

Control of cell polarity and mitotic spindle positioning in animal cells

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Cell polarity is an essential feature of many animal cells. It is critical for epithelial formation and function, for correct partitioning of fate-determining molecules, and for individual cells to chemotax or grow in a defined direction. For some of these processes, the position and orientation of the mitotic spindle must be coupled to cell polarity for correct positioning of daughter cells and inheritance of localised molecules. Recent work in several different systems has led to the realisation that similar mechanisms dictate the establishment of polarity and subsequent spindle positioning in many animal cells.

Microtubules and conserved PAR proteins are essential mediators of cell polarity, and mitotic spindle positioning depends on heterotrimeric G protein signalling and the microtubule motor protein dynein.

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Abbreviations

APC	anaphase-promoting complex
aPKC	atypical protein kinase C
Dsh	Dishevelled
GFP	green fluorescent protein
MT	microtubule
MTOC	MT-organising centre

Introduction

The processes of cell polarisation and asymmetric cell division can be divided into a set of general steps irrespective of the mechanisms used (Figure 1). First, an initially unpolarised cell needs to receive an external polarising signal. This could be contact from another cell, ligand binding, pressure, light, fertilisation, etc. Second, cellular response to the signal should create an asymmetry within the cell and this polarised state must be maintained. Third, components to be asymmetrically segregated need to be localised along the polarity-axis. Fourth, the orientation of the mitotic spindle must be coupled to overall cell polarity. *A priori*, different cells could use different mechanisms at each point in this process.

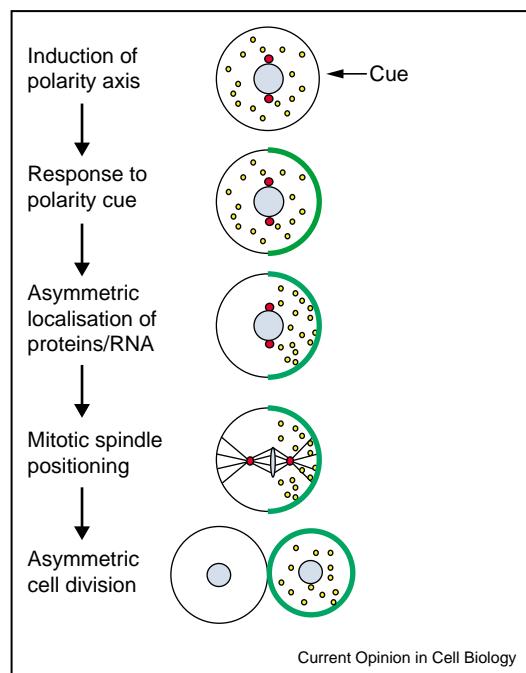
Indeed, initial polarity cues vary between different cell types. However, a wealth of studies from many systems has shown that the same series of molecules is used repeatedly after the polarity cue is delivered. The PAR proteins and microtubules are widely used for polarity establishment in animal cells, and heterotrimeric G proteins and dynein for positioning and orienting the mitotic spindle.

Role and function of PAR proteins

The PAR proteins, together with atypical protein kinase C (aPKC) and the small G protein Cdc42, appear to play central roles in establishing and maintaining the polarity of many, if not most, animal cells (Table 1). Although there is clear support for their requirement for polarity, much less is known about how they carry out this function. Here, I review their roles and biochemical activities and discuss recent findings that are beginning to shed light on their mechanisms of action.

Many of these conserved polarity molecules were initially identified in *Caenorhabditis elegans* through mutants disrupting the asymmetry of the first cell division. Lack of any of the six PAR proteins, PKC-3 or CDC-42 disrupt the overall polarity of the embryo [1–6]. The PDZ-domain-containing proteins PAR-3 and PAR-6 are localised to the anterior cortex together with PKC-3, a serine/threonine kinase ([3,7,8]; Figure 2a). PAR-1 (a serine/threonine kinase) and PAR-2 (a RING-finger protein) localise to the posterior cortex ([9–11]; Figure 2a). PAR-4 (a serine/threonine kinase) and PAR-5 (a 14-3-3 protein) are cortically associated but not asymmetrically localised [12,13]. The localisation pattern of the small G protein CDC-42 in the early embryo is not yet known, but it binds to PAR-6 and appears to be required for the activity of PAR-3, PAR-6, and PKC-3 [5,6].

In polarised mammalian epithelial cells, PAR-3/ASIP, PAR-6 and aPKC (homologues of *C. elegans* PAR-3, PAR-6 and PKC-3, respectively) form a complex that is localised to the tight junction ([14–18]; Figure 2c). Functional studies indicate that this complex is important in establishing epithelial cell polarity through tight junction formation. Expression of dominant-negative aPKC or of different forms of PAR-6 disrupts tight junctions, whereas overexpression of PAR-3/ASIP promotes tight-junction formation [19[•],20[•]–22[•]]. In addition, aPKC activity is important for maturation of *de novo* tight junctions [23[•]]. It appears that an important output of these protein interactions is regulation of the kinase activity of aPKC. Binding of Cdc42 to PAR-6 appears to potentiate the

Figure 1

General steps in cell polarisation and asymmetric cell division.

kinase activity of aPKC, whereas PAR-3 inhibits aPKC activity [17,18,20*]. In *Drosophila*, homologues of PAR-3, PAR-6 and PKC-3 function together in several different cell types (e.g. in oocyte and neuroblast polarity) [24–27]. One function in neuroblasts seems to be to localise the scaffold protein Inscuteable to orient mitotic spindles (Figure 2b; see below) [28]. In zebrafish, an aPKC homologue called *heart and soul* is required for the formation

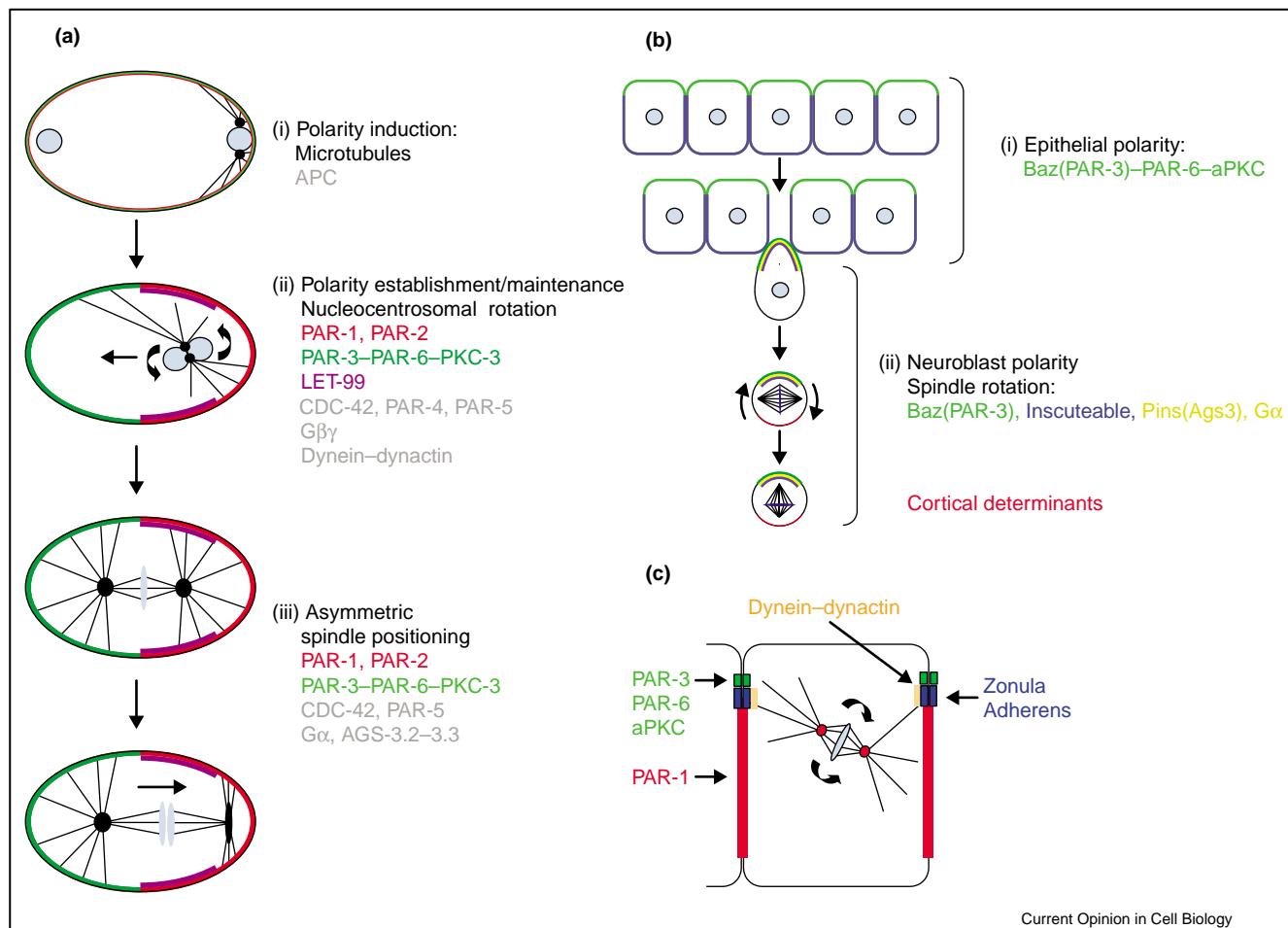
and maintenance of zonula adherens junctions during early epithelial development, and also has a role in spindle orientation in the retina [29*]. A critical problem now is to identify the targets of aPKC; so far, none are known.

Mammalian PAR-1 (mPAR-1) also appears to have a conserved function in cell polarity. In polarised epithelial cells, mPAR-1 is localised to the lateral membrane domain (Figure 2c), and expression of a version of mPAR-1 lacking the kinase domain disrupts cell adhesion and cell polarity [30]. Studies of PAR-1 in *Drosophila* led to the first hint that establishing embryonic polarity might be a conserved process [31,32]. As in *C. elegans* embryos, PAR-1 is localised to the posterior end of the *Drosophila* oocyte. Analyses of *par-1* mutants revealed two distinct functions in oocyte development. PAR-1 is required early for oocyte determination and later for establishing anterior-posterior polarity in the oocyte (see below) [31–34]. Lack of the later PAR-1 function results in disorganisation of the oocyte microtubule cytoskeleton, suggesting that microtubules or associated proteins might be PAR-1 kinase targets [31,33,34]. Indeed, in mammalian cells, PAR-1-like kinases called microtubule-affinity regulating kinases (MARKs) have been shown to phosphorylate microtubule-associated proteins, causing their dissociation from microtubules and increasing microtubule dynamics [35]. However, no microtubule-associated targets have yet been identified in *Drosophila*. Furthermore, the recent identification of two PAR-1 targets indicates that it has additional roles besides controlling the microtubule cytoskeleton. First, PAR-1 phosphorylates the Oskar protein, leading to its stabilisation at the posterior of the oocyte, where it directs pole cell formation [36]. Second, PAR-1 appears to phosphorylate the Dishevelled (Dsh) protein and act as a regulator of Wnt signalling [37].

Table 1
PAR and related polarity proteins in *C. elegans*, *Drosophila* and mammals.

<i>C. elegans</i>	<i>Drosophila</i>	Mammals	Protein motifs/biochemical function	Binding partners	Function
PAR-1	PAR-1	MARKs, C-TAK1, mPAR-1	Ser/Thr protein kinase	PAR-5	Phosphorylation of 14-3-3 binding sites; PAR-1 family kinase targets include MTs, Osk, Dsh, CDC25, KSR
PAR-2	?	?	RING finger	?	Localisation of PAR-3/PAR-6/PKC-3 complex in <i>C. elegans</i> embryos
PAR-3	Bazooka	PAR-3/ASIP	PDZ domain	PAR-6, aPKC, Inscuteable	Regulation of PKC-3 activity, apical localisation of Inscuteable in <i>Drosophila</i> neuroblasts
PAR-4	LKB1	LKB1, STK11	Ser/Thr protein kinase	?	No targets known
PAR-5	14-3-3 ϵ	14-3-3	14-3-3 protein	PAR-1	Regulates activity or localisation of many proteins through binding phosphorylated target sites
PAR-6	PAR-6	PAR-6	PDZ domain, semi-CRIB domain	PAR-3, aPKC	Regulation of PKC-3 activity
PKC-3	aPKC	aPKC, PKC ζ	Ser/Thr protein kinase	PAR-3, PAR-6	No targets known
CDC-42	Cdc42	Cdc42	Small GTPase	PAR-6	Regulation of PKC-3 activity/cytoskeleton regulation/signal transduction

See text for references. CRIB, Cdc42/Rac-interactive binding; C-TAK1, Cdc25C-associated kinase 1; KSR, kinase suppressor of Ras; MARK, microtubule-affinity regulating kinase.

Figure 2

Cell polarity and spindle positioning in different animal cells. **(a)** *C. elegans* one-cell embryo. (i) Polarity induction is through interaction of the sperm astral microtubules with the cortex. APC activity is also essential for initial polarisation. (ii) Polarity is established and maintained through the PAR proteins, some of which are asymmetrically localised. Nucleocentrosomal rotation requiring LET-99, G β γ , dynein and the dynactin complex orients the first division. (iii) Asymmetric spindle positioning requires PAR proteins, G α (GOA-1 and GPA-16), and AGS-3.2-3.3. **(b)** *Drosophila* epithelial cell and neuroblast polarity. (i) Baz(PAR-3)-PAR-6-aPKC is required for epithelial cell polarity. Neuroblasts delaminate from this layer, inheriting PAR polarity. (ii) During delamination, Inscuteable, Pins and a G α subunit become asymmetrically localised apically, together with the PAR-3-PAR-6-aPKC complex, and are required for localisation of cortical determinants and spindle rotation. **(c)** Mammalian epithelial cells. PAR-3, PAR-6 and aPKC are localised to the tight junction and PAR-1 to the lateral membrane. Dynein and the dynactin complex, involved in spindle positioning, are found in a circumferential ring below the tight junction. Distributions of proteins in grey are not shown.

New studies on *Drosophila* PAR-1 provide a clearer view of its biochemical function. Benton and St Johnston sought PAR-1 binding partners and identified a homologue of *C. elegans* PAR-5, a 14-3-3 protein [38**]. 14-3-3 proteins regulate numerous cell processes through binding phosphorylated consensus sites in target proteins (see [39] for a review). However, *Drosophila* PAR-5 binds PAR-1 in a region other than its phosphobinding pocket. The authors showed that PAR-1 phosphorylates 14-3-3 consensus sites in target proteins, generating a binding site for 14-3-3 [38**]. Association between PAR-1 and PAR-5 thus appears to allow delivery of PAR-5 to the site after phosphorylation. Consistent with this model, the 30-amino-acid region of Dsh mapped to contain the phos-

phorylated site has a 14-3-3 consensus sequence within it. Further, C-TAK1 (Cdc25C-associated kinase 1), a mammalian PAR-1-like kinase, generates 14-3-3 binding sites upon phosphorylation of the target proteins Cdc25 or KSR (kinase suppressor of Ras) [40,41]. Global searches of 14-3-3 consensus sites should now help in defining the targets of PAR-1 kinases.

So far, no clear homologues of PAR-2 have been found in other organisms. It may be that PAR-2 has a specific role in maintaining localisation of the PAR-3-PAR-6-PKC-3 complex in the one-cell *C. elegans* embryo. Homologues of PAR-4 exist in *Drosophila*, *Xenopus*, mouse and human [42-44]. In humans, a PAR-4 homologue LKB1 (or

STK11) is mutated in Peutz–Jeghers syndrome, a disorder with predisposition to gastrointestinal polyposis and cancer [42,43]. A role for PAR-4 in cell polarity has been recently been shown in *Drosophila* (S Martin and D St Johnston, personal communication), but as yet there is no data on a polarity role from a vertebrate system.

Microtubules and polarity establishment

The proteins discussed above are involved in establishing and maintaining cell polarity, but how is polarity initiated? Evidence from *C. elegans*, *Drosophila* and mouse points to a role for microtubules in the initiation of embryonic polarity in each of these organisms.

In *C. elegans*, oocytes do not have predetermined polarity. Entry of the sperm at fertilisation is the essential polarity-inducing event [45]. The sperm brings in the centrosomes, which nucleate microtubules, and polarity appears to be induced through interaction of the sperm asters with the cortex [46,47]. This interaction determines the posterior end and leads to the asymmetric localisation the PAR proteins as described above. Weak mutants of components of the anaphase promoting complex (APC) or RNA interference of the gene encoding separin show impaired association of the sperm aster/pronuclear complex with the cortex [48^{*}]. In these mutants, PAR-2 is found in cytoplasmic puncta and PAR-3 is uniformly distributed at the cortex, suggesting that cortical interaction requiring the APC and separin might deliver PAR-2 or clear PAR-3, or both.

A new study provides insights into how the sperm asters cause the asymmetric localisation of the PAR proteins. Seydoux and co-workers [49^{*}] looked at GFP fusions of PAR-2 and PAR-6 in live embryos during polarity establishment. In early one-cell embryos, these two PAR proteins show uniform cortical localisation; however, at the time of sperm aster growth, GFP:PAR-6 is excluded from the cortex at the region of aster contact. This exclusion is independent of PAR-2. By contrast, GFP:PAR-2 becomes enriched where the sperm asters contact the cortex. They further show that NMY-2, a non-muscle myosin required for embryonic polarity, is essential for the initial exclusion of PAR-6 [49^{*}]. The authors speculate that interaction of sperm astral microtubules with the cortical actin cytoskeleton removes PAR-6 and facilitates the localisation of PAR-2 at the posterior. Previous work indicates that PAR-2 and the PAR-3–PAR-6–PKC-3 complex mutually exclude each other [3,7,8,11], and the new study [49^{*}] suggests that this is part of a maintenance phase of PAR asymmetry. An open question is how interaction of microtubules with the cortex affects PAR localisation. Are microtubule-binding proteins involved? Also, how do the PAR proteins bind the cortex? None of them are predicted to be membrane bound, so they must interact with as yet unknown cortical proteins. In mammalian epithelial cells, ASIP/PAR-3

binds to JAM-1, a transmembrane junction protein involved in cell adhesion [50,51], suggesting that JAM-1 might target the complex to the tight junction.

In contrast to *C. elegans*, where the oocyte is not polarised, *Drosophila* oocytes are highly polarised cells. Polarity arises during oocyte development. Incomplete, asymmetric divisions of germ-line stem cells produce a cyst of 16 interconnected cells [52]. One of these is chosen as the oocyte, possibly although asymmetric inheritance of a membrane-rich structure called the ‘fusome’ [53,54]. The other 15 cells become nurse cells that provide components to the growing oocyte [52]. An intact microtubule cytoskeleton is needed for oocyte determination, as disruption of microtubules leads to the production of 16 nurse cells and no oocyte [55].

The initial anterior–posterior polarisation of the oocyte critically depends on a polarised array of MTs; a stable MTOC formed at the posterior directs the posterior movement of oocyte specific factors such as Orb, Bicaudal D and Egalitarian (reviewed in [56,57]). Later, the microtubule cytoskeleton is repolarised to direct the asymmetric localisation of the determinants Bicoid and Oskar to the anterior and posterior ends of the oocyte, respectively [56,57]. Initial oocyte polarisation requires PAR-1, PAR-5, BAZ/PAR-3, PAR-6 and aPKC [24,27,33,34,58^{*}]. Recently, it was shown that MTs are required for the accumulation of a newly detected isoform of PAR-1 (N1S) in the oocyte [58^{*}]. Remarkably, after oocyte determination, the localisation of this PAR-1 isoform to the posterior of the early oocyte is complementary to that of Baz/PAR-3 at the anterior, similar to that seen for PAR-1 and PAR-3 in *C. elegans* embryos. Thus in *Drosophila*, as in *C. elegans*, MTs appear to be important for establishing asymmetric PAR distributions.

In the mouse, the first cleavage divides the embryo into two cells with different fates. One cell gives rise primarily to the embryonic part of the blastocyst (and tends to divide first) and the other cell primarily to the abembryonic (extraembryonic) part [59]. New work has shown that the orientation of the first mitotic spindle is defined by the site of the last meiotic division [60^{**}]. Using a special fixation condition, ‘stable’ microtubules of both mitotic asters can be observed to contact the region of the meiotic midbody. In time-lapse recordings, the spindle can be seen to move to a region parallel to the meiotic midbody before division. Thus, interaction of microtubules with a cortical site defines the first cleavage plane, which divides the embryo into two cells of different fates. These data imply that the one-cell mouse embryo has localised components that are differentially partitioned by the oriented first cell division. In this model, one could imagine that the microtubule-containing cortical site of the last meiotic division (the meiotic midbody) might have a role in both organising this polarity and orienting the division.

Polarised early cleavages imply the existence of polarity-establishing molecules, and it will be of great interest to determine whether PAR proteins are involved and asymmetrically localised in early mouse development. It should be kept in mind, however, that this ‘asymmetric’ division is not determinative. Early mouse development is highly regulative, as individual blastomeres can go on to form a complete mouse (see [61] for a review). How this regulation occurs is not known. One very speculative possibility suggested by these new results is that spindle orientation via interaction with a mitotic midbody could re-establish asymmetries during regulative development.

Dynein and spindle positioning

Once polarity is established, what is the mechanism by which spindles are oriented and positioned before cell division? This obviously must involve a link between microtubules and the cell cortex. Work in a wide variety of systems implicate the minus-end-directed microtubule motor protein dynein and the functionally linked dynactin complex in orienting mitotic spindles.

A clear role for dynein in controlling spindle positioning comes from work in budding yeast. There, dynein and the dynactin complex are both required for astral-microtubule sliding on the cortex [62]. In dynein and dynactin mutant cells, mitotic spindle positioning into the bud neck is defective (the spindle is enclosed in the nucleus in yeast) and MT organisation is abnormal, with overgrown and buckled MTs. This work suggests that dynein and dynactin mediate interactions between MTs and the cortex which are required to move the spindle into the bud neck.

In *C. elegans*, after polarity establishment by the sperm asters, the maternal pronucleus migrates to meet the paternal one in the posterior. The pronuclei and associated centrosomes then migrate to the centre of the cell. This migration is accompanied by a 90° nucleocentrosomal rotation event that orients the centrosomes to lie along the anterior–posterior axis (Figure 2a). Reducing the activity of dynein or components of the dynactin complex inhibit this rotation [63,64].

Studies in mammalian cells and in *Drosophila* add further support to a general role for dynein in spindle orientation. In astrocytes, dynein is required for MTOC reorientation, a process that is also controlled by CDC-42 and aPKC [65]. In polarised mammalian epithelial cells, dynein is localised in a circumferential belt at the adherens junction [66,67*] (Figure 2c). Overexpression of LIS1 perturbs dynein and dynactin localisation and leads mitotic spindle misorientation [68]. Similarly, in non-columnar rat epithelial cells, the orientation of the mitotic spindle along the long axis is inhibited by injection of antibodies against dynein [69]. Finally, dynein is required for correct spindle orientations during germline stem cell divisions in

Drosophila [70]. Thus, roles for dynein and dynactin in spindle position and orientation are well established, but how are these roles coupled to cell polarity?

Spindle regulation via heterotrimeric G proteins

Spatial control of spindle orientation and positioning must respond to the overall polarity of the cell. Work in several systems points to heterotrimeric G proteins as transducers of polarity information to the mitotic spindle. Targets of the G proteins are not yet known, but widespread roles for dynein in spindle positioning make it a good candidate.

In *C. elegans*, besides dynein and the dynactin complex, several other molecules are needed for the rotation event that orients the first mitotic spindle. In mutants of several of the *par* genes, the first rotation sometimes fails, suggesting that these polarity molecules are required to localise rotation activity [12,71]. One regulator of rotation appears to be a G β subunit of a heterotrimeric G protein: in G β mutants, rotation sometimes fails, but the localisation and other activities of the PAR proteins are unaffected, suggesting that G β acts downstream of the PARs [72]. Mutations in the *let-99* gene result in a phenotype very similar to that of G β mutants, with loss of rotation of the pronuclear/centrosomal complex [73]. Instead of rotation, *let-99* mutant embryos display exaggerated movements of the pronuclear/centrosomal complex towards different cortical regions, suggesting that it might directly regulate interaction of microtubules and the cortex [73,74*]. Insight into this mechanism was provided by the recent cloning and molecular characterisation of *let-99* [74*]. The LET-99 protein contains a DEP (Dishevelled/EGL-10/pleckstrin) domain, which is found in numerous regulators of G proteins [75]. LET-99 is enriched in a band encircling the one-cell embryo within the PAR-2 region (Figure 2a), and its localisation there depends on PAR protein function [74*]. The exaggerated movements of the pronuclear/centrosomal complex in *let-99* mutants are further shown to require dynein activity. This has led to a model whereby LET-99 (possibly together with G β) localises dynein-mediated pulling forces by inhibiting them in the LET-99 band region ([74*]; Figure 2a).

After rotation of the pronuclear/centrosomal complex, the first mitotic spindle initially sets up centrally. The spindle is then displaced towards the posterior in metaphase and anaphase. This leads to an asymmetric first division, with a larger anterior blastomere (AB) and a smaller posterior one (P1), each with distinct fates [76,77]. The PAR proteins control this spindle asymmetry through regulation of pulling forces acting at the poles. Grill *et al.* [78] showed that in wild-type embryos there are strong pulling forces acting on the posterior aster and weak ones on the anterior one; in *par-2* and *par-3* mutants, the forces are the same at each pole, with weak ones at both poles in *par-2* mutants and strong ones in *par-3* mutants.

The PAR proteins might control spindle forces through regulation of two redundant G α subunits of a heterotrimeric G protein. When G α function is reduced, the mitotic spindle fails to move towards the posterior, but other aspects of cell polarity are normal [79*,80]. The heterotrimeric G protein appears to be regulated through a receptor-independent mechanism. Inhibition of *ags-3.2* and *ags-3.3*, two distant homologues of Ags3, a mammalian receptor-independent activator of G protein signalling, leads to a spindle-positioning phenotype identical to that of loss of G α ([81]; M Gotta and J Ahringer, unpublished data). In addition, spindle elongation at anaphase fails to occur, and spindle forces are weak or absent (M Gotta and J Ahringer, unpublished data). Thus, PAR polarity appears to be transduced to the mitotic spindle through G α and Ags3 molecules, which control forces acting on the spindle poles by an as yet unknown mechanism.

Control of spindle positioning through heterotrimeric G proteins and Ags3-like molecules is conserved, as they are involved in this process in *Drosophila* neuroblasts [82,83*,84]. The PAR proteins Baz/PAR-3, PAR-6 and aPKC are required for epithelial cell polarity. Neuroblasts delaminate from a sheet of epithelial cells and inherit PAR polarity from the mother cells (Figure 2b). In these neuroblasts, a 90°-rotation event orients the mitotic spindles to be perpendicular to those in the epithelial sheet [85]. Both the polarity of the neuroblast (judged by asymmetric localisation of cortical determinants) and the spindle rotation are dependent on a heterotrimeric G protein and an Ags3-like molecule called Pins (Partner of Inscuteable) [82,83*,84].

Thus, in contrast to *C. elegans*, heterotrimeric G proteins affect both spindle positioning and overall polarity in *Drosophila* cells. One way to reconcile these observations is suggested by experiments showing that the mitotic spindle itself might induce polarity. In some polarity mutants of *Drosophila* (e.g. *inscuteable* and *pins*), crescents of cortical determinants are formed that are not aligned with the abnormally oriented mitotic spindle [82,84,86]. However, just before division, in a process termed ‘telophase rescue’, the crescents do become aligned, and it has been suggested that polarity cues from the spindle orient the crescent of localised determinants [87]. Evidence for this being a conserved activity of the spindle comes from recent work on the *C. elegans* gene *spn-4* [88*]. In *spn-4* mutants, rotation of the nucleocentrosomal complex fails in the P1 cell. In these embryos, PAR-2 and germline P granules are initially localised normally to the posterior of the P1 cell (but lateral to the misaligned spindle); however, just before division they become redistributed to one of the two spindle poles, such that they are correctly partitioned into only one daughter cell. Redistribution of P granules fails to occur in G β mutants [72], suggesting that heterotrimeric G proteins might

control spindle polarity. Consistent with this, overexpression of G αi in *Drosophila* neuroblasts also prevents telophase rescue [83*].

Conclusions

This is an exciting time for studies of cell polarity and asymmetric cell division. Over the past few years, we have seen the enormous benefit of studying these processes in different systems. Conservation of the molecules involved means that findings in one system can be applied to make progress in others. Although our understanding of these processes has improved recently, we still have only a basic understanding of their mechanisms. For example, it is not yet known how PAR polarity information is transduced within the cell, or how heterotrimeric G proteins signal to the spindle. With many leads and tools now in hand, the answers to these questions should soon be rolling in.

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