Cell polarity: the PARty expands

Chris Q. Doe

Recent work has revealed an evolutionarily conserved trio of proteins that regulate cell polarity in epithelial cells, embryonic blastomeres and neural precursors. This common cell-polarity mechanism is used in cell-specific ways, as highlighted by the recent finding that at least two different types of asymmetric division are observed in *Drosophila* neural precursors.

Despite the importance of establishing and maintaining cell polarity for cell function and development, relatively little is known about how metazoan cells establish polarity or whether there is a common mechanism used by different cell types and organisms. Studies by Petronczki and Knoblich[1], on page 43 of this issue, and by Wodarz *et al.*[2] have shown that a protein complex that is known to regulate cell polarity in *Caenorhabditis elegans* and mammalian epithelia is also required for cell polarity in *Drosophila* epithelia and neural precursors (Fig. 1). The existence of a conserved cell-polarity mechanism does not mean that it is used in the same way by all cells, however, and indeed two papers[3,4], on pages 50 and 58 of this issue, show that cells in the *Drosophila* cell lineage for external sense organs use two different modes of asymmetric division to generate cell diversity (Fig. 2). Together, these results emphasize the emergence of an evolutionarily conserved mechanism that regulates cell polarity in metazoans, as well as the potential for cell-type-specific responses to these cell-polarity cues.

The *C. elegans* embryo is a model system for studying cell polarity. Over the past decade several proteins that are essential for establishing anterior/posterior cell polarity of the zygote have been identified (reviewed in ref. 5). Three proteins are localized to the anterior cortex of the one-cell embryo — PAR-3, a protein with three PDZ (Psd95, Discs Large, ZO-1) domains; PAR-6, which contains a single PDZ domain and a small, G-protein-binding CRIB-like domain; and PKC-3, which is an atypical protein kinase C (aPKC). All three proteins are required for establishing anterior/posterior cell polarity in the zygote, although the molecular mechanism of their localization and function remains unclear. Mammalian homologues of PAR-3, PAR-6 and PKC-3 (ASIP, Par6, and PKCζ or PKCλ, respectively; hereafter referred to as PAR-3, PAR-6, and aPKC for simplicity) are also part of a multiprotein complex. They are apically localized in epithelia and are required for control of epithelial cell polarity and growth (reviewed in ref. 6). In *Drosophila*, the PAR-3 related protein Bazooka is also localized to the apical cortex of epithelia and is required for epithelial cell polarity. These findings raised the possibility that the PAR-3–PAR-6–aPKC protein complex is an evolutionarily conserved regulator of cell polarity (Fig. 1).

The recent finding that *Drosophila* contains proteins related to PAR-6 and aPKC, which form a complex with Bazooka to regulate apical/basal polarity in epithelial and non-epithelial cell types, has strengthened this speculation. Petronczki and Knoblich have characterized the *Drosophila par-6* gene, whereas Wodarz and colleagues carried out a similar analysis of the *Drosophila aPKC* gene. Both PAR-6 and aPKC are present at the apical cortex of epithelial cells, as well as in apical crescents in dividing neuroblasts, which are neural precursors that have an epithelial origin. The subcellular distribution of PAR-6 and aPKC is identical to that of Bazooka; in fact, both PAR-6 and aPKC can bind to Bazooka *in vitro* and *in vivo*, and both proteins require Bazooka for their apical localization. All three proteins

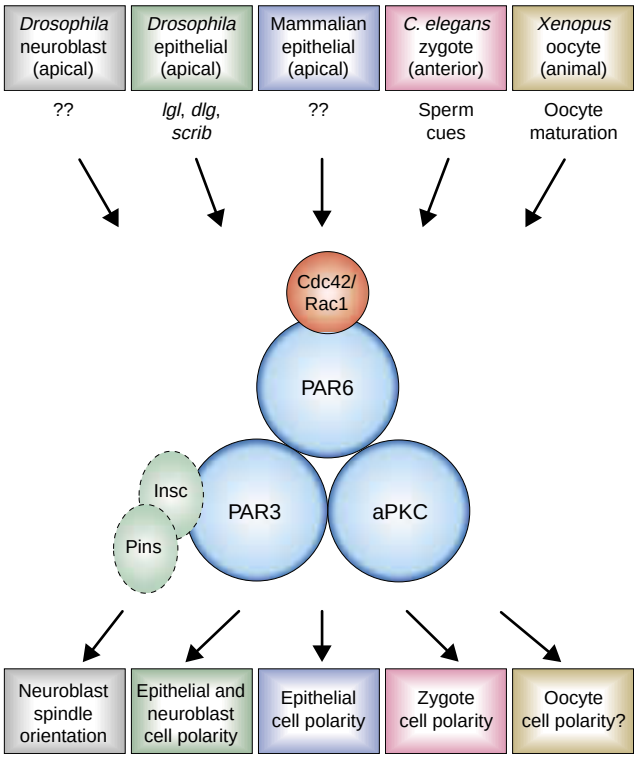

Figure 1 **The PAR-3–PAR-6–aPKC protein complex. The complex exhibits polarized localization in a variety of cell types and organisms. Cdc42 and Rac1 are known to bind to mammalian PAR-6 but have not been tested in other organisms; Insc/Pins proteins bind to PAR-3 (Bazooka) but have not been identified in other organisms to date. In *Drosophila* neuroblasts (grey), the complex uses Inscuteable and Pins to determine orientation of the miotic spindle and to regulate apical/basal polarity. In *Drosophila* epithelial cells (green) and mamalian epithelial cells (blue), the complex regulates apical/basal cell polarity. In *C. elegans* (pink), the complex is involved in establishing anterior/posterior zygote polarity in response to sperm cues. In *Xenopus* (brown), the complex is localized to the animal pole in response to oocyte maturation, but it's function is unknown.**

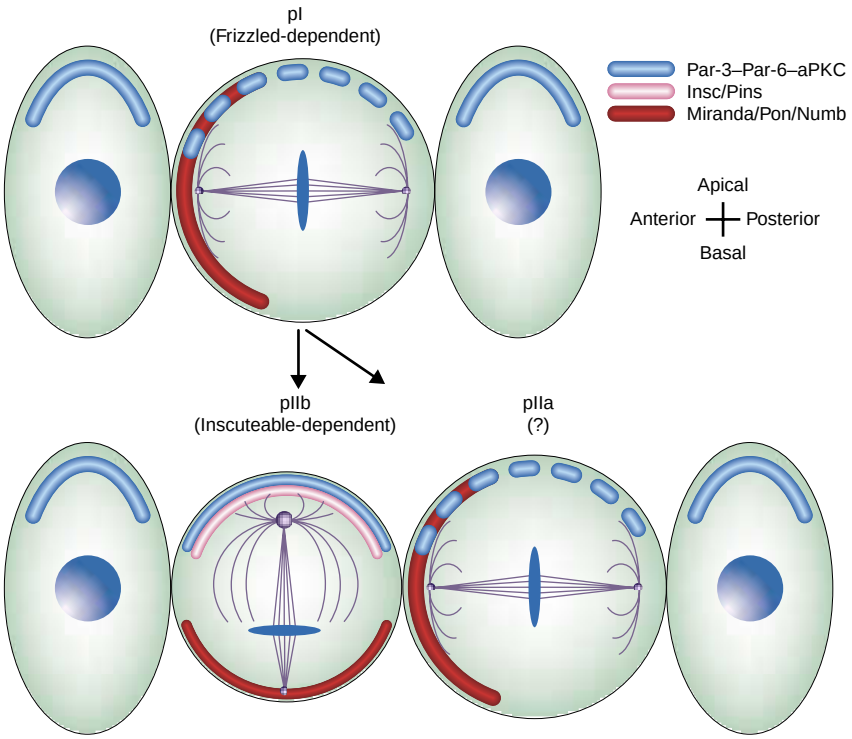

Figure 2 **Two types of asymmetric division in the *Drosophila* cell lineage for external sense organs. pI and pIIa cells divide along the anterior/posterior axis with spindle orientation regulated by Frizzled (Fz) signalling in pI cells and by or an unknown signal in pIIa cells. In contrast, pIIb cells divide along the apical/basal axis with spindle orientation regulated by Inscuteable (Insc; pink) and probably PAR-3–PAR-6–aPKC (blue). In addition, pI/pIIa and pIIb cells differ in spindle morphology (purple) and in the localization of the cell-fate determinants Pon, Miranda and Numb (brown). pI and pIIa cells are likely to contain apical PAR-3–PAR-6–aPKC (dashed crescent), although this has not been proven.**

are interdependent for apical targeting; indicating that formation of a protein complex may be necessary for stable apical localization. Embryos that lack maternal and zygotic PAR-6 or aPKC show similar striking defects in epithelial-cell polarity — cells are rounded rather than columnar, do not form a monolayer, and show mislocalization of apical proteins (Bazooka is cytoplasmic; Armadillo is uniformally cortical) and basal proteins (Neurotactin is uniformally cortical). Neuroblasts lacking PAR-6 or aPKC also show loss of apical/basal polarity — the mitotic spindle is randomly orientated instead of aligned with the apical/basal axis, the normally basal Miranda and Numb proteins are uniformly cortical, and the normally apical Bazooka and Inscuteable proteins are cytoplasmic. These epithelial and neuroblast phenotypes are similar to those of embryos lacking maternal and zygotic Bazooka function[7], indicating that complex formation may be required for the function of PAR-3, PAR-6 and aPKC.

How is the asymmetric localization of PAR-3–PAR-6–aPKC regulated? In *C. elegans*, the anterior localization of this complex depends on sperm cues and the function of several *par* genes (reviewed in ref. 5). In *Drosophila* epithelia, its apical localization requires the function of the cortical Scribble, Discs Large and Lethal Giant Larvae tumour-suppressor proteins[8], and this may also be the case in *C. elegans* epithelia, as a Scribble-related protein, Let-413, is required for epithelial cell polarity[9]. In mammals, only activated (GTP-bound) Cdc42 and Rac1 associate with the PAR-6, indicating that polarized activation of small G proteins may lead to PAR-3–PAR-6–aPKC localization or activation (reviewed in ref. 6). And in *Xenopus*, oocyte maturation triggers localization of PAR-3–PAR-6–aPKC related proteins to the animal pole (reviewed in ref. 5). It will be interesting to see which, if any, of the above-mentioned regulators are conserved between organisms or cell types, and how they work at a mechanistic level to localize PAR-3–PAR-6–aPKC.

Even less is known about the downstream effectors of the PAR-3–PAR-6–aPKC complex. In *Drosophila*, this complex binds to the Inscuteable/Pins proteins, which are necessary for spindle orientation, although the link between Insc/Pins and the spindle has not been defined (reviewed in ref. 5; see Fig. 1). In mammals, it is thought that aPKC is inactive when bound to PAR-3, but active when bound to PAR-6, and that inhibition of aPKC function is necessary to restrain cell proliferation (reviewed in ref. 6). This is an attractive model, in light of the observation that mutations in *scribble*, *discs large* or *lethal giant larvae* lead to delocalization of at least one component of the PAR-3–PAR-6–aPKC complex (Bazooka) and result in epithelial tumours in *Drosophila*[8]. Are these tumours due to unrestrained aPKC activity?

The existence of a conserved cell-polarity mechanism involving PAR-3–PAR-6–aPKC does not necessarily mean that it is used in the same way by all cells, as shown by two more papers in this issue. Bellaiche *et al.*[3] and Roegiers *et al.*[4] report on the mechanisms that regulate asymmetric cell division within the well-characterized *Drosophila* cell lineage for adult external sense organs, and show that two types of asymmetric cell division occur within this lineage (Fig. 2). External sense organs (innervated bristles) develop from a precursor called pI, which divides to form pIIa and pIIb cells; pIIa produces the external bristle and socket cells, whereas pIIb generates the internal neuron, sheath cell, and a migrating glial cell[10].

The authors of these two papers show that the asymmetric division of pI cells has the following characteristics: first, it divides within the plane of the epithelium; second, the mitotic spindle forms at a random position but becomes 'anchored' along the anterior/posterior axis at early anaphase; third, spindle orientation is regulated by the transmembrane Frizzled (Fz) protein and not by Inscuteable (Insc); and fourth, the spindle itself is symmetric but is eccentrically positioned close to the anterior cell cortex, giving rise to to a slightly smaller anterior daughter cell. Roegiers and colleagues go on to show that the division of pIIb cells is different in many respects — cell division occurs along the apical/basal axis rather then the anterior/posterior axis, spindle orientation is regulated by apical Insc (and presumably by the PAR-3–PAR-6–aPKC complex, although this was not directly assayed) and not by Fz activity, and the mitotic spindle itself is asymmetric with a profusion of astral microtubules at the apical pole. This reveals that division of pIIb cells is quite similar to that of embryonic neuroblasts[11].

One explanation for the difference in spindle orientation between pI and pIIb/neuroblast cell types is that pI does not express Insc, and thus cannot respond to the apical PAR-3–PAR-6–aPKC cue. This could be tested by misexpressing Insc in pI cells, which has not been done (although

ectopic Insc can induce apical/basal spindle orientation in embryonic epithelia[12]). However, Roegiers and colleagues have carried out the converse experiment of removing Insc from pIIb cells, which causes the division axis to switch to an anterior/posterior orientation, presumably in response to Fz-dependent polarity cues. A simple model is that there is a hierarchy of polarity cues available to pI cells and its sibling cell types — if Insc is present, cell division will respond to PAR-3–PAR-6–aPKC cues and occur in the apical/basal axis; if Insc is absent, cell division will respond to Fz cues and occur in the anterior/posterior axis.

One response seems to be the same for both pI and pIIb/neuroblasts — one pole of the mitotic spindle is always associated with the cortical 'crescent' of the cell-fate determinants Partner of Numb (Pon), Numb and Miranda. These proteins are co-localized to the anterior cortex of mitotic pI cells and to the basal cortex of mitotic pIIb cells (and neuroblasts). How is spindle orientation and Pon/Miranda localization coordinated? Both Bellaiche *et al.* and Roegiers *et al.* use elegant live-imaging methods to show that localization of Pon tagged with green fluorescent protein (GFP) occurs at pI metaphase, before the final 'anchoring' of the GFP-labelled mitotic spindle in early anaphase. Roegiers and colleagues propose that this anchoring of the mitotic spindle occurs at the anterior cortex in the centre of the Pon crescent, on the basis of two observations — first, that only the anterior pole of the mitotic spindle is tightly associated with the cell cortex during wild-type pI division, and second, that *fz* mutants can occasionally exhibit 'bent' spindles in which both spindle poles are in contact with the cortex within the Pon crescent, indicating that both spindle poles are physically attached to the Pon-containing cell cortex. The identity of the cortical-anchoring cue remains mysterious, but it may be restricted to the anterior pI cortex (and perhaps to the basal pII and neuroblast cortex) by the same mechanism that localizes Pon and Miranda, thereby ensuring coordinated control of the localization of cell-fate determinants and spindle orientation.

Recent progress in understanding cell polarity and asymmetric division has been phenomenal, and shows no sign of abating. It will be fascinating to see which inputs and outputs of the PAR-3–PAR-6–aPKC complex are conserved between cell types and organisms, and how they are used in different contexts to regulate cell function, growth control and asymmetric cell division. □

Chris Doe is in the Institute of Molecular Biology and the Institute of Neuroscience, HHMI, University of Oregon, Eugene, Oregon 97403, USA
e-mail: cdoe@uoneuro.uoregon.edu

1. Petronczki, M. & Knoblich, J. A. *Nature Cell Biol.* 3, 43–49 (2001).
2. Wodarz, A., Ramrath, A., Grimm, A., & Knust, E. *J. Cell Biol.* 150, 1361–1374 (2000).
3. Bellaiche, Y., Gho, M., Kaltschmidt, J. A., Brand, A. H. & Schweisguth, F. *Nature Cell Biol.* 3, 50–57 (2001).
4. Roegiers, F., Younger-Shepard, S., Jan, L. & Jan, Y. N. *Nature Cell Biol.* 3, 58–67 (2001).
5. Doe, C. Q. & Bowerman, B. *Curr. Opin. Cell Biol.* in the press.
6. Kim, S. K. *Nature Cell Biol.* 2, E143–E145 (2000).
7. Schober, M., Schaefer, M. & Knoblich, J. A. *Nature* 402, 548–551 (1999).
8. Bilder, D., Li. M. & Perrimon, N. *Science* 289, 113–116 (2000).
9. Legouis, R. *et al. Nature Cell Biol.* 2, 415–422 (2000).
10. Gho, M., Bellaiche, Y. & Schweisguth, F. *Development* 126, 3573–3584 (1999).
11. Kaltschmidt, J. A., Davidson, C. M., Brown, N. H. & Brand, A. H. *Nature Cell Biol.* 2, 7–12 (2000).
12. Kraut, R., Chia, W., Jan, L. Y., Jan, Y. N. & Knoblich, J. A. *Nature* 383, 50–55 (1996).