rat retina, where mInsc localizes apically and directs mitotic spindle orientation along the AB axis (Zigman et al., 2005). More recently, studies in embryonic mouse skin progenitors have illustrated the conservation of the Insc-Pins-Gαi-NuMA-Dynein-Dynactin cascade to regulate AB division in these cells (Figure 2B). In dividing skin progenitors, the analysis of the distribution of mInsc, Pins, and NuMA reveals that all proteins are localized in an apical domain in a subset of cells dividing along the AB axis (Figure 2B). Their apical localizations are under the control of β1-integrin and α-catenin (Lechler and Fuchs, 2005). Furthermore, Gαi3 and Dynactin (Dctn1) are localized apically, with Dcnt1 also localizing on the centrosomes (Williams et al., 2011). Thus, the mInsc, Pins, NuMA, and Dynein proteins selectively partition to the basal progenitor daughter cell in response to its AB polarization. Gαi3 controls the Pins localization, which itself regulates the NuMA apical localization. Remarkably, loss of Pins, NuMA, or Dctn1 function induces planar cell division (Williams et al., 2011) (Figure 2B), suggesting that an additional mechanism regulates planar spindle orientation in skin progenitors.

Collectively, studies in *Drosophila* NBs and skin progenitors point toward a general role of Insc as a cell-type-specific regulator of the apical localization of Gαi, Pins, and NuMA, which therefore triggers the AB orientation of the mitotic spindle. Accordingly, overexpression of Insc is sufficient to induce more AB division in skin progenitors (Poulson and Lechler, 2010; Williams et al., 2011). Insc also induces AB division in epithelial cells that normally divide in a planar fashion, such as *Drosophila* embryonic epithelial cells (Kraut et al., 1996) or vertebrate neuroepithelial progenitors (Konno et al., 2008).

Planar Spindle Orientation of Progenitor Division

In many tissues with an epithelial organization, progenitors divide parallel to the plane of the tissue (thereafter referred to as planar orientation). We first review the planar division of the vertebrate neuroepithelium progenitors, whose orientation is planar but random relative to the animal anterior-posterior (a-p) and dorsal-ventral axes. We then review the *Drosophila* sensory organ progenitor division, whose orientation is planar and also controlled along the a-p axis of the *Drosophila* dorsal thorax.

Vertebrate Neural Progenitors. The roles of Gαi, Pins, and NuMA have been studied in vertebrate neural progenitors, which divide either symmetrically or asymmetrically during neurogenesis (see below). Remarkably, in the mouse and chick neuroepithelium, the complex was shown to regulate planar cell divisions (Morin et al., 2007; Konno et al., 2008). Pins and NuMA form a ring at the lateral cell cortex in chick neuroepithelial cells (Peyre et al., 2011) (Figure 2C). This contrasts with the apical polarized distribution observed in fly NBs and mouse skin progenitors (Figures 2A and 2B). The relevance of the distribution in a ring was explored using real-time imaging (Peyre et al., 2011). In neuroepithelial cells, the spindle forms with a random orientation and undergoes a rapid rotation to align with the apical surface, with both spindle poles located underneath the Pins-NuMA ring. The spindle is then maintained in this plane, in which it rotates freely until anaphase. All spindle movements are lost upon depletion of Pins or NuMA; conversely, overexpression of $G\alpha i_{GDP}$ homogenizes Pins around the cell cortex and results in

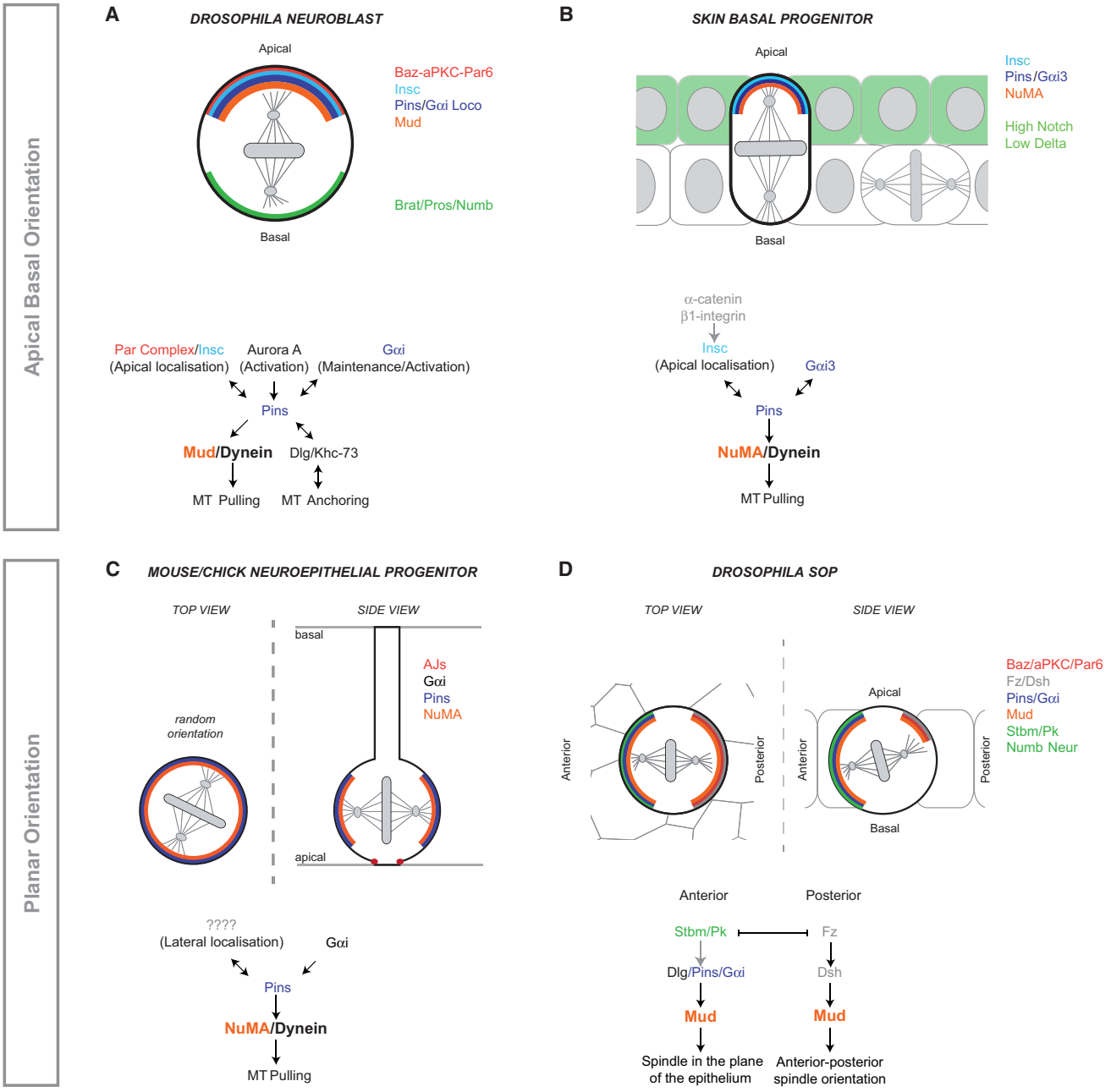

Figure 2. Diverse Polarity Cues Converge on NuMA and the Dynein-Dynactin Complex to Control Mitotic Spindle Orientation

(A) Top: the AB localization of the relevant polarity markers is shown in a NB at metaphase. Bottom: the molecular pathways leading to the regulation of Dynein-Dynactin complex via the $Pins^{TPR}$ pathway and to the regulation of Khc-73 via the $Pins^{LINKER}$ pathway. The Par3-Par6-aPKC (Par complex) interacts with Insc, which regulates the apical localization of Pins during the first NB ACD. Note that Dlg is enriched at the apical NB cortex. Khc-73 likely localizes to the plus-end of astral MTs. Other molecules depicted in black are uniformly localized. Ric-8 and the GoLoco and RGS domain protein Locomotion defects (Loco) are also required for mitotic spindle orientation, suggesting that Loco-$G\alpha o_{GDP}$ complex and the GDP-GTP cycle of Gαi and Gαo are also needed for mitotic spindle positioning (Yu et al., 2005; Hampoelz et al., 2005; Wang et al., 2005). Besides, Ric8 is required for Gαi cortical anchoring. See Figure 3 for the mechanisms likely regulating cortical polarization in subsequent cell divisions.

(B) Top: localization of the relevant polarity markers in the asymmetrically dividing basal progenitor cells in the mouse skin. Bottom: molecular pathway leading to mitotic spindle orientation along the AB axis.

(C) Top view shows localization of the relevant polarity markers in a dividing vertebrate neuroepithelial progenitor shown in a top view (left) and a side view (right). Note that Gαi is localized uniformly at the cell membrane, whereas Pins and NuMA are enriched in a lateral ring. Bottom view illustrates molecular pathway leading to mitotic spindle orientation along the plane of the epithelium axis. The orientation along the a-p and dorsal-ventral axes of the neural tube is random.

(D) Top view shows localization of the relevant polarity markers in a dividing *Drosophila* pI progenitor shown in a top view (left) and a side view (right). Bottom view shows molecular pathway leading to mitotic spindle orientation in the plane of the epithelium axis and along the a-p axis. The orientation along the a-p axis is controlled by the Fz pathway, with Fz and Dsh localizing at the posterior apical cortex and Stbm and Prickle (Pk). Pins counteracts the AB tilt induced by Fz pathway to maintain the spindle in the plane of the epithelium (Bellaïche et al., 2004). The cell fate determinants Numb and Neuralized (Neur) are localized at the anterior lateral cell cortex (for review see Bardin et al., 2004).

random spindle movements, indicating that the complex is necessary and permissive for spindle movements and that its restricted localization is instructive to orient these movements (Peyre et al., 2011). Although Gαi subunits are required for the lateral recruitment of Pins and NuMA, they are homogeneous at the cell cortex, indicating that a yet unknown mechanism restricts Pins and NuMA localization in a ring. Like in invertebrates, NuMA is likely to regulate mitotic spindle orientation via the Dynein-Dynactin complex, whose components Lis1 and Huntingtin (Htt) were shown to control the planar orientation of the mitotic spindle of mouse neuroepithelial progenitors (Yingling et al., 2008; Godin et al., 2010). Htt localizes both at the centrosomes and at the cell cortex with Dynein and NuMA, and its loss of function perturbs the distribution of NuMA and Dynein on the spindle in cultured cells (Godin et al., 2010).

Drosophila *Sensory Organ Precursor Cell Division*. In the dorsal thorax (notum) of the *Drosophila* pupa, SOP (or pI) cells divide asymmetrically to produce a posterior cell, pIIa, and an anterior cell, pIIb, which will further divide to give rise to a mechanosensory organ (Gho et al., 1999; Fichelson and Gho, 2003). During the pI division, the cell fate determinants Numb and Neuralized localize at the anterior pI cell cortex and segregate exclusively to the anterior pIIb cell (for review see Bardin et al., 2004). Accordingly, the mitotic spindle aligns with the a-p axis of the fly body by rotation in late prophase (Gho and Schweisguth, 1998; Gho et al., 1999; Bellaïche et al., 2001a). The spindle is also slightly tilted along the AB axis (Gho et al., 1999; David et al., 2005) (Figure 2D). The pI has provided an excellent model to study planar mitotic spindle orientation along a tissue polarity axis in response to Frizzled (Fz) planar cell polarity (PCP) pathway, which signals in part via the Dishevelled (Dsh) protein (for review see Goodrich and Strutt, 2011).

Fz and Dsh localize at the posterior apical pI cell cortex, and they are essential for the correct orientation of the mitotic spindle along the a-p axis (Gho and Schweisguth, 1998; Bellaïche et al., 2001a, 2004). During pI cell division, Fz colocalizes at the posterior apical cortex with Mud (Ségalen et al., 2010). Accordingly, Dsh and a C-terminal domain of Mud can form a complex, and Dsh regulates the posterior apical localization of Mud (Ségalen et al., 2010). As observed in *fz* or *dsh* mutant pI cells, the a-p orientation of the mitotic spindle is lost in *mud* mutant pI cells (Ségalen et al., 2010). Although the role of the Dynein-Dynactin complex has not been studied in the pI cell, it is likely to function with Mud downstream of Fz because Dynein is needed for the correct orientation of the mitotic spindle during the *C. elegans* EMS cell division, which is also polarized by Fz signaling (Zhang et al., 2008).

The mechanisms maintaining the mitotic spindle in the plane of the epithelium during pI cell division are also partially understood. In pI cells, the Fz and Dsh signaling positions the Par complex at the posterior lateral cortex, and Pins and Gαi are restricted to the anterior lateral cortex (Bellaïche et al., 2001b) (Figure 2D). Strikingly, neither Par3 nor Pins is required to orient the mitotic spindle along the a-p axis (David et al., 2005). However, loss of Pins results in an increased tilting of the spindle toward an AB orientation, whereas *fz* and *dsh* mutant pI cells show a more planar spindle orientation in pI (David et al., 2005). Hence, Fz and Dsh signaling aligns the spindle with the a-p axis but concomitantly tilts it relative to the AB axis of the epithelium; the activity of Pins counterbalances the AB tilting induced by PCP signaling and therefore maintains the spindle in the plane of the epithelium. Because Pins and Mud colocalize at the anterior cortex and Fz-Dsh colocalize with Mud at the apical posterior cortex (Ségalen et al., 2010), the Fz-Dsh pathway and the Pins pathway act cooperatively through Mud to orient the mitotic spindle along the a-p axis, while maintaining the mitotic spindle in the plane of the epithelium.

In conclusion, the study of different models of asymmetric division in *Drosophila*, *C. elegans*, and vertebrate systems shows the existence of a diverse range of cortical cues that polarize dividing cells and orient their axis of division. Remarkably, either through Pins-Gαi or the Fz signaling pathway, they converge on members of the NuMA family, which emerges as a central regulator of mitotic spindle orientation during ACD.

Propagation of Mitotic Spindle Orientation from One Division to the Next by Spindle Polarity

In the previous section, we have described the classical linear view in which intrinsic or extrinsic cortical cues instruct cell division orientation. Here, we describe an additional mechanism whereby the intrinsic asymmetry of the spindle might be used to define cortical cues and to maintain cell division orientation from one division to the next. In animal cells, the interphase centrosome generally contains two closely apposed centrioles, which duplicate for the next round of division. Each daughter inherits a centrosome formed of a mature and a newly synthesized centriole, which will again duplicate for the next division. Therefore, the mitotic spindle is intrinsically asymmetric because one spindle pole is formed of a centrosome composed of a "grandmother" centriole and a daughter centriole, and the other pole of a "mother" centriole and a daughter centriole (for review see Strnad and Gönczy, 2008). The intrinsic spindle polarity may play distinct roles during division, in particular in the regulation of cell fate specification (Wang et al., 2009) and in mitotic spindle orientation.

The first evidence of a link between asymmetry in centriole age and spindle orientation in asymmetric division came from studies in stem cells of the *Drosophila* male germline (Yamashita et al., 2007) where the "grandmother" centriole is inherited by the stem cell. More recently, studies in fly larval NBs have suggested that the role of spindle asymmetry is to perpetuate polarity and spindle orientation from one cell cycle to the next. During the first division of the embryonic NBs after they delaminate from the neurectoderm, the two centrosomes first locate laterally on either side of the nucleus, and the spindle rotates 90° as it forms in prometaphase (Kaltschmidt et al., 2000). However, in the subsequent embryonic and all larval NB divisions, the mitotic spindle forms roughly aligned with its final position from prophase onward, and only slightly rotates or rocks during prometaphase and metaphase (Rebollo et al., 2007, 2009; Rusan and Peifer, 2007). How cell division orientation is regulated in these divisions has been revealed by real-time imaging of centrosomes in wild-type and mutant conditions (Rebollo et al., 2007, 2009; Rusan and Peifer, 2007). Immediately after cytokinesis, the NB centrosome splits in two before centriole duplication (Januschke et al., 2011). One of the resulting centrosomes remains associated with the apical cortex, organizing an MT apical network, whereas the other centrosome does not organize MTs and is

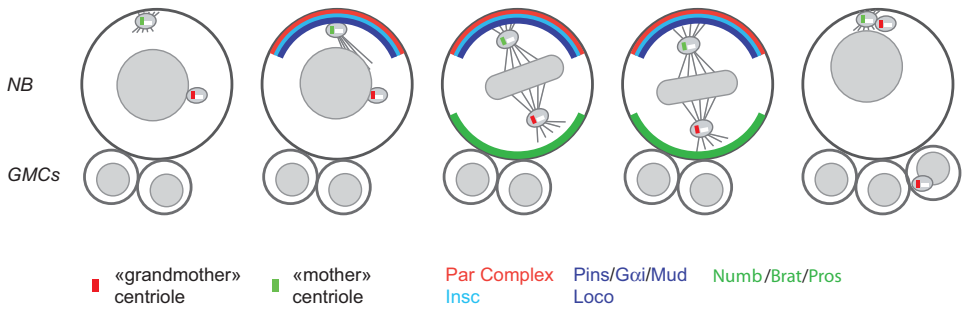

Figure 3. Spindle Polarity in *Drosophila* NBs

In larval NB divisions, except the first one, the mitotic spindle forms roughly aligned with its final position from prophase onward, and only slightly rotates or rocks during prometaphase and metaphase (Rebollo et al., 2007, 2009; Rusan and Peifer, 2007). In *Drosophila* NBs, the two centrosomes are characterized by different behaviors during the cell cycle. After cytokinesis, the centrosome inherited by the NB splits in two. One centrosome remains associated with the apical cell cortex through a dense MT network. The other centrosome, containing the oldest "grandmother" centriole (red), sheds its pericentriolar material. It shows intense movements throughout interphase and moves away to the basal pole of the cell. The spindle assembles with its near-definitive orientation. The centrosome containing the "mother" centriole (green) is inherited by the self-renewing NB.

highly mobile, eventually moving to the opposite half of the cell (Rebollo et al., 2007; Rusan and Peifer, 2007) (Figure 3). The "active" apical centrosome is labeled by Polo kinase (Rusan and Peifer, 2007) and is positioned in close contact with the region of the cortex where the apical Par and Pins proteins were located in the previous mitosis (Januschke and Gonzalez, 2010). Upon entry into the next mitosis, it remains in this position, and it specifies the position of apical Pins asymmetric accumulation (Januschke and Gonzalez, 2010). Upon nuclear envelope breakdown, the second centrosome also becomes active, and the mitotic spindle forms; the spindle slightly rotates during metaphase to its final anaphase orientation. Thus, the successive NB cell divisions are polarized by the apical centrosome that maintains its position from one division to the next (Rebollo et al., 2007; Rusan and Peifer, 2007; Januschke and Gonzalez, 2010). However, unlike in the *Drosophila* male germline stem cells, recent evidence based on real-time imaging of differentially labeled centrioles in larval NBs shows that the active apical centrosome is composed of the "mother" centriole produced during the previous division (Conduit and Raff, 2010; Januschke et al., 2011). Hence, the mitotic spindle has an intrinsic polarity, and the NB always inherits the youngest centrosome, which may perpetuate cell polarization from one division to the next (Figure 3).

The mechanisms controlling the intrinsic spindle centriolar asymmetry and centrosome anchoring are not fully understood. Although Pins is not necessary for the maintenance of a larger aster of MTs over the apical centrosome, it is necessary for subsequent maintenance of the apical centrosome at the apical cortex (Rebollo et al., 2007). The role of Khc-73 and Dlg in mitotic spindle orientation and cortical polarization (Siegrist and Doe, 2005) could suggest a possible function in the regulation of centrosome polarization. It will be interesting to determine whether the $Pins^{LINKER}$ pathway holds the apical centrosome in place during mitosis, while the $Pins^{TPR}$ pathway finely aligns the mitotic spindle with the cortical Par complex. More generally, analyzing the role of cortical polarity complexes and Mud differentially in the first versus the subsequent NB divisions might reveal how polarity is perpetuated from one division to the next and how centrosome segregation is controlled.

Future analysis in vertebrates should explore whether "spindle polarity" is a conserved mechanism to perpetuate cell polarization and mitotic spindle orientation from one division to the next. Of note, either the "grandmother" or the "mother" centriole associates with the self-renewing cell in different stem cell populations (Yamashita et al., 2007; Wang et al., 2009; Conduit and Raff, 2010; Januschke et al., 2011), suggesting that distinct mechanisms might link centriole age with cell fate determination and spindle orientation.

Does Mitotic Spindle Orientation Control Binary Cell Fate Decision?

The notion that mitotic spindle orientation controls binary fate choices derives largely from early studies of invariant lineages in *C. elegans* and *Drosophila*, in which a clear correlation between cell polarity, spindle orientation, and asymmetric distribution of cell fate determinants has been described. This connection is at the root of the idea that spindle orientation is essential for the maintenance of stem cell populations, and that its deregulation may be a cause of tumorigenesis (Caussinus and Gonzalez, 2005; Knoblich, 2010). In vertebrates, the notion of ACD is not as clearly defined as in invertebrates. Indeed, the existence of invariant cell lineages in vertebrates is a matter of debate (Jones and Simons, 2008). Nonetheless, a correlation between cell division and the acquisition of different fates, suggestive of the existence of ACDs, has been shown in progenitor cells in muscle (Shinin et al., 2006), skin, and the developing nervous system (as discussed above). By analogy with the fly NB, it has been proposed that the orientation of cell division may regulate the identity of the progeny in a binary way in these tissues. We review here recent results that (1) reevaluate the contribution of spindle orientation to asymmetric fate choices in *Drosophila* NBs, (2) support the conservation of a functional relationship between spindle orientation and cell fate decisions in the embryonic mouse epidermis, (3) analyze the role of spindle orientation in vertebrate ventricular progenitors of the neuroepithelium.

Asymmetric Division of Drosophila NBs

Uncovering the specific contribution of mitotic spindle orientation in NBs versus GMC fate decisions has been hampered by

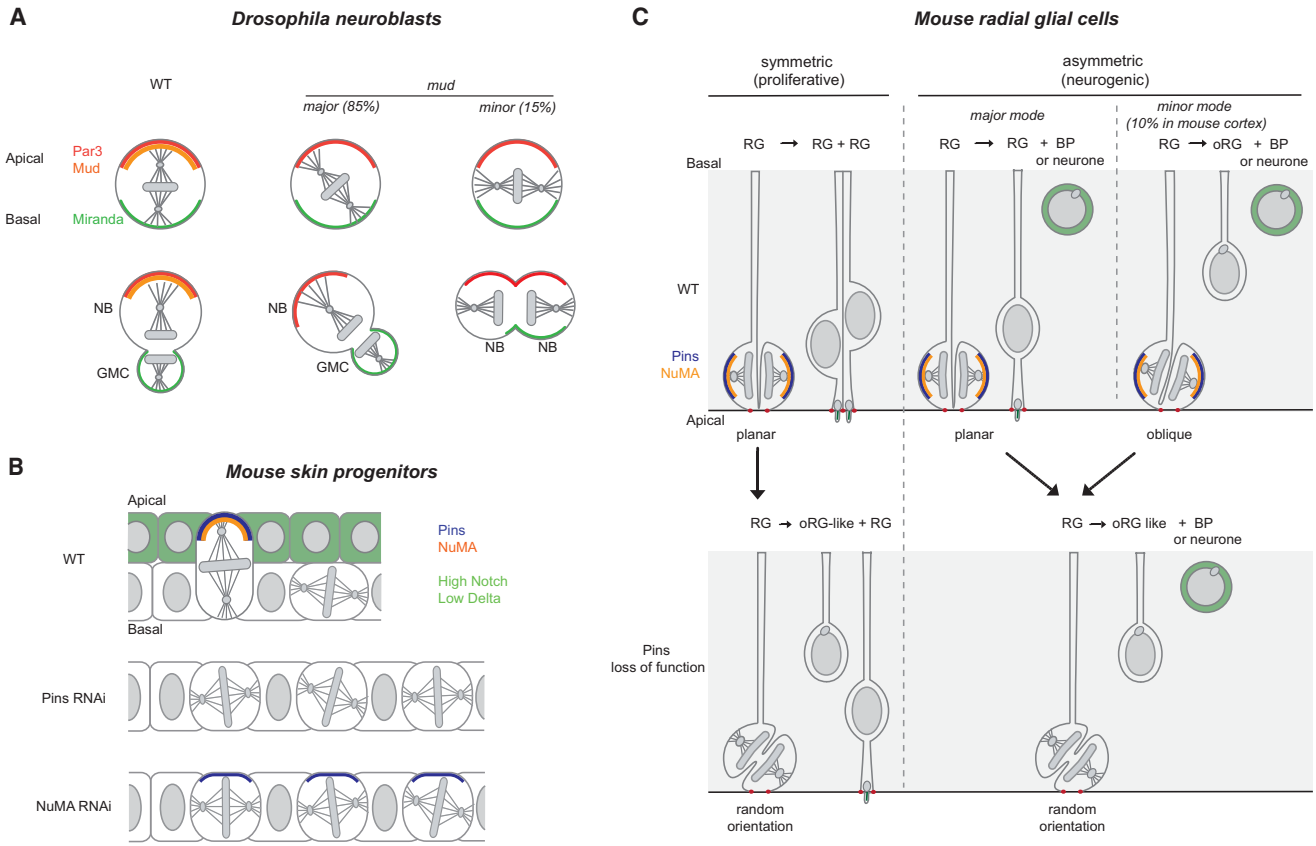

Figure 4. Role of Mitotic Spindle Orientation in Binary Cell Fate Specification

(A) In *Drosophila* NB, spindle orientation is correlated with the AB axis of the cell and the asymmetric localization of fate determinants. In *mud* mutant NBs, spindle orientation is randomized, while polarity is not affected in metaphase. Yet, in the majority of mutant NBs in anaphase (left), fate determinants segregate mostly in the basal daughter cell, a process known as "telophase rescue." Accordingly, the apical cell adopts the NB fate, whereas the basal one adopts a GMC fate. In a minority of *mud* NBs, the spindle is perpendicular to its wild-type orientation (right). "Telophase rescue" does not occur in this context, and both daughters adopt the NB identity, despite their inheritance of GMC fate determinants. Miranda is an adaptor protein required for the basal segregation of the cell fate determinant Pros (Ikeshima-Kataoka et al., 1997), and was used as a reporter for basal segregation in the study by Cabernard and Doe (2009).

(B) AB versus planar orientation of the division of skin basal progenitors regulates the asymmetric versus symmetric nature of the fate decisions, respectively, and simultaneously promotes the stratification versus elongation of the tissue. Loss of Pins or NuMA function disrupts both asymmetric division and stratification.

(C) In the top row, in mouse radial glial cells, both symmetric proliferative (left) and asymmetric neurogenic (middle) divisions are planar. In most divisions, the two daughter cells inherit subapical junctions, which maintain their position next to the ventricular surface. In neurogenic divisions, one of the two sisters retracts its apical attachment, delaminates to migrate away from the ventricular surface, and differentiates as a neuron or becomes a basal progenitor (BP) that will usually undergo a terminal division (Shitamukai et al., 2011). A minority of divisions is slightly oblique (right), so that the cell that inherits the basal process loses the apical attachment. This cell retains the molecular signature of RG and is proposed to become an outer radial glia (oRG) (Shitamukai et al., 2011). It is not clear whether oRG and RG are a single cell type with two different localizations or whether they have different properties. oRG cells are present in low quantity in the mouse cortex (Shitamukai et al., 2011; Wang et al., 2011) but much more frequent in the ferret and primates (Hansen et al., 2010; Fietz et al., 2010). The sister cell probably delaminates and becomes a neuron or a basal progenitor (although Wang et al. [2011] propose that it remains a RG). Bottom row shows that loss of Pins function results in random spindle orientation. Clonal analysis of the fate and position of the progeny shows that random spindle orientation does not change the symmetric versus asymmetric nature of the division but affects the position of one daughter cell: scattered cells expressing markers of ventricular progenitors are found away from the ventricular surface. In the mouse cortex, spindle randomization favors the oRG cell localization at the expense of RG.

the fact that Par complex components, Gαi, Pins, and Insc control cell polarity, spindle orientation, and the distribution of fate determinants. Remarkably, mutations in *mud* specifically affect spindle orientation and not AB polarity (Bowman et al., 2006; Izumi et al., 2006; Siller et al., 2006). This has allowed a more refined analysis of the role of spindle orientation (Bowman et al., 2006; Izumi et al., 2006). Cabernard and Doe (2009) used live imaging to follow the distribution of apical polarity markers and basal fate determinants between daughters of *mud* mutant NBs (Figure 4A). This study reveals two things. First, in the majority of *mud* mutant NBs, fate specification is correct even though spindle orientation is defective. This can be attributed to the existence of a "telophase rescue" phenomenon, which redistributes fate determinants in accordance with spindle orientation immediately before cytokinesis in the majority of mutant NBs, irrespective of the AB polarity axis. The mechanisms of "telophase rescue" are not entirely clear and may act through cortical polarization by the Dlg-Khc-73 pathway (Siegrist and Doe, 2005). In addition, in cases of imperfect distribution of fate determinants at the time of cytokinesis, subtle differences in their amount inherited by sister cells may be sufficient to trigger an amplification loop that ultimately resolves the binary fate

choice between sisters. Such a phenomenon has been observed in the *Drosophila* pI cell via the Notch signaling pathway (for review see Bardin et al., 2004). Second, a minority of *mud* mutant NBs divide with their axis of division perpendicular to the AB axis. These cells always generate two equal-sized daughters with a NB identity. Strikingly, basal fate determinants still segregate asymmetrically in most of these divisions but fail to promote a GMC fate (Figure 4A). Cabernard and Doe (2009) observed that the apical marker Par3 is always inherited by both sisters and might overrule the basal determinants to dictate a NB fate. Nonetheless, overexpression of the basal determinant Prospero can switch both sisters from an NB to a GMC identity. Hence, these data indicate that it is the ratio of basal versus apical determinants in the daughter cells, more than the strict binary distribution of fate determinants between sister cells, that controls their GMC versus NB fate. In summary, the precise orientation of the mitotic spindle appears to be one of several mechanisms, which concur to facilitate the regulation of asymmetric fate in the NB progeny.

Asymmetric Division in Vertebrate Skin Progenitor Cells

During embryonic mouse skin development, the single-layered surface ectoderm covering the mouse embryo must initiate stratification and terminal differentiation to develop a functional epidermis (Lechler and Fuchs, 2005). A shift from planar to predominantly perpendicular basal cell division coincides with stratification and the formation of suprabasal differentiated cells (Figure 2B). The reduction in AB cell division caused by the loss of Pins, NuMA, or dnct1 function is associated with defects in stratification, differentiation, and barrier formation of the epithelial tissue (Figure 4B), indicating that orientation of the mitotic spindle is important for correct specification of suprabasal (differentiated) daughter cells (Williams et al., 2011). The correct specification of the suprabasal cell layer was shown to depend on Notch signaling activity. Notch ligands Dl2 and jag2 are expressed in the basal cell, whereas Notch2 and Notch3 receptors as well as the Notch target gene HES1 are expressed in the suprabasal cells. Loss of Pins function is associated with a decrease in Notch signaling activity in suprabasal cells. The stratification and differentiation defects observed in the Pins mutant embryos are reminiscent of the ones observed in mutant embryos for Rbpj, an obligatory DNA binding partner of Notch intracellular domain (de la Pompa et al., 1997). In conclusion, in this system the orientation of cell divisions provides a regulatory role in cell fate decisions controlling the differentiation of mouse epidermis progenitor cells. Whether this corresponds to a strict requirement remains to be elucidated, for example using live analyses and fate mapping of sister cells to compare the wild-type and mutant spindle orientation situations. In addition, spindle orientation plays an essential role in the organization of the tissue and promotes stratification by controlling the relative position of the different cell types. This double role in fate determination and stratification may explain why the phenotype of Pins, NuMA, and Gαi loss of function appears much more dramatic in the vertebrate skin than in *Drosophila* NBs.

Mitotic Spindle Orientation of Neuroepithelial Progenitors during Vertebrate Neurogenesis

Ventricular neuroepithelial progenitors are highly polarized cells that compose the pseudostratified neuroepithelium. They harbor a small cortical apical domain ("apical endfoot") and a basal-lateral domain that includes a thin and extended basal process connected to the pial surface of the tissue (Figure 4C). During an initial proliferative phase, neuroepithelial progenitors amplify their pool through symmetric (proliferative) divisions. They later switch to a neurogenic phase during which they divide asymmetrically to renew a ventricular progenitor (the radial glia, RG) and produce a more committed daughter cell, which migrates basally. Initial observations in the ferret neocortex suggested that AB divisions were asymmetric and neurogenic, whereas planar divisions were symmetric and proliferative (Chenn and McConnell, 1995). However, the vast majority of neural progenitors divide with a near planar orientation even at stages where asymmetric divisions predominate (Kosodo et al., 2004; Noctor et al., 2008). This suggested that minor shifts in spindle orientation may regulate symmetric versus asymmetric division by causing the cleavage plane to respectively either bisect or bypass the apical domain, whose constituents could act as cell fate determinant(s) maintaining the RG fate (Kosodo et al., 2004; Marthiens and ffrench-Constant, 2009).

A prediction of this model is that the loss of planar spindle orientation should favor asymmetric divisions and lead to accelerated neurogenesis. Indeed, studies analyzing the loss of function of a number of different genes have described a correlation between spindle orientation defects and premature neuronal differentiation at the expense of RG cells in the cortex (Feng and Walsh, 2004; Fish et al., 2006; Gauthier-Fisher et al., 2009; Godin et al., 2010). However, in the mouse cortex and in the chick spinal cord, the high proportion of oblique divisions resulting from randomization of spindle orientation by Pins or NuMA loss of function did not accelerate neurogenesis but caused the scattering of progenitors in the subventricular zone (Morin et al., 2007; Konno et al., 2008; Peyre et al., 2011). Clonal fate analysis in vivo showed that these ectopic progenitors retain the molecular signature of their ventricular counterpart, indicating that they have not changed their identity (Figure 4C) (Morin et al., 2007; Konno et al., 2008; Shitamukai et al., 2011).

In conclusion, in the context of the divisions of NBs and skin progenitors, the role of mitotic spindle orientation in cell fate determination is established. So far, in the vertebrate neuroepithelium, the published data demonstrate a role of planar spindle orientation in the organization of the ventricular proliferation zone. Whether spindle orientation also has a direct instructive role on cell fate specification is still unclear because none of the studies in which the spindle is misoriented has addressed the distribution of fate determinants. Clearly, the unambiguous identification of fate determinants, and of their distribution in asymmetrically dividing RG, is needed to solve this long-standing question.

Having reviewed the mechanisms and roles of spindle orientation in the context of cell fate specification, we will now address the role of mitotic spindle orientation in the context of tissue architecture and tissue morphogenesis. Strikingly, in this context divisions are mostly symmetric, yet some of the mechanisms described above are also at play to regulate spindle orientation.

Planar Orientation of Symmetric Cell Division and Epithelial Tissue Architecture

During growth and homeostasis of epithelial tissues, the newborn cells remain in the epithelial plane, and this is achieved

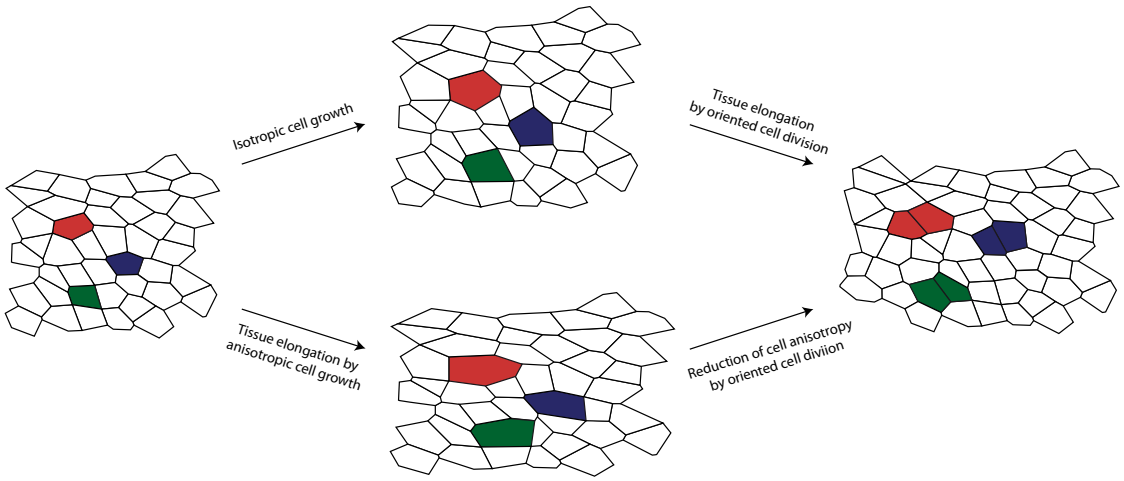

Figure 5. Two Possible Models by which OCD Might Contribute to Tissue Elongation

Cell division orientation is the main "driving force" for tissue elongation (top path). Cell growth is isotropic (red, green, and blue cells) during interphase, but upon cell division the positioning of the two daughter cells in the tissue leads to a local elongation. In such a model, blocking cell division prevents tissue elongation. Anisotropic cell growth drives tissue elongation (bottom path). Cell growth is anisotropic (red, green, and blue cells) either due to an increase of cortical tension perpendicular to the tissue elongation axis or to a global anisotropic constraint along the tissue elongation axis. Cell division oriented along the cell long axis will reduce cell anisotropy and maintain tissue packing. In such a model, blocking cell division does not prevent tissue elongation. Note that the two models are not mutually exclusive: OCD itself might generate a local elongation of neighboring cells, and this elongation might in return trigger an anisotropic cell growth.

by the orientation of the mitotic spindle in this plane. The mechanisms regulating planar orientation have yet to be studied in vivo, but they might be dependent upon the activity of Pins/Gαi/NuMA/Dynein, as shown by studies on MDCK cells and MDCK cyst formation (Reinsch and Karsenti, 1994; Busson et al., 1998; Zheng et al., 2010; Hao et al., 2010). In MDCK cysts, the distribution of Gαi, Pins, and NuMA is similar to the one described in dividing neurepithelial cells. Gαi is homogeneous at the cortex, whereas Pins and NuMA are restricted to the lateral cell cortex. This lateral restriction has been investigated in this model: direct phosphorylation of Pins by apical aPKC increases its affinity for a 14-3-3 protein. 14-3-3 competes with $G\alpha i_{GDP}$ subunits for the interaction with Pins, leading to the release of Pins from the apical cortex and its localization as a ring-like structure where it could recruit NuMA and the Dynein-Dynactin complex (Hao et al., 2010). It will be important to determine whether these mechanisms are used in the context of developing and adult epithelial tissues. In vivo, an obvious challenge will be to distinguish a direct effect on mitotic spindle orientation from a more indirect defect on AB polarity, which in turn compromises planar mitotic spindle orientation.

Orientation of Symmetric Cell Division and Tissue Morphogenesis

The stereotypical orientation of symmetric cell divisions during tissue morphogenesis in multiple tissues has led to the proposal that OCDs participate in tissue morphogenesis, such as neural tube formation in the zebrafish (Ciruna et al., 2006; Tawk et al., 2007; Quesada-Hernández et al., 2010; Žigman et al., 2011) or tissue elongation. Here, we review the role and mechanisms of OCD in the context of tissue elongation that has been the focus of many recent studies.

OCD may contribute to tissue elongation by two distinct but nonmutually exclusive mechanisms: (1) cell growth is isotropic, and OCD drives tissue elongation by positioning daughter cells along the axis of elongation (for review see Keller, 2006; Lecuit and Le Goff, 2007) (Figure 5); and (2) growth is anisotropic in the direction of tissue elongation. OCD along the cell long axis (so-called "Hertwig rule") would therefore reduce cell elongation to restore isotropic cell shape; anisotropic cell growth may be intrinsic or may be the result of a global tissue anisotropic stress (Figure 5). In order to understand whether OCD drives or results from tissue elongation, a prerequisite is to identify the molecular pathways, which regulate cell division orientation during symmetric cell division. The mechanisms controlling OCD in tissue morphogenesis were first identified in the zebrafish gastrula and were shown to depend on the Wnt-Fz PCP pathway (Gong et al., 2004). Later, the Fat-Dachsous (Ds) pathway was shown to be essential in orienting cell division during tissue morphogenesis in both *Drosophila* and mouse (Baena-López et al., 2005; Saburi et al., 2008). The recent studies reviewed below have further characterized the mechanism of mitotic spindle orientation; they indicate that the role of cell division orientation in tissue elongation varies between tissues and suggest novel roles for mitotic spindle orientation in tissue development.

The Wnt-Fz Signaling Pathway

OCDs have been described in the zebrafish embryo (Concha and Adams, 1998; Gong et al., 2004; Ciruna et al., 2006; Tawk et al., 2007; Žigman et al., 2011). In particular, cell divisions are oriented along the embryo's a-p axis in the epiblast, which dramatically elongates along the embryo's a-p axis and gives rise to the neural ectoderm and the epidermis (Concha and Adams, 1998; Gong et al., 2004). The epiblast elongation is known to depend on cell-cell intercalation regulated by the Wnt-Fz PCP pathway (Heisenberg et al., 2000). Strikingly, a disruption of the PCP pathway by loss of function of Wnt-11, Frizzled7 (Fz7), Dishevelled (Dvl), or Strabismus (Stbm) affects

cell division orientation and correlates with a reduction in elongation of the epiblast, suggesting a possible link between OCD and tissue elongation (Gong et al., 2004; Quesada-Hernández et al., 2010). Testing the role of OCD in epiblast elongation was made possible only recently by finding that Dynein and NuMA act downstream of the PCP pathway to regulate OCD (Quesada-Hernández et al., 2010; Ségalen et al., 2010). Loss of Dynein or NuMA function randomizes cell division orientation in the epiblast. Furthermore, NuMA was shown to interact with Dvl and to be recruited to the cortex upon overexpression of Dvl (Ségalen et al., 2010). Hence, much like in the *Drosophila* pI asymmetric division, NuMA and Dynein act downstream of the Wnt-Fz PCP pathway to regulate mitotic spindle orientation in symmetric cell divisions. Strikingly, NuMA or Dynein losses of function as well as the inhibition of cell division do not perturb tissue elongation (Quesada-Hernández et al., 2010; Ségalen et al., 2010). The results collectively demonstrate that cell division is not instructive for tissue elongation during gastrulation in zebrafish embryos.

In parallel, recent work in the developing limb bud mesenchyme of the zebrafish, chick, and mouse embryos has shown that cells elongate and preferentially divide along the proximodistal axis of the limb bud in a Wnt-5a-dependent manner, and that this orientation parallels the axis of elongation of the structure (Wyngaarden et al., 2010; Gros et al., 2010). These studies did not determine, however, whether cell elongation or OCDs are the cause of tissue elongation.

Fat-Ds Pathway

Fat and Ds are two heterophilic atypical cadherins. They have recently emerged as components of a conserved signaling pathway, the Fat-Ds pathway (also known as the Fat-Ds/Four-jointed [Fj] pathway), controlling tissue size, tissue planar polarity, and tissue shape in *Drosophila* and vertebrates (Reddy and Irvine, 2008). Fat and Ds bind to each other, and their binding is regulated by the Golgi protein kinase Fj (Ishikawa et al., 2008; Simon et al., 2010). In *Drosophila*, the *ds* and *fj* genes are expressed in opposing gradients within the tissue, which promote the graded activation of the Fat-Ds pathway and directional information within the tissue (Reddy and Irvine, 2008).

The role of Fat-Ds signaling in the regulation of mitotic spindle orientation was first identified in *Drosophila*, whose wings have an elongated shape along their proximal-distal (p-d) axis. Somatic clones in the wing are elongated along the p-d axis, indicating that the growth of this tissue is larger along the p-d axis (Figure 5). Furthermore, cell division orientation in the wing imaginal disc is also preferentially oriented along the p-d axis during wing development (Baena-López et al., 2005). In either *fat* or *ds* mutant wings, cell division orientation is random relative to the p-d axis, somatic clones adopt a rounder shape, and elongation of the wing along the p-d axis is reduced (Baena-López et al., 2005).

The Fat-Ds pathway has a conserved role in the orientation of cell division in vertebrates. During mouse postnatal nephron maturation, kidney tubules elongate dramatically while maintaining a constant diameter. Somatic clones indicate an oriented elongation along the axis of tubule lengthening. The orientation of cell divisions is strongly biased along the direction of kidney tubule elongation (Fischer et al., 2006), and disruption of the *Fat4* gene results both in cell division misorientation and in shorter and enlarged tubules (Saburi et al., 2008). Strikingly, the tubule elongation phenotype of $Fat4^{-/-}$ mice is enhanced by removing one copy of the Wnt-Fz PCP pathway gene Vangl2 (Saburi et al., 2008). The synergistic effect between the Wnt-Fz and Fat-Ds pathway might be due to an earlier function of the Wnt-Fz PCP pathway in cell division orientation. Indeed, during embryonic development, Wnt7b, secreted by the ureteric epithelium, regulates cell division orientation by activating the expression of PCP Wnts (Wnt5a, Wnt11, Wnt4) in the interstitial cells (Yu et al., 2009). Therefore, both Wnt-Fz and Fat-Ds pathways regulate cell division orientation at different stages of the development of the kidney tubule.

Collectively, the studies demonstrate that the Fat-Ds pathway contributes to both tissue elongation and orientation of cell divisions in the *Drosophila* wing and in mouse kidney. These data, however, do not directly show that the Fat-Ds pathway drives tissue elongation through OCD.

Regulation of OCD by the Dachs Myosin and Local Cell Topology

As exemplified in the context of Wnt-Fz signaling pathway in the fish epiblast, the characterization of mechanisms by which the Fat-Ds pathway regulates mitotic spindle orientation will permit us to test more directly the role of OCD in tissue elongation. Although these mechanisms have yet to be fully understood, the Fat-Ds pathway was recently shown to regulate mitotic spindle orientation in *Drosophila* wing imaginal discs via the Dachs unconventional myosin (Mao et al., 2011). In parallel, in the same tissue, it was proposed that cell division orientation is controlled by an unforeseen mechanism involving local cell topology (Gibson et al., 2011).

The graded activation of the Fat-Ds pathway is reflected by the polarized enrichment of Dachs at the proximal edge of the cells in around 50% of epithelial cells (Mao et al., 2006; Rogulja et al., 2008; Schwank et al., 2011). In *dachs* mutant wings, cell division orientation is random relative to the p-d axis and the elongation of the wing is reduced (Mao et al., 2006, 2011). Analysis of cell shape and computer simulation reveals that Dachs could regulate mitotic spindle orientation by regulating cell shape downstream of the Fat-Ds pathway. Indeed, in the wing epithelial tissue, cell division orientation is biased along the long cell axis. In *dachs* mutant tissue, the apical domain of cells is larger, suggesting that Dachs might control apical cell shape by regulating cortical tension (Mao et al., 2011). Accordingly, computer simulations show that a polarized cortical tension along a given axis and an orientation of cell division relative to the cell long axis are sufficient to elongate a tissue perpendicular to the axis of polarized cortical tension (Mao et al., 2011). Further analyses correlating Dachs polarization, cortical tension, cell shape, and mitotic spindle orientation will elucidate the role of Dachs myosin polarization in the regulation of tissue morphogenesis via cell elongation or OCD.

Within a proliferative monolayered epithelial tissue, the number of sides of the apex of the cell (one aspect of cell topology) adopts a given distribution: six-sided cells are the most frequent, five- and seven-sided cells are frequent, and four- and eight-sided cells are rare (Gibson et al., 2006; Farhadifar et al., 2007; Aegerter-Wilmsen et al., 2010; Staple et al., 2010). A recent study has analyzed whether the local topology of a dividing cell (i.e., the number of sides of its immediate

neighbors) influences its interphasic shape and therefore provides a cue to orient the mitotic spindle according to the "Hertwig rule" (Gibson et al., 2011). An ordered mechanical model shows that a central cell, surrounded by mostly hexagonal cells and by one small four-sided cell, tends to elongate in an orientation orthogonal to the position of the four-sided cell; on the contrary, if a large eight-sided cell replaces the four-sided cell, the central cell elongates in the direction parallel to the position of the eight-sided cell. This suggests that a cell should preferentially divide parallel to the position of its four-sided neighbors and orthogonal to the position of its eight-sided neighbors. Accordingly, in the *Drosophila* imaginal wing tissue, in late telophase the chance of finding a four-sided cell near the telophase bridge is much higher than the chance of finding an eight-sided cell. This observation and the mechanical model concur to show that the local cell topology biases cell division orientation. Nevertheless, it would be interesting to analyze how four- and eight-sided cells, which are rare in proliferative tissues, impact on the overall distribution of the cell division orientation (Gibson et al., 2006; Farhadifar et al., 2007; Aegerter-Wilmsen et al., 2010; Staple et al., 2010). A local cell topology rule cannot explain a bias of cell division orientation along a tissue symmetry axis. Further studies should therefore explore the interplay between the local cell topology rule and the Fat-Ds signaling pathway orienting cell division along tissue symmetry axis.

Interplay between Fz PCP and Fat/Ds Pathway: A Novel Role for OCD during Tissue Morphogenesis

In the context of an extensive study of the mechanisms of planar cell polarization by the Fat-Ds pathway, Aigouy et al. (2010) have revealed a possible and unexpected role of OCD in linking tissue elongation and tissue planar polarization. During pupal development, the *Drosophila* wing blade drastically elongates along its p-d axis, concomitant with the contraction of the proximal wing hinge. Prior to hinge contraction and wing elongation, Fz and Stbm (a PCP pathway component) planar cell distribution is oriented at around 60° relative to the p-d axis. Strikingly, p-d elongation of the wing blade correlates with the reorientation of Fz and Stbm planar cell polarization along the p-d axis, suggesting a coupling between tissue elongation and tissue PCP. Accordingly, mechanical severing of the hinge or loss of Ds function abrogates tissue elongation and affects PCP reorientation. This model is supported by additional experimental observations and computer simulations. The hinge contraction occurs concomitantly with the p-d elongation of wing blade cells and their division along the p-d axis. Furthermore, upon cell division, PCP proteins are not relocalized to the interface formed between the two daughter cells. Because cell divisions are oriented and PCP proteins do not reassemble at the newly formed interfaces, cell divisions thereby modify the orientation of PCP protein localization. Accordingly, computer simulations including cell and PCP protein dynamics demonstrate that OCDs and the exclusion of PCP from the newly formed interfaces are sufficient ingredients to reorient PCP either parallel or orthogonally to the tissue elongation axis. In summary this elegant study reveals a possible novel role for OCD during tissue elongation, i.e., coupling tissue elongation and planar polarization along the same axis.

Collectively, these results indicate that a correlation between orientation of cell division and tissue elongation is not an absolute indication of a function of cell division in tissue elongation and that therefore the role of OCD might vary from tissue to tissue, with OCD being either a cause or a consequence of tissue elongation. Furthermore, it will be important in the future to integrate the role of OCD with other morphogenetic events, such as cell-cell rearrangements and cell shape changes.

From the Empirical Cell Long Axis Rule to the Prediction of Mitotic Spindle Orientation

A problem in understanding mitotic spindle orientation in the study of symmetric cell division in tissue or cell culture is the usual absence of obvious cortical landmarks, which have been instrumental in deciphering mitotic spindle orientation in models of ACD. For more than a century, the "Hertwig rule," which states that cells divide along their long cell axis, was the only rule to predict cell division orientation in cell culture or in embryos. This rule poses at least two questions: (1) What are the underlying biological or biophysical mechanisms orienting division along the interphasic long axis of the cell? and (2) How do mitotic rounded cells "remember" their interphasic cell shape? By using microfabrication techniques, two experimental and theoretical studies have addressed these questions.

Using micropatterning techniques, the geometry of the cell adhesion pattern (i.e., where the cell attaches to the substratum) can be reproducibly defined (Théry et al., 2005). By changing the geometry of the adhesion pattern without changing the overall shape of the cell, it was demonstrated that the geometry of the adhesion pattern, rather than cell shape, dictates the mitotic spindle orientation (Théry et al., 2005). Cells round up during mitosis but remain connected to the adhesive substrate by retraction fibers, whose distribution during mitosis is dictated by the geometry of the micropattern during interphase (Figure 6A). Strikingly, assuming that the distribution of retraction fibers defines the distribution of FGs at the cortex of the dividing cell is sufficient to predict cell division orientation (Théry et al., 2007). This provides an elegant model by which cells "remember" their adhesion pattern and divide.

Round one-cell stage sea urchin embryos are devoid of retraction fibers, and the orientation of cell division cannot be dictated by adhesion to the substratum. Nevertheless, using microfabricated 3D molds, in which the sea urchin zygote is gently inserted, its shape can be reproducibly defined (Minc et al., 2011). Strikingly, sea urchin embryos respond to this deformation by reproducibly modulating the shape of their nucleus and by dividing along a specific orientation. Although the "Hertwig rule" applies to most shapes, it is not sufficient to predict mitotic spindle orientation in several specific shapes. To generate a theoretical model of mitotic spindle orientation, Minc et al. (2011) made the elegant assumption that the pulling force generated on the spindle pole by each MT scales with its length, as proposed in the context of *C. elegans* zygote division (Grill and Hyman, 2005; Kimura and Onami, 2005). Strikingly, using a single adjustable parameter that reflects the strength of coupling between the MT and FGs as well as the noise of the system, the theoretical model faithfully reproduces the distribution of mitotic spindle orientation in all tested shapes imposed to the sea urchin embryo (Figure 6B) (Minc et al., 2011). The scaling between MT length and pulling force is not easily explained by a limited number of FGs at the cell cortex. This raises the interesting notion that FGs might also be present in the cytoplasm (Wühr et al., 2010).

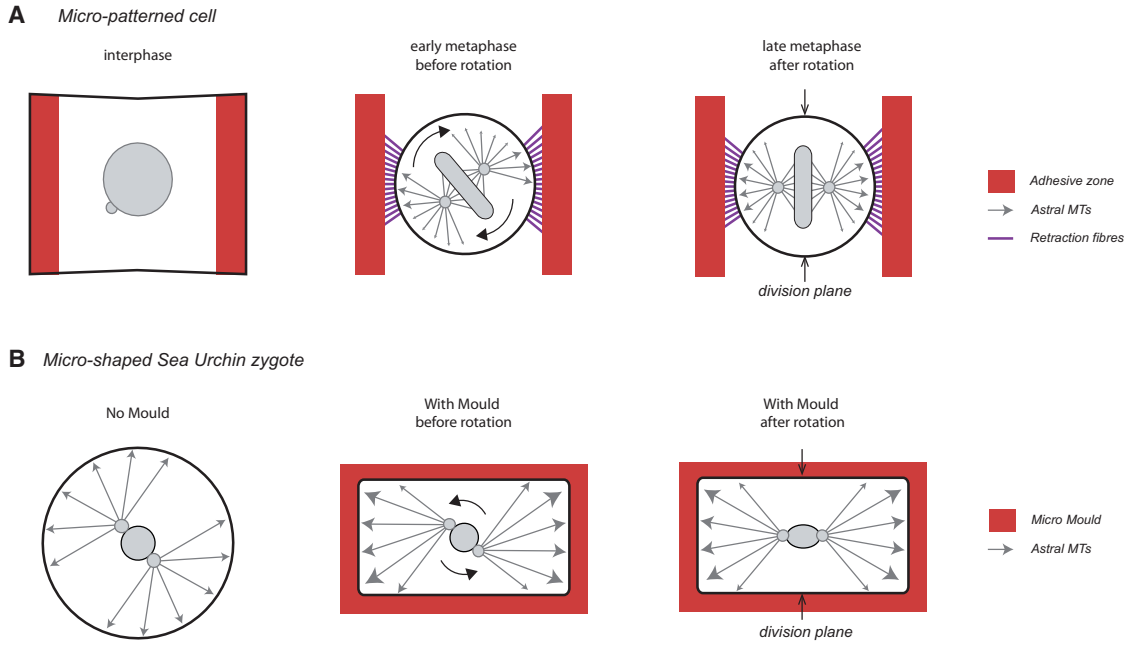

Figure 6. Predicting Cell Division Orientation

(A) Micropatterned cell in mitosis. The adhesive pattern (red) dictates the shape of the interphase cell (left) as well as the distribution of retraction fibers (purple) of the round mitotic cell (middle and right). The distribution of retraction fibers controls the position of FGs, which regulate the distribution of pulling forces on astral MTs (gray arrows). Imbalance in pulling forces produces a torque (black arrows), which results in the rotation of the spindle (middle). Arrows emanating from the centrosomes schematize the astral MTs. The size of the arrowhead indicates the strength of the pulling forces induced by the distribution of FGs. The position of the division plane is also indicated.

(B) Left: spherical sea urchin embryos divide without spindle rotation. Middle and right: microshaped sea urchin zygote. A microfabricated mold (red) controls the elongated shape of the sea urchin embryo. Long astral MTs (gray arrows) are pulled with a larger force than the one pulling on short MTs, which generate a force imbalance and a torque (middle) resulting in rotation and nucleus elongation (right). The size of the arrowhead indicates the strength of the pulling forces induced by the lengths of the MTs. The distribution of forces orients the mitotic spindle and elongates the nucleus. The position of the division plane is also indicated.

Collectively, the two studies have provided an elegant explanation of the empirical "Hertwig rule." Importantly, they demonstrate that the century-old rule is a consequence of either the geometry of cortical landmark position in interphase or of the existence of a correlation between the length of the astral MTs and the forces applied. Finally, they demonstrate that distinct biological or biophysical mechanisms could concur to generate a bias in mitotic spindle orientation relative to cell shape. These two studies also pave the way for understanding the mitotic spindle orientation in complex multicellular tissues (Minc et al., 2011).

Pulling on Microtubules? Not So Simple!

The genetic and biochemical data all add up to a model whereby the Dynein-Dynactin complex anchored at the cell cortex walks along MTs to pull on the spindle poles. To complete this model in both symmetric and ACD, it is essential to add at least three additional elements: (1) the cell cortical tension, (2) the dynamics of MTs, and (3) a restoring force preventing the collapse of the mitotic spindle on the cortex (Grill and Hyman, 2005). The stereotypical posterior movement of the anaphase aster in the *C. elegans* zygote has been extensively studied by high temporal and spatial resolution optical methods, laser ablation, computer simulation, and theoretical approaches to integrate these elements in mitotic spindle positioning.

Cell Cortical Tension

The presence of FGs attached to the plasma membrane and pulling on the MTs supposes the existence of tension on the membrane to prevent membrane invagination. Work in the *C. elegans* zygote has demonstrated that the actin-myosin network prevents FGs from deforming the plasma membrane (Redemann et al., 2010). Upon partial deletion of Myosin II function, long membrane invaginations are pulled from the plasma membrane toward the spindle pole. The number of invaginations nearly matches the number of FGs determined by centrosome laser ablation. Loss of Gα, GPR-1/2, or LIN-5 suppresses their formation. This indicates that MT pulling forces are associated with membrane invagination and that the cortex rigidity might balance plasma membrane invagination to promote spindle positioning. In agreement with the potential role of cortical tension in mitotic spindle orientation, Myosin was previously shown to regulate both mitotic spindle positioning and nuclear centration, a process requiring MT-cortex interaction to position the nucleus at the center of the *C. elegans* zygote (Severson and Bowerman, 2003; Goulding et al., 2007).

Does higher cortical tension correlate with higher pulling forces on MTs? In the *C. elegans* zygote, the posterior cortex is characterized by a lower cortical tension relative to the anterior cortex (Munro et al., 2004; Mayer et al., 2010). However, the posterior cortex is associated with a net larger MT pulling force (Grill et al., 2001, 2003). Accordingly, it as been proposed that

a softer deformable cortex permits a longer association between FGs and the MTs, thus providing sustained pulling forces on the MTs (Kozlowski et al., 2007).

Collectively, these results suggest that cortical tension has to be finely regulated during mitosis to provide a balancing tension to MT pulling forces, while allowing sustained association between FGs and the MTs. Nevertheless, the role of cortical tension remains challenging to decipher because several regulators of actin or Myosin also affect the polarization of both Par proteins and GPR-1/2.

MT Dynamics and Restoring Force

Quantifications of MT dynamics using the EB1 plus-end marker or α-tubulin fused to GFP during anaphase of the *C. elegans* zygote division have shown that: (1) the number of MTs at the cortex is similar at the anterior and posterior poles of the zygote (Kozlowski et al., 2007); and (2) MT catastrophe is seldom in the cytoplasm, but MTs briefly contact the cortex (between 0.1 and 1 s) in an end-on configuration (the MTs remain orthogonal and do not slide on the cortex) prior to undergoing catastrophe (Labbé et al., 2003; Srayko et al., 2005; Kozlowski et al., 2007). The brief interaction between the cortex and the MT suggests that MTs rapidly depolymerize upon touching the cortex, and that FGs function by attaching to the depolymerizing MTs. Quantification of MT dynamics has also indicated the origin of the restoring force, which prevents the collapse of the spindle pole on the cortex (Kozlowski et al., 2007). Individual MTs are very dynamic but tend to regrow along preexisting MTs, therefore forming stable (but dynamic) MT fibers extending from the centrosome to the cortex. Furthermore, each movement of the aster toward the cortex is concomitant with the bending of perpendicular MT fibers. This suggests that the pulling force generated by FGs at the cell cortex is balanced by the bending of the perpendicular MT fibers, hence preventing the collapse of the aster into the cortex (Kozlowski et al., 2007). In agreement with these conclusions, 3D computer simulation suggests that a ”touch-and-pull” mechanism might be sufficient to explain the oscillation and posterior displacement of the mitotic spindle, and that the restoring force can be produced by the bending of lateral MT (Kozlowski et al., 2007). Strikingly, computer simulations also show that posterior pole displacement and oscillation could be generated either by a 50% increase of the FG attachment rate at the posterior cortex or by a 50% decrease in posterior cortex rigidity. As stated above, this indicates that not only MT-cortex interaction regulates pulling force but that cortical tension provides an additional level of control to position or orient the mitotic spindle (Kozlowski et al., 2007).

A role of MT depolymerization in aster pulling is supported by: (1) in vitro force measurements demonstrating that attachment to depolymerizing MTs can generate a force up to 50 pN (above the 7–8 pN force generated by Dynein walking on MTs [Grishchuk et al., 2005; Toba et al., 2006]); and (2) the pharmacological blockage of MT depolymerization, which abolishes pulling forces in the *C. elegans* zygote (Nguyen-Ngoc et al., 2007). Yet, the mechanisms regulating MT depolymerization remain to be better characterized. A putative regulator might be efa-6, an ARF6 GEF (O'Rourke et al., 2010). Efa-6 is localized at the cell cortex and slightly enriched at the anterior cortex. Loss of Efa-6 function induces longer MTs near the cell cortex, suggesting that cortical Efa-6 promotes, for example, MT catastrophe (O'Rourke et al., 2010). Although Efa-6 would be an excellent candidate to experimentally test the role of MT depolymerization in pulling forces, the interpretation of its phenotype is complicated by the fact that it both increases and decreases astral pulling forces (O'Rourke et al., 2010): indeed, Efa-6 loss of function increases centrosome separation during anaphase, suggesting that Efa-6 reduces aster pulling forces; nevertheless, its loss of function also abolishes the posterior aster oscillation, suggesting that Efa-6 function increases aster pulling forces. This suggests that the role of MT depolymerization in the regulation of pulling forces might be far more complex than just providing a dynamic anchoring to FGs or allowing the Dynein motor to walk on astral MTs. The study of additional regulators of MT dynamics (Srayko et al., 2005) might clarify the role of MT dynamics in astral pulling force generation.

Collectively, the studies challenge a model whereby the Dynein-Dynactin walking force on MTs is sufficient to orient the mitotic spindle. They demonstrate that we are far from understanding how FGs are produced on the cortex and that both temporal and spatial MT dynamics are key actors of mitotic spindle position. Finally, they raise several interesting questions regarding the mechanisms of cortical elasticity regulation and the molecular nature of MT-cortex adaptors that control the MT attachment rate at the cell cortex.

Conclusions and Open Questions

The progress achieved in our understanding of mitotic spindle orientation in the last decade has been enormous. It has been possible through a combination of genetics and biochemical approaches favorably complemented by real-time imaging, force measurements, and modeling.

In the case of ACD, the *Drosophila* and *C. elegans* models have been instrumental in discovering the conserved mechanisms controlling mitotic spindle orientation. Although polarizing cues are diverse, NuMA and the Dynein-Dynactin complex lie now at the heart of mitotic spindle orientation in ACD in both invertebrates and vertebrates models. Nonetheless, we still have little understanding of how NuMA binds to or activates the Dynein-Dynactin complex. As described in the last section, we have yet to understand the exact role of this complex and how its function is positioned with respect to the mechanisms regulating MT depolymerization and cortex tension. Nevertheless, the identifications of NuMA and Pins have permitted a more direct assessment of the role of mitotic spindle orientation in cell fate specification and the illustration that mitotic spindle orientation is likely to be but one of the regulators of cell fate specification, and that additional ‘‘backup’’ mechanisms can control or overrule the function of mitotic spindle orientation. Understanding the interplay between mitotic spindle orientation and these backup mechanisms will be necessary to further decipher the mechanisms of cell fate specification. A role for mitotic spindle orientation in stem cell biology and in cancer biology is now emerging, and it will be an important direction for future research. Finally, analyses of ACDs in *C. elegans* and *Drosophila* have also illuminated some fundamental principles of basic cell biology, as illustrated by the discovery of mechanisms regulating MT dynamics, centrosome inheritance, or the cortical cytokinesis pathway. It is clear that in the future ACD will be a major system to uncover

and dissect fundamental unforeseen central mechanisms of cell division.

In the case of proliferating tissues where cells divide symmetrically, the PCP pathway seems to be an important actor that specifies the orientation of symmetric cell division along a tissue symmetry axis. The characterization of the function of NuMA and Dynein as a downstream effector of Fz PCP pathway indicates that cortical cues might converge on NuMA and Dynein during both asymmetric and symmetric cell divisions. Fundamental questions remain nevertheless unanswered. Whether mechanical constraints also converge on NuMA and Dynein has yet to be addressed. While Pins and NuMA are known to control the planar orientation of both symmetric and ACDs in the neuroepithelium, in most of the other epithelial tissues, we are still lacking an understanding of how the mitotic spindle orientation is maintained in the epithelial plane. Furthermore, we need to further characterize how OCD intertwines with cell-cell rearrangements and cell morphogenesis to define tissue shape. The possibility to produce a ''constant'' cell via microfabrication has permitted us to go beyond the ''Hertwig rule'' and to predict the distribution of cell division orientation. In the future, it will be central to analyze how cell signaling, geometry, and topology, as well as mechanical constraints, convey spindle orientation and might define the architecture and the shape of tissues.

ACKNOWLEDGMENTS

We thank anonymous reviewers and our colleagues A. Bardin, F. Bosveld, A. Bouisson, N. Christophorou, F. Graner, N. Minc, and S. Tozer for critical comments on the manuscript. Work in Y.B.'s laboratory is supported by grants from the HFSP, the ANR (BLAN07-3-207540), the CNRS, INSERM, ERC Starting Grant (CePoDro 209718), and the Curie Institute. Work in X.M.'s laboratory is supported by an INSERM Avenir Grant (R08221JS), the Fondation pour la Recherche Medicale (FRM implantation nouvelle équipe), and institutional grants from INSERM, CNRS, and the ENS.

REFERENCES

Aegerter-Wilmsen, T., Smith, A.C., Christen, A.J., Aegerter, C.M., Hafen, E., and Basler, K. (2010). Exploring the effects of mechanical feedback on epithelial topology. Development *137*, 499–506.

Afshar, K., Willard, F.S., Colombo, K., Johnston, C.A., McCudden, C.R., Siderovski, D.P., and Gönczy, P. (2004). RIC-8 is required for GPR-1/2-dependent Galpha function during asymmetric division of *C. elegans* embryos. Cell *119*, 219–230.

Aigouy, B., Farhadifar, R., Staple, D., Sagner, A., Röper, J.C., Jülicher, F., and Eaton, S. (2010). Cell flow reorients the axis of planar polarity in the wing epithelium of *Drosophila*. Cell *142*, 773–786.

Baena-López, L.A., Baonza, A., and García-Bellido, A. (2005). The orientation of cell divisions determines the shape of *Drosophila* organs. Curr. Biol. *15*, 1640–1644.

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

Bellaïche, Y., Gho, M., Kaltschmidt, J.A., Brand, A.H., and Schweisguth, F. (2001a). Frizzled regulates localization of cell-fate determinants and mitotic spindle rotation during asymmetric cell division. Nat. Cell Biol. *3*, 50–57.

Bellaïche, Y., Radovic, A., Woods, D.F., Hough, C.D., Parmentier, M.L., O'Kane, C.J., Bryant, P.J., and Schweisguth, F. (2001b). The Partner of Inscuteable/Discs-large complex is required to establish planar polarity during asymmetric cell division in *Drosophila*. Cell *106*, 355–366.

Bellaïche, Y., Beaudoin-Massiani, O., Stuttem, I., and Schweisguth, F. (2004). The planar cell polarity protein Strabismus promotes Pins anterior localization during asymmetric division of sensory organ precursor cells in *Drosophila*. Development *131*, 469–478.

Bowman, S.K., Neumüller, R.A., Novatchkova, M., Du, Q., and Knoblich, J.A. (2006). The *Drosophila* NuMA Homolog Mud regulates spindle orientation in asymmetric cell division. Dev. Cell *10*, 731–742.

Busson, S., Dujardin, D., Moreau, A., Dompierre, J., and De Mey, J.R. (1998). Dynein and dynactin are localized to astral microtubules and at cortical sites in mitotic epithelial cells. Curr. Biol. *8*, 541–544.

Cabernard, C., and Doe, C.Q. (2009). Apical/basal spindle orientation is required for neuroblast homeostasis and neuronal differentiation in *Drosophila*. Dev. Cell *17*, 134–141.

Caussinus, E., and Gonzalez, C. (2005). Induction of tumor growth by altered stem-cell asymmetric division in *Drosophila melanogaster*. Nat. Genet. *37*, 1125–1129.

Chell, J.M., and Brand, A.H. (2010). Nutrition-responsive glia control exit of neural stem cells from quiescence. Cell *143*, 1161–1173.

Chenn, A., and McConnell, S.K. (1995). Cleavage orientation and the asymmetric inheritance of Notch1 immunoreactivity in mammalian neurogenesis. Cell *82*, 631–641.

Ciruna, B., Jenny, A., Lee, D., Mlodzik, M., and Schier, A.F. (2006). Planar cell polarity signalling couples cell division and morphogenesis during neurulation. Nature *439*, 220–224.

Colombo, K., Grill, S.W., Kimple, R.J., Willard, F.S., Siderovski, D.P., and Gönczy, P. (2003). Translation of polarity cues into asymmetric spindle positioning in *Caenorhabditis elegans* embryos. Science *300*, 1957–1961.

Concha, M.L., and Adams, R.J. (1998). Oriented cell divisions and cellular morphogenesis in the zebrafish gastrula and neurula: a time-lapse analysis. Development *125*, 983–994.

Conduit, P.T., and Raff, J.W. (2010). Cnn dynamics drive centrosome size asymmetry to ensure daughter centriole retention in *Drosophila* neuroblasts. Curr. Biol. *20*, 2187–2192.

Conklin, E.G. (1905). The organization and cell-lineage of the ascidian embryo. J. Acad. Nat. Sci. Phila. *13*, 1–119.

Couwenbergs, C., Spilker, A.C., and Gotta, M. (2004). Control of embryonic spindle positioning and Galpha activity by *C. elegans* RIC-8. Curr. Biol. *14*, 1871–1876.

Couwenbergs, C., Labbé, J.C., Goulding, M., Marty, T., Bowerman, B., and Gotta, M. (2007). Heterotrimeric G protein signaling functions with dynein to promote spindle positioning in *C. elegans*. J. Cell Biol. *179*, 15–22.

David, N.B., Martin, C.A., Segalen, M., Rosenfeld, F., Schweisguth, F., and Bellaïche, Y. (2005). *Drosophila* Ric-8 regulates Galphai cortical localization to promote Galphai-dependent planar orientation of the mitotic spindle during asymmetric cell division. Nat. Cell Biol. *7*, 1083–1090.

de la Pompa, J.L., Wakeham, A., Correia, K.M., Samper, E., Brown, S., Aguilera, R.J., Nakano, T., Honjo, T., Mak, T.W., Rossant, J., and Conlon, R.A. (1997). Conservation of the Notch signalling pathway in mammalian neurogenesis. Development *124*, 1139–1148.

Du, Q., and Macara, I.G. (2004). Mammalian Pins is a conformational switch that links NuMA to heterotrimeric G proteins. Cell *119*, 503–516.

Du, Q., Stukenberg, P.T., and Macara, I.G. (2001). A mammalian Partner of inscuteable binds NuMA and regulates mitotic spindle organization. Nat. Cell Biol. *3*, 1069–1075.

Dujardin, D.L., and Vallee, R.B. (2002). Dynein at the cortex. Curr. Opin. Cell Biol. *14*, 44–49.

Etemad-Moghadam, B., Guo, S., and Kemphues, K.J. (1995). Asymmetrically distributed PAR-3 protein contributes to cell polarity and spindle alignment in early *C. elegans* embryos. Cell *83*, 743–752.

Farhadifar, R., Röper, J.C., Aigouy, B., Eaton, S., and Jülicher, F. (2007). The influence of cell mechanics, cell-cell interactions, and proliferation on epithelial packing. Curr. Biol. *17*, 2095–2104.

Feng, Y., and Walsh, C.A. (2004). Mitotic spindle regulation by Nde1 controls cerebral cortical size. Neuron *44*, 279–293.

Fichelson, P., and Gho, M. (2003). The glial cell undergoes apoptosis in the microchaete lineage of *Drosophila*. Development *130*, 123–133.

Fietz, S.A., Kelava, I., Vogt, J., Wilsch-Bräuninger, M., Stenzel, D., Fish, J.L., Corbeil, D., Riehn, A., Distler, W., Nitsch, R., and Huttner, W.B. (2010). OSVZ progenitors of human and ferret neocortex are epithelial-like and expand by integrin signaling. Nat. Neurosci. *13*, 690–699.

Fischer, E., Legue, E., Doyen, A., Nato, F., Nicolas, J.F., Torres, V., Yaniv, M., and Pontoglio, M. (2006). Defective planar cell polarity in polycystic kidney disease. Nat. Genet. *38*, 21–23.

Fish, J.L., Kosodo, Y., Enard, W., Pääbo, S., and Huttner, W.B. (2006). Aspm specifically maintains symmetric proliferative divisions of neuroepithelial cells. Proc. Natl. Acad. Sci. USA *103*, 10438–10443.

Galli, M., and van den Heuvel, S. (2008). Determination of the cleavage plane in early *C. elegans* embryos. Annu. Rev. Genet. *42*, 389–411.

Gauthier-Fisher, A., Lin, D.C., Greeve, M., Kaplan, D.R., Rottapel, R., and Miller, F.D. (2009). Lfc and Tctex-1 regulate the genesis of neurons from cortical precursor cells. Nat. Neurosci. *12*, 735–744.

Gho, M., and Schweisguth, F. (1998). Frizzled signalling controls orientation of asymmetric sense organ precursor cell divisions in *Drosophila*. Nature *393*, 178–181.

Gho, M., Bellaïche, Y., and Schweisguth, F. (1999). Revisiting the *Drosophila* microchaete lineage: a novel intrinsically asymmetric cell division generates a glial cell. Development *126*, 3573–3584.

Gibson, M.C., Patel, A.B., Nagpal, R., and Perrimon, N. (2006). The emergence of geometric order in proliferating metazoan epithelia. Nature *442*, 1038–1041.

Gibson, W.T., Veldhuis, J.H., Rubinstein, B., Cartwright, H.N., Perrimon, N., Brodland, G.W., Nagpal, R., and Gibson, M.C. (2011). Control of the mitotic cleavage plane by local epithelial topology. Cell *144*, 427–438.

Glotzer, M. (2009). The 3Ms of central spindle assembly: microtubules, motors and MAPs. Nat. Rev. Mol. Cell Biol. *10*, 9–20.

Godin, J.D., Colombo, K., Molina-Calavita, M., Keryer, G., Zala, D., Charrin, B.C., Dietrich, P., Volvert, M.L., Guillemot, F., Dragatsis, I., et al. (2010). Huntingtin is required for mitotic spindle orientation and mammalian neurogenesis. Neuron *67*, 392–406.

Gönczy, P. (2008). Mechanisms of asymmetric cell division: flies and worms pave the way. Nat. Rev. Mol. Cell Biol. *9*, 355–366.

Gong, Y., Mo, C., and Fraser, S.E. (2004). Planar cell polarity signalling controls cell division orientation during zebrafish gastrulation. Nature *430*, 689–693.

Goodrich, L.V., and Strutt, D. (2011). Principles of planar polarity in animal development. Development *138*, 1877–1892.

Goranov, A.I., and Amon, A. (2010). Growth and division—not a one-way road. Curr. Opin. Cell Biol. *22*, 795–800.

Gotta, M., Dong, Y., Peterson, Y.K., Lanier, S.M., and Ahringer, J. (2003). Asymmetrically distributed *C. elegans* homologs of AGS3/PINS control spindle position in the early embryo. Curr. Biol. *13*, 1029–1037.

Goulding, M.B., Canman, J.C., Senning, E.N., Marcus, A.H., and Bowerman, B. (2007). Control of nuclear centration in the *C. elegans* zygote by receptor-independent Galpha signaling and myosin II. J. Cell Biol. *178*, 1177–1191.

Grill, S.W., and Hyman, A.A. (2005). Spindle positioning by cortical pulling forces. Dev. Cell *8*, 461–465.

Grill, S.W., Gönczy, P., Stelzer, E.H., and Hyman, A.A. (2001). Polarity controls forces governing asymmetric spindle positioning in the *Caenorhabditis elegans* embryo. Nature *409*, 630–633.

Grill, S.W., Howard, J., Schäffer, E., Stelzer, E.H., and Hyman, A.A. (2003). The distribution of active force generators controls mitotic spindle position. Science *301*, 518–521.

Grill, S.W., Kruse, K., and Jülicher, F. (2005). Theory of mitotic spindle oscillations. Phys. Rev. Lett. *94*, 108104.

Grishchuk, E.L., Molodtsov, M.I., Ataullakhanov, F.I., and McIntosh, J.R. (2005). Force production by disassembling microtubules. Nature *438*, 384–388.

Gros, J., Hu, J.K., Vinegoni, C., Feruglio, P.F., Weissleder, R., and Tabin, C.J. (2010). WNT5A/JNK and FGF/MAPK pathways regulate the cellular events shaping the vertebrate limb bud. Curr. Biol. *20*, 1993–2002.

Hampoelz, B., Hoeller, O., Bowman, S.K., Dunican, D., and Knoblich, J.A. (2005). *Drosophila* Ric-8 is essential for plasma-membrane localization of heterotrimeric G proteins. Nat. Cell Biol. *7*, 1099–1105.

Hansen, D.V., Lui, J.H., Parker, P.R., and Kriegstein, A.R. (2010). Neurogenic radial glia in the outer subventricular zone of human neocortex. Nature *464*, 554–561.

Hao, Y., Du, Q., Chen, X., Zheng, Z., Balsbaugh, J.L., Maitra, S., Shabanowitz, J., Hunt, D.F., and Macara, I.G. (2010). Par3 controls epithelial spindle orientation by aPKC-mediated phosphorylation of apical Pins. Curr. Biol. *20*, 1809–1818.

Heisenberg, C.P., Tada, M., Rauch, G.J., Saúde, L., Concha, M.L., Geisler, R., Stemple, D.L., Smith, J.C., and Wilson, S.W. (2000). Silberblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. Nature *405*, 76–81.

Hertwig, O. (1884). Das Problem der Befruchtung und der Isotropie des Eies, eine Theory der Vererbung. Jenaische Zeitschrift fuer Naturwissenschaft *18*, 21–23.

Hess, H.A., Röper, J.C., Grill, S.W., and Koelle, M.R. (2004). RGS-7 completes a receptor-independent heterotrimeric G protein cycle to asymmetrically regulate mitotic spindle positioning in *C. elegans*. Cell *119*, 209–218.

Ikeshima-Kataoka, H., Skeath, J.B., Nabeshima, Y., Doe, C.Q., and Matsuzaki, F. (1997). Miranda directs Prospero to a daughter cell during *Drosophila* asymmetric divisions. Nature *390*, 625–629.

Ishikawa, H.O., Takeuchi, H., Haltiwanger, R.S., and Irvine, K.D. (2008). Four-jointed is a Golgi kinase that phosphorylates a subset of cadherin domains. Science *321*, 401–404.

Izumi, Y., Ohta, N., Hisata, K., Raabe, T., and Matsuzaki, F. (2006). *Drosophila* Pins-binding protein Mud regulates spindle-polarity coupling and centrosome organization. Nat. Cell Biol. *8*, 586–593.

Januschke, J., and Gonzalez, C. (2010). The interphase microtubule aster is a determinant of asymmetric division orientation in *Drosophila* neuroblasts. J. Cell Biol. *188*, 693–706.

Januschke, J., Llamazares, S., Reina, J., and Gonzalez, C. (2011). *Drosophila* neuroblasts retain the daughter centrosome. Nat. Commun. *2*, 243.

Johnston, C.A., Hirono, K., Prehoda, K.E., and Doe, C.Q. (2009). Identification of an Aurora-A/PinsLINKER/Dlg spindle orientation pathway using induced cell polarity in S2 cells. Cell *138*, 1150–1163.

Jones, P., and Simons, B.D. (2008). Epidermal homeostasis: do committed progenitors work while stem cells sleep? Nat. Rev. Mol. Cell Biol. *9*, 82–88.

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

Keller, R. (2006). Mechanisms of elongation in embryogenesis. Development *133*, 2291–2302.

Kimura, A., and Onami, S. (2005). Computer simulations and image processing reveal length-dependent pulling force as the primary mechanism for *C. elegans* male pronuclear migration. Dev. Cell *8*, 765–775.

Knoblich, J.A. (2010). Asymmetric cell division: recent developments and their implications for tumour biology. Nat. Rev. Mol. Cell Biol. *11*, 849–860.

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

Kosodo, Y., Röper, K., Haubensak, W., Marzesco, A.M., Corbeil, D., and Huttner, W.B. (2004). Asymmetric distribution of the apical plasma membrane during neurogenic divisions of mammalian neuroepithelial cells. EMBO J. *23*, 2314–2324.

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

Kraut, R., Chia, W., Jan, L.Y., Jan, Y.N., and Knoblich, J.A. (1996). Role of inscuteable in orienting asymmetric cell divisions in *Drosophila*. Nature *383*, 50–55.

Krueger, L.E., Wu, J.C., Tsou, M.F., and Rose, L.S. (2010). LET-99 inhibits lateral posterior pulling forces during asymmetric spindle elongation in *C. elegans* embryos. J. Cell Biol. *189*, 481–495.

Labbé, J.C., Maddox, P.S., Salmon, E.D., and Goldstein, B. (2003). PAR proteins regulate microtubule dynamics at the cell cortex in *C. elegans*. Curr. Biol. *13*, 707–714.

Lechler, T., and Fuchs, E. (2005). Asymmetric cell divisions promote stratification and differentiation of mammalian skin. Nature *437*, 275–280.

Lecuit, T., and Le Goff, L. (2007). Orchestrating size and shape during morphogenesis. Nature *450*, 189–192.

Mao, Y., Rauskolb, C., Cho, E., Hu, W.L., Hayter, H., Minihan, G., Katz, F.N., and Irvine, K.D. (2006). Dachs: an unconventional myosin that functions downstream of Fat to regulate growth, affinity and gene expression in *Drosophila*. Development *133*, 2539–2551.

Mao, Y., Tournier, A.L., Bates, P.A., Gale, J.E., Tapon, N., and Thompson, B.J. (2011). Planar polarization of the atypical myosin Dachs orients cell divisions in *Drosophila*. Genes Dev. *25*, 131–136.

Marthiens, V., and ffrench-Constant, C. (2009). Adherens junction domains are split by asymmetric division of embryonic neural stem cells. EMBO Rep. *10*, 515–520.

Mayer, M., Depken, M., Bois, J.S., Jülicher, F., and Grill, S.W. (2010). Anisotropies in cortical tension reveal the physical basis of polarizing cortical flows. Nature *467*, 617–621.

Merdes, A., Ramyar, K., Vechio, J.D., and Cleveland, D.W. (1996). A complex of NuMA and cytoplasmic dynein is essential for mitotic spindle assembly. Cell *87*, 447–458.

Minc, N., Burgess, D., and Chang, F. (2011). Influence of cell geometry on division-plane positioning. Cell *144*, 414–426.

Morin, X., Jaouen, F., and Durbec, P. (2007). Control of planar divisions by the G-protein regulator LGN maintains progenitors in the chick neuroepithelium. Nat. Neurosci. *10*, 1440–1448.

Munro, E., Nance, J., and Priess, J.R. (2004). Cortical flows powered by asymmetrical contraction transport PAR proteins to establish and maintain anterior-posterior polarity in the early *C. elegans* embryo. Dev. Cell *7*, 413–424.

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

Noctor, S.C., Martínez-Cerdeño, V., and Kriegstein, A.R. (2008). Distinct behaviors of neural stem and progenitor cells underlie cortical neurogenesis. J. Comp. Neurol. *508*, 28–44.

O'Rourke, S.M., Christensen, S.N., and Bowerman, B. (2010). *Caenorhabditis elegans* EFA-6 limits microtubule growth at the cell cortex. Nat. Cell Biol. *12*, 1235–1241.

Panbianco, C., Weinkove, D., Zanin, E., Jones, D., Divecha, N., Gotta, M., and Ahringer, J. (2008). A casein kinase 1 and PAR proteins regulate asymmetry of a PIP(2) synthesis enzyme for asymmetric spindle positioning. Dev. Cell *15*, 198–208.

Park, D.H., and Rose, L.S. (2008). Dynamic localization of LIN-5 and GPR-1/2 to cortical force generation domains during spindle positioning. Dev. Biol. *315*, 42–54.

Pecreaux, J., Röper, J.C., Kruse, K., Jülicher, F., Hyman, A.A., Grill, S.W., and Howard, J. (2006). Spindle oscillations during asymmetric cell division require a threshold number of active cortical force generators. Curr. Biol. *16*, 2111–2122.

Peyre, E., Jaouen, F., Saadaoui, M., Haren, L., Merdes, A., Durbec, P., and Morin, X. (2011). A lateral belt of cortical LGN and NuMA guides mitotic spindle movements and planar division in neuroepithelial cells. J. Cell Biol. *193*, 141–154.

Poulson, N.D., and Lechler, T. (2010). Robust control of mitotic spindle orientation in the developing epidermis. J. Cell Biol. *191*, 915–922.

Quesada-Hernández, E., Caneparo, L., Schneider, S., Winkler, S., Liebling, M., Fraser, S.E., and Heisenberg, C.P. (2010). Stereotypical cell division orientation controls neural rod midline formation in zebrafish. Curr. Biol. *20*, 1966–1972.

Radulescu, A.E., and Cleveland, D.W. (2010). NuMA after 30 years: the matrix revisited. Trends Cell Biol. *20*, 214–222.

Rebollo, E., Sampaio, P., Januschke, J., Llamazares, S., Varmark, H., and González, C. (2007). Functionally unequal centrosomes drive spindle orientation in asymmetrically dividing *Drosophila* neural stem cells. Dev. Cell *12*, 467–474.

Rebollo, E., Roldán, M., and Gonzalez, C. (2009). Spindle alignment is achieved without rotation after the first cell cycle in *Drosophila* embryonic neuroblasts. Development *136*, 3393–3397.

Reddy, B.V., and Irvine, K.D. (2008). The Fat and Warts signaling pathways: new insights into their regulation, mechanism and conservation. Development *135*, 2827–2838.

Redemann, S., Pecreaux, J., Goehring, N.W., Khairy, K., Stelzer, E.H., Hyman, A.A., and Howard, J. (2010). Membrane invaginations reveal cortical sites that pull on mitotic spindles in one-cell *C. elegans* embryos. PLoS One *5*, e12301.

Reinsch, S., and Karsenti, E. (1994). Orientation of spindle axis and distribution of plasma membrane proteins during cell division in polarized MDCKII cells. J. Cell Biol. *126*, 1509–1526.

Rogulja, D., Rauskolb, C., and Irvine, K.D. (2008). Morphogen control of wing growth through the Fat signaling pathway. Dev. Cell *15*, 309–321.

Rusan, N.M., and Peifer, M. (2007). A role for a novel centrosome cycle in asymmetric cell division. J. Cell Biol. *177*, 13–20.

Saburi, S., Hester, I., Fischer, E., Pontoglio, M., Eremina, V., Gessler, M., Quaggin, S.E., Harrison, R., Mount, R., and McNeill, H. (2008). Loss of Fat4 disrupts PCP signaling and oriented cell division and leads to cystic kidney disease. Nat. Genet. *40*, 1010–1015.

Schaefer, M., Shevchenko, A., Shevchenko, A., and Knoblich, J.A. (2000). A protein complex containing Inscuteable and the Galpha-binding protein Pins orients asymmetric cell divisions in *Drosophila*. Curr. Biol. *10*, 353–362.

Schwank, G., Tauriello, G., Yagi, R., Kranz, E., Koumoutsakos, P., and Basler, K. (2011). Antagonistic growth regulation by Dpp and Fat drives uniform cell proliferation. Dev. Cell *20*, 123–130.

Ségalen, M., Johnston, C.A., Martin, C.A., Dumortier, J.G., Prehoda, K.E., David, N.B., Doe, C.Q., and Bellaïche, Y. (2010). The Fz-Dsh planar cell polarity pathway induces oriented cell division via Mud/NuMA in *Drosophila* and zebrafish. Dev. Cell *19*, 740–752.

Severson, A.F., and Bowerman, B. (2003). Myosin and the PAR proteins polarize microfilament-dependent forces that shape and position mitotic spindles in *Caenorhabditis elegans*. J. Cell Biol. *161*, 21–26.

Shinin, V., Gayraud-Morel, B., Gomès, D., and Tajbakhsh, S. (2006). Asymmetric division and cosegregation of template DNA strands in adult muscle satellite cells. Nat. Cell Biol. *8*, 677–687.

Shitamukai, A., Konno, D., and Matsuzaki, F. (2011). Oblique radial glial divisions in the developing mouse neocortex induce self-renewing progenitors outside the germinal zone that resemble primate outer subventricular zone progenitors. J. Neurosci. *31*, 3683–3695.

Siegrist, S.E., and Doe, C.Q. (2005). Microtubule-induced Pins/Galphai cortical polarity in *Drosophila* neuroblasts. Cell *123*, 1323–1335.

Siller, K.H., and Doe, C.Q. (2008). Lis1/dynactin regulates metaphase spindle orientation in *Drosophila* neuroblasts. Dev. Biol. *319*, 1–9.

Siller, K.H., and Doe, C.Q. (2009). Spindle orientation during asymmetric cell division. Nat. Cell Biol. *11*, 365–374.

Siller, K.H., Serr, M., Steward, R., Hays, T.S., and Doe, C.Q. (2005). Live imaging of *Drosophila* brain neuroblasts reveals a role for Lis1/dynactin in spindle assembly and mitotic checkpoint control. Mol. Biol. Cell *16*, 5127–5140.

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

Simon, M.A., Xu, A., Ishikawa, H.O., and Irvine, K.D. (2010). Modulation of fat: dachsous binding by the cadherin domain kinase four-jointed. Curr. Biol. *20*, 811–817.

Sousa-Nunes, R., Cheng, L.Y., and Gould, A.P. (2010). Regulating neural proliferation in the *Drosophila* CNS. Curr. Opin. Neurobiol. *20*, 50–57.

Sousa-Nunes, R., Yee, L.L., and Gould, A.P. (2011). Fat cells reactivate quiescent neuroblasts via TOR and glial insulin relays in *Drosophila*. Nature *471*, 508–512.

Speicher, S., Fischer, A., Knoblich, J., and Carmena, A. (2008). The PDZ protein Canoe regulates the asymmetric division of *Drosophila* neuroblasts and muscle progenitors. Curr. Biol. *18*, 831–837.

Srayko, M., Kaya, A., Stamford, J., and Hyman, A.A. (2005). Identification and characterization of factors required for microtubule growth and nucleation in the early *C. elegans* embryo. Dev. Cell *9*, 223–236.

Srinivasan, D.G., Fisk, R.M., Xu, H., and van den Heuvel, S. (2003). A complex of LIN-5 and GPR proteins regulates G protein signaling and spindle function in *C elegans*. Genes Dev. *17*, 1225–1239.

Staple, D.B., Farhadifar, R., Röper, J.C., Aigouy, B., Eaton, S., and Jülicher, F. (2010). Mechanics and remodelling of cell packings in epithelia. Eur. Phys. J. E Soft Matter *33*, 117–127.

Strnad, P., and Gönczy, P. (2008). Mechanisms of procentriole formation. Trends Cell Biol. *18*, 389–396.

Tanaka, T.U. (2010). Kinetochore-microtubule interactions: steps towards bi-orientation. EMBO J. *29*, 4070–4082.

Tawk, M., Araya, C., Lyons, D.A., Reugels, A.M., Girdler, G.C., Bayley, P.R., Hyde, D.R., Tada, M., and Clarke, J.D. (2007). A mirror-symmetric cell division that orchestrates neuroepithelial morphogenesis. Nature *446*, 797–800.

Théry, M., and Bornens, M. (2006). Cell shape and cell division. Curr. Opin. Cell Biol. *18*, 648–657.

Théry, M., Racine, V., Pépin, A., Piel, M., Chen, Y., Sibarita, J.B., and Bornens, M. (2005). The extracellular matrix guides the orientation of the cell division axis. Nat. Cell Biol. *7*, 947–953.

Théry, M., Jiménez-Dalmaroni, A., Racine, V., Bornens, M., and Jülicher, F. (2007). Experimental and theoretical study of mitotic spindle orientation. Nature *447*, 493–496.

Toba, S., Watanabe, T.M., Yamaguchi-Okimoto, L., Toyoshima, Y.Y., and Higuchi, H. (2006). Overlapping hand-over-hand mechanism of single molecular motility of cytoplasmic dynein. Proc. Natl. Acad. Sci. USA *103*, 5741–5745.

Uemura, T., Shepherd, S., Ackerman, L., Jan, L.Y., and Jan, Y.N. (1989). numb, a gene required in determination of cell fate during sensory organ formation in *Drosophila* embryos. Cell *58*, 349–360.

Wang, H., Ng, K.H., Qian, H., Siderovski, D.P., Chia, W., and Yu, F. (2005). Ric-8 controls *Drosophila* neural progenitor asymmetric division by regulating heterotrimeric G proteins. Nat. Cell Biol. *7*, 1091–1098.

Wang, X., Tsai, J.W., Imai, J.H., Lian, W.N., Vallee, R.B., and Shi, S.H. (2009). Asymmetric centrosome inheritance maintains neural progenitors in the neocortex. Nature *461*, 947–955.

Wang, X., Tsai, J.W., Lamonica, B., and Kriegstein, A.R. (2011). A new subtype of progenitor cell in the mouse embryonic neocortex. Nat. Neurosci. *14*, 555–561.

Williams, S.E., Beronja, S., Pasolli, H.A., and Fuchs, E. (2011). Asymmetric cell divisions promote Notch-dependent epidermal differentiation. Nature *470*, 353–358.

Wühr, M., Tan, E.S., Parker, S.K., Detrich, H.W., 3rd, and Mitchison, T.J. (2010). A model for cleavage plane determination in early amphibian and fish embryos. Curr. Biol. *20*, 2040–2045.

Wyngaarden, L.A., Vogeli, K.M., Ciruna, B.G., Wells, M., Hadjantonakis, A.K., and Hopyan, S. (2010). Oriented cell motility and division underlie early limb bud morphogenesis. Development *137*, 2551–2558.

Yamashita, Y.M., Mahowald, A.P., Perlin, J.R., and Fuller, M.T. (2007). Asymmetric inheritance of mother versus daughter centrosome in stem cell division. Science *315*, 518–521.

Yingling, J., Youn, Y.H., Darling, D., Toyo-Oka, K., Pramparo, T., Hirotsune, S., and Wynshaw-Boris, A. (2008). Neuroepithelial stem cell proliferation requires LIS1 for precise spindle orientation and symmetric division. Cell *132*, 474–486.

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

Yu, F., Wang, H., Qian, H., Kaushik, R., Bownes, M., Yang, X., and Chia, W. (2005). Locomotion defects, together with Pins, regulates heterotrimeric G-protein signaling during *Drosophila* neuroblast asymmetric divisions. Genes Dev. *19*, 1341–1353.

Yu, F., Kuo, C.T., and Jan, Y.N. (2006). *Drosophila* neuroblast asymmetric cell division: recent advances and implications for stem cell biology. Neuron *51*, 13–20.

Yu, J., Carroll, T.J., Rajagopal, J., Kobayashi, A., Ren, Q., and McMahon, A.P. (2009). A Wnt7b-dependent pathway regulates the orientation of epithelial cell division and establishes the cortico-medullary axis of the mammalian kidney. Development *136*, 161–171.

Zhang, H., Skop, A.R., and White, J.G. (2008). Src and Wnt signaling regulate dynactin accumulation to the P2-EMS cell border in *C. elegans* embryos. J. Cell Sci. *121*, 155–161.

Zheng, Z., Zhu, H., Wan, Q., Liu, J., Xiao, Z., Siderovski, D.P., and Du, Q. (2010). LGN regulates mitotic spindle orientation during epithelial morphogenesis. J. Cell Biol. *189*, 275–288.

Zigman, M., Cayouette, M., Charalambous, C., Schleiffer, A., Hoeller, O., Dunican, D., McCudden, C.R., Firnberg, N., Barres, B.A., Siderovski, D.P., and Knoblich, J.A. (2005). Mammalian inscuteable regulates spindle orientation and cell fate in the developing retina. Neuron *48*, 539–545.

Žigman, M., Trinh le, A., Fraser, S.E., and Moens, C.B. (2011). Zebrafish neural tube morphogenesis requires Scribble-dependent oriented cell divisions. Curr. Biol. *21*, 79–86.