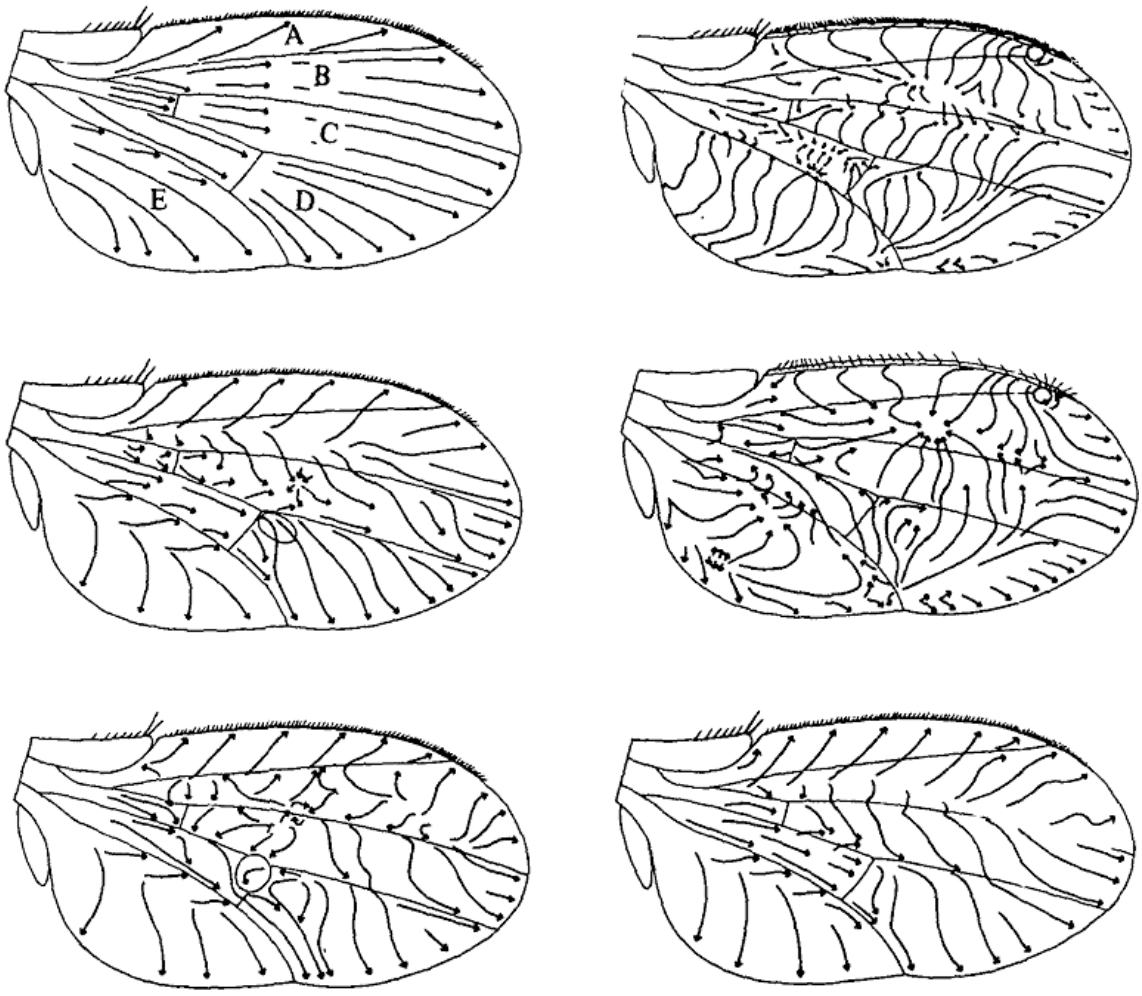
Putative Roles of Planar Cell Polarity Proteins in the Establishment and Regulation of Asymmetric Cell Division

Cancer Genomics & Developmental Biology Master Thesis

By Marten Hornsveld



cancer genomics & developmental biology

The Master's Programme

Universiteit Utrecht

Putative Roles of Planar Cell Polarity Proteins in the Establishment and Regulation of Asymmetric Cell Division

Cancer genomics & developmental biology master thesis
By Marten Hornsveld
Studentnr. 3203166

July 2010

Supervised by Prof. Dr. Sander van den Heuvel

Department of Developmental Biology
University Utrecht

* Cover figure: Early drawings showing hair polarity patterns in *Drosophila* mutant wings. Figure adapted from Gubb et. al 1982

Abstract

During animal development, cells need to grow, divide and differentiate according to the body plan. To do so, cells utilize complex mechanisms to polarize themselves asymmetrically on the apical-basal axis, but also within the plane of the tissue, known as planar cell polarity (PCP). In order to establish an axis for oriented division, asymmetrically segregate cell fate determinants and orient the mitotic spindle, the cells use PCP to align themselves with the body plan. How PCP regulates asymmetric division and spindle orientation is unknown at the moment. Recent data suggest an important role for the PCP core proteins Frizzled, Van Gogh/Strabismus and Flamingo/Starry Night in aligning the spindle to the axis of division. However, the exact mechanism remains to be resolved. In this thesis, current models on how cells establish polarity, PCP and orient the mitotic spindle to ensure correct asymmetric division will be reviewed. Furthermore, current important questions in the field of PCP research nowadays will be discussed.

Cell polarity in development and disease

To get from a fertilized single cell zygote to a multi-cellular organism like a human being, millions of well coordinated cell divisions are needed. Before each division a cell determines the direction in which to divide and what fate to adopt. To do so, cells need to interpret the body plan using axes generated by molecular signaling pathways. Establishing this orientation is known as cell polarity. Defects in establishing cell polarity can lead to different diseases ranging from birth defects to cancer. Even though cell polarity got attention from many scientists for over a century, mechanisms by which cells establish their polarity and regulate their directional growth are not fully understood (Axelrod, 2009b).
During embryonic development as well as tissue homeostasis in adult organisms, stem cells are responsible for the supply of new tissue. After a stem cell divides, the two new daughter cells can adopt different fates, resulting mostly in one daughter maintaining the stem cell properties and one daughter which will differentiate. Determination of daughter cell fate can be mediated by cell intrinsic asymmetric division or by extrinsic signaling from the environment to one of the daughter cells. Several steps are involved in becoming intrinsically asymmetric; at first a cell must become polarized amongst the axis on which it is going to divide; secondly the cell needs to segregate cell fate determinants to a specific pole of the cell; and thirdly the cell needs to align the mitotic spindle with the axis of division to ensure that cleavage correctly partitions the fate determinants into only one of the daughter cells (Gonczy, 2008).
Cells can polarize with respect to two axes, namely the apical-basal (A-B) axis and axes in the plane of the tissue. A-B polarity is most evident in epithelial cells were the apical side usually faces lumen, for example in endothelial cells, or the exterior like in the epidermis, whereas the basal side faces internally. Orthogonal to the A-B axis cells can establish axes which lie in the plane of the tissue. This is known as planar cell polarity (PCP), which can define the Anterior-Posterior (A-P) axis for example. PCP is established by cell extrinsic polarization cues, which allow cells to align their polarity trough cell-cell contact with their neighbors and thereby to the symmetry axis of the organism. PCP signaling is highly conserved and evident in many different tissues like the scales of fish, feathers of birds and hairs of mammals and allows these tissues to be organized in the same direction (Segalen and Bellaiche, 2009).
The development of model organisms like *Drosophila melanogaster* and *Caenorhabditis elegans* has been extensively used to study intrinsically asymmetric cell divisions. The results in these model organisms provided the solid basis for theories on the mechanisms of cell polarity. One of many challenges in cell polarity research is to understand how cells establish their correct orientation for asymmetric division relative to the body axis defined by PCP signaling pathways. Recently, several molecular pathways were uncovered and detailed insight into PCP signaling is acquired in *Drosophila* wings and eyes (Wu and Mlodzik, 2009).
I will review existing models describing how cell polarity orients the direction of cell division and merge these models with recent data in search for a mechanism of spindle positioning during the subsequent divisions of the mechanosensory organ in *Drosophila*. Insights from *Drosophila* can provide a fundamental basis for cell polarity research in more complex organisms. Ultimately, these insights result in a full understanding of how multi-cellular organisms develop and contribute to the development of cures for multiple human diseases.

Establishment of asymmetry by competitive polarity complexes

In order to elucidate the mechanism by which cells organize their polarity and use polarity to orient their spindle, understanding of how polarity is established is essential. Epithelial cells are highly polarized with distinct apical, lateral and basal domains. A-B polarity is established by multiple complexes including the adherens junctions, Crumbs, Par and Scribbled complexes. Adherens junctions consist of E-cadherin, α- and β-catenin, and mediate cell-cell contact. At the junctions, polarity complexes repel each other from the cell cortex resulting in distinct localization patterns for each complex (Humbert et al., 2006).
The Par and Crumbs complexes localize apical of the adherens junctions and define the apical domain of the cell. Together with the atypical protein kinase C (aPKC in *Drosophila*, PKC-3 in *C. elegans*), Par proteins are a core component of cell polarity and are highly conserved throughout metazoa. PAR proteins were discovered in *C. elegans* where they play an important role in specification of the A-P axis and in ensuring asymmetric division in the embryo (Kemphues et al., 1988). Additionally, Par proteins function in establishing A-B polarity in epithelial cells, oriented cell migration and neuronal-axon specification (Wu and Mlodzik, 2009).
The *C. elegans* studies identified six *par* genes. *par-1* and *par-4* (*LKB1* in mammals) code for protein kinases (Guo and Kemphues, 1995; Morton et al., 1992; Watts et al., 2000), *par-3* (*bazooka* in *Drosophila*) and *par-6* code for PDZ containing proteins, *par-5* encodes a 14-3-3 protein (Morton et al., 2002) and *par-2* encodes a *C. elegans* specific zinc-finger protein (Boyd et al., 1996). PAR-6 and PKC-3 bind each other through their N-terminal domains (Suzuki et al. 2003; Suzuki et al., 2001). PAR-6 can also interact with the Rho kinase family member CDC-42 which is indicated as an important player in establishing polarity, linking its activity to PKC-3 (Garrard et al., 2003; Gotta et al., 2001; Hutterer et al., 2004).PAR-3 dynamically interacts with PAR-6/PKC-3 by binding to the kinase domain of PKC-3. Upon phosphorylation by PKC-3, PAR-3 loses its affinity for PKC-3 and dissociates from the complex (Izumi et al., 1998; Nagai-Tamai et al., 2002). In *C. elegans* embryos PAR-3/PAR-6/PKC-3 defines the anterior domain, PAR-1 and PAR-2 define the posterior domain.

In epithelial cells of *Drosophila*, but also in mammals, the Par-3/Par-6/aPKC complex localizes apical of the cell junctions, Par-1 localizes to the basal membrane and no asymmetric localization of Par-4 and Par-5 is found. Localization of Par-6/aPKC to the cell junctions is mediated by Par-3 which localizes to the junctions just prior to Par-6/aPKC (Itoh et al., 2001; Takekuni et al., 2003). At the junctions of the cell aPKC antagonizes the binding of Par1 to the membrane and thereby restricts Par-1 to the basal membranes of the cell. Conversely, Par-1 phosphorylates Par-3 resulting in the dissociation of the Par-3/Par-6/aPKC complex at basal membrane of epithelial cells, or posterior membrane in *Drosophila* oocytes (Benton and St Johnston, 2003; Suzuki et al., 2004). These antagonizing interactions are assisted by Par-5 which can bind to the phosphorylated forms of Par-3 and Par-1 (Suzuki et al., 2004; Suzuki and Ohno, 2006).
In *Drosophila* and mammalian epithelial cells, Par-3/Par-6/aPKC also interacts with the Scribble complex in order to establish A-B polarity. The Scribble complex consists of the three proteins, Scribble, Disc large (Dlg; DLG1-4 in mammals) and Lethal giant larvae (Lgl; LGL1 and LGL2 in mammals). All of these proteins are suggested to function as scaffold proteins and regulate protein-protein interactions (Humbert et al., 2006). Scribble and Dlg co-localize at the lateral membrane in

overlap with Lgl which is localized basolaterally (Bilder and Perrimon, 2000; Wirtz-Peitz and Knoblich, 2006). Correct localization of the complex is mutually dependent on all three proteins and mutation in members of the complex results in loss of A-B polarity and lethal tissue overgrowth (Bilder, 2004; Bilder et al., 2000; Zeitler et al., 2004). Dlg and Scribbled are also known to function in Wnt signaling, Rho kinase regulation and G-protein coupled receptor (GPCR) binding. Lgl has been found to bind to myosin II, t-SNARE components involved in vesicle trafficking and importantly, binding to Par-3/Par-6/aPKC (Wirtz-Peitz and Knoblich, 2006). Establishment of the apical Par and the basal Scribble domain is dependent on interaction of Lgl with Par-6/aPKC (Hutterer et al., 2004). Lgl competes with Par-3 for Par-6/aPKC binding thereby moving Par-3 from the complex. Upon binding of Lgl to Par-6/aPKC, Lgl becomes phosphorylated and undergoes a conformational change which inhibits Lgl binding to the membrane or cytoskeleton (Figure 1) (Betschinger et al., 2005; Yamanaka et al., 2003).

A third set of proteins cooperating with Par-3/Par-6/aPKC to establish A-B polarity in epithelial cells is the apically localized Crumbs complex. The Crumbs complex consists of transmembrane protein Crumbs (Crb), and the scaffold proteins Patj (Pals-associated tight-junction protein), Stardust (Sdt) (Bachmann et al., 2001). Mutation in *crb* and *sdt* results in the loss of epithelial polarity in the *Drosophila* embryo and delocalization of the other complex members (Bilder et al., 2003; Tanentzapf and Tepass, 2003; Tepass and Knust, 1993; Tepass et al., 1990).

Many of the interactions between the multiple complexes leading to the establishment of cell polarity still need to be elucidated. However, a common theme is that polarity complexes can interact with each other, thereby limiting the localization of the complexes to specific domains and create molecular asymmetry within a cell (Suzuki and Ohno, 2006; Wu and Mlodzik, 2009).

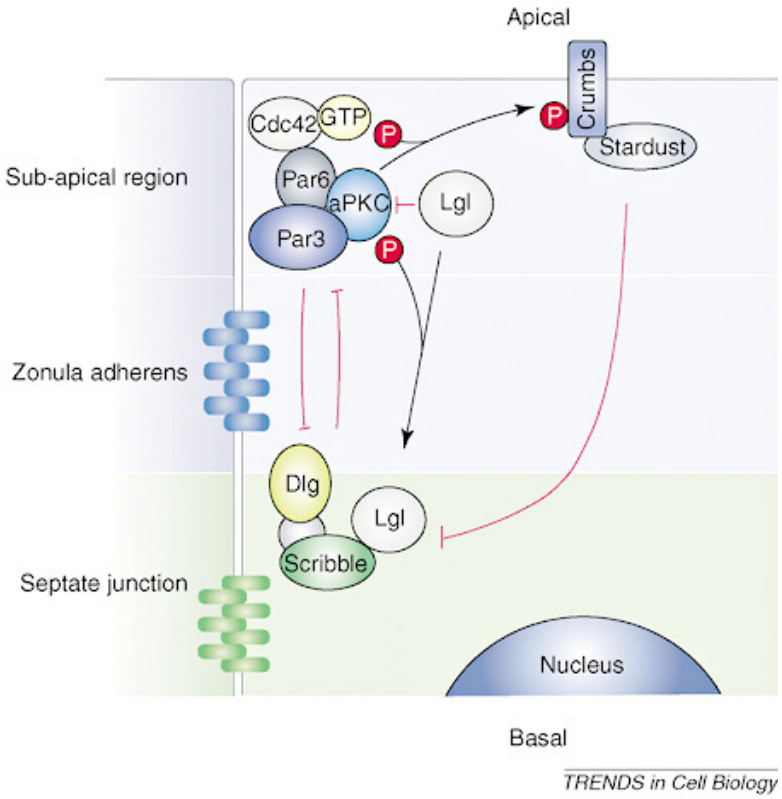


Figure 1: **Polarity establischment in epithelial cells.** Polarity complexes interact at the zonula adherens and restrict each others localization to specific domains in epithelial cells. Par-3/Par-6/aPKC cooperates with the crumbs complex to restrict the scribbled to the basal-lateral cortex. Visa versa the scribbled complex restricts Par-3/Par-6/aPKC to the sub apical region. Red lines indicate functional antagonism, black lines indicate activation. (Figure adapted from Humbert et al 2009)

A global tissue polarity cue connects cells to the axis of the body

During development, cells need to interpret the body plan in order to divide with correct orientation and generate specific types of tissue. To do so, cells communicate PCP information to each other. PCP enables cells to adapt and organize polarity in the plane of the tissue, perpendicularly to the A-B axis. PCP was initially described in *Drosophila* and primarily studied in epithelia of the wing, eye and the bristles on the notum. Two mechanisms exist to establish PCP, a global mechanism which links the direction of polarization to the axis of the body and a core module regulating cellular asymmetries and alignment of the cells to the axis of the global module in a domino like fashion. As the cell interprets its axis, down stream effectors of the PCP proteins can organize the above described polarity complexes, regulate cell-type specific morphological polarity and undergo directed cell division or migration (Axelrod, 2009a).
The global PCP module consists of three proteins, the atypical cadherins Fat (Ft) and Dachsous (Ds) and the Golgi resident kinase Four-jointed (Fj). Ft and Ds form heterodimers by heterotypical interaction bridging cell boundaries. Fj mediates the binding affinity of Ds and Ft for each other by lowering Ds affinity for Ft and increasing Ft affinity for Ds. Since Fj is expressed in a gradient throughout the tissue, differences in Fj activity between cells results in different production of Ft-Ds or Ds-Ft heterodimers at the cell boundary (Adler et al., 1998; Clark et al., 1995; Lawrence et al., 2004; Mahoney et al., 1991; Yang et al., 2002; Zeidler et al., 2000). This mechanism potentially provides cells with a directional cue to establish their polarity on. Loss of Fj results in uncoupling of the global directional cue from the core module, allowing cells to align with each other but in a random orientation in respect to the actual body axis. The mechanistic link between the global and local PCP mechanisms remains to be found however (Axelrod, 2009a; Vladar et al., 2009).

PCP establishment at the cellular level

On the level of single cells, PCP establishment is better understood. PCP is mediated through an evolutionary highly conserved set of “core” proteins namely; the seven-transmembrane receptor Frizzled (Fz); Fz downstream effector multi-domain protein Dishevelled (Dsh); the Lim domain protein Prickle (Pk); the 4-pass transmembrane protein Van Gogh (Vang, also known as Strabismus/Stbm), the akyrin repeat protein Diego (Dgo) and the seven-transmembrane atypical cadherin Flamingo (Fmi, also known as Starry Night/Stan) (Seifert and Mlodzik, 2007; Strutt, 2003; Wu and Mlodzik, 2009; Zallen, 2007).
Fz is known to function in the canonical Wnt/β-catenin pathway which regulates transcription by the T-cell transcription factor (TCF). When Fz is unbound serine/threonine kinases Casein Kinase I and GSK-3 phosphorylate β-catenin and a complex with Axin and Adenomatous Polyposis coli (APC) facilitates the destruction of β-catenin resulting in no transcription by TCF. Binding of Wnt to Fz leads to the activation of Dsh which inhibits the destruction of β-catenin and therefore allows β-catenin to bind TCF and activate transcription. The Wnt/β-catenin pathway is essential during development where it plays an important role in tissue specification and segmentation. Next to canonical Wnt signaling, Fz is also involved several non-canonical Wnt-signaling pathways (Clevers, 2006). It is unknown if Fz non-canonical

signaling in PCP is Wnt dependent. No Wnt ligands have been indicated to influence PCP establishment in *Drosophila* yet, therefore the function of Fz in PCP might be independent of Wnt/Fz canonical and non-canonical signaling pathways (Wu and Mlodzik, 2009).

PCP is best studied in the wings and eyes of *Drosophila*. Cells of the wing are hexagonal shaped and from each cell a single actin-rich trichome grows from the distal side and points distally. In genetic mosaics, clones mutant for PCP proteins changed the orientation of the hairs, therefore position and direction of hair growth from the wing cells provides an elegant system to study PCP signaling and lead to the present models (Adler, 1992; Gubb and Garcia-Bellido, 1982). It was found that localization of PCP proteins becomes asymmetric in wing cells during pupal formation. Initially, the core PCP proteins are uniformly distributed at the sub-apical ring near the adherens junctions, at later stages Fz together with Dsh and Dgo localizes at the distal edge of the cells as Vang and Pk localize to the proximal edge of the cell (Axelrod, 2001; Bastock et al., 2003; Das et al., 2004; Strutt, 2001; Tree et al., 2002). Fmi co-localizes with both the proximal and distal complexes (Figure 2) (Usui et al., 1999). Similar localization of the PCP core proteins is seen in vertebrates, which suggests that both polarized localization as well as PCP signaling are evolutionary conserved (Ciruna et al., 2006; Yin et al., 2008).

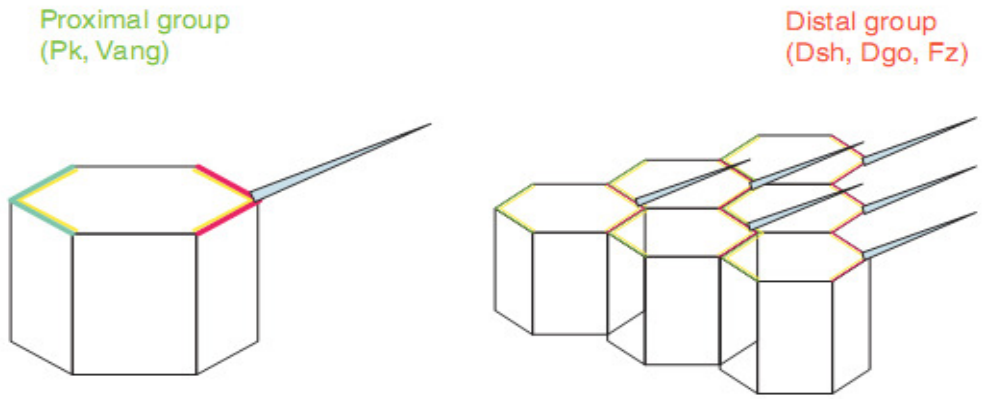


Figure 2: Localization of core PCP proteins in the drosophila wing. Vang together with Pk localizes to the apical proximal side of the cell (green). Fz together with Dsh and Dgo localize to the apical distal side of the cell (red). Fmi is localized both proximal and distal (yellow) (Figure adapted from Wu & Mlodzik 2009)

Members of the PCP core proteins can be subdivided into cell autonomous and non-autonomous acting factors. *dsh*, *dgo*, and *pk* mutant clones only affect PCP in a cell autonomous way and are needed to establish PCP within the cell. Whereas *fz*, *fmi* and *vang* mutant clones also show changes in polarity of neighboring wild-type cells and therefore also play a key role in communicating PCP between cells (Lawrence et al., 2004; Lee and Adler, 2002; Strutt and Strutt, 2002; Taylor et al., 1998; Vinson and Adler, 1987).

PCP proteins connect cells to the body axes

How PCP core proteins signal planar polarity information in and between cells remains to be elucidated, but great advances have been made recently in understanding the mechanism of non-autonomous signaling. Clonal analysis of *Drosophila* wing cells lacking *fz* or *vang,* lead to the finding that not only fz^- or $vang^-$ cells but also neighboring wild-type cells alter their polarity (Vinson and Adler, 1987). Similar effects were found in the eye and abdomen, indicating a general role for Fz and Vang in non-autonomous cell-to-cell communication (Lawrence et al., 2004; Strutt and Strutt, 2002; Zheng et al., 1995).
fz^- clones in the wing repolarize neighboring wild-type cells resulting in the trichome to point towards the clones instead of distally. The other way around, if *fz* is over-expressed, the neighboring wild-type cells point their trichome away from the clone (Lawrence et al., 2004). These non-autonomous effects in the wing indicate that cells acquire their proximal-distal polarization by orienting away from high Fz levels and towards low Fz levels at the cell boundaries (Adler et al., 1997). $Vang^-$ clones show an opposite effect as neighboring wild-type cells point their trichomes away from the clone (Taylor et al., 1998). $Fz^-/Vang^-$ double mutants show phenotypes similar to Fz^- single mutants suggesting that Fz level at the cell boundary is indeed mediating the orientation of neighboring cells in a non-autonomous fashion (Wu and Mlodzik, 2009).
Fmi plays an essential role in Fz mediated non-autonomous signaling. fmi^- clones do not show alterations in cell polarity of neighboring cells, suggesting that Fmi it-self has no function in non-autonomous signaling. However, if *Fz* or *Vang* is overexpressed in fmi^- clones, neighboring cells are unaffected showing that Fmi is essential in sending and receiving non-autonomous signaling (Lawrence et al., 2004). The extracellular domain (ECD) of Fmi was found to interact homophilicaly in trans with the ECD of Fmi presented on neighboring cell membranes. Also, Fmi only localizes to the membrane parts where Fmi from a neighboring cell is present. This leads to the model that Fmi-Fmi interaction stabilizes Fmi protein complexes with Fz or Vang around the adherens junctions where cells contact each other (Usui et al., 1999). On the other hand, Fz and Vang are needed for correct Fmi localization since localization of Fmi at the junctions is reduced in Fz^- and $Vang^-$ cells and even more in $Fz^-/Vang^-$ double mutant cells (Strutt and Strutt, 2008). Physical interaction of Fmi with Vang and Fz was also confirmed by co-immunoprecipitation (Chen et al., 2008). Collectively, these results suggests that during PCP establishment Fmi interaction with Fz and Vang is essential for stabilization and correct localization of the complexes at the junctions (Figure 3)(Wu and Mlodzik, 2009).

It is clear that Fmi-Fz and Fmi-Vang binding and localization is essential in propagating polarity throughout the tissue. However, the exact mechanism of how these complexes establish and communicate the polarity information remains to be elucidated and is a subject of intense debate. Several recent studies proposed interesting models of how this mechanism could work. A recent study of Chen and co-workers proposed that Fmi can exist in two forms within the cell, namely the V-Fmi which binds to Vang, and the F-Fmi form which changes conformation upon binding to Fz. At the cell boundaries, V-Fmi would preferentially interact with F-Fmi of the neighboring cell and thereby the complex would be able to recruit Fz and communicate asymmetrically. Based on the fact that when *fmi* is overexpressed a similar phenotype as in Fz^- mutants is found (which is the recruitment of Fz to the

boundary of the neighboring Wild-type cells) it is proposed that V-Fmi is the normal form (Usui et al., 1999). This model is supported by the fact that in a *Fz*$^{-}$ background Fz is recruited to the boundary of the neighboring cells by Vang-Fmi (Chen et al., 2008). An alternative to this model is proposed by Strutt & Strutt who show that Fmi has an intrinsic preference for Fz over Vang. Fmi binding to Fmi-Fz at cell boundaries is suggested to be more stable compared to Vang-Fmi binding. Therefore, in *Fz*$^{-}$ clones Fmi preferentially binds to Fz-Fmi from neighboring cells. The finding that over expression of Fmi results in stabilization of Fz-Fmi at all junctions of the mutant cell in combination with the fact that levels of Fmi-Vang are reduced, supports the preference of Fmi to bind Fz. Interestingly, overexpressed *Fmi* in *Fz*$^{-}$ cells is unable to stabilize Vang at the cells junctions which suggests a co-factor or modification of Fmi is needed. The C-terminal domain of Fmi is indicated to function in this process since Fmi lacking the C-terminus shows increased Fz binding (Strutt and Strutt, 2008). The models of Chan and Strutt & Strutt both propose that Fmi is the essential player to mediate cell-to-cell communication. However, no evidence exists indicating or arguing against a signaling function for Fmi, leaving a major gap in our understanding of PCP signaling. Additionally, non-autonomous phenotypes for Fmi are only seen when *Fmi* is overexpressed and not when it is lost. This suggests a complex signaling mechanism between Fz, Vang and Fmi of neighboring cells to establish PCP, and probably is dependent on additional factors (Wu and Mlodzik, 2009).

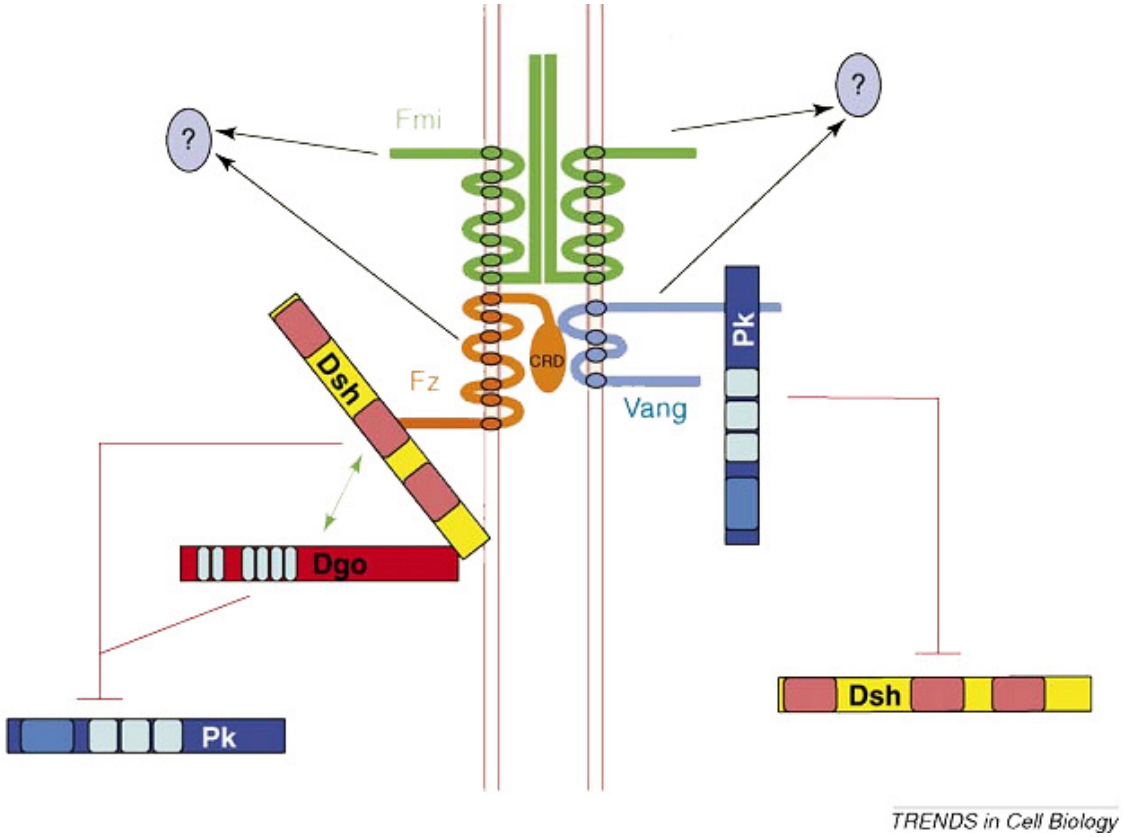


Figure 3: Schematic presentation of interactions between Fmi, Fz, Vang and their intercellular partners. Fmi (green) at the distal membrane is bound to Fz (orange) and with Vang (light blue) at the proximal membrane of the wing cell. Fmi interacts homophilically with Fmi at the neighboring cell membrane. Fz binds to Vang through its CRD. Fz-Fmi and Vang-Fmi transduce non-autonomous signaling of PCP information through an unknown effector (Black arrows). Dsh and Dgo interact downstream of Fz and antagonize Pk localization at the distal membrane. Conversely Pk downstream of Vang antagonizes Dsh localization at the proximal membrane. (Figure adapted from Wu & Mlodzik 2009)

Another model is proposed by Wu & Mlodzik in which Vang and Fz mediate signaling by direct interaction. The extra-cellular domain of Fz contains a cystein-rich domain which can interact with the extra-cellular domain of Vang across cell membranes. The cystein-rich domain was also found to be essential in signaling since mutation or deletion of this domain eliminates the non-autonomous features of Fz (Wu and Mlodzik, 2008). Findings supporting this mechanism are that Fz non-autonomous effects are not seen when *Fz* mutant clones are analyzed in flies with a *vang*$^{-}$ background, and that Fz defective in its cystein-rich domain do not rescue *fz*$^{-}$ non-autonomous phenotypes (Chen et al., 2004; Lawrence et al., 2004; Taylor et al., 1998; Wu and Mlodzik, 2008). Despite this controversy in the proposed models it is clear that it is a complex interplay between Fz, Vang and Fmi that mediates PCP. The mechanism of PCP establishment, direction of PCP signaling and interpretation of this polarity cue by cells remains to be elucidated to allow a comprehensive model.

Polarity complexes in regulating spindle orientation

Spindle Positioning in budding yeast

As a cell determines its axis of division, it needs to organize polarity complexes, segregate cell-fate determinants and position the mitotic spindle. PCP core proteins play an essential but mysterious role in communicating polarity information to the cell. Insight on the mechanism used by different organisms to orient the mitotic spindle might point to likely downstream candidates for PCP signaling and help elucidate these pathways.
Single-cell organisms do not use the complex mechanisms as described above to establish polarity but still need to position their spindle correctly to ensure equal segregation of the genome. The mechanism of spindle positioning is well understood in the budding yeast S*accharomyces cerevisiae*. Many proteins used by budding yeast to position its spindle are evolutionary conserved and used throughout the animal kingdom. Therefore, studies in budding yeast can provide insight in basic mechanisms to position the spindle. Budding yeast uses its polarity axis to direct polarized growth of a bud, later becoming the daughter cell, as well as for positioning the mitotic spindle to ensure equal segregation of the genome between the mother and the bud (Siller and Doe, 2009).
Localized activation of Cdc42 marks the bud site and initiates the establishment of polarity. Activated Cdc42 organizes polarized actin cables which extend from the bud into the mother cell and, subsequently, septin proteins accumulate at the bud-mother cell border and form a collar marking the bud neck. Positioning of one spindle pole body (SPB) at the bud neck is the first step in positioning the spindle. Positioning of the SPB is mediated by the APC-related protein Kar9 which is localized to the microtubule plus tips by the EB1-related protein Bim1. Kar9 binds Myo2, a class V myosin, which mediates polarized transport of microtubule plus tips towards the bud neck and into the bud (Hwang et al., 2003). During microtubule transport along the actin fibers, microtubules do not shrink indicating that Myo2 is the force responsible for spindle movement. After actin dependent spindle alignment and microtubule attachment to the bud neck and tip, microtubules start to depolymerize resulting in the final positioning of the spindle at the bud entry (Adames and Cooper, 2000; Kusch et al., 2002). Attachment of microtubules to the cortex of the bud and the bud neck

requires the actin- forming-binding protein Bud6 (Huisman et al., 2004; Segal et al., 2000). Depolymerization of the attached microtubules is promoted by the PAR-1 related septin associated kinases Hsl1 and Gin4. It is unclear if Hsl1 and Gin4 affect microtubules directly or trough associated proteins, and how Bud6 mediates microtubule attachment. It is clear however that actin- and microtubule based motors in combination with the interaction of microtubules with the cell cortex are essential for spindle positioning (Figure 4a) (Siller and Doe, 2009).
During anaphase, a second pathway is involved in positioning the spindle. The SPB located at the bud neck is pulled into the bud to establish its final position on the axis. Pulling on the microtubules resulting in the translocation of the SPB is mediated by the Dynein-Dynactin motor protein complex (Eshel et al., 1993; Li et al., 1993). First Dynein-Dynactin is recruited to the SPB by the CLIP-170 related protein Bik1 which subsequently localizes Dynein-Dynactin to the microtubule plus tip through binding to the microtubule plus end directed kinesin Kip2 (Miller et al., 2006). As the Dynein-Dynactin complex reaches the cortex of the bud, Dynein is activated by the membrane bound protein Num1. Dynein then pulls the SPB into the bud using its minus end directed motor activity and places the SPB at its final position (Figure 4b) (Farkasovsky and Kuntzel, 2001; Sheeman et al., 2003). Multi-cellular organisms use strategies similar to budding yeast to position the mitotic spindle, although with greater complexity and involvement of cell-cell signaling.

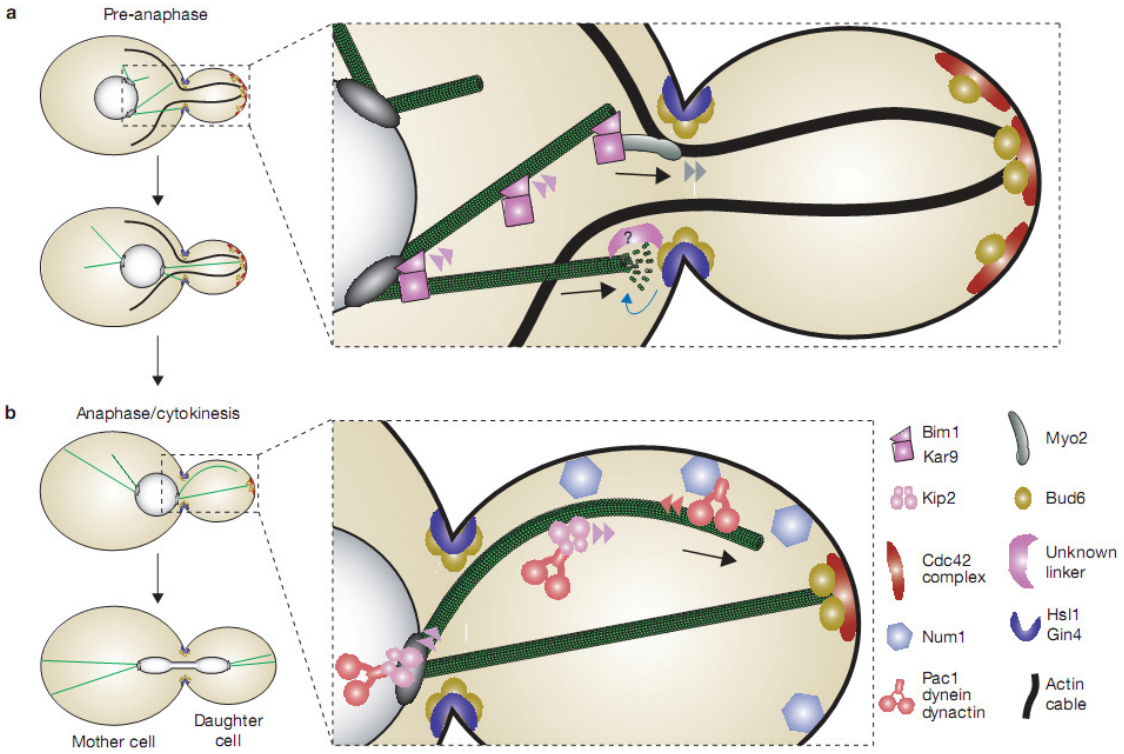


Figure 4: Spindle positioning in S. cerevisiae. (a) Before anaphase Bim1–Kar9 are recruited to the SPB and translocate to microtubule plus-ends (pink arrowheads) where they associate with Myo2. Myo2 motor activity (grey arrowheads) pulls attached microtubules into the bud resulting in positioning of the SPB at the bud neck. The bud neck kinases Hsl1and Gin4 promote microtubule shortening (blue arrow), which results in spindle alignment with the bud and SPB localization to the bud neck. (**b**) During anaphase Kip2 transports Pac1–Dynein–Dynactin to microtubule plus-ends. When the dynein complex reaches the cortex it is activated by Num1. Cortical Dynein–Dynactin than pulls the daughter SPB into the bud. (Figure adapted from Siller & Doe 2009)

Preparing the cell for asymmetric division

During development of multi-cellular organisms, cells need to divide according to the body plan as well as generate numerous different cell-types. This is only possible with tight regulation of cell fate and spindle orientation, leading asymmetric cell division. During prophase polarity complexes like Par-3/Par-6/aPKC and cell fate determinants are assembled in a crescent shape over one pole of the cell. Cell fate determinants enable that the identity of the two daughter cells can be different and give rise to different types of tissue after asymmetric division. One way to regulate cell fate is the differential localization of proteins at one of the poles of the dividing cell. Similarly, differential localization of mRNA is seen in the *Drosophila* oocyte, here oskar mRNA is localized at the posterior side and is coupled to translational repression. Differential protein stability in for example *C. elegans* embryo's where the germline specifying protein PIE-1 is degraded in somatic cells is also a mechanism to regulate cell fate (DeRenzo et al., 2003; Johnstone and Lasko, 2001).
The exact mechanism of how the cell fate determinants are segregated is under heavy investigation. However, it is known that mechanisms similar to the localization of polarity complexes like Par-3/Par-6/aPKC play an important role in restricting cell fate determinants to a specific pole of the cell. aPKC is known to phosphorylate fate determinants directly and thereby excluding them from the aPKC pole. aPKC also phosphorylates substrates like Lgl, a protein also indicated in recruiting fate determinants to the cell pole opposite of aPKC (Betschinger et al., 2003; Plant et al., 2003; Smith et al., 2007; Yamanaka et al., 2003).
After the cell establishes its polarity and correctly localized its cell fate determinants at the specific cell poles, it needs to ensure that these poles end up in the right daughter cell. To ensure the asymmetric inheritance of the cell fate determinants the cell needs to align the mitotic spindle with the polarity axis and thereby position the cleavage plane at the cell equator, which mostly is at the border of the poles.
The position of the spindle is controlled in a variety of ways, for example in *Drosophila* germ and larval neuroblast cells the centrosome at one pole of the spindle has a fixed position (Rebollo et al., 2007; Yamashita et al., 2007). However, in many other cell types centrosomes are not fixed and the cell needs to actively orient the spindle to ensure correct alignment with the asymmetry axis. Many aspects of spindle positioning and how spindle positioning is regulated by polarity complexes are still unclear, but studies in *Drosophila* and *C. elegans* provided great insights on how it probably works.

Spindle positioning by force in the C. elegans embryo

During development of the *C. elegans* embryo, positioning of the spindle is tightly controlled and division follows a reproducible pattern. Therefore, *C. elegans* provides a helpful and elegant model to study mechanisms of spindle positioning and asymmetric cell division (Galli and van den Heuvel, 2008). The *C. elegans* oocyte is not polarized until fertilization determines the anterior-posterior axis with the entry site of the sperm determining the posterior (Goldstein and Hird, 1996). After fertilization the oocyte nucleus undergoes meiosis I and II in close proximity of the cell cortex generating two polar bodies outside the zygote. Subsequently, the cortex wrinkles by contraction and relaxation of the actomyosin network. Asymmetric localization of the PAR proteins and smoothening of the cortex starts with retraction of the actomyosin network towards the anterior, away from the sperm entry site and

expands anterior resulting in a wrinkled anterior and smooth posterior cortex. PAR-3/PAR-6/PKC-3 initially localize uniformly on the cortex but move to the anterior side of the zygote and PAR-1 and PAR-2 localization at the posterior side in initialized. When the smooth and wrinkled domains reach equilibrium at roughly half way the zygote the anterior cell cortex smoothens and a platform for asymmetric division is established (Galli and van den Heuvel, 2008).

After the zygote established its A-P polarity it needs to segregate cell fate determinants to the specific poles and align the mitotic spindle with the axis. During prophase, the maternal and paternal nuclei move to the centre of the zygote and the centrosomes are positioned on the A-P axis (Figure 5). Secondly, the mitotic spindle formed during pro-metaphase needs to be positioned more posterior of the zygote centre to ensure that cleavage results in a larger anterior blastomere cell (AB) and smaller posterior cell (P1). Positioning of the spindle is mediated by pulling forces on the astral microtubules at the cell cortex which are stronger at the posterior side of the zygote (Grill et al., 2001; Labbe et al., 2004).

PAR proteins were found to play a key role in establishing differential pulling forces at the cell cortex. In the wild-type zygote one pole of the mitotic spindle travels faster and further towards the posterior and one slower towards the anterior resulting in the more posterior cleavage plane. In *par-2* mutant zygotes both spindle poles move equally fast and far as the wild-type anterior pole, as in *par-3* mutants both poles move as fast and far as the posterior wild-type pole, both resulting in a centrally positioned spindle and symmetric division. This finding showed that the asymmetric distribution of the PAR proteins is involved in spindle positioning (Grill et al., 2001). Laser severing of microtubules at different positions in the mitotic spindle showed that posterior positioning of the spindle is mediated by pulling forces at the cell cortex, indicating that an effector downstream of the PAR polarity would be located at the cell cortex (Grill et al., 2001; Grill et al., 2003).

Heterotrimeric G-proteins (HGP) were found to play a key role in spindle positioning. (Gotta and Ahringer, 2001; Miller and Rand, 2000; Zwaal et al., 1996). The HGP complex consists of the three subunits Gα (GOA-1 and GPA-16 in C.elegans), Gβ and Gγ (together Gβγ) and is tethered to the plasma membrane by lipid modifications on Gα and Gγ. Activation of the HGP signaling pathway can be initiated by G-protein coupled receptors (GPCR), which upon ligand binding promotes the dissociation of active Gα-GTP from Gβγ. Secondly, HGP signaling can be activated in a receptor independent way, which uses the guanine exchange factor RIC-8 to generate Gα-GTP and subsequently a GTPase activating protein RGS-7 to generate the Gα-GDP which is the form needed for spindle orientation (Kimple et al., 2002; Tall and Gilman, 2005; Tall et al., 2003). Gα-GDP is able to bind to the GoLoco domain of GPR-1 and GPR-2 (together GPR-1/2). GPR-1/2 binding keeps Gα active by displacing Gβγ and Gα activates GPR-1/2 by preventing intermolecular interaction of the GoLoco domain with the TPR domain of GPR-1/2 itself. Subsequently the free TPR domain of GPR-1/2 is able to bind to the coiled-coil protein LIN-5 (Mud in *Drosophila*, NuMa in human) (Colombo et al., 2003; Du and Macara, 2004; Gotta et al., 2003; Lorson et al., 2000; Nipper et al., 2007; Srinivasan et al., 2003).

The LIN-5/GPR-1/2/Gα complex mediates spindle positioning by binding to the minus end directed motor protein dynein and its activator LIS-1. Cortical Dynein can bind the plus ends of the astral microtubules and a combination of microtubule depolymerization with dyneins minus end directed motor activity allows the complex

to pull the microtubule towards the cell cortex (Couwenbergs et al., 2007; Kozlowski et al., 2007; Nguyen-Ngoc et al., 2007).
Asymmetry in pulling force resulting in the posterior placement of the spindle can arise from the slightly increased concentration of GPR-1/2 at the posterior cortex which intuitively results in increased posterior pulling force (Gotta et al., 2003). Secondly the DEP domain G-protein regulator LET-99 plays a central role. In response to the PAR domains LET-99 localizes to a lateral cortical belt and mediates exclusion of GPR-1/2 from this domain (Park and Rose, 2008; Tsou et al., 2002; Tsou et al., 2003). It was recently proposed that this exclusion of pulling forces at the central-posterior LET-99 domain removes the counter productive pulling at the lateral astral microtubules of the posterior spindle pole resulting in a stronger posterior net force (Figure 4)(Krueger et al., 2010).

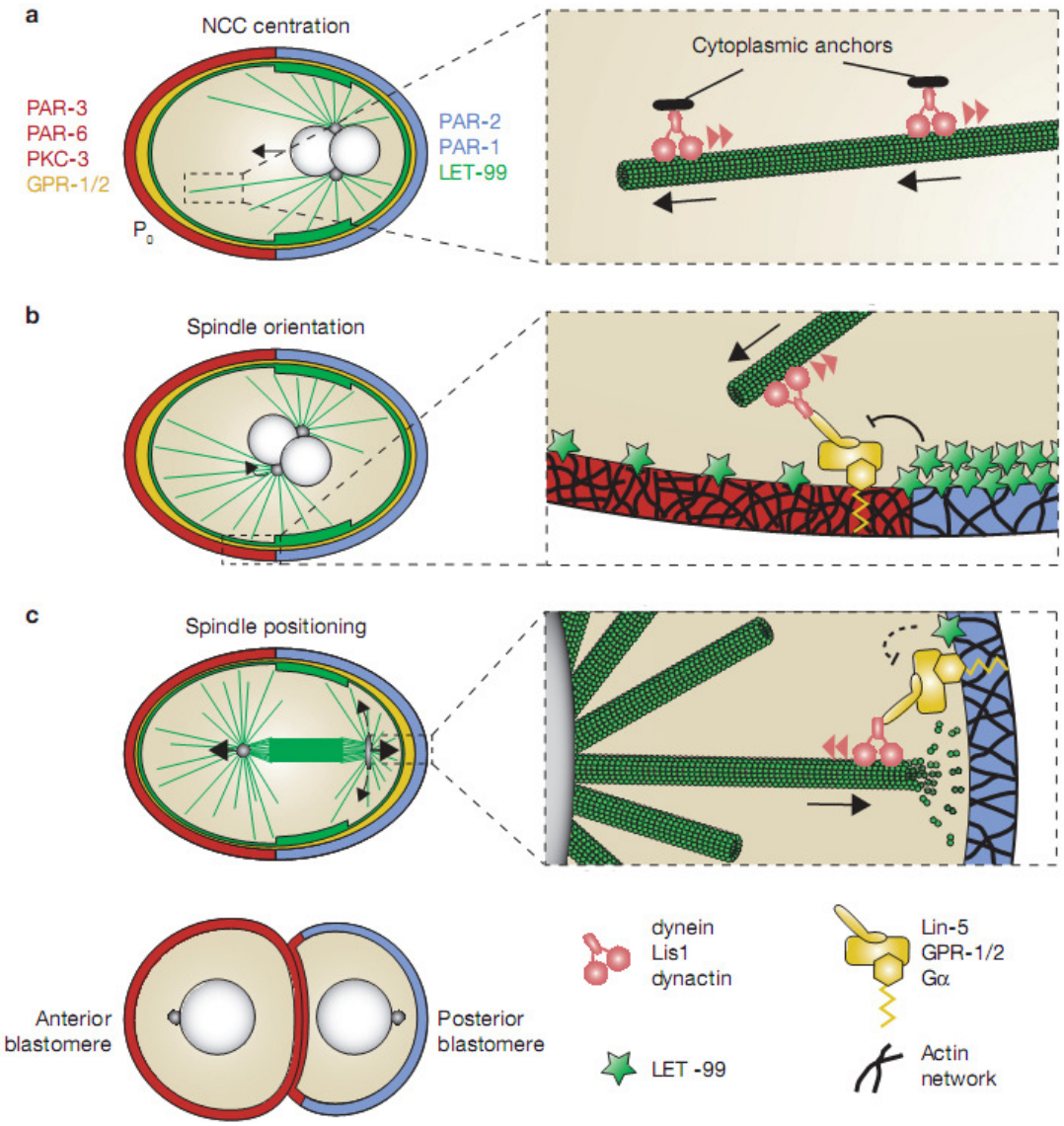


Figure 5: Spindle positioning in the *C. elegans* zygote. (a) When the zygote established its polarity, the nucleus/centrosomal complex (NCC) moves anteriorly to the cell centre. (**b**) At the cell center the centrosomes become aligned along the anterior-posterior axis due to the activity of the cortical GPR-1/2 complex and associated dynein–dynactin. (**c**) At anaphase, GPR-1/2 is enriched at the posterior cortex, where it activates cortical dynein resulting in posterior spindle displacement and generation of a larger anterior AB cell and a smaller posterior P1 cell. LET-99 is enriched in a lateral cortical belt and restricts cortical GPR-1/2 localization to the posterior. Light red arrowheads show the direction of dynein motion; black arrows show the direction of the net spindle positioning force. (Figure adapted from Siller & Doe 2009)

In subsequent divisions of the *C. elegans* embryo an extra level of complexity is added in respect to spindle positioning. The cells need to position the spindle to generate cells of unequal size, now the spindle needs to be rotated to ensure division in the right direction. Following the two cell stage the P1 blastomere needs to divide on the A-P axis and gives rise to the P2 and EMS cells. The AB cell gives rise to a anterior ABa and a dorsal ABp daughter. In turn, ABa and ABp divide on the left-right axis with the spindle tilted in a 20˚ angle relatively to the A-P axis, resulting in a more anterior position of the left daughter cell relatively to the right daughter cell (Bergmann et al., 2003). Similar as in the zygote, the P1 cell asymmetrically localizes the PAR proteins and restricts LET-99 to a cortical band. Orientation of the spindle on the A-P axis is also dependent on LIN-5/GPR-1/2/Gα and its connection to Dynein (Lorson et al., 2000; Nguyen-Ngoc et al., 2007; Srinivasan et al., 2003).

As early as the four-cell stage embryo, signaling between cells plays an important role in orienting the spindle and in specification of cell fate. The EMS cell divides to give rise to the E and MS cell, which form endoderm and mesoderm respectively. It was found that EMS contact with the P2 cell is essential for E cell specification, but also for orientation of the EMS spindle to ensure a proper division on the A-P axis (Bei et al., 2002; Goldstein, 1992; Labbe and Goldstein, 2002; Schlesinger et al., 1999; Thorpe et al., 1997). Both induction of the E cell fate in the posterior daughter cell and orientation of the spindle are regulated by members of the Wnt/Fz pathway. MOM-2/Wnt produced in the P2 cell induces transcription of TCF target genes and therefore endoderm specification in the E cell, but MOM-5/Fz is also involved in spindle orientation in a non-canonical fashion (Bei et al., 2002; Schlesinger et al., 1999).
The presence of P2 as well as MOM-5/Fz and DSH-3/MIG-5(Dsh) are essential for EMS spindle positioning. MOM-5/Fz is proposed to signal through downstream effectors of the Wnt/Fz pathway, leading to GSK-3 activation, which was also found to be involved in regulating spindle rotation together with the Casein kinase I (Schlesinger et al., 1999). Wnt/Fz signalling together with the SRC-1 kinase pathway mediates the orientation of the spindle in the EMS cell (Bei et al., 2002). SRC-1 is suggested to exclude LET-99 from the EMS-P2 contact site and therefore allowing the LIN-5/GRP-1/2/Gα to be recruited at the cell boundaries (Tsou et al., 2003). Dynein-Dynactin is recruited to the P2-EMS border by MOM-5/Fz and SRC-1, were it is able to bind LIN-5/GRP-1/2/Gα and generate force to position the spindle (Schlesinger et al., 1999; Zhang et al., 2008). It is currently not known if MOM-5/Fz signaling at the P2-EMS border is similar to PCP signaling, and how MOM-5/Fz mediates spindle orientation. However, it is clear that in *C. elegans* endoderm induction, MOM-5/Fz plays an important role in MOM-2/Wnt mediated transcription initiation as well as in spindle positioning. These two functions of MOM-5/Fz are a commonly used throughout the animal kingdom and highlight the importance of MOM-5/Fz in cell polarity. *C. elegans* is a very elegant system to study cell polarity and spindle orientation, but to get a better understanding on how Fz signaling orients the spindle in PCP, studies in *Drosophila* are of better help since PCP is more evident in its epithelia compared to the *C. elegans* embryo.

Spindle positioning in *Drosophila* neuroblasts

The proteins and mechanisms used by *C. elegans* and *S. Cerevisiae* to position the spindle are almost entirely conserved in *Drosophila*. Asymmetric cell division and positioning of the spindle are best studied in the embryonic and larval neuroblast and the sensory organ precursor (SOP) cells, which give rise to the external sensory organs. Embryonic neuroblasts delaminate from the neurectoderm with established A-B polarity and contact the neurectoderm apically. Larval neuroblasts derive from the embryonic neuroblasts but contact a glial cell instead of the neurectoderm (Doe, 2008).
Larval neuroblasts show no polarization until late interphase/early prophase when polarity is established by Par-3 (Bazooka) (Schober et al., 1999; Wodarz et al., 1999), Par-6 (Petronczki and Knoblich, 2001) and aPKC (Rolls et al., 2003; Wodarz et al., 2000), which localize to the apical cortex. Similar to *C. elegans*, positioning of the spindle is mediated by a HGP, Gαi, and a GoLoco domain protein, Partner of inscutable (Pins, GPR-1/2 in *C. elegans*). In *Drosophila* neuroblasts, Inscutable plays a central but tissue specific role by binding to Par-3 and Pins resulting in the coupling of the two complexes and recruiting Gαi/Pins to the apical (Knoblich, 2008; Kraut et al., 1996). Spindle orientation in embryonic neuroblasts relative to the neurectoderm is lost in Par-3 and Inscutable mutants (Kraut et al., 1996; Wodarz et al., 1999). In larval neuroblasts, reduction of Pins or Gαi results in the uncoupling of spindle orientation relative to the A-B axis, but loss of Par-6 or aPKC has no effect on spindle orientation (Nipper et al., 2007; Rolls et al., 2003; Siegrist and Doe, 2005). Combined loss of Par-3 and Pins results in loss of spindle pole asymmetry and the production of equal sized daughter cells after division (Cai et al., 2003; Fuse et al., 2003). This data shows that Par-3, Inscutable, Pins and Gαi are the key polarity proteins regulating spindle positioning likely through multiple pathways (Siller and Doe, 2009).
Prior to mitosis, in both embryonic and larval neuroblasts one active centrosome associates with the apical cortex throughout interphase and one inactive centriole pair moves through the cell. During prophase the inactive centriole pair localizes basally and becomes activated to become a functional centrosome, which results in the formation of a bipolar spindle. During pro-metaphase the spindle moves and astral microtubules probe the cell cortex and exert pulling forces, but the spindle never moves far from the A-B axis. In embryonic neuroblasts both centriole pairs can become the active apical centrosome, in larval neuroblasts however, the old centrosome always becomes the apical active centrosome suggesting spindle orientation is fixed throughout the cell cycle in these cells (Figure 6) (Knoblich, 2008; Rebollo et al., 2007; Rusan and Peifer, 2007; Siegrist and Doe, 2006)

Two pathways are known to regulate spindle orientation in neuroblasts. As in *C. elegans,* the conserved complex of Gαi, Pins and Mud (LIN-5 in *C. elegans*, NuMa in humans) is involved in the receptor independent G-protein pathway. Pins can bind Gαi through its GoLoco domain and Mud through its TPR domain. Linkage of the Gαi/Pins/Mud complex to the Par-3/Par-6/aPKC complex by Inscutable suggests a model wherein Mud binding to Dynein-Dynactin can fix the spindle apically, since dynactin-dynein is involved in spindle orientation and movement in neuroblasts (Siller and Doe, 2008; Siller et al., 2005) However, unlike in *C. elegans* no Dynein-Dynactin localization is seen at the cell cortex and no interaction of Mud with Dynein-Dynactin has been documented in *Drosophila*(Siller and Doe, 2009)

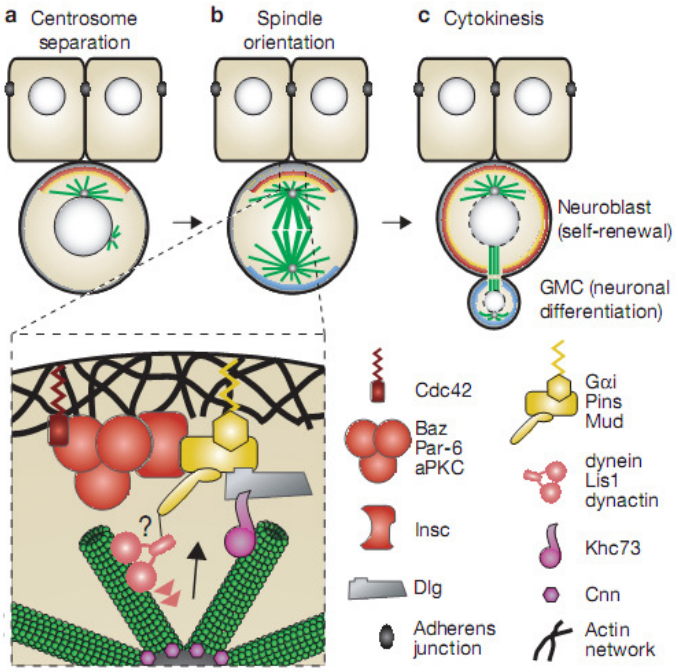


Figure 6: Spindle orientation in drosophila the neuroblast. (**a**) Early in prophase centrosomes separate and one remains closely to the apical pole as the other migrates basally. (**b**) At prometaphase/metaphase the spindle is aligned at the A-B axis and cell fate determinants are localized at the basal pole (blue). (**c**) During anaphase/telophase the apical spindle pole remains tightly associated with the apical cortex and the basal pole displaces more basally resulting in a large apical neuroblast and a small basal GMC. (Figure adapted from Siller & Doe 2009)

A second spindle orientation pathway consists of Pins, Dlg, and the microtubule plus-end directed motor Kinesin heavy chain 73 (Khc73). Khc73, localized at the astral microtubule plus tips, is able to bind to cortical Dlg, which in turn is able to recruit cytoplasmic Pins to the cell cortex (Bellaiche et al., 2001). Binding of Khc73 to Dlg induces a conformational change in Dlg, which allows oligomerization of Dlg proteins and generate clusters of concentrated Dlg for cytoplasmic Pins to bind to (McGee and Bredt, 1999). These interactions allow the cell to recruit polarity complexes over one of the spindle poles. This mechanism is also known as telophase rescue since this pathway is active only from metaphase. Khc73/Pins/Dlg hereby allows the cells to repolarize and position cell fate determinants based on the spindle pole positions, ensuring correct asymmetric division. Why clustering of polarity complexes over only one spndle pole occurs remains unclear, but it might be a result of a difference in centrosome composition or because other polarity proteins favor polarity crescent formation over one of the poles (Lambert and Nagy, 2002; Siegrist and Doe, 2005).

PCP and its role in spindle positioning

As described above, studies on PCP in *Drosophila* eye and wing epithelia proposed a mechanism for how cells can communicate polarity throughout the tissue. In addition, great insight in polarity establishment, cell fate determinant segregation and spindle orientation was obtained in studies of *Drosophila*, *C. elegans* and yeast. However, the mechanism by which cells communicate the PCP cue within the cell to ensure correct cell fate determinant segregation and spindle orientation remains to be solved. The experimental tools as described above are all unsuitable to answer this question. Yeast is a single-cell organisms with no need for PCP and *C. elegans* embryo's are to early in development for clear PCP signaling. *Drosophila* wing and eye studies focus on

trichome and omatidal growth direction, not cell division, and neuroblast polarity establishment uses Inscutable which complicates interpretation of possible separate signaling pathways.
A very helpful system to study PCP signaling in polarity establishment and spindle orientation is the development of sensory organs of *Drosophila*. Sensory organ precursor (SOP or pI) cells lay in the epithelium and give rise to a four cell organ. These cells obtain a different fate through a highly reproducible pattern of divisions. SOP cells are not stem cells since these organs do not maintain a stem cell population. However, they go through a highly regulated series of asymmetric cell divisions. Interestingly, SOP cells divide with evident PCP resulting in establishment of asymmetry, spindle alignment and division on the A-P axis. Therefore sensory organ development provides a good model to study PCP signaling and its role during cell division compared to the other models described.

Before division, a SOP cell delaminates from the epithelium and subsequently divides to give rise to an anterior pIIb and posterior pIIa cell. Next the pIIa cell divides again in the plane of the epithelium to give rise to the posterior socket cell and anterior hair cell, whereas the pIIb cell divides on the A-B axis to give rise to a neuron and a sheath cell (Knoblich, 2008; Segalen and Bellaiche, 2009). Cell fate of the SOP daughter cells is determined by regulation of the Notch signaling pathway through directional signaling to pIIa from pIIb and the asymmetric inheritance of fate determinants Numb and Neuralized by the latter (Le Borgne et al., 2005; Schweisguth, 2004).
Many of the polarity complexes used by *Drosophila* neuroblasts are also functioning in SOPs, however a major difference is the absence of Inscutable in SOPs resulting in Dlg/Pins/Gαi localization at the anterior cortex and Par-3/Par-6/aPKC at the posterior cortex in a PCP dependent manner (Bellaiche et al., 2004; Roegiers et al., 2001; Schaefer and Knoblich, 2001). PCP establishment in SOP cells is controlled by the core PCP proteins Fz, Dsh, Fmi, Vang and Pk. Fz and Dsh localize to the apical posterior cortex, Vang and Pk to the anterior apical cortex and Fmi is localized uniformly (Segalen and Bellaiche, 2009). Genetic studies revealed that Vang and Fz/Dsh cooperate to localize Dlg/Pins/gαi through recruitment of Pins by Vang and exclusion of Pins by Dsh at the anterior and posterior cortex respectively (Bellaiche et al., 2004). Dlg/Pins/Gαi excludes Par-3/Par-6/aPKC from the anterior cortex which in turn restricts Numb to the anterior cortex by aPKC mediated phosphorylation (Smith et al., 2007). How PCP core proteins regulate Dlg/Pins/Gαi and Par-3/Par-6/aPKC mechanistically remains to be elucidated. However, interaction of Dsh with aPKC was recently found in mammalian neuronal development and indicates there might be Fz/Dsh mediated recruitment or interaction connecting Par-3/Par-6/aPKC regulation to PCP signaling (Zhang et al., 2007). If an interaction between Dsh and aPKC would also occur in SOP cells, this might mediate the recruitment of the Par-3/Par-6/aPKC to the posterior cortex and thereby facilitate the localization of Dlg/Pins/Gα to the anterior. The posterior exclusion of Pins combined with the recruitment of Par-3/Par-6/aPKC to the posterior cortex might illustrate the key role of Fz/Dsh in communicating PCP to the cell.
Even though a mechanistic link remains to be described it is clear that PCP signaling is essential for maintaining correct A-P polarity and division in SOP cells. *Drosophila* pupae SOP cells mutant for *fz* or *dsh* are still able to localize their cell fate determinants asymmetrically, but in a random orientation towards the A-P axis (Gho and Schweisguth, 1998). Spindle orientation is also random to the A-P axis but

remains oriented relative to the cell fate determinants. Similar results were found for the other PCP genes *fmi*, *vang* and *pk* (Bellaiche et al., 2004; Lu et al., 1999). Interestingly, the spindle remains oriented parallel to the plane of the tissue in these mutants. This suggests that the remaining core PCP proteins are sufficient to maintain the spindles planar orientation, or a PCP independent spindle orientation mechanism exists. Both scenarios are probably true and act in a redundant fashion. It was proposed that Vang and Fz mediate planar spindle orientation independently of each other, suggesting two redundant pathways and thereby ensuring a robust system for correct asymmetric division. Indeed SOP clones mutant for *fz*, *dsh* or *vang* are still able to polarize asymmetrically and to divide on the A-P axis in response to the PCP cue provided by surrounding wild-type cells (Gomes et al., 2009).

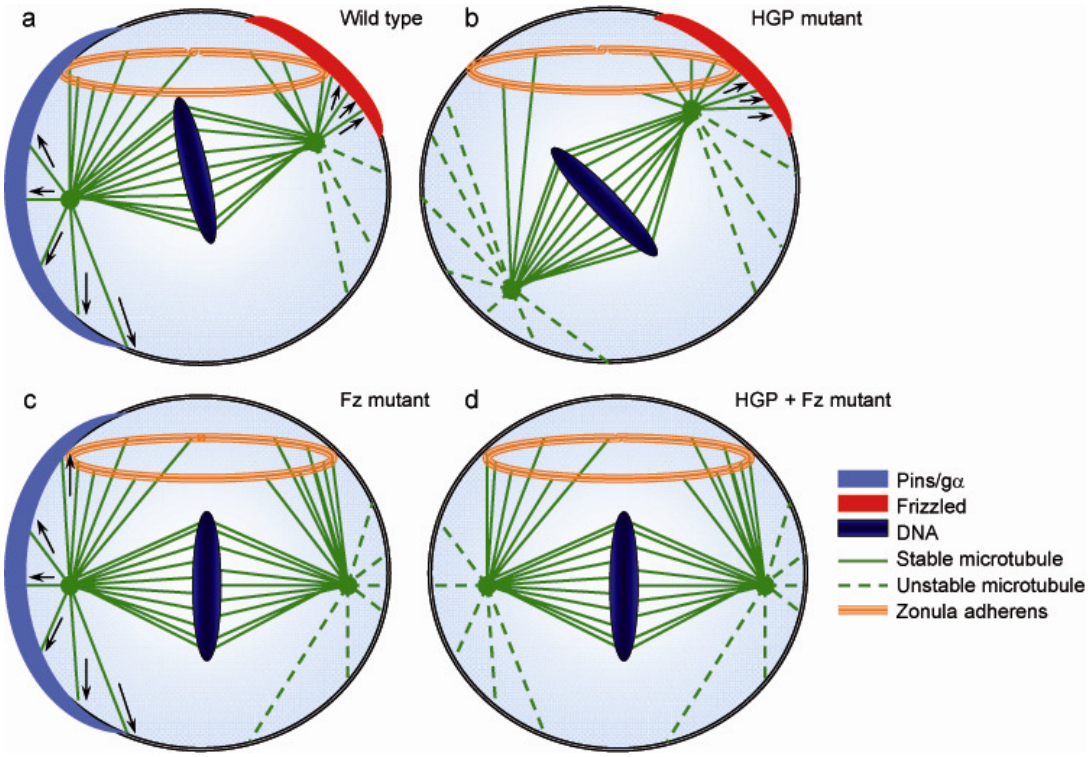


Figure 7: Spindle position in different mutant SOP cells. (**a**) In wild type SOP cells the spindle is oriented on the A-P axis and in the plane of the tissue. The spindle is tilted slightly apical at the posterior side, since Fz is involved in force generation at the apical posterior side. (**b**) In SOP cells mutant for HGP genes, *Ric-8* or *Pins*, the anterior spindle orientation domain is lost resulting in a more apical tilted spindle but still on the A-P axis due to normal PCP signaling. (**c**) *fz* loss in SOP cells results in the loss of the apical posterior spindle orientation domain leading to spindle orientation parallel to the plane of the tissue but random relative to the A-P axis. (**d**) Double mutants for HGP genes and *Fz* still orient the spindle parallel to the plane of the tissue by a mechanism that might involve microtubule attachment to the junctions located in the zonula adherens.

How can Fz and Vang regulate spindle orientation independently? In order to position the spindle on the A-P axis as well as in the plane of the tissue the cell needs to create two spindle positioning domains on the cortex able to regulate pulling forces on astral microtubules. The anterior Dlg/Pins/Gαi cortical domain and apical posterior Fz/Dsh domain are proposed to provide a platform for microtubules to bind to (David et al., 2005). In pupae mutant for *Gαi*, *Gγ*, *pins* or *ric-8* the spindle is still oriented on the A-

P axis but tilts to a more A-B oriented position since Fz is localized at the apical posterior cortex. This shows that HGP proteins and Pins compensate the force generated at the apical-posterior location of Fz to maintain the spindle in the plane of the epithelium. Mutants for *Fz* or *Fz* and HGP genes combined, show random spindle positioning relatively to the A-P axis but still parallel to the plane of the tissue (David et al., 2005; Hampoelz et al., 2005; Wang et al., 2005). These data show that Fz is needed to establish a microtubule binding domain at the apical posterior cortex. However, another redundant mechanism appears to align the spindle with the plane of the epithelium when PCP signaling is lost. The role for Fz in generating an apical posterior domain able to attach and modulate microtubules is unexplored up to today and needs to be pursued to get insight in how PCP signaling could regulate spindle forces (Figure 7).
The role of Vang in positioning the spindle is even more obscure. The apical anterior localization of Vang makes it attractive to suggest a role for Vang in the recruitment of an anterior spindle positioning domain. Indeed Vang has been implicated to be involved in the recruitment of Pins to the anterior cortex (Bellaiche et al., 2004). Direct interaction between Vang and Pins has not been demonstrated and it is likely that there are additional players involved in recruiting Pins to the anterior cortex. A major problem in most studies on PCP signaling is the fact that Vang, Fz and Fmi are mutually dependent on each other at cell-cell contact sites to establish a planar polarity axis, something which is difficult to account for experimentally (Chen et al., 2008; Strutt and Strutt, 2008; Wu et al., 2008). Loss of one of the core PCP proteins, in for example mutant pupae, results in loss of coordination between the cell division axis and planar axis, but the cells ability to establish asymmetry remains. Therefore it is not necessarily true that if Pins or HGP are mislocalized (but still asymmetric) in studies on loss of either Vang or Fz, this is due to a direct downstream effect of Vang or Fz signaling in the cell (Bellaiche et al., 2004; David et al., 2005; Hampoelz et al., 2005; Wang et al., 2005). Since in these mutants wild-type proteins for other PCP core proteins are still active, these proteins might be able to initiate the establishment of polarity but without locking polarity on a certain axis. Experiments in which SOP clones mutant for polarity proteins are surrounded by wild-type cells might therefore shed more light on the downstream effects of the PCP core proteins.

Fz and Fmi as potential GPCRs

Most studies on PCP in spindle positioning and polarity establishment focus on the function of Fz/Dsh and Vang. However, Fmi recently emerged as a third essential player in PCP establishment and signaling. No downstream effector is known for Fmi and only binding to Vang and Fz has been described. Interestingly this binding to Vang and Fz is essential for establishing PCP and PCP signaling (Chen et al., 2008; Lawrence et al., 2004; Strutt and Strutt, 2007; Strutt and Strutt, 2008; Usui et al., 1999; Wu et al., 2008). In SOP cells, *Fmi* shows a similar loss-of function phenotype as *Fz* and *Vang* and is proposed to function downstream of Fz since Fz and Dsh are required for correct Fmi localization (Lu et al., 1999). If Fmi is truly downstream of Fz in SOP cells is unclear however, since the effects of Fmi loss on Fz are unknown. Also, the interaction between Vang and Fmi in SOP cells is uncharacterized. It is more likely that Fmi, Vang and Fz are mutually dependent on each other like in wing and eye cells of *Drosophila* (Chen et al., 2008; Lawrence et al., 2004; Strutt and Strutt, 2007; Strutt and Strutt, 2008; Usui et al., 1999; Wu et al., 2008).

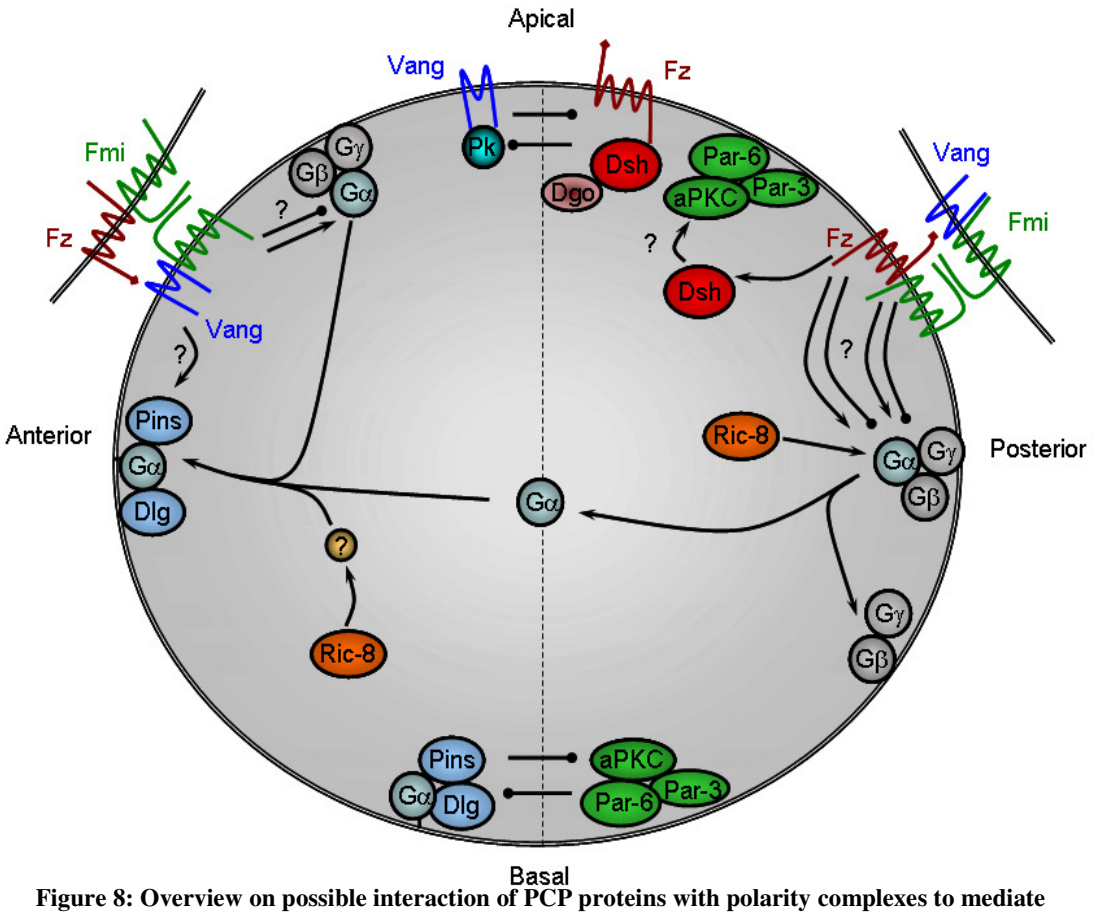


Figure 8: Overview on possible interaction of PCP proteins with polarity complexes to mediate spindle positioning in SOP cells. At anterior cell-cell contact site, apical localized Vang-Fmi interacts with Fz-Fmi of the neighboring cell. At the posterior end of the cell, apical localized Fz-Fmi interacts with Vang-Fmi of the neighboring cell. These interactions might inhibit or initiate inter-cellular signaling of Fmi, Fz or Vang, potentially resulting in initiation of Gα signaling, or recruitment of Par-3/Par-6/aPKC to the posterior and Pins/Dlg/Gα to the anterior, respectively. Gα signaling could also be initiated in a receptor independent way by Ric-8. At the cell equator, Pins/Dlg/Gα and Par-3/Par-6/aPKC as well as Vang/Pk and Fz/Dsh/Dgo antagonize each other, hereby restricting each others localization. Arrows show activating interactions, blunt ends show antagonistic interactions. Question marks indicate proposed possible interactions which remain to be verified scientifically

If Fmi is the effector in PCP signaling, what could the role of Fmi be? Both Fz and Fmi are 7-pass transmembrane proteins and therefore potential GPCRs. Especially since Gαi and Gαo (together: Gα) play an important role in transducing Wnt signaling and spindle positioning it is attractive to suggest that Fz functions as a GPCR mediating Gα release and thereby signaling. Indeed in SOPs with mutant Gαi or Gαo, defects in aligning the spindle with the A-P axis and establishing asymmetry are found and Gα signaling is proposed as the transducer of the PCP signal (Katanaev et al., 2005; Katanaev and Tomlinson, 2006). Unexpectedly however, up to now no biochemical evidence has been found to support direct interaction between Gα and Fz, and it is becoming more and more unlikely that Fz is truly a GPCR for Gα. If the PCP properties of Gα are coupled to a receptor, Fmi might be a good candidate GPCR. Fmi is a 7-pass transmembrane protein of the adhesion-GPCR family of proteins (Bjarnadottir et al., 2004). As a GPCR, Fmi could regulate the release of Gαi and Gαo (Usui et al., 1999). It has been shown that the intracellular C-terminus of Fmi is essential to communicate PCP in and between cells and has a role in directed dendrite elongation, which is in support of the idea that Fmi might have a signaling

function(Chen et al., 2008; Kimura et al., 2006). As with Fz up to now no biochemical evidence exists on whether Fmi interacts with Gα or not and the question remains if Fmi is a GPCR at all, or if Gα function in PCP is connected to a receptor. Since Fmi is a cadherin it could also be mediating cell adhesion at the junctions, or play a role in maintaining tissue integrity. Fmi could thereby facilitate Fz and Vang interaction at the junctions, resulting in the establishment of PCP. Up to now, the role of Fmi remains obscure and it is of great importance for the understanding of PCP establishment to elucidate the exact function of this protein.
Gα signaling might also be initialized in a receptor independent way by its GEF Ric-8. Ric-8 is able to bind Gα and exchange its Gα-GDP into Gα-GTP which could release Gα from the HGP complex. Next to being a GEF for Gα, Ric-8 is also essential for membrane localization of Gαi by an unknown mechanism which likely involves another substrate (David et al., 2005; Hampoelz et al., 2005; Wang et al., 2005). Gα appears to be a key player in autonomous PCP establishment, and how Gα is regulated remains a critical question in understanding PCP (Figure 8).

The default spindle orientation mechanism

SOPs lacking PCP signaling in combination with *Gα* or *pins* deletion still align their spindle parallel to the plane of the tissue. This suggests that next to an anterior Gα/Pins domain and the apical posterior Fz/Dsh domain a third redundant spindle aligning mechanism might exist (David et al., 2005). The tumor suppressor gene *apc* has been indicated to be involved in spindle alignment among many other functions like migration, Wnt signaling, chromosome segregation and apoptosis (McCartney and Nathke, 2008). Apc is able to bind and stabilize microtubule plus ends through interaction with EB1, as well as binding to actin and actin regulating proteins (Kawasaki et al., 2000; Moseley et al., 2007; Okada et al., ; Su et al., 1995; Watanabe et al., 1996). This indicates that APC can be brought in close proximity of the cell cortex by astral microtubule plus ends. If Apc can bind to the cortex or cortical actin while remaining its binding with microtubules, this mechanism could link the spindle to the cortex. Interestingly EB1 is able to bind members of the Dynein-Dynactin complex and thereby might contribute to cortical binding of astral microtubules and spindle positioning (Berrueta et al., 1999; McCartney et al., 2001; Strickland et al., 2005). In support of the idea that Apc plays a role in positioning the spindle, spindles in *Drosophila* spermatocytes mutant for *apc* lose their strict orientation towards the stem cell niche (Yamashita et al., 2003).
A similar role for Apc was shown in syncytial embryos of *Drosophila* where Apc together with α- and β-catenin localize to the sites cortical spindle attachment (McCartney et al., 2001). In these studies it is shown that Apc binds to α- and β-catenin which are associated with E-cadherin in spermatocytes and epithelial cells in *Drosophila*. Disruption of E-cadherin junctions in epithelial cells results in loss of cell polarity and Apc localization at the apical lateral membrane, suggesting Apc interacts with E-cadherin at the junctions (Lu et al., 2001; Yamashita et al., 2003). In SOP cells E-cadherin is essential to ensure correct asymmetric division as well, probably in thight cooperation with the PCP core proteins which localize at these junctions (Le Borgne et al., 2002; Wu and Mlodzik, 2009). Now we know that spindles remain oriented parallel to the plane of the tissue even in the absence of PCP core proteins or polarity complexes like Gα/Pins, there might be a role for E-cadherin with Apc in this

process. As the SOP cell divides an apical ring of E-cadherin keeps the cell connected to the surrounding tissue (Le Borgne et al., 2002). If Apc at these junctions is able to stabilize microtubules, an apical ring like domain arises for microtubules to bind and stabilize at, resulting in a planar oriented spindle (Figure 7d). The shape of the cell can also be of great importance for spindle position, since space limitation could force a spindle to adapt its orientation to this limited space. Apc is a potential candidate to be involved in spindle positiong. However, experiments confirming the ability of Apc to lock microtubules at the cell cortex have not been published and it remains to be established in what context Apc could function in this process**.**

Concluding remarks

After more than a century of research, our understanding on how cells establish polarity in order to control cell fate, oriented division and directional growth, gets better. But many questions on these mechanisms remain. It is clear that cells are able to establish polarity by the use of competitive protein complexes. Secondly, we now know that cells are capable to communicate polarity to each other, using the PCP core proteins Fz, Vang and Fmi. And thirdly, we know that polarity proteins determine cell fate, as well as the position of the mitotic spindle. Thereby giving rise to multiple types of tissue and allowing cells to divide directionally. Connecting cell polarity establishment with the downstream mechanisms of cell fate specification and spindle orientation is the next step in understanding development. Hence, we need to elucidate how communication between cells is mediated by Vang, Fz and Fmi, and especially what their down stream targets are that mediate polarity and asymmetric division. Also, elucidation of the physical interactions between known and putative polarity proteins, for example APC, is essential to understand polarity establishment. All together, many questions remain to be answered in the coming century before we can completely understand cell polarity and ultimately animal development.

Acknowledgements

Hereby, I would like to thank Prof. Dr. Sander van den Heuvel for putting time and effort in critically supervising me during the course of writing this thesis. Also I would like to thank Dr. Mike Boxem for reviewing the thesis.

Literature

Adames, N.R., and Cooper, J.A. (2000). Microtubule interactions with the cell cortex causing nuclear movements in Saccharomyces cerevisiae. J Cell Biol *149*, 863-874.

Adler, P.N. (1992). The genetic control of tissue polarity in *Drosophila*. Bioessays *14*, 735-741.

Adler, P.N., Charlton, J., and Liu, J. (1998). Mutations in the cadherin superfamily member gene dachsous cause a tissue polarity phenotype by altering frizzled signaling. Development *125*, 959-968.

Adler, P.N., Krasnow, R.E., and Liu, J. (1997). Tissue polarity points from cells that have higher Frizzled levels towards cells that have lower Frizzled levels. Curr Biol *7*, 940-949.

Axelrod, J.D. (2001). Unipolar membrane association of Dishevelled mediates Frizzled planar cell polarity signaling. Genes Dev *15*, 1182-1187.

Axelrod, J.D. (2009a). Progress and challenges in understanding planar cell polarity signaling. Semin Cell Dev Biol *20*, 964-971.

Axelrod, J.D. (2009b). Studies of epithelial PCP. Semin Cell Dev Biol *20*, 956.

Bachmann, A., Schneider, M., Theilenberg, E., Grawe, F., and Knust, E. (2001). *Drosophila* Stardust is a partner of Crumbs in the control of epithelial cell polarity. Nature *414*, 638-643.

Bastock, R., Strutt, H., and Strutt, D. (2003). Strabismus is asymmetrically localised and binds to Prickle and Dishevelled during *Drosophila* planar polarity patterning. Development *130*, 3007-3014.

Bei, Y., Hogan, J., Berkowitz, L.A., Soto, M., Rocheleau, C.E., Pang, K.M., Collins, J., and Mello, C.C. (2002). SRC-1 and Wnt signaling act together to specify endoderm and to control cleavage orientation in early *C. elegans* embryos. Dev Cell *3*, 113-125.

Bellaiche, 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.

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

Benton, R., and St Johnston, D. (2003). *Drosophila* PAR-1 and 14-3-3 inhibit Bazooka/PAR-3 to establish complementary cortical domains in polarized cells. Cell *115*, 691-704.

Bergmann, D.C., Lee, M., Robertson, B., Tsou, M.F., Rose, L.S., and Wood, W.B. (2003). Embryonic handedness choice in *C. elegans* involves the Galpha protein GPA-16. Development *130*, 5731-5740.

Berrueta, L., Tirnauer, J.S., Schuyler, S.C., Pellman, D., and Bierer, B.E. (1999). The APC-associated protein EB1 associates with components of the dynactin complex and cytoplasmic dynein intermediate chain. Curr Biol *9*, 425-428.

Betschinger, J., Eisenhaber, F., and Knoblich, J.A. (2005). Phosphorylation-induced autoinhibition regulates the cytoskeletal protein Lethal (2) giant larvae. Curr Biol *15*, 276-282.

Betschinger, J., Mechtler, K., and Knoblich, J.A. (2003). The Par complex directs asymmetric cell division by phosphorylating the cytoskeletal protein Lgl. Nature *422*, 326-330.

Bilder, D. (2004). Epithelial polarity and proliferation control: links from the *Drosophila* neoplastic tumor suppressors. Genes Dev *18*, 1909-1925.

Bilder, D., Li, M., and Perrimon, N. (2000). Cooperative regulation of cell polarity and growth by *Drosophila* tumor suppressors. Science *289*, 113-116.

Bilder, D., and Perrimon, N. (2000). Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. Nature *403*, 676-680.

Bilder, D., Schober, M., and Perrimon, N. (2003). Integrated activity of PDZ protein complexes regulates epithelial polarity. Nat Cell Biol *5*, 53-58.

Bjarnadottir, T.K., Fredriksson, R., Hoglund, P.J., Gloriam, D.E., Lagerstrom, M.C., and Schioth, H.B. (2004). The human and mouse repertoire of the adhesion family of G-protein-coupled receptors. Genomics *84*, 23-33.

Boyd, L., Guo, S., Levitan, D., Stinchcomb, D.T., and Kemphues, K.J. (1996). PAR-2 is asymmetrically distributed and promotes association of P granules and PAR-1 with the cortex in *C. elegans* embryos. Development *122*, 3075-3084.

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

Chen, C.M., Strapps, W., Tomlinson, A., and Struhl, G. (2004). Evidence that the cysteine-rich domain of *Drosophila* Frizzled family receptors is dispensable for transducing Wingless. Proc Natl Acad Sci U S A *101*, 15961-15966.

Chen, W.S., Antic, D., Matis, M., Logan, C.Y., Povelones, M., Anderson, G.A., Nusse, R., and Axelrod, J.D. (2008). Asymmetric homotypic interactions of the atypical cadherin flamingo mediate intercellular polarity signaling. Cell *133*, 1093-1105.

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.

Clark, H.F., Brentrup, D., Schneitz, K., Bieber, A., Goodman, C., and Noll, M. (1995). Dachsous encodes a member of the cadherin superfamily that controls imaginal disc morphogenesis in *Drosophila*. Genes Dev *9*, 1530-1542.

Clevers, H. (2006). Wnt/beta-catenin signaling in development and disease. Cell *127*, 469-480.

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

Couwenbergs, C., Labbe, 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.

Das, G., Jenny, A., Klein, T.J., Eaton, S., and Mlodzik, M. (2004). Diego interacts with Prickle and Strabismus/Van Gogh to localize planar cell polarity complexes. Development *131*, 4467-4476.

David, N.B., Martin, C.A., Segalen, M., Rosenfeld, F., Schweisguth, F., and Bellaiche, 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.

DeRenzo, C., Reese, K.J., and Seydoux, G. (2003). Exclusion of germ plasm proteins from somatic lineages by cullin-dependent degradation. Nature *424*, 685-689.

Doe, C.Q. (2008). Neural stem cells: balancing self-renewal with differentiation. Development *135*, 1575-1587.

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

Eshel, D., Urrestarazu, L.A., Vissers, S., Jauniaux, J.C., van Vliet-Reedijk, J.C., Planta, R.J., and Gibbons, I.R. (1993). Cytoplasmic dynein is required for normal nuclear segregation in yeast. Proc Natl Acad Sci U S A *90*, 11172-11176.

Farkasovsky, M., and Kuntzel, H. (2001). Cortical Num1p interacts with the dynein intermediate chain Pac11p and cytoplasmic microtubules in budding yeast. J Cell Biol *152*, 251-262.

Fuse, N., Hisata, K., Katzen, A.L., and Matsuzaki, F. (2003). Heterotrimeric G proteins regulate daughter cell size asymmetry in *Drosophila* neuroblast divisions. Curr Biol *13*, 947-954.

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

Garrard, S.M., Capaldo, C.T., Gao, L., Rosen, M.K., Macara, I.G., and Tomchick, D.R. (2003). Structure of Cdc42 in a complex with the GTPase-binding domain of the cell polarity protein, Par6. EMBO J *22*, 1125-1133.

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

Goldstein, B. (1992). Induction of gut in Caenorhabditis elegans embryos. Nature *357*, 255-257.

Goldstein, B., and Hird, S.N. (1996). Specification of the anteroposterior axis in Caenorhabditis elegans. Development *122*, 1467-1474.

Gomes, J.E., Corado, M., and Schweisguth, F. (2009). Van Gogh and Frizzled act redundantly in the *Drosophila* sensory organ precursor cell to orient its asymmetric division. PLoS One *4*, e4485.

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

Gotta, M., Abraham, M.C., and Ahringer, J. (2001). CDC-42 controls early cell polarity and spindle orientation in *C. elegans*. Curr Biol *11*, 482-488.

Gotta, M., and Ahringer, J. (2001). Axis determination in *C. elegans*: initiating and transducing polarity. Curr Opin Genet Dev *11*, 367-373.

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.

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

Gubb, D., and Garcia-Bellido, A. (1982). A genetic analysis of the determination of cuticular polarity during development in *Drosophila* melanogaster. J Embryol Exp Morphol *68*, 37-57.

Guo, S., and Kemphues, K.J. (1995). par-1, a gene required for establishing polarity in *C. elegans* embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. Cell *81*, 611-620.

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.

Huisman, S.M., Bales, O.A., Bertrand, M., Smeets, M.F., Reed, S.I., and Segal, M. (2004). Differential contribution of Bud6p and Kar9p to microtubule capture and spindle orientation in *S. cerevisiae*. J Cell Biol *167*, 231-244.

Humbert, P.O., Dow, L.E., and Russell, S.M. (2006). The Scribble and Par complexes in polarity and migration: friends or foes? Trends Cell Biol *16*, 622-630.

Hutterer, A., Betschinger, J., Petronczki, M., and Knoblich, J.A. (2004). Sequential roles of Cdc42, Par-6, aPKC, and Lgl in the establishment of epithelial polarity during *Drosophila* embryogenesis. Dev Cell *6*, 845-854.

Hwang, E., Kusch, J., Barral, Y., and Huffaker, T.C. (2003). Spindle orientation in Saccharomyces cerevisiae depends on the transport of microtubule ends along polarized actin cables. J Cell Biol *161*, 483-488.

Itoh, M., Sasaki, H., Furuse, M., Ozaki, H., Kita, T., and Tsukita, S. (2001). Junctional adhesion molecule (JAM) binds to PAR-3: a possible mechanism for the recruitment of PAR-3 to tight junctions. J Cell Biol *154*, 491-497.

Izumi, Y., Hirose, T., Tamai, Y., Hirai, S., Nagashima, Y., Fujimoto, T., Tabuse, Y., Kemphues, K.J., and Ohno, S. (1998). An atypical PKC directly associates and colocalizes at the epithelial tight junction with ASIP, a mammalian homologue of Caenorhabditis elegans polarity protein PAR-3. J Cell Biol *143*, 95-106.

Johnstone, O., and Lasko, P. (2001). Translational regulation and RNA localization in *Drosophila* oocytes and embryos. Annu Rev Genet *35*, 365-406.

Katanaev, V.L., Ponzielli, R., Semeriva, M., and Tomlinson, A. (2005). Trimeric G protein-dependent frizzled signaling in *Drosophila*. Cell *120*, 111-122.

Katanaev, V.L., and Tomlinson, A. (2006). Dual roles for the trimeric G protein Go in asymmetric cell division in *Drosophila*. Proc Natl Acad Sci U S A *103*, 6524-6529.

Kawasaki, Y., Senda, T., Ishidate, T., Koyama, R., Morishita, T., Iwayama, Y., Higuchi, O., and Akiyama, T. (2000). Asef, a link between the tumor suppressor APC and G-protein signaling. Science *289*, 1194-1197.

Kemphues, K.J., Kusch, M., and Wolf, N. (1988). Maternal-effect lethal mutations on linkage group II of Caenorhabditis elegans. Genetics *120*, 977-986.

Kimple, R.J., Kimple, M.E., Betts, L., Sondek, J., and Siderovski, D.P. (2002). Structural determinants for GoLoco-induced inhibition of nucleotide release by Galpha subunits. Nature *416*, 878-881.

Kimura, H., Usui, T., Tsubouchi, A., and Uemura, T. (2006). Potential dual molecular interaction of the *Drosophila* 7-pass transmembrane cadherin Flamingo in dendritic morphogenesis. J Cell Sci *119*, 1118-1129.

Knoblich, J.A. (2008). Mechanisms of asymmetric stem cell division. Cell *132*, 583-597.

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.

Kusch, J., Meyer, A., Snyder, M.P., and Barral, Y. (2002). Microtubule capture by the cleavage apparatus is required for proper spindle positioning in yeast. Genes Dev *16*, 1627-1639.

Labbe, J.C., and Goldstein, B. (2002). Embryonic development: a new SPN on cell fate specification. Curr Biol *12*, R396-398.

Labbe, J.C., McCarthy, E.K., and Goldstein, B. (2004). The forces that position a mitotic spindle asymmetrically are tethered until after the time of spindle assembly. J Cell Biol *167*, 245-256.

Lambert, J.D., and Nagy, L.M. (2002). Asymmetric inheritance of centrosomally localized mRNAs during embryonic cleavages. Nature *420*, 682-686.

Lawrence, P.A., Casal, J., and Struhl, G. (2004). Cell interactions and planar polarity in the abdominal epidermis of *Drosophila*. Development *131*, 4651-4664.

Le Borgne, R., Bardin, A., and Schweisguth, F. (2005). The roles of receptor and ligand endocytosis in regulating Notch signaling. Development *132*, 1751-1762.

Le Borgne, R., Bellaiche, Y., and Schweisguth, F. (2002). *Drosophila* E-cadherin regulates the orientation of asymmetric cell division in the sensory organ lineage. Curr Biol *12*, 95-104.

Lee, H., and Adler, P.N. (2002). The function of the frizzled pathway in the *Drosophila* wing is dependent on inturned and fuzzy. Genetics *160*, 1535-1547.

Li, Y.Y., Yeh, E., Hays, T., and Bloom, K. (1993). Disruption of mitotic spindle orientation in a yeast dynein mutant. Proc Natl Acad Sci U S A *90*, 10096-10100.

Lorson, M.A., Horvitz, H.R., and van den Heuvel, S. (2000). LIN-5 is a novel component of the spindle apparatus required for chromosome segregation and cleavage plane specification in Caenorhabditis elegans. J Cell Biol *148*, 73-86.

Lu, B., Roegiers, F., Jan, L.Y., and Jan, Y.N. (2001). Adherens junctions inhibit asymmetric division in the *Drosophila* epithelium. Nature *409*, 522-525.

Lu, B., Usui, T., Uemura, T., Jan, L., and Jan, Y.N. (1999). Flamingo controls the planar polarity of sensory bristles and asymmetric division of sensory organ precursors in *Drosophila*. Curr Biol *9*, 1247-1250.

Mahoney, P.A., Weber, U., Onofrechuk, P., Biessmann, H., Bryant, P.J., and Goodman, C.S. (1991). The fat tumor suppressor gene in *Drosophila* encodes a novel member of the cadherin gene superfamily. Cell *67*, 853-868.

McCartney, B.M., McEwen, D.G., Grevengoed, E., Maddox, P., Bejsovec, A., and Peifer, M. (2001). *Drosophila* APC2 and Armadillo participate in tethering mitotic spindles to cortical actin. Nat Cell Biol *3*, 933-938.

McCartney, B.M., and Nathke, I.S. (2008). Cell regulation by the Apc protein Apc as master regulator of epithelia. Curr Opin Cell Biol *20*, 186-193.

McGee, A.W., and Bredt, D.S. (1999). Identification of an intramolecular interaction between the SH3 and guanylate kinase domains of PSD-95. J Biol Chem *274*, 17431-17436.

Miller, K.G., and Rand, J.B. (2000). A role for RIC-8 (Synembryn) and GOA-1 (G(o)alpha) in regulating a subset of centrosome movements during early embryogenesis in Caenorhabditis elegans. Genetics *156*, 1649-1660.

Miller, R.K., D'Silva, S., Moore, J.K., and Goodson, H.V. (2006). The CLIP-170 orthologue Bik1p and positioning the mitotic spindle in yeast. Curr Top Dev Biol *76*, 49-87.

Morton, D.G., Roos, J.M., and Kemphues, K.J. (1992). par-4, a gene required for cytoplasmic localization and determination of specific cell types in Caenorhabditis elegans embryogenesis. Genetics *130*, 771-790.

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

Moseley, J.B., Bartolini, F., Okada, K., Wen, Y., Gundersen, G.G., and Goode, B.L. (2007). Regulated binding of adenomatous polyposis coli protein to actin. J Biol Chem *282*, 12661-12668.

Nagai-Tamai, Y., Mizuno, K., Hirose, T., Suzuki, A., and Ohno, S. (2002). Regulated protein-protein interaction between aPKC and PAR-3 plays an essential role in the polarization of epithelial cells. Genes Cells *7*, 1161-1171.

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

Nipper, R.W., Siller, K.H., Smith, N.R., Doe, C.Q., and Prehoda, K.E. (2007). Galphai generates multiple Pins activation states to link cortical polarity and spindle orientation in *Drosophila* neuroblasts. Proc Natl Acad Sci U S A *104*, 14306-14311.

Okada, K., Bartolini, F., Deaconescu, A.M., Moseley, J.B., Dogic, Z., Grigorieff, N., Gundersen, G.G., and Goode, B.L. Adenomatous polyposis coli protein nucleates actin assembly and synergizes with the formin mDia1. J Cell Biol.

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.

Petronczki, M., and Knoblich, J.A. (2001). DmPAR-6 directs epithelial polarity and asymmetric cell division of neuroblasts in *Drosophila*. Nat Cell Biol *3*, 43-49.

Plant, P.J., Fawcett, J.P., Lin, D.C., Holdorf, A.D., Binns, K., Kulkarni, S., and Pawson, T. (2003). A polarity complex of mPar-6 and atypical PKC binds,

phosphorylates and regulates mammalian Lgl. Nat Cell Biol *5*, 301-308.

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

Roegiers, F., Younger-Shepherd, S., Jan, L.Y., and Jan, Y.N. (2001). Two types of asymmetric divisions in the *Drosophila* sensory organ precursor cell lineage. Nat Cell Biol *3*, 58-67.

Rolls, M.M., Albertson, R., Shih, H.P., Lee, C.Y., and Doe, C.Q. (2003). *Drosophila* aPKC regulates cell polarity and cell proliferation in neuroblasts and epithelia. J Cell Biol *163*, 1089-1098.

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

Schaefer, M., and Knoblich, J.A. (2001). Protein localization during asymmetric cell division. Exp Cell Res *271*, 66-74.

Schlesinger, A., Shelton, C.A., Maloof, J.N., Meneghini, M., and Bowerman, B. (1999). Wnt pathway components orient a mitotic spindle in the early Caenorhabditis elegans embryo without requiring gene transcription in the responding cell. Genes Dev *13*, 2028-2038.

Schober, M., Schaefer, M., and Knoblich, J.A. (1999). Bazooka recruits Inscuteable to orient asymmetric cell divisions in *Drosophila* neuroblasts. Nature *402*, 548-551.

Schweisguth, F. (2004). Regulation of notch signaling activity. Curr Biol *14*, R129-138.

Segal, M., Bloom, K., and Reed, S.I. (2000). Bud6 directs sequential microtubule interactions with the bud tip and bud neck during spindle morphogenesis in Saccharomyces cerevisiae. Mol Biol Cell *11*, 3689-3702.

Segalen, M., and Bellaiche, Y. (2009). Cell division orientation and planar cell polarity pathways. Semin Cell Dev Biol *20*, 972-977.

Seifert, J.R., and Mlodzik, M. (2007). Frizzled/PCP signalling: a conserved mechanism regulating cell polarity and directed motility. Nat Rev Genet *8*, 126-138.

Sheeman, B., Carvalho, P., Sagot, I., Geiser, J., Kho, D., Hoyt, M.A., and Pellman, D. (2003). Determinants of *S. cerevisiae* dynein localization and activation: implications for the mechanism of spindle positioning. Curr Biol *13*, 364-372.

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

Siegrist, S.E., and Doe, C.Q. (2006). Extrinsic cues orient the cell division axis in *Drosophila* embryonic neuroblasts. Development *133*, 529-536.

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.

Smith, C.A., Lau, K.M., Rahmani, Z., Dho, S.E., Brothers, G., She, Y.M., Berry, D.M., Bonneil, E., Thibault, P., Schweisguth, F.*, et al.* (2007). aPKC-mediated phosphorylation regulates asymmetric membrane localization of the cell fate determinant Numb. EMBO J *26*, 468-480.

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.

Strickland, L.I., Wen, Y., Gundersen, G.G., and Burgess, D.R. (2005). Interaction between EB1 and p150glued is required for anaphase astral microtubule elongation and stimulation of cytokinesis. Curr Biol *15*, 2249-2255.

Strutt, D. (2003). Frizzled signalling and cell polarisation in *Drosophila* and vertebrates. Development *130*, 4501-4513.

Strutt, D., and Strutt, H. (2007). Differential activities of the core planar polarity proteins during *Drosophila* wing patterning. Dev Biol *302*, 181-194.

Strutt, D.I. (2001). Asymmetric localization of frizzled and the establishment of cell polarity in the *Drosophila* wing. Mol Cell *7*, 367-375.

Strutt, H., and Strutt, D. (2002). Nonautonomous planar polarity patterning in *Drosophila*: dishevelled-independent functions of frizzled. Dev Cell *3*, 851-863.

Strutt, H., and Strutt, D. (2008). Differential stability of flamingo protein complexes underlies the establishment of planar polarity. Curr Biol *18*, 1555-1564.

Su, L.K., Burrell, M., Hill, D.E., Gyuris, J., Brent, R., Wiltshire, R., Trent, J., Vogelstein, B., and

Kinzler, K.W. (1995). APC binds to the novel protein EB1. Cancer Res *55*, 2972-2977.

Suzuki, A., Hirata, M., Kamimura, K., Maniwa, R., Yamanaka, T., Mizuno, K., Kishikawa, M., Hirose, H., Amano, Y., Izumi, N.*, et al.* (2004). aPKC acts upstream of PAR-1b in both the establishment and maintenance of mammalian epithelial polarity. Curr Biol *14*, 1425-1435.

Suzuki, A., and Ohno, S. (2006). The PAR-aPKC system: lessons in polarity. J Cell Sci *119*, 979-987.

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

Tall, G.G., and Gilman, A.G. (2005). Resistance to inhibitors of cholinesterase 8A catalyzes release of Galphai-GTP and nuclear mitotic apparatus protein (NuMA) from NuMA/LGN/Galphai-GDP complexes. Proc Natl Acad Sci U S A *102*, 16584-16589.

Tall, G.G., Krumins, A.M., and Gilman, A.G. (2003). Mammalian Ric-8A (synembryn) is a heterotrimeric Galpha protein guanine nucleotide exchange factor. J Biol Chem *278*, 8356-8362.

Tanentzapf, G., and Tepass, U. (2003). Interactions between the crumbs, lethal giant larvae and bazooka pathways in epithelial polarization. Nat Cell Biol *5*, 46-52.

Taylor, J., Abramova, N., Charlton, J., and Adler, P.N. (1998). Van Gogh: a new *Drosophila* tissue polarity gene. Genetics *150*, 199-210.

Tepass, U., and Knust, E. (1993). Crumbs and stardust act in a genetic pathway that controls the organization of epithelia in *Drosophila* melanogaster. Dev Biol *159*, 311-326.

Tepass, U., Theres, C., and Knust, E. (1990). crumbs encodes an EGF-like protein expressed on apical membranes of *Drosophila* epithelial cells and required for organization of epithelia. Cell *61*, 787-799.

Thorpe, C.J., Schlesinger, A., Carter, J.C., and Bowerman, B. (1997). Wnt signaling polarizes an early *C. elegans* blastomere to distinguish endoderm from mesoderm. Cell *90*, 695-705.

Tree, D.R., Shulman, J.M., Rousset, R., Scott, M.P., Gubb, D., and Axelrod, J.D. (2002). Prickle mediates feedback amplification to generate asymmetric planar cell polarity signaling. Cell *109*, 371-381.

Tsou, M.F., Hayashi, A., DeBella, L.R., McGrath, G., and Rose, L.S. (2002). LET-99 determines spindle position and is asymmetrically enriched in response to PAR polarity cues in *C. elegans* embryos. Development *129*, 4469-4481.

Tsou, M.F., Hayashi, A., and Rose, L.S. (2003). LET-99 opposes Galpha/GPR signaling to generate asymmetry for spindle positioning in response to PAR and MES-1/SRC-1 signaling. Development *130*, 5717-5730.

Usui, T., Shima, Y., Shimada, Y., Hirano, S., Burgess, R.W., Schwarz, T.L., Takeichi, M., and Uemura, T. (1999). Flamingo, a seven-pass transmembrane cadherin, regulates planar cell polarity under the control of Frizzled. Cell *98*, 585-595.

Vinson, C.R., and Adler, P.N. (1987). Directional non-cell autonomy and the transmission of polarity information by the frizzled gene of *Drosophila*. Nature *329*, 549-551.

Vladar, E.K., Antic, D., and Axelrod, J.D. (2009). Planar Cell Polarity Signaling: The Developing Cell's Compass. Cold Spring Harb Perspect Biol *1*, a002964.

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.

Watanabe, M., Kakiuchi, H., Kato, H., Shiraishi, T., Yatani, R., Sugimura, T., and Nagao, M. (1996). APC gene mutations in human prostate cancer. Jpn J Clin Oncol *26*, 77-81.

Watts, J.L., Morton, D.G., Bestman, J., and Kemphues, K.J. (2000). The *C. elegans* par-4 gene encodes a putative serine-threonine kinase required for establishing embryonic asymmetry. Development *127*, 1467-1475.

Wirtz-Peitz, F., and Knoblich, J.A. (2006). Lethal giant larvae take on a life of their own. Trends Cell Biol *16*, 234-241.

Wodarz, A., Ramrath, A., Grimm, A., and Knust, E. (2000). *Drosophila* atypical protein kinase C associates with Bazooka and controls polarity of epithelia and neuroblasts. J Cell Biol *150*, 1361-1374.

Wodarz, A., Ramrath, A., Kuchinke, U., and Knust, E. (1999). Bazooka provides an apical cue for Inscuteable localization in *Drosophila* neuroblasts. Nature *402*, 544-547.

Wu, J., Jenny, A., Mirkovic, I., and Mlodzik, M. (2008). Frizzled-Dishevelled signaling specificity outcome can be modulated by Diego in *Drosophila*. Mech Dev *125*, 30-42.

Wu, J., and Mlodzik, M. (2008). The frizzled extracellular domain is a ligand for Van Gogh/Stbm during nonautonomous planar cell polarity signaling. Dev Cell *15*, 462-469.

Wu, J., and Mlodzik, M. (2009). A quest for the mechanism regulating global planar cell polarity of tissues. Trends Cell Biol *19*, 295-305.

Yamanaka, T., Horikoshi, Y., Sugiyama, Y., Ishiyama, C., Suzuki, A., Hirose, T., Iwamatsu, A., Shinohara, A., and Ohno, S. (2003). Mammalian Lgl forms a protein complex with PAR-6 and aPKC independently of PAR-3 to regulate epithelial cell polarity. Curr Biol *13*, 734-743.

Yamashita, Y.M., Jones, D.L., and Fuller, M.T. (2003). Orientation of asymmetric stem cell division by the APC tumor suppressor and centrosome. Science *301*, 1547-1550.

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.

Yang, C.H., Axelrod, J.D., and Simon, M.A. (2002). Regulation of Frizzled by fat-like cadherins during planar polarity signaling in the *Drosophila* compound eye. Cell *108*, 675-688.

Yin, C., Kiskowski, M., Pouille, P.A., Farge, E., and Solnica-Krezel, L. (2008). Cooperation of polarized cell intercalations drives convergence and extension of presomitic mesoderm during zebrafish gastrulation. J Cell Biol *180*, 221-232.

Zallen, J.A. (2007). Planar polarity and tissue morphogenesis. Cell *129*, 1051-1063.

Zeidler, M.P., Perrimon, N., and Strutt, D.I. (2000). Multiple roles for four-jointed in planar polarity and limb patterning. Dev Biol *228*, 181-196.

Zeitler, J., Hsu, C.P., Dionne, H., and Bilder, D. (2004). Domains controlling cell polarity and proliferation in the *Drosophila* tumor suppressor Scribble. J Cell Biol *167*, 1137-1146.

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.

Zhang, X., Zhu, J., Yang, G.Y., Wang, Q.J., Qian, L., Chen, Y.M., Chen, F., Tao, Y., Hu, H.S., Wang, T.*, et al.* (2007). Dishevelled promotes axon differentiation by regulating atypical protein kinase C. Nat Cell Biol *9*, 743-754.

Zheng, L., Zhang, J., and Carthew, R.W. (1995). frizzled regulates mirror-symmetric pattern formation in the *Drosophila* eye. Development *121*, 3045-3055.

Zwaal, R.R., Ahringer, J., van Luenen, H.G., Rushforth, A., Anderson, P., and Plasterk, R.H. (1996). G proteins are required for spatial orientation of early cell cleavages in *C. elegans* embryos. Cell *86*, 619-629.