Asymmetric cell division: fly neuroblast meets worm zygote

Chris Q Doe* and Bruce Bowerman†

Both *Drosophila* neuroblasts and *Caenorhabditis elegans* zygotes use a conserved protein complex to establish cell polarity and regulate spindle orientation. Mammalian epithelia also use this complex to regulate apical/basal polarity. Recent results have allowed us to compare the mechanisms regulating asymmetric cell division in *Drosophila* neuroblasts and the *C. elegans* zygote.

Addresses
*Institute of Molecular Biology, Institute of Neuroscience, Howard Hughes Medical Institute, 1254 University of Oregon, Eugene, Oregon 97403, USA; e-mail: cdoe@uoneuro.uoregon.edu
†Institute of Molecular Biology, 1370 Franklin Boulevard, University of Oregon, Eugene, Oregon 97403, USA;
e-mail: bbowerman@molbio.uoregon.edu

Current Opinion in Cell Biology 2001, 13:68–75

0955-0674/01/$ - see front matter

Abbreviations

AB	anterior blastomere at the two-cell stage
a–p	anterior–posterior
aPKC	atypical protein kinase C
Baz	Bazooka
dlg	*discs large*
GFP	green fluorescent protein
GMCs	ganglion mother cells
lgl	*lethal giant larvae*
Insc	Inscuteable
mex-5	muscle excess
MF	microfilament
MLC-4	non-muscle myosin regulatory light chain
MT	microtubule
NMY-2	type II non-muscle myosin heavy chain
P_0	one-cell zygote
P_1	posterior blastomere at two-cell stage
par	partitioning-defective mutant phenotype
PDZ	PSD-95/Discs large/ZO-1
PIE-1	pharyngeal and intestinal excess 1
Pins	Partner of Inscuteable
pod	*partitioning–osmotic defective*
Pon	Partner of Numb

Introduction

Embryonic *Drosophila* neuroblasts (neuronal precursor cells) develop from an apical/basal polarized epithelium called the ventral neuroectoderm (Figure 1). Individual neuroblasts move out of the epithelium and come to lie adjacent to the basal surface of the neuroectoderm, losing their epithelial morphology but retaining aspects of apical/basal polarity. Neuroblasts repeatedly divide asymmetrically along their apical/basal axis. These unequal divisions produce larger apical daughters that retain the stem-cell-like properties of a neuroblast and smaller, fate-committed basal daughter cells called ganglion mother cells (GMCs) that produce neurons or glia.

In another example of asymmetric cell division, the one-cell *Caenorhabditis elegans* embryo, called P_0, divides along its anterior–posterior (a–p) axis to produce a large anterior blastomere at the two-cell stage (AB) and a small posterior P_1 blastomere (Figure 2). In addition to their unequal size, these two cells have different cell cycle times and are born committed to distinct fates [1–3]. AB generates predominantly ectodermal cell types, whereas P_1 generates mesoderm, endoderm and germline cells. P_1 and its descendants undergo repeated asymmetric divisions that are largely responsible for patterning the early embryo [4], but we focus here on the first division of P_0.

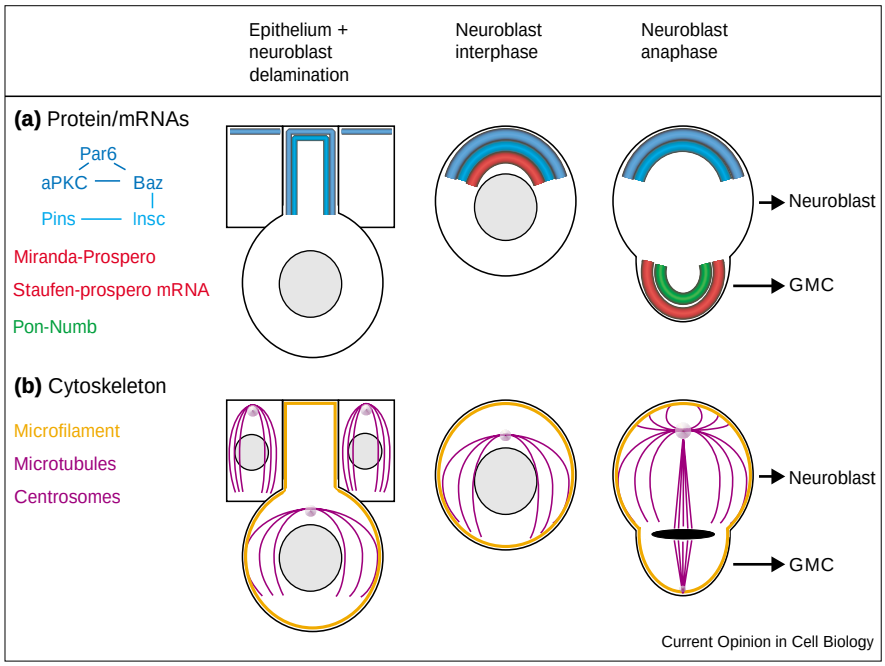
In spite of the apparently substantial differences between a fly neuroblast and a one-cell worm embryo, recent work has revealed extensive parallels in the mechanisms regulating protein localization and spindle orientation during asymmetric cell divisions. Furthermore, vertebrate epithelia also use related mechanisms to establish or maintain apical/basal polarity. Previous reviews have covered earlier work on the asymmetric division of neuroblasts and early *C. elegans* blastomeres, as well as germ cell and post-embryonic asymmetric division in *Drosophila* and *C. elegans* [3,5–8]. Here we present recent results and highlight common features of cell polarity and asymmetric division in fly neuroblasts and the worm zygote.

Establishing cell polarity: a conserved apical/anterior protein complex in flies, worms and vertebrates

The initial apical/basal polarity of a *Drosophila* neuroblast is inherited, at least in part, from the epithelium that generates it. A newly delaminated neuroblast maintains apical enrichment of several proteins found at the apical cortex of epithelial cells. These proteins include the multi-PDZ (PSD-95/Discs large/ZO-1) domain protein Bazooka (Baz), the single-PDZ domain protein DmPAR-6 and an atypical protein kinase C, aPKC [9–11,12••,13••]. These three proteins are conserved in *C. elegans* (where they are called Par-3, Par-6 and aPKC); they are localized to the anterior cell cortex in early blastomeres and are required for establishing cell polarity, see below) (reviewed in [3,5]). This protein trio is also conserved in *Xenopus*, where they are localized to the animal pole of mature oocytes and epithelial cell contact sites [14•,15•], and in mammals where they are localized to apical tight junctions in epithelia and are necessary for normal tight junctions [16•–19•]. In *Drosophila*, all three proteins physically interact, as shown by *in vitro* and *in vivo* experiments [12••,13••], and all are apical from late interphase until late anaphase, at which time they are degraded or delocalized [9–11,12••,13••]. Thus with every neuroblast division, apical localization of Baz, Par6 and aPKC must be re-established.

Two mechanisms are probably used for Baz, Par6 and aPKC localization: an epithelial-based mechanism for the initial, pre-division apical localization in newly delaminated

Figure 1

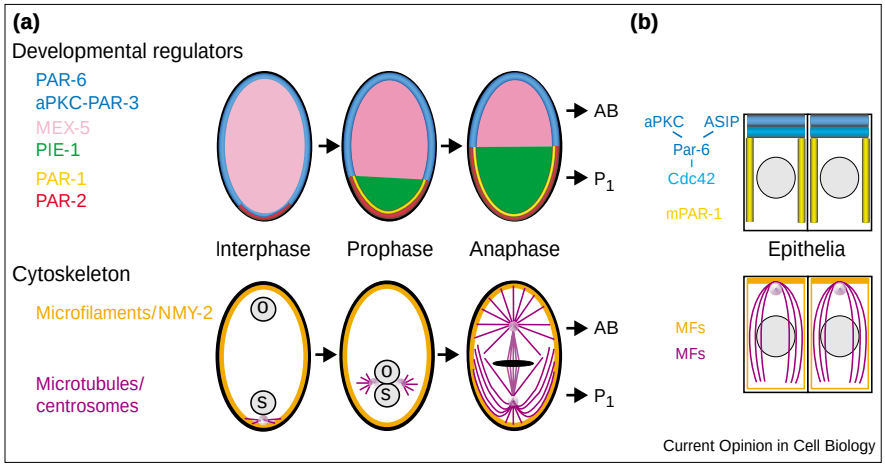


Formation and asymmetric division of *Drosophila* neuroblasts. The cells are oriented so that the apical region of each cell points towards the top of the page. Proteins and mRNAs known to have a polarized localization are show in (**a**), with physical interactions indicated by dashes between the proteins in the key. The cytoskeleton is shown in (**b**). The organization of the MT cytoskeleton in the ectoderm and delaminating neuroblast (left panel) is based on our unpublished data (S Siegrist, K Siller, CQ Doe). Basal centrosomes have been observed in the ectoderm as well [40•]. See text for further details.

neuroblasts, and a different mechanism for apical localization following each neuroblast cell division. The epithelial mechanism of apical protein localization probably requires the cortical tumor suppressor genes *discs large* (*dlg*), *lethal giant larvae* (*lgl*), and *scribble* (*scrib*), which control the apical targeting of many epithelial proteins [20•] (see also Update). On the basis of *in vitro* drug studies, the non-epithelial mechanism of apical protein localization clearly requires microfilaments (MFs), and microtubules (MTs) may also play a role [21].

Although Baz, DmPar-6 and aPKC are the first apical proteins detected in delaminating neuroblasts [9–11,12••,13••], the Inscuteable (Insc) and Partner of Inscuteable (Pins) proteins join the apical protein complex during or just after delamination and are required to maintain apical localization of the entire complex [22,23••–25••]. It is not known whether Baz, DmPar-6 and aPKC are apically localized before Insc/Pins in subsequent neuroblast divisions. Insc contains a central domain with five ankyrin-like repeats that is sufficient and necessary for its localization and

Figure 2



Polarization of the (**a**) *C. elegans* zygote and of (**b**) mammalian epithelia. For *C. elegans*, the cells are oriented so that the anterior region of each cell points towards the top of the page; for epithelia, the apical region points toward the top of the page. Developmental regulators are show in the upper panels and the cytoskeleton in the lower panels. Color coding is used throughout, with protein identities listed to the left in each panel. Pronuclei and nuclei are shown as grey circles; s is for sperm and o for oocyte pronuclei. In a *C. elegans* zygote, recruitment of PAR-2 to the cortex (upper panel) by sperm pronucleus-associated astral MTs (lower panel) may initiate axis formation. The recruitment of PAR-1 to this patch may downregulate cytoplasmic MEX-5, permitting expression of the germline determinant PIE-1 posteriorly. This region, in a MF-requiring process, expands to encompass the posterior half of the zygote. See text for details.

function [26,27]. The Pins amino terminus binds Insc and contains seven tetratrico-peptide repeats [24••], whereas its carboxyl terminus binds to Gα proteins *in vitro* and *in vivo* [23••,25••]; however, a role for G-protein function has yet to be demonstrated in neuroblasts.

The five proteins in the apical complex are all required for the maintenance of the others at the apical neuroblast cortex; without Baz, DmPar-6 or aPKC the apical protein complex never forms, and without Insc or Pins the complex forms, but at a very reduced level. All apical complex single mutants have the same phenotype: randomized spindle orientation and failure to reliably localize cell fate determinants to the basal cortex of the neuroblast (see below). This suggests that apical localization of these five proteins is essential for their function — otherwise, single mutants would have distinctive phenotypes — and makes it difficult to determine which apical protein is most directly required for spindle orientation or basal protein localization.

Baz, DmPar-6 and aPKC are apical in the *Drosophila* neuroblast, and a related complex of cortical proteins — PAR-3, PAR-6 and PKC-3 — is restricted to the anterior half of the *C. elegans* zygote, shortly after fertilization and in response to sperm cues. These three proteins all share a common boundary roughly midway along the a–p axis (Figure 2), and consistent with their precise colocalization, PAR-3 and PKC-3 bind to each other *in vitro*. Two recent observations suggest that the sperm-donated centrosome is required for initiation of a–p axis formation in *C. elegans* and thereby specifies the anterior localization of PAR-3, PAR-6 and PKC-3. The first observation was that anucleate mutant sperm could still fertilize oocytes, donate their single centrosome and specify a normal a–p axis [28•]. Also, sperm-aster-directed MT assembly appears to be necessary for specification of the posterior pole [29•,30••].

Although sperm-derived MT organizing centers may initiate the establishment of a posterior pole, MFs are required to elaborate the putative sperm astral signal into a fully polarized a–p axis (see final section below). Although both the *C. elegans* zygote and *Drosophila* neuroblasts require MFs for asymmetric protein localization, a role for MTs in neuroblasts is less clear. Given that for many years experimental manipulations implicated only MFs, and not MTs, in the formation of the a–p axis in *C. elegans*, this difference between the fly and worm could prove to be transient.

In addition to requiring the MT- and MF-dependent specification of a posterior pole, anterior cortical protein localization in the *C. elegans* zygote requires further regulatory processes, including interactions among the anterior proteins themselves. Both PAR-6 and PKC-3 absolutely require PAR-3 for cortical localization, whereas some cortical PAR-3 can be transiently detected in the absence of PAR-6 or PKC-3 in early P_0 embryos [31]. Thus PAR-3 may initiate assembly of an anterior cortical complex, with maintenance being more interdependent. This interdependence is similar to that reported for apical complex proteins in *Drosophila* neuroblasts.

Functions of the apical/anterior protein complex

In *Drosophila* embryonic neuroblasts, the Baz, DmPar-6 and aPKC apical complex regulates basal protein localization and spindle orientation; in *C. elegans* zygotes, the PAR-3, PAR-6 and PKC-3 anterior complex has similar but distinct roles in posterior protein localization and spindle orientation. In the following two sections we discuss similarities and differences in how this evolutionarily conserved protein complex functions in the neuroblast and zygote.

Basal/posterior protein localization

Wild-type *Drosophila* neuroblasts have two protein complexes that are basally localized during mitosis: the 'Miranda complex' and the 'Numb complex.' The Miranda complex contains Miranda (a cortically associated coiled-coil domain protein), Prospero (a homeodomain-containing transcription factor that binds to Miranda and requires it for cortical association), Staufen (an RNA-binding protein that also binds to Miranda and requires it for cortical localization), and *prospero* mRNA (which requires Staufen for its localization). It is not known whether both Prospero and Staufen can bind to Miranda simultaneously. The Miranda mRNA–protein complex is apical at late interphase, and it translocates to the basal cortex during prophase of mitosis [6,8]. The 'Numb' complex contains Numb protein, which can affect cell fate by binding to and inhibiting Notch signaling, and its cortical-anchoring protein Partner of Numb (Pon); these proteins are cytoplasmic at interphase and are recruited to the basal cortex during early mitosis [6,8].

In neuroblasts lacking the Baz, DmPar-6, aPKC, Insc and Pins apical complex, Miranda and Numb complexes either become uniformly cortical or form randomly positioned crescents that frequently are not associated with spindle poles [9–11,12••,13••,22,23••,24••]. Similarly, larval neuroblasts with reduced Pins levels show defects in Prospero localization, and possibly an equalization of neuroblast/GMC sizes [25••]. Intriguingly, the movement of Miranda complex proteins from the apical side of interphase neuroblasts to the basal cortex of mitotic neuroblasts fails to occur in G_2-arrested neuroblasts, revealing a role for the cell cycle in regulating basal protein transport [32,33]. Aside from these requirements, the mechanism of basal protein localization remains a mystery (see also Update). It is unlikely that apical and basal protein domains are formed by mutual exclusion, as appears to take place with the anterior and posterior cortical protein domains in *C. elegans* zygotes (see below). It is unlikely for three reasons: a sizeable gap occurs between the detectable boundaries of apical and basal protein crescents in neuroblasts, which would not be expected if basal proteins were excluded from the cortex by apical proteins or vice versa; fly embryos lacking apical proteins often still form randomly positioned crescents of the normally basal protein complexes; and the mutational elimination of basal complex

proteins does not perturb either apical complex formation or its boundaries.

When the *C. elegans* PAR-3, PAR-6 and PKC-3 complex becomes localized to the anterior cortex of the zygote, a ring-finger protein called PAR-2 and a serine/threonine kinase called PAR-1 accumulate in the posterior cortex [34,35]. The posterior boundary of the PAR-3, PAR-6 and PKC-3 complex abuts precisely with the anterior boundary of PAR-2 (Figure 2), and removal of PAR-2, but not PAR-1, results in a posterior expansion of PAR-3 [31,36,37]. Conversely, the PAR-1 and PAR-2 boundaries, which presumably coincide, move towards the anterior following inactivation of PAR-3 [31,35,36]. These results are consistent with the anterior and posterior membrane domains establishing their abutting boundary in part by mutual exclusion, which may involve at least PAR-2 and PAR-3. The anterior expansion of cortical PAR-2 in *par-3*, *par-6* or *pkc-3* mutants is similar to the expansion of basal proteins into the apical cortex of *Drosophila* neuroblasts after mutational inactivation of apical complex proteins [9–11,12••,13••,22,23••,24••].

Other genes are required for the mutually exclusive distributions of some PAR proteins. The *par-4* gene encodes a putative serine/threonine kinase that is present at the cortex throughout P_0. The cortical localization of PAR-4 is unaffected by the removal of the other PAR proteins [38•], but in *par-4* mutants, PAR-3 and PAR-6 expand posteriorly to cover roughly 85% of egg length [37] without affecting PAR-2 distribution [35], thereby abolishing the mutual exclusivity of PAR-2 and PAR-3. The mutual exclusivity of PAR-2 and PAR-3 also requires a gene called *pod-1* (*partitioning–osmotic defect 1*): both PAR-3 and PAR-1 are uniformly cortical in *pod-1* mutant embryos [29•]. POD-1 is related to coronin, and, like coronin, it is a MF-binding protein. It is anteriorly localized, with a boundary that appears to coincide with that of PAR-3, PAR-6 and PKC-3. Intriguingly, POD-1 seems to function primarily in membrane trafficking, thus implicating both secretion and MFs in the establishment or maintenance of a–p polarity [39]. Perhaps the anteriorly restricted POD-1 somehow polarizes membrane trafficking or protein transport to establish or maintain the asymmetric distributions of at least some PAR proteins.

Spindle orientation

For reliable asymmetric cell division, the mitotic spindle must be aligned with the axis of cell polarization, which leads to the question of how this is accomplished in the neuroblast or zygote. In *Drosophila*, time-lapse imaging with a GFP-tagged MT-binding protein was used to show that the neuroblast spindle initially aligns perpendicular to the apical/basal axis, but then rotates 90° during metaphase to align with the apical/basal axis [40•]. Spindle rotation is rapid and occurs after the apical protein complex is established, and at about the same time as basal protein localization occurs. Neuroblasts lacking functional apical complex genes (*baz*/*DmPar-6*/*aPKC*/*insc*/*pins*) show random spindle orientation [9–11,12••,13••,22,23••,24••].

In epithelia, Baz, DmPar-6 and aPKC are apically localized, but Insc is not expressed and Pins is cytoplasmic, and the spindle is perpendicular to the apical/basal axis. However, misexpression of Insc in epithelia can induce apical localization of Pins and reorientation of mitotic spindles along the apical/basal axis [9–11,12••,13••,22,23••,24••]. Thus, Insc and Pins are necessary for the correct spindle orientation in neuroblasts, and, along with Baz, DmPar-6 and aPKC, are sufficient to induce apical/basal spindle orientation in epithelia. It is not known whether Insc or Pins provides the most direct link to the mitotic spindle.

In the post-fertilization *C. elegans* zygote, the maternal pronucleus migrates to meet its paternal partner near the posterior pole, followed by formation of the first mitotic spindle [41] (Figure 2). The sperm-derived asters first align perpendicular to the a–p axis, but as the spindle forms it moves towards the center of the zygote and rotates 90° to lie along the a–p axis. The spindle then elongates during anaphase and moves posteriorly, resulting in the birth of the larger, anterior AB blastomere and the smaller posterior P_1 blastomere. A reduced level of either dynein or dynactin (a dynein cofactor) prevents rotation of the first mitotic spindle [42,43]. The heavy chain of dynein, a multi-protein minus-end-directed MT motor, is cytoplasmic but enriched at the periphery of the maternal and paternal pronuclei during pronuclear migration and is detected at the cell cortex during rotation of the first mitotic spindle [43].

Loss of anterior protein complex function (PAR-3, PAR-6 and PKC-3) does not affect spindle rotation in P_0, but does prevent posterior spindle displacement, resulting in daughter cells of equal size. In wild-type embryos, the larger AB divides before P_1, and the mitotic spindle in P_1 rotates to lie along the a–p axis, orthogonal to the transversely oriented spindle in AB. In *par-3*, *par-6* or *pkc-3* mutant embryos, both two-cell stage daughters divide synchronously, and both their mitotic spindles rotate and align with the a–p axis. Thus, the *C. elegans* apical complex is required both to alter spindle positioning along the a–p axis without affecting rotation in P_0, and to block spindle rotation in AB. This is quite different from the function of the apical protein complex in *Drosophila* neuroblasts, which is required for aligning the spindle along the apical/basal axis but not for cell size asymmetry. How this conserved protein complex has evolved so that it influences spindle orientation in these different ways is not understood. Perhaps the function of Insc and Pins accounts for these variations, as these genes have not been identified in *C. elegans*.

In summary, there are clear similarities between the function of anterior/apical protein complexes in *Drosophila* and *C. elegans*, but there are key differences. Similarities include the role of both protein complexes in regulating the mitotic spindle position (although in different ways) and in polarizing protein localization to the opposite side of the cell.

Differences include the precise effect on the complex on the spindle, the cues that initiate apical/anterior complex localization, and the identity of the basal/posterior proteins that require the apical complex for proper localization.

Spindle asymmetry and unequal cell division

In neuroblasts, the mitotic spindle becomes asymmetric soon after it aligns along the apical/basal axis. In both embryonic and larval neuroblasts, the basal centrosome and astral MTs disappear by late telophase [40•]; this explains why larval GMCs, which inherit the basal spindle pole, divide with an asterless, centrosome-free spindle [44•]. In addition, both embryonic and larval neuroblasts appear to have longer kinetochore MTs in the apical half of the spindle, thereby shifting the spindle midbody towards the basal side of the cell (Figure 1; [40•,44•]). Unequal midbody positioning may trigger eccentric cleavage furrow positioning and thus cause the physically asymmetric neuroblast division. A similar asymmetry in astral MTs is observed during *C. elegans* early embryonic divisions (see below), but there does not appear to be any difference in kinetochore MT length, and thus asymmetric division is solely due to eccentric positioning of the symmetrical spindle in worms [41].

What is the mechanism for shifting the spindle towards the posterior cortex in P_0? Mammalian Par-1 homologues can destabilize MTs by phosphorylating MT-associated proteins [45]; therefore perhaps *C. elegans* PAR-1 destabilizes posterior astral MTs, thereby shifting the spindle posteriorly. A quantitative analysis of astral MT length and dynamics in wild-type and *par-1* mutant embryos is not yet available but would address this possibility. It is intriguing that *Drosophila* Par-1 is posteriorly localized in the oocyte, regulates MT organization and is required for specification of the posterior pole [46•,47•]. Finally, recent genetic screens have identified several *C. elegans* mutants with mitotic spindle assembly and positioning defects [48–51]. As the sequences of these newly identified loci are revealed, the mechanisms that coordinate the polarization of developmental potential along the a–p axis with mitotic spindle alignment in the early *C. elegans* embryo should become clearer.

Microfilaments and the establishment of cell polarity

Drug studies first showed that MFs are essential for establishing a–p polarity in *C. elegans* [52]. More recently, further insight into the role that MFs play in establishing an a–p axis has come from an analysis of both NMY-2, a type II non-muscle myosin heavy chain that binds to PAR-1, and MLC-4, the non-muscle myosin regulatory light chain. If either NMY-2 or MLC-4 function is eliminated, many, but not all signs, of a–p polarity are lost [53,54]. In particular, PAR-2 still localizes to the zygote cortex posteriorly, but only in a small patch near the sperm pronucleus [54]. Thus sperm astral MTs may promote an association of PAR-2 with the adjacent cortex [29•,30••], but MF motor activity is required for the patch to expand [29•,54]. Intriguingly, PAR-2 is required for the cortical localization of PAR-1, and the carboxyl terminus of PAR-1 can bind to NMY-2. Once a sufficiently large PAR-2 patch assembles over the sperm asters, perhaps it can recruit enough PAR-1 to the posterior cortex to influence NMY-2 function.

At the anterior cortex, a different mechanism may regulate the MF cytoskeleton. The *C. elegans* anterior complex proteins, PAR-3, PAR-6 and PKC-3, have vertebrate homologues, ASIP, Par-6 and aPKC, which are apically localized in epithelia (Figure 2). Vertebrate Par-6 can bind to the small G proteins Cdc42 and Rac1, which are ubiquitous MF regulators [16•–19•]. These protein interactions appear to be important both for epithelial cell polarity [16•], and for preventing abnormal cell proliferation [19•]. It would be worth exploring whether *C. elegans* PAR-6 binds to *C. elegans* Cdc42 or Rac1 proteins to regulate the MF cytoskeleton at the anterior cortex. Perhaps these two different links to cortical MFs — PAR-1/NMY-2 and PAR-6/CDC-42 — mediate the mutual exclusion of PAR-2 and PAR-3, and the expansion of an initially small patch of cortical PAR-1/PAR-2 to fully and exclusively encompass the posterior pole (Figure 2).

In *Drosophila*, MFs are essential for apical localization of Insc and the basal localization of Miranda complex proteins [21]; but the MF dependence of Baz, DmPar-6 and aPKC has not been assayed. Intriguingly, the conserved apical protein complex in *Drosophila* neuroblasts contains both DmPar-6 and Pins (which binds to Gα proteins *in vivo* and *in vitro*). It will be interesting to determine if DmPar-6 and/or Pins recruit or modulate small G protein functions to regulate the MF cytoskeleton and cell polarity in *Drosophila* neuroblasts.

Generation of cell diversity

The ultimate role of asymmetric cell division is to create two distinct cell types. How are the distinct neuroblast/GMC or AB/P_1 cell fates established? There are no cell fate determinants known to be localized to the neuroblast during asymmetric division: all apical proteins are degraded or delocalized before cleavage. In contrast, several cell fate determinants localize to the basal cortex of mitotic neuroblasts and segregate specifically into the GMC. The best characterized example is Prospero, which is required for GMC-specific gene expression and triggering exit from the mitotic cell cycle [6,8]. The Numb cell fate determinant is also segregated into the GMC, where it can be asymmetrically localized in the following GMC division to distinguish sibling neuron fates [55].

In *C. elegans*, PAR-1 controls the distribution of two nearly identical and partially redundant 'CCCH finger' proteins, MEX-5 and MEX-6 [56••,57]. These two proteins then influence the function of multiple embryonic determinants of cell fate. MEX-5 itself is polarized to the anterior cytoplasm of P_0 as it divides (Figure 2). The MEX-5 boundary coincides precisely with the boundary of the abutting cortical PAR domains, and PAR-1 is required to

exclude MEX-5 from P_1. Finally, a deletion analysis of the transcription repressor PIE-1 (pharyngeal and intestinal excess 1), one target of MEX-5 and MEX-6, strongly implicates differential protein stability as a mechanism for generating asymmetric distributions of PIE-1 and other determinants in response to MEX-5 and MEX-6 [57,58••]. These downstream pathways are only beginning to be defined in both flies and worms, but it seems likely that they will diverge substantially given the very different cellular and embryonic contexts in which they occur.

Conclusions and future directions

Recent work has revealed a conserved apical/anterior protein complex that regulates cell polarity in *Drosophila* neuroblasts, *Drosophila* epithelia, mammalian epithelia and *C. elegans* zygotes. Different proteins are known to interact with this protein complex in each cell-type, and this may allow a common cell polarity system to regulate cell-type-specific properties such as maintaining different membrane domains in epithelia or regulating spindle orientation and the generation of cell diversity in neuroblasts and zygotes.

Despite considerable recent progress in the understanding of asymmetric cell division, plenty of questions remain. In *Drosophila* neuroblasts, no single protein maintains an apical localization past telophase, so how is apical/basal polarity re-established at each neuroblast cell division? What are the anchors and motors that bind to Miranda or Pon and deliver them to the basal cortex, and how is this process triggered by entry into mitosis? In both *Drosophila* and *C. elegans*, how do apical/anterior proteins drive spindle rotation, and what are the cortical anchors and what are the motors? What is the cause and effect of each aspect of spindle asymmetry? Given the rapid pace of this field, we expect answers to many of these questions soon, perhaps by the time you read this review!

Update

Recent work reveals a role for the tumor suppressor genes *lgl* and *dlg* in establishing a neuroblast cell polarity. Although they function in epithelial cells to restrict proteins to the apical membrane domain [20•], in neuroblasts they are required to restrict proteins such as Miranda, Prospero and Pon to the basal cortex. These proteins relocate uniformly around the cortex or onto the mitotic spindle and centrosomes in the absence of *lgl* or *dlg* function [59•,60•].

Acknowledgements

We thank Ken Kemphues at Cornell University for sharing ideas and information, and Ken Kemphues, Rebecca Lyczak, Aaron Severson, and Karsten Siller for comments on the manuscript.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Sulston JE, Schierenberg E, White JG, Thomson JN: The embryonic cell lineage of the nematode ***Caenorhabditis elegans***. *Dev Biol* 1983, 100:64-119.

2. Priess JR, Thomson JN: Cellular interactions in early ***C. elegans*** embryos. *Cell* 1987, 48:241-250.

3. Bowerman B, Shelton CA: Cell polarity in the early ***Caenorhabditis elegans*** embryo. *Curr Opin Genet Dev* 1999, 9:390-395.

4. Schierenberg E: Reversal of cellular polarity and early cell-cell interaction in the embryos of ***Caenorhabditis elegans***. *Dev Biol* 1987, 122:452-463.

5. Hawkins N, Garriga G: Asymmetric cell division: from A to Z. *Genes Dev* 1998, 12:3625-3638.

6. Jan YN, Jan L: Polarity in cell division: what frames thy fearful asymmetry? *Cell* 2000, 100:599-602.

7. Lin H: The self-renewing mechanism of stem cells in the germline. *Curr Opin Cell Biol* 1998, 10:687-693.

8. Matsuzaki F: Asymmetric division of ***Drosophila*** neural stem cells: a basis for neural diversity. *Curr Opin Neurobiol* 2000, 10:38-44.

9. Kuchinke U, Grawe F, Knust E: Control of spindle orientation in ***Drosophila*** by the Par-3-related PDZ-domain protein Bazooka. *Curr Biol* 1998, 8:1357-1365.

10. Schober M, Schaefer M, Knoblich JA: Bazooka recruits Inscuteable to orient asymmetric cell divisions in ***Drosophila*** neuroblasts. *Nature* 1999, 402:548-551.

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

12. •• Petronczki M, Knoblich JA: DmPAR-6 directs epithelial polarity and asymmetric cell division of neuroblasts in ***Drosophila***. *Nat Cell Biol* 2000, in press.
See annotation to [13••].

13. •• Wodarz A, Ramath A, Grimm A, Knust E: ***Drosophila*** atypical protein kinase C associates with Bazooka and controls polarity of epithelia and neuroblasts. *J Cell Biol* 2000, 150:1361-1374.
These two papers [12••,13••] identify *Drosophila* proteins (DmPar-6 and aPKC) related to the *C. elegans* Par-3 binding proteins, Par-6 and PKC-3, respectively. Both *Drosophila* proteins bind Baz and are apically localized in neuroblasts and epithelia; these proteins appear to be interdependent for localization and required for the subsequent localization of Insc and Pins in neuroblasts. In epithelia, they are required for maintaining apical/basal polarity, and in neuroblasts they are necessary for spindle orientation and basal protein localization.

14. • Nakaya M, Fukui A, Izumi Y, Akimoto K, Asashima M, Ohno S: Meiotic maturation induces animal-vegetal asymmetric distribution of aPKC and ASIP/PAR-3 in ***Xenopus*** oocytes. *Development* 2000, 127:5021-5031.
See annotation to [15•].

15. • Choi S, Ki J, Han J: Identification and developmental expression of par-6 gene in ***Xenopus laevis***. *Mech Dev* 2000, 91:347-350.
These papers [14•,15•] show that the Par-3/Par-6/aPKC proteins are localized asymmetrically in *Xenopus* oocytes (to the animal pole), extending the number of organisms and developmental contexts where these proteins may act to regulate cell polarity. The function of these proteins in *Xenopus* has not yet been tested, however.

16. • Joberty G, Petersen C, Gao L, Macara IG: The cell-polarity protein Par6 links Par3 and atypical protein kinase C to Cdc42. *Nat Cell Biol* 2000, 2:531-539.
The authors identify human PAR-6 family members that form a multiprotein complex with human PAR-3, aPKC isoforms and Cdc42. Overexpression of human PAR-6 (but not of a mutant with an altered PDZ domain that prevents interaction with PAR-3), of the amino-terminal fragment of PAR-3, or of an activated Cdc42 in cultured epithelial cells leads to a loss these and other proteins from tight junctions and disruption of cell contacts.

17. • Johansson A, Driessens M, Aspenstrom P: The mammalian homologue of the ***Caenorhabditis elegans*** polarity protein PAR-6 is a binding partner for the Rho GTPases Cdc42 and Rac1. *J Cell Sci* 2000, 113:3267-3275.
The authors identify a mammalian PAR-6 homologue that can bind a relative of PAR-3, Cdc-42 and Rac1. In cultured epithelial cells, endogenous PAR-6 was found not only at cell junctions, but also in the nucleus, whereas PAR-3 was present only at cell junctions, colocalized with PAR-6.

18. • Lin D, Edwards AS, Fawcett JP, Mbamalu G, Scott JD, Pawson T: A mammalian PAR-3-PAR-6 complex implicated in Cdc42/Rac1 and aPKC signalling and cell polarity. *Nat Cell Biol* 2000, 2:540-547.
The authors identify mouse PAR-3 and PAR-6 relatives that bind to each other through PDZ domains and exhibit similar distribution and subcellular

localization within the CNS. mPAR-3 and mPAR-6 independently bind atypical protein kinase C isoforms, and mPAR-6 binds to Cdc42 and Rac1 GTPases. mPAR-3 can act as both a substrate and inhibitor of an atypical protein kinase C *in vitro*.

19. Qiu RG, Abo A, Steven Martin G: A human homolog of the
• ***C. elegans*** polarity determinant Par-6 links Rac and Cdc42 to PKCzeta signaling and cell transformation. *Curr Biol* 2000, 10:697-707.
The authors identify a human PAR-6 that can bind to Cdc42. The human PAR-6 binds to both Cdc42 and Rac1 in a GTP-dependent manner and can also bind an atypical protein kinase C isoform. These proteins can form a stable ternary complex, stimulating aPKC activity *in vitro*. Human PAR-6 also potentiates the ability of Rac1/Cdc42 to transform cultured cells into a more proliferative state. This potentiation requires the Rac1/Cdc42-interacting CRIB domain of hPAR-6.

20. Bilder D, Li M, Perrimon N: Cooperative regulation of cell polarity
• and growth by ***Drosophila*** tumor suppressors. *Science* 2000, 289:113-116.
This work describes the function of the *discs large, lethal giant larvae*, and *scribble* tumor suppressor genes in regulating apical, but not basolateral, protein localization in *Drosophila* epithelia.

21. Broadus J, Doe CQ: Extrinsic cues, intrinsic cues and microfilaments regulate asymmetric protein localization in ***Drosophila*** neuroblasts. *Curr Biol* 1997, 7:827-835.

22. Kraut R, Chia W, Jan LY, Jan YN, Knoblich JA: Role of inscuteable in orienting asymmetric cell divisions in ***Drosophila***. *Nature* 1996, 383:50-55.

23. Schaefer M, Shevchenko A, Knoblich JA: A protein complex
•• containing Inscuteable and the Galpha-binding protein Pins orients asymmetric cell divisions in ***Drosophila***. *Curr Biol* 2000, 10:353-362.
See annotation to [25••].

24. Yu F, Morin X, Cai Y, Yang X, Chia W: Analysis of partner of
•• inscuteable, a novel player of ***Drosophila*** asymmetric divisions, reveals two distinct steps in inscuteable apical localization. *Cell* 2000, 100:399-409.
See annotation to [25••].

25. Parmentier ML, Woods D, Greig S, Phan PG, Radovic A, Bryant P,
•• O'Kane CJ: Rapsynoid/Partner of Inscuteable controls asymmetric division of larval neuroblasts in ***Drosophila***. *J Neurosci* 2000, 20:RC84.
The authors of these three papers [23••–25••] use preparative immunoprecipitation and mass spectroscopy [23••] or yeast two-hybrid [24••,24••] experiments to identify the Insc-binding Pins protein and the Giα protein. Pins binds directly to Insc, and Giα binds Pins. Removal of maternal and zygotic Pins leads to failure to maintain Baz/Insc apical localization and defects in spindle orientation. The localization and function of Giα was not determined.

26. Knoblich JA, Jan LY, Jan YN: Deletion analysis of the ***Drosophila*** Inscuteable protein reveals domains for cortical localization and asymmetric localization. *Curr Biol* 1999, 9:155-158.

27. Tio M, Zavortink M, Yang X, Chia W: A functional analysis of inscuteable and its roles during ***Drosophila*** asymmetric cell divisions. *J Cell Sci* 1999, 112:1541-1551.

28. Sadler PL, Shakes DC: Anucleate ***Caenorhabditis elegans*** sperm
• can crawl, fertilize oocytes and direct anterior-posterior polarization of the 1-cell embryo. *Development* 2000, 127:355-366.
The authors show that the *emb-27* and *emb-30* genes are required paternally for embryogenesis in *C. elegans*. Males with mutations in these genes produce mostly anucleate sperm that nevertheless retain a centriole. In addition to activating the completion of meiosis by the maternal pronucleus, and the production of an eggshell by the zygote, these anucleate sperm can also induce an apparently normal a–p axis.

29. O'Connell KF, Maxwell KN, White JG: The spd-2 gene is required
• for polarization of the anteroposterior axis and formation of the sperm asters in the ***Caenorhabditis elegans*** zygote. *Dev Biol* 2000, 222:55-70.
The *C. elegans* gene *spd-2* is required maternally. In *spd-2* mutant embryos, pronuclei migrate and meet in the middle of the zygote, but the sperm asters fail to develop during this time. In addition to their failure to form sperm asters in early zygotes, *spd-2* mutant embryos suffer an extensive loss of a–p polarity. In addition, cytoplasmic streaming fails, P-granules are not localized posteriorly, PAR-3 remains cortical throughout the zygote and PAR-2 is present either at both poles or distributed throughout the cortex. These findings provided the first genetic evidence that sperm aster development is required for establishment of the a–p axis in *C. elegans*.

30. Wallenfang MR, Seydoux G: Polarization of the anterior-posterior
•• axis of ***C. elegans*** is a microtubule-directed process. *Nature* 2000, 408:89-92.
Using a genetic approach, the authors document a requirement for sperm aster MTs in specifying the posterior pole. They show that in mutant embryos arrested at metaphase in meiosis, sperm astral MTs do not form, and the persistent meiotic spindle can specify some but not all features of a posterior pole. This reverses the normal axis of polarity, as the maternal pronucleus is usually present at the pole opposite the sperm pronucleus [25••]. Chemical depolymerization of MTs blocks the specification of a posterior pole by the meiotic spindle in these mutants. Genetically preventing entry into meiosis by reducing the function of a cdc2 homolog, also blocks the specification of a posterior pole by the maternal pronucleus in meiotic metaphase arrest mutants. Finally, axis formation is prevented entirely by reducing the function of an Aurora kinase family member that is required for duplication of the sperm-donated centrosome and assembly of the first mitotic spindle.

31. Tabuse Y, Izumi Y, Piano F, Kemphues KJ, Miwa J, Ohno S: Atypical protein kinase C cooperates with PAR-3 to establish embryonic polarity in ***Caenorhabditis elegans***. *Development* 1998, 125:3607-3614.

32. Knoblich JA, Jan LY, Jan YN: Asymmetric segregation of Numb and Prospero during cell division. *Nature* 1995, 377:624-627.

33. Spana EP, Doe CQ: The prospero transcription factor is asymmetrically localized to the cell cortex during neuroblast mitosis in ***Drosophila***. *Development* 1995, 121:3187-3195.

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

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

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

37. Hung TJ, Kemphues KJ: PAR-6 is a conserved PDZ domain-containing protein that colocalizes with PAR-3 in ***Caenorhabditis elegans*** embryos. *Development* 1999, 126:127-135.

38. Watts JL, Morton DG, Bestman J, Kemphues KJ: The ***C. elegans***
• par-4 gene encodes a putative serine-threonine kinase required for establishing embryonic asymmetry. *Development* 2000, 127:1467-1475.
The authors report the sequencing and localization of PAR-4, a predicted Ser/Thr kinase. Its closest relatives are *Xenopus* and human proteins of unknown function, although mutations in the human gene are associated with Peutz-Jeghers syndrome. PAR-4, like other PAR proteins, is enriched at the cell cortex by the time pronuclei meet. The distribution of PAR-4 is not polarized; it is present throughout the cortex of all embryonic cells. Mutations in the other *par* genes do not affect PAR-4 localization.

39. Rappleye CA, Paredez AR, Smith CW, McDonald KL, Aroian RV: The coronin-like protein POD-1 is required for anterior-posterior axis formation and cellular architecture in the nematode ***Caenorhabditis elegans***. *Genes Dev* 1999, 13:2838-2851.

40. Kaltschmidt JA, Davidson CM, Brown NH, Brand AH: Rotation and
• asymmetry of the mitotic spindle direct asymmetric cell division in the developing central nervous system. *Nat Cell Biol* 2000, 2:7-12.
This paper and that of Bonaccorsi *et al.* [44•] show that the mitotic spindle is clearly asymmetric in embryonic [40•] and larval [44•] neuroblasts, although the functional significance of the asymmetries was not investigated. In this paper, GFP–tau labeled MTs are imaged in living embryos to reveal that the neuroblast spindle is first oriented perpendicular to the apical/basal axis before rotating to an apical/basal orientation. Both papers show that the basal side of the spindle (destined for the GMC) has shorter kinetochore and astral MTs and a smaller or undetectable centrosome. In addition, reference [44•] shows that the larval GMC spindle appears to be anastral.

41. Albertson DG, Thomson JN: Segregation of holocentric chromosomes at meiosis in the nematode, ***Caenorhabditis elegans***. *Chromosome Res* 1993, 1:15-26.

42. Skop AR, White JG: The dynactin complex is required for cleavage plane specification in early ***Caenorhabditis elegans*** embryos. *Curr Biol* 1998, 8:1110-1116.

43. Gonczy P, Pichler S, Kirkham M, Hyman AA: Cytoplasmic dynein is required for distinct aspects of MTOC positioning, including centrosome separation, in the one cell stage ***Caenorhabidits elegans*** embryo. *J Cell Biol* 1999, 147:135-150.

44. Bonaccorsi S, Giansanti MG, Gatti M: Spindle assembly in
• ***Drosophila*** neuroblasts and ganglion mother cells. *Nat Cell Biol* 2000, 2:54-56.
See annotation [36•].

45. Drewes G, Ebneth A, Preuss U, Mandelkow EM, Mandelkow E: MARK, a novel family of protein kinases that phosphorylate microtubule-associated proteins and trigger microtubule disruption. *Cell* 1997, 89:297-308.

46. Shulman JM, Benton R, St Johnston D: The ***Drosophila*** homolog of
• ***C. elegans*** PAR-1 organizes the oocyte cytoskeleton and directs oskar mRNA localization to the posterior pole. *Cell* 2000, 101:377-388.
See annotation [47•].

47. Tomancak P, Piano F, Riechmann V, Gunsalus KC, Kemphues KJ,
• Ephrussi A: A ***Drosophila melanogaster*** homologue of ***Caenorhabditis elegans*** par-1 acts at an early step in embryonic-axis formation. *Nat Cell Biol* 2000, 2:458-460.
These two papers [46•,47•] identify a *Drosophila* PAR-1 relative that is localized to the posterior pole of the fly oocyte and, as in worms, is required for establishing the posterior pole of the body axis. Consistent with a role in regulating MT dynamics, abnormalities in MT organization are observed in mutant fly embryos lacking PAR-1 function.

48. O'Connell KF, Leys CM, White JG: A genetic screen for temperature-sensitive cell-division mutants of ***Caenorhabditis elegans***. *Genetics* 1998, 149:1303-1321.

49. Gonczy P, Schnabel H, Kaletta T, Amores AD, Hyman T, Schnabel R: Dissection of cell division processes in the one cell stage ***Caenorhabditis elegans*** embryo by mutational analysis. *J Cell Biol* 1999, 144:927-946.

50. Pichler S, Gonczy P, Schnabel H, Pozniakowski A, Ashford A, Schnabel R, Hyman AA: OOC-3, a novel putative transmembrane protein required for establishment of cortical domains and spindle orientation in the P(1) blastomere of ***C. elegans*** embryos. *Development* 2000, 127:2063-2073.

51. Basham SE, Rose LS: Mutations in ooc-5 and ooc-3 disrupt oocyte formation and the reestablishment of asymmetric PAR protein localization in two-cell ***Caenorhabditis elegans*** embryos. *Dev Biol* 1999, 215:253-263.

52. Hill DP, Strome S: Brief cytochalasin-induced disruption of microfilaments during a critical interval in 1-cell ***C. elegans*** embryos alters the partitioning of developmental instructions to the 2-cell embryo. *Development* 1990, 108:159-172.

53. Guo S, Kemphues KJ: A non-muscle myosin required for embryonic polarity in ***Caenorhabditis elegans***. *Nature* 1996, 382:455-458.

54. Shelton CA, Carter JC, Ellis GC, Bowerman B: The nonmuscle myosin regulatory light chain gene mlc-4 is required for cytokinesis, anterior-posterior polarity, and body morphology during ***Caenorhabditis elegans*** embryogenesis. *J Cell Biol* 1999, 146:439-451.

55. Buescher M, Yeo SL, Udolph G, Zavortink M, Yang X, Tear G, Chia W: Binary sibling neuronal cell fate decisions in the ***Drosophila*** embryonic central nervous system are nonstochastic and require inscuteable-mediated asymmetry of ganglion mother cells. *Genes Dev* 1998, 12:1858-1870.

56. Schubert CM, Lin R, de Vries CJ, Plasterk RH, Priess JR: MEX-5 and
•• MEX-6 function to establish soma/germline asymmetry in early ***C. elegans*** embryos. *Mol Cell* 2000, 5:671-682.
The authors analyze two nearly identical and partially redundant 'CCCH finger' proteins called MEX-5 and MEX-6. These regulators appear to transduce cortical PAR polarity into an asymmetric distribution of downstream embryonic determinants. The cytoplasmic MEX-5 protein is polarized in distribution as the one-cell zygote divides. MEX-5 is present at high levels anteriorly and apparently excluded posteriorly. Exclusion of MEX-5 protein from posterior embryonic cells requires the posteriorly localized cortical kinase PAR-1. The cytoplasmic boundary of MEX-5 in the zygote coincides precisely with the abutting PAR domain's boundary at the cortex. High levels of anterior MEX-5 exclude posterior determinants and also promote high levels of at least some anterior determinants. Intriguingly, at least three targets of MEX-5/6 regulation – the posterior determinants PIE-1, POS-1 and MEX-1 – are CCCH finger proteins (reviewed in [57]).

57. Bowerman B: Embryonic polarity: Protein stability in asymmetric cell division. *Curr Biol* 2000, 10:R637-R641.

58. Reese KR, Dunn MA, Waddle JA, Seydoux G: Asymmetric
•• segregation of PIE-1 in ***C. elegans*** is mediated by two complementary mechanisms that act through separate PIE-1 protein domains. *Molecular Cell* 2000, in press.
PIE-1 is a *C. elegans* CCCH finger protein that is segregated to posterior daughters during the asymmetric division of germline precursors in the early embryo. PIE-1 is required for germline fate and may act by repressing all or nearly all Pol II transcription in germline precursors. The authors analyze the expression of a collection of PIE-1–GFP translational fusions in transgenic worms. Using time-lapse digital imaging of GFP in live embryos, the authors define two protein domains required for the asymmetric distribution of PIE-1. One includes the first CCCH finger and promotes the degradation of residual PIE-1 in somatic daughters (after the asymmetric division of germline precursors into germline and somatic daughters). Two related embryonic determinants, POS-1 and MEX-1, also contain CCCH fingers, which mediate degradation in somatic daughters. A second, more carboxy-terminal PIE-1 domain includes the second CCCH finger, which is required for enrichment of PIE-1 in the posterior cytoplasm just prior to the asymmetric division of germline precursors. This posterior enrichment could involve protein movement or differential protein stability.

59. OhShiro T, Yagami T, Zhong C, Matsuzaki F: Role of cortical tumor
• suppressor proteins in asymmetric division of ***Drosophila*** neuroblast. *Nature* 2000, in press.
See annotation [60•].

60. Peng C-Y, Manning L, Albertson R, Doe CQ: The tumor suppressor
• genes ***lgl*** and ***dlg*** regulate basal protein targeting in ***Drosophila*** neuroblasts. *Nature* 2000, in press.
These papers [59•,60•] show the *lgl* and *dlg* tumor suppressor genes have a role in establishing cell polarity in neuroblasts, in addition to their previously defined role in epithelial cell polarity.