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Molecular control of cell polarity and asymmetric cell division in *Drosophila* neuroblasts

Andreas Wodarz

In the embryonic central nervous system of the fruit fly *Drosophila*, most neurons and glial cells are generated by asymmetric division of neural stem cells called neuroblasts. Several genes have been identified that are required for the establishment of neuroblast polarity, for the asymmetric segregation of cell fate determinants and for the proper orientation and geometry of the mitotic spindle. However, little was known about the interactions between these genes and their respective gene products. It has emerged that most of the relevant proteins are assembled into three major protein complexes whose molecular interactions are conserved in evolution.

Addresses
Abteilung Stammzellbiologie, Georg-August-Universität Göttingen, Justus-von-Liebig-Weg 11, 37077 Göttingen, Germany

Corresponding author: Wodarz, Andreas (awodarz@gwdg.de)

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Introduction

The *Drosophila* neuroblast (NB) has served for more than a decade now as an excellent model system to study the molecular control of cell polarity and asymmetric cell division in a genetically accessible organism. NBs delaminate from the epithelium of the ventral neuroectoderm into the interior of the embryo where they enter mitosis and start to divide repeatedly in an asymmetric fashion. In each division, another NB and a smaller ganglion mother cell (GMC) are generated. While the NB continues to divide, the GMC divides only once more and generates a pair of neurons or glial cells.

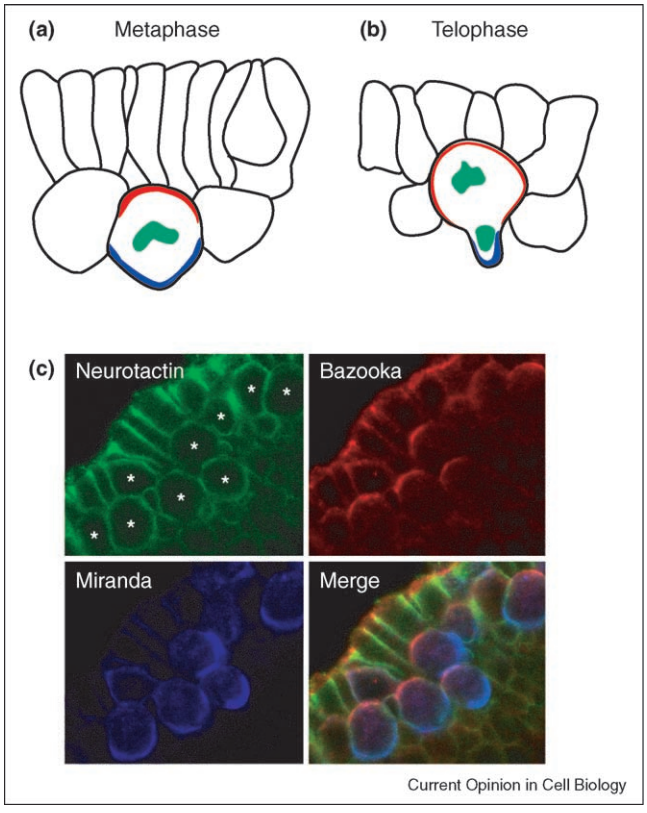
During delamination and division, NBs retain apical–basal polarity, which becomes apparent from the polarized localization of several proteins and mRNAs to the apical or basal cortex of the NB [1,2] (Figure 1). The establishment and maintenance of apical–basal cell polarity is controlled by proteins that are themselves localized in a polar fashion in the NB cortex. Three of these proteins, named Bazooka/PAR-3, PAR-6 and atypical protein kinase C (aPKC), are localized apically (Figure 1) and are associated in the so-called PAR/aPKC complex, which is highly conserved in evolution [3,4]. In the past three years it has become much clearer how the PAR/aPKC complex is integrated in a network of protein–protein interactions with other protein complexes involved in the regulation of cell polarity. One of the interacting complexes contains the proteins Discs Large (Dlg), Lethal giant larvae (Lgl) and Scribble (Scrib), and a second complex contains the proteins Partner of Inscuteable (Pins) and Gαi, a Gα subunit of heterotrimeric G-proteins. These interactions have not only been uncovered in *Drosophila* NBs but also in *Drosophila* and mammalian epithelia, the early *Xenopus* embryo and the *C. elegans* zygote, demonstrating that these protein complexes and their molecular interactions form an evolutionarily conserved network that controls cell polarity in many different cell types and organisms.

Here I will review recent results regarding the interactions between these complexes in *Drosophila* and how they relate to findings in other model organisms, especially *C. elegans* and vertebrates.

Interactions between the PAR/aPKC complex and Lgl control the subcellular localization of cell fate determinants

To generate daughter cells of different developmental potential, cell fate determinants including the transcription factor Prospero, the Notch regulator Numb and their binding partners Miranda and Partner of Numb (Pon) are localized to the basal NB cortex in metaphase and segregate exclusively into the GMC upon cytokinesis (Figure 1). The basal localization of the determinants depends on the function of the PAR/aPKC complex in the apical cortex and on the function of the Dlg/Lgl/Scrib complex, which is more ubiquitously localized around the cell cortex [1,2]. Several recent papers now show that these two protein complexes can be placed in a functional hierarchy by genetic and molecular approaches. The link between the two complexes was uncovered by the discovery that the Lgl protein coimmunoprecipitated with PAR-6 and aPKC in embryonic cell lysates [5••]. The same paper also showed that Lgl is phosphorylated by aPKC and that this phosphorylation inactivates Lgl. Consequently, Lgl promotes cortical localization of cell fate determinants only in the basal NB cortex, where aPKC is absent. Strong support for this model comes from a

Figure 1



Regulators of cell polarity and cell fate determinants are asymmetrically localized in *Drosophila* neuroblasts (NBs). **(a)** shows drawings of *Drosophila* NBs in metaphase and **(b)** shows drawings of *Drosophila* NBs in telophase, both based on confocal images. The subcellular localization of components of the PAR/aPKC complex and of the Pins/Gαi complex is indicated in red. The subcellular localization of the cell fate determinants Prospero and Numb and their adaptor proteins Miranda and Partner of Numb is drawn in blue. DNA is drawn in green. The outlines of adjacent neuroectodermal cells and NBs are shown in black. Note that in telophase NBs (b) the proteins that form an apical crescent in metaphase (a) expand to the whole NB cortex (red), whereas proteins that form a basal crescent in metaphase are pushed into the budding GMC (blue). Apical is to the top in (a) and (b). **(c)** Confocal images of dividing NBs labeled with antibodies against Neurotactin (green), Bazooka (red) and Miranda (blue). Neurotactin is a transmembrane protein that labels the basolateral membrane of the neuroectodermal epithelium and the whole membrane of the NBs (asterisks). Bazooka labels the apicolateral cortex of neuroectodermal epithelial cells and forms an apical crescent in NBs. Miranda is an adaptor protein for the cell fate determinant Prospero and forms a basal crescent in mitotic NBs. Apical is to the upper left corner.

second paper from the same group showing that phosphorylation of Lgl by aPKC leads to a conformational change in the protein that prevents its association with the cytoskeleton [6]. Genetic interaction studies demonstrated that the PAR/aPKC complex antagonizes the function of the Dlg/Lgl/Scrib complex both in NBs [7] and in embryonic epithelia [8•,9•,10], consistent with the model based on molecular data. The association between Lgl, aPKC and PAR-6 is conserved in vertebrates and is required for the separation of the apical and the basolateral plasma membrane domain in early blastomeres and epithelial tissues [11•,12•,13•]. Interestingly, either Lgl or Baz/PAR-3, but not both together, can form a complex with PAR-6 and aPKC, indicating that the PAR/aPKC complex is actually quite dynamic and changes its composition during cell polarization [11•,12•].

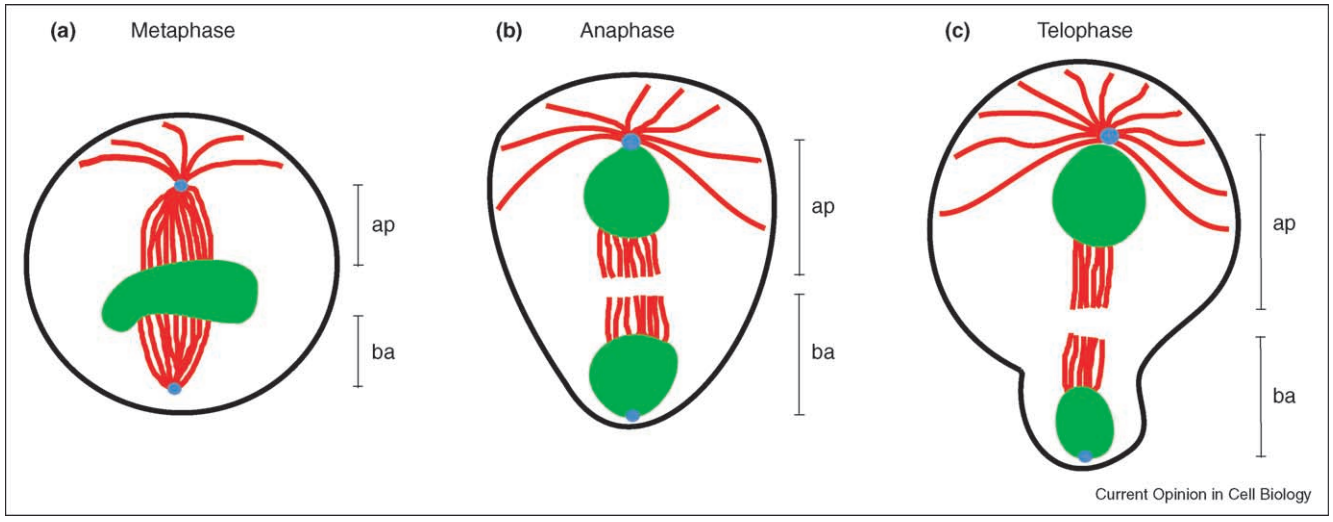
The findings presented so far all show that Lgl is inactivated by aPKC in the apical cytocortex and that Lgl activity is required for basolateral localization of cell fate determinants. But what is the precise function of Lgl at the basolateral cortex? It has been shown that the homologs of Lgl in bakers yeast bind to the t-SNARE protein Sec9 and promote the fusion of exocytic vesicles with the plasma membrane [14]. Mlgl, a mouse homolog of Lgl, binds to Syntaxin 4, a mammalian t-SNARE, at the basolateral membrane [15]. Thus, Lgl may be involved in basolateral targeting of exocytic vesicles containing proteins and lipids that are required for the localization of cell fate determinants.

In 1994 Lgl was found to be in a complex with non-muscle myosin II [16], suggesting that Lgl has a function in actin-dependent transport processes. An intact actin cytoskeleton is indeed required for the basal localization of cell fate determinants [1,2]. Genetic interaction studies showed that the phenotype of mutations in *lgl* can be partially suppressed by mutations in *zipper*, the *Drosophila* non-muscle myosin II heavy chain gene [17–19]. Consistent with this genetic interaction, mutations in *lgl* lead to delocalization of myosin II, which in wild type NBs accumulates in the apical cortex in prophase and metaphase and moves towards the site of cytokinesis in anaphase and telophase [20••]. Myosin II is required for basal localization of cell fate determinants, but surprisingly is not colocalized with the determinants at any time during mitosis. Instead, myosin II seems to push the determinants basally in a dynamic fashion. The authors of this study propose an interesting model in which myosin II prevents binding of the adaptor protein Miranda to actin, thus leading to exclusion of Miranda and Prospero from cortical regions where myosin II is present [20••]. The asymmetric localization of Miranda in mitotic NBs also depends on the function of myosin VI [21]. Myosin VI binds directly to Miranda and shows partial colocalization with Miranda in cytoplasmic particles. These findings have been taken as evidence that Miranda may be a cargo for myosin VI and is transported basally along actin filaments [21].

Heterotrimeric G-proteins and the control of spindle geometry in neuroblasts

Although the PAR/aPKC complex is essential to control the basal localization of cell fate determinants in NBs, it appears to act redundantly with heterotrimeric G-proteins in the control of spindle orientation and the generation of

Figure 2



Spindle asymmetry in *Drosophila* NBs. **(a)** In a metaphase NB, the apical half of the spindle (ap) has roughly the same length as the basal half (ba). However, at this stage astral microtubules have already formed at the apical centrosome, whereas very few if any astral microtubules are visible at the basal centrosome. Also, the distance from the apical centrosome to the cortex is longer than the distance from the basal centrosome to the cortex. In anaphase **(b)** and telophase **(c)** the difference in length between the apical and the basal half of the spindle becomes more pronounced and astral microtubules continue to grow at the apical, but not at the basal centrosome. DNA is shown in green, microtubules in red and centrosomes in blue. The drawings are based on confocal images. Apical is to the top.

two daughter cells of unequal size. The rotation of the mitotic spindle, which results in its alignment with the apical–basal axis of the NB, is essential for the proper segregation of the cell fate determinants to the basally located GMC. The pronounced asymmetry in cell size between the NB and the GMC (Figures 1,2) is probably important for keeping the volume of the NB large enough to allow repeated divisions without cell growth.

The first indication that heterotrimeric G-proteins are involved in the control of NB division was the isolation of a protein complex containing the proteins Inscuteable (Insc), Partner of Inscuteable (Pins) and the Gαi subunit of heterotrimeric G-proteins [22]. All three proteins are colocalized in the apical cortex of NBs and are connected to the PAR/aPKC complex by binding of Insc to Baz [23,24]. In single mutants for all three genes the mitotic spindle fails to rotate. However, *insc*, *pins* and *Gαi* mutants show different phenotypes with respect to the localization of cell fate determinants. While cell fate determinants and their adaptor proteins are still asymmetrically localized in the majority of *pins* and *Gαi* mutant NBs, they are mislocalized in *insc* mutants [22,25,26•,27,28••]. Neither single mutants of *pins*, *Gαi* or *insc* nor single mutants of any component of the PAR/aPKC complex show a loss of cell size asymmetry between the NB and the GMC with high penetrance. By contrast, any double mutant combination between either *pins* or *Gαi* and a component of the PAR/aPKC complex or *insc* leads to the formation of daughter cells with almost equal size in all NB divisions [26•,28••,29•,30•]. The easiest explanation for these observations is as follows. Cell size asymmetry depends on the presence of an apical cue that can either be provided by the PAR/aPKC complex including Insc or by the Pins/Gαi complex in a redundant fashion. Only when both cues are absent, for example in a double mutant for *baz* and *pins*, are two daughter cells of equal size formed. Consistent with this interpretation is the finding that the PAR/aPKC complex and the Pins/Gαi complex are independent of each other with respect to their subcellular localization in the apical NB cortex [28••,29•].

How is cell size asymmetry generated? In wild-type anaphase NBs, the apical half of the mitotic spindle becomes longer than the basal half, resulting in positioning of the cleavage plane closer to the basal centrosome than to the apical centrosome [31] (Figure 2). In addition, the apical centrosome enlarges, moves away from the plasma membrane and nucleates numerous astral microtubules that touch the cortex, whereas the basal centrosome remains small, lies much closer to the plasma membrane and is almost devoid of astral microtubules (Figure 2). Several recent papers show that the different behaviors of the centrosomes are controlled redundantly by the activity of the PAR/aPKC complex and by G-protein signaling. In mutants for the genes encoding Gβ13F and Gγ1, the G-protein subunits that form a complex with Gαi, both centrosomes develop aster microtubules resembling those that are present only at the apical centrosome in wild-type NBs [26•,29•,30•]. The same phenotype has been described for double mutants

of components of the PAR/aPKC complex and the Pins/Gαi complex (e.g. *baz;pins* double mutants) [26•]. Conversely, overexpression of Gβ13F and Gγ1 together or of a membrane-tethered form of Gβ13F alone suppresses the formation of aster microtubules at both centrosomes, indicating that active Gβ13F antagonizes the formation of aster microtubules [30•].

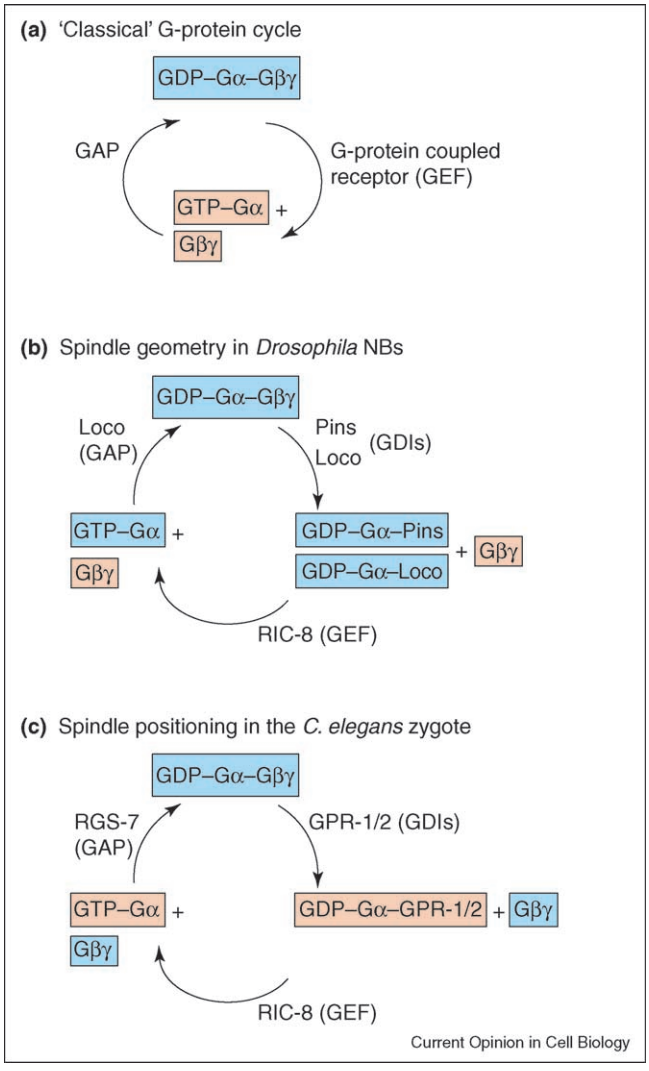
It seems that both the activity of the PAR/aPKC complex and signaling by heterotrimeric G-proteins affect the properties of the centrosomes in NBs. Because both complexes are localized asymmetrically in wild-type NBs, only one of the two centrosomes is within the reach of their signaling activity and thus adopts different properties from the other centrosome. This model raises the question of whether there is a common target of both signaling complexes that is responsible for controlling the position, size and microtubule-nucleating activity of the centrosome. Such a target molecule could either be localized to the centrosome itself or to the astral microtubules. The latter possibility appears more likely, because a protein present on the plus ends of astral microtubules could directly interact with the PAR/aPKC complex and with the Pins/Gαi complex in the apical cortex. Such an interaction might promote the growth of apical aster microtubules, which would lead to the generation of a force that pushes the apical centrosome away from the cortex, thus leading to basal displacement of the spindle.

Activation of G-protein signaling by a receptor-independent mechanism

In 'classical' textbook models of signaling by heterotrimeric G-proteins, the Gα subunit alternates between an inactive GDP-bound state, in which it is associated in a complex with the Gβγ subunits, and the active GTP-bound state, which allows it to interact with downstream signaling components (Figure 3a). The free Gβγ subunits can also transmit signals by distinct signaling pathways. The activation of G-protein signaling in this 'classical' model occurs by ligand binding of G-protein-coupled receptors, which catalyzes the exchange of GDP for GTP on the Gα subunit.

In *Drosophila* NBs, this 'classical' mode of G-protein signaling has apparently been modified and works independently of G-protein-coupled receptors. Instead, the dissociation of the heterotrimeric G-protein complex is triggered by binding of Pins to GDP–Gαi [32] (Figure 3b). Interestingly, overexpression in NBs of a constitutively GTP-bound form of Gαi causes only subtle dominant phenotypes, whereas overexpression of wild-type Gαi, which can be bound either to GDP or to GTP, leads to the formation of equal-sized daughter cells [26•,32]. This phenomenon could be caused by the titration of free Gβγ subunits by an excess of GDP–Gαi [26•,32]. Consistent with this interpretation, over-

Figure 3



Cycling of heterotrimeric G-proteins can be regulated in receptor-dependent and receptor-independent ways. **(a)** In the 'classical' case, heterotrimeric G-proteins are activated by seven-pass transmembrane receptors that act as G-protein coupled receptors (GEFs) and catalyze the exchange of GDP for GTP on Gα and the release of the free Gβγ subunits. Both GTP–Gα and Gβγ are active and trigger independent signaling pathways. **(b)** The G-protein cycle that controls the geometry of the mitotic spindle in *Drosophila* NBs is activated by binding of the GDIs Pins and Loco to GDP–Gαi. This leads to the release of the Gβγ subunits from the complex and to the activation of unknown effector molecules that affect the properties of the centrosome, which lies within the range of active Gβγ. The analysis of loss-of-function mutants and overexpression experiments indicate that the free Gβγ complex rather than Gαi is crucial for the formation of an asymmetric spindle. **(c)** The G-protein cycle that determines the position of the mitotic spindle in the *C. elegans* zygote is regulated in a very similar way to the G-protein cycle in *Drosophila* NBs. However, for spindle positioning in the worm, Gα seems to be more important than the free Gβγ complex. It is unclear at present which form of Gα is the signal for generating unequal pulling forces on the spindle poles. Inactive forms of G-protein subunits are boxed in blue and forms that are presumed to be active are boxed in orange (see text for details).

expression of another Gα subunit, Gαo47A, causes a phenotype very similar to overexpression of Gαi, although complete loss-of-function of Gαo47A in NBs does not cause any defect in asymmetric cell division [26•]. Mutation of *Gβ13F* and *Gγ1* leads to essentially the same phenotype as overexpression of Gαi, again demonstrating that Gβγ function is essential for asymmetric NB division [29•,30•]. The mutant phenotypes of *Gβ13F* and *Gγ1* are stronger than the phenotype of Gαi mutants, most likely because the localization of all apical proteins considered so far is affected in mutants for both genes. Gαi levels are strongly reduced in *Gβ13F* and *Gγ1* mutants, Pins is mislocalized to the cytoplasm and the amount of PAR/aPKC complex present in the apical NB cortex is also drastically reduced [26•,29•,30•]. Nonetheless, there is some residual activity of the PAR/aPKC complex in *Gβ13F* and *Gγ1* mutants, as cell fate determinants are still asymmetrically localized despite the loss of cell size asymmetry [26•,29•,30•,32]. Only in double mutants for *Gβ13F* or *Gγ1* and a component of the PAR/aPKC complex is the asymmetric localization of cell fate determinants completely abolished [29•,30•].

The G-protein cycle in *Drosophila* neuroblasts

Heterotrimeric G-proteins depend on a variety of regulators to cycle between the GTP-bound and the GDP-bound state. Among these regulators are guanine nucleotide exchange factors (GEFs) that catalyze the exchange of GDP for GTP, GTPase activating proteins (GAPs) that accelerate the hydrolysis of GTP bound to the Gα subunit, and guanine nucleotide dissociation inhibitors (GDIs) that keep the Gα subunit in the GDP-bound state (Figure 3b). One GDI that has already been discussed above is Pins. If the model stating that binding of Pins to GDP–Gαi is required for the release of the Gβγ subunits from Gαi were correct, then the *pins* loss-of-function phenotype should be very similar to the phenotype of mutants in Gβ or Gγ. However, this is not the case, raising the question of whether additional GDI proteins exist that may act redundantly with Pins. Indeed, a recent paper shows that the protein encoded by the *locomotion defects* (*loco*) gene is a GDI that binds Gαi and functions together with Pins in regulating the levels of free Gβγ [33••] (Figure 3b). *loco*, *pins* double mutants show essentially the same phenotype as mutants in Gβ or Gγ, consistent with their function as redundantly acting GDIs for Gαi [33••].

How is the G-protein cycle completed, enabling another round of signaling to start, once the complex between the GDIs and GDP–Gαi has been formed? In the search for a GEF that acts on Gαi, the *Drosophila* homolog of the RIC-8 gene from *C. elegans* was cloned and mutants of this gene were isolated (H Wang, KH Ng, H Qian, DP Siderovski, W Chia and F Yu, personal communication). As expected for a GEF, DmRIC-8 binds to GDP–Gαi *in vitro* and forms a complex with Gαi and Pins *in vivo*. In *DmRIC-8* mutants, Gαi, Pins and Gβ13F are mislocalized and spindle orientation is randomized. Thus, both the biochemical properties and the mutant phenotype of *DmRIC*-8 indicate that this protein is a GEF for Gαi that functions in the G-protein cycle in NBs (Figure 3b). Finally, there should be a GAP that facilitates GTP hydrolysis on GTP–Gαi to regenerate the heterotrimeric GDP–Gαi–Gβγ complex. This GAP activity may be provided by the Loco protein, which contains a regulator of G-protein signaling (RGS) domain and shows GAP activity towards GTP–Gαi *in vitro* [33••] (Figure 3b).

Control of spindle positioning by G-proteins in *C. elegans* and vertebrates

Heterotrimeric G-proteins are also essential for the asymmetric positioning of the mitotic spindle in the one cell-embryo of the nematode *C. elegans*. Two functionally redundant Gα subunits, GOA-1 and GPA-16, are required for the generation of the unequal pulling forces that lead to posterior displacement of the spindle, which results in the formation of a larger anterior and a smaller posterior daughter cell [34]. In contrast to the situation in *Drosophila*, the corresponding Gβγ subunits are dispensable for spindle positioning in the zygote but function later in development in the regulation of spindle rotation [34]. As in *Drosophila*, activation of the Gα subunits is carried out in a receptor-independent way by the GDIs GPR-1/GPR-2, two nearly identical proteins that represent the *C. elegans* orthologs of *Drosophila* Pins and mammalian AGS-3 [35•–37•] (Figure 3c). GPR-1/GPR-2 are enriched at the posterior cortex of the one-cell embryo and are thus in the right place to activate the Gα subunits in an asymmetric fashion, resulting in unequal pulling forces on the mitotic spindle [35•–37•]. The asymmetric localization of GPR-1/GPR-2 is under the control of the *PAR* genes, which can be placed upstream of G-protein signaling by genetic epistasis analyses [35•–37•]. The G-protein cycle in the *C. elegans* zygote is completed by the activities of the GEF RIC-8 and the GAP RGS-7, both of which are required for the generation of spindle asymmetry [38•,39•,40••] (Figure 3c). It is unclear at present which form of Gα is actually sending the signal that generates the unequal pulling forces acting on the spindle poles. The active form of Gα could be GDP–Gα–GPR-1/2, GTP–Gα or even GTP–Gα–RGS-7.

A second important question in this context is how G-proteins control the forces that act on the spindle poles. One hint may come from studies on LGN, a mammalian Pins homolog. LGN binds to NuMA, a microtubule-associated protein that is localized at spindle poles and controls the formation of aster microtubules [41]. A subsequent study by the same group showed that LGN functions as a conformational switch that can bind simultaneously to NuMA and Gαi in its active conformation, thus providing a link between G-proteins and microtubules [42••].

Conclusions

Cell polarity and asymmetric division of *Drosophila* NBs are controlled by at least three evolutionarily conserved protein complexes that are linked to each other by direct protein–protein interactions. Of central importance is the PAR/aPKC complex, which, together with the Dlg/Lgl/Scrib complex, regulates the asymmetric localization of cell fate determinants. The key event in the interaction between these two complexes is the phosphorylation of Lgl by aPKC, which leads to local inactivation of Lgl and restricts the activity of Lgl to the basal cortex of the NB. The PAR/aPKC complex, together with heterotrimeric G-proteins, also controls the orientation and geometry of the mitotic spindle. The targets of the PAR/aPKC complex and of G-protein signaling at the spindle poles or on the spindle itself are not yet known. Proteins that are localized to the plus ends of astral microtubules could directly bind to both complexes at the apical cortex, which might cause spindle rotation and protect apical aster microtubules from shrinking. The identification of such target proteins is one of the major challenges for the near future. A second important goal will be to figure out how the PAR/aPKC complex and the Pins/Gαi complex are localized to the apical cytocortex. The answers to these questions will be important to understand the mechanisms that control cell polarity and asymmetric cell division in many different cell types.

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