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Asymmetric cell division: recent developments and their implications for tumour biology

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Abstract | The ability of cells to divide asymmetrically is essential for generating diverse cell types during development. The past 10 years have seen tremendous progress in our understanding of this important biological process. We have learned that localized phosphorylation events are responsible for the asymmetric segregation of cell fate determinants in mitosis and that centrosomes and microtubules play important parts in this process. The relevance of asymmetric cell division for stem cell biology has added a new dimension to the field, and exciting connections between asymmetric cell division and tumorigenesis have begun to emerge.

Centrosome
(Also called the microtubule-organizing centre or spindle pole). A structure that nucleates microtubules and is important for signalling processes.

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The development of multicellular organisms involves the specification of diverse cell types from a single fertilized egg. To generate this diversity, some cells can undergo an asymmetric cell division, during which they differentially segregate protein or RNA determinants into the two daughter cells, thereby determining distinct cell fates.

The process of asymmetric cell division was originally described almost 100 years ago by Conklin[1], who found that during division of early ascidian embryos an area of yellow cytoplasm always co-segregates with cells that will become muscle cells. It was not until 1994, however, that an asymmetrically segregating cell fate determinant from *Drosophila melanogaster*, called Numb, was functionally and molecularly characterized[2]. During mitosis, Numb was found to localize to one edge of the cell, forming a crescent-shaped pattern, and to segregate into only one of the two daughter cells[2,3] (Supplementary information S1 (movie)); in the absence of Numb, normally different cells assume the same fate in *D. melanogaster* external sensory organs[4]. These observations suggested that high levels of Numb in one of the two daughter cells cause the division to become asymmetric.

In *Caenorhabditis elegans,* a similar asymmetric localization was found for partitioning defective (Par) proteins, which are also involved in other processes that require polarization[5–7]. During the first division of the *C. elegans* zygote, PAR-3 (REF. 8), PAR-6 (REF. 9) and protein kinase C-like 3 (PKC-3)[10] accumulate at the anterior cell cortex, and PAR-1 (REF. 11) and PAR-2 (REF. 12) accumulate posteriorly. Thus, these proteins differentially segregate into one of the two daughter cells. In contrast to *D. melanogaster* Numb, however, Par proteins are also required for other aspects of asymmetric cell division, including the establishment of different daughter cell sizes and the orientation and position of the mitotic spindle in *C. elegans*[6,7]. In fact, it is the *D. melanogaster* homologues of the anterior Par proteins that direct the asymmetric localization of Numb into one of the two daughter cells[13–17].

A simple model of asymmetric cell division postulates that it is a three-step process: in interphase, Par proteins set up a polarity axis[18]; in mitosis, this axis is used for spindle orientation and for the asymmetric localization of cell fate determinants; and in telophase, the tight coordination of spindle orientation and asymmetric localization ensures that cell fate determinants are inherited by only one of the two daughter cells.

Since this model was first proposed almost 10 years ago[18], new findings have emerged. In this Review I highlight the recent discoveries that have changed our view of how determinants are asymmetrically localized. I also summarize recent findings revealing a surprising role for centrosomes in maintaining the polarity axis over many divisions. Finally, I describe how the connections between asymmetric cell division and tumorigenesis have opened unexpected and challenging avenues for this dynamic and rapidly moving field.

Asymmetric cell division: the basics

The mechanisms of asymmetric cell division have been derived from studies of invertebrates — specifically, *D. melanogaster* and *C. elegans*. Below, I describe the basic principles of this process in these organisms.

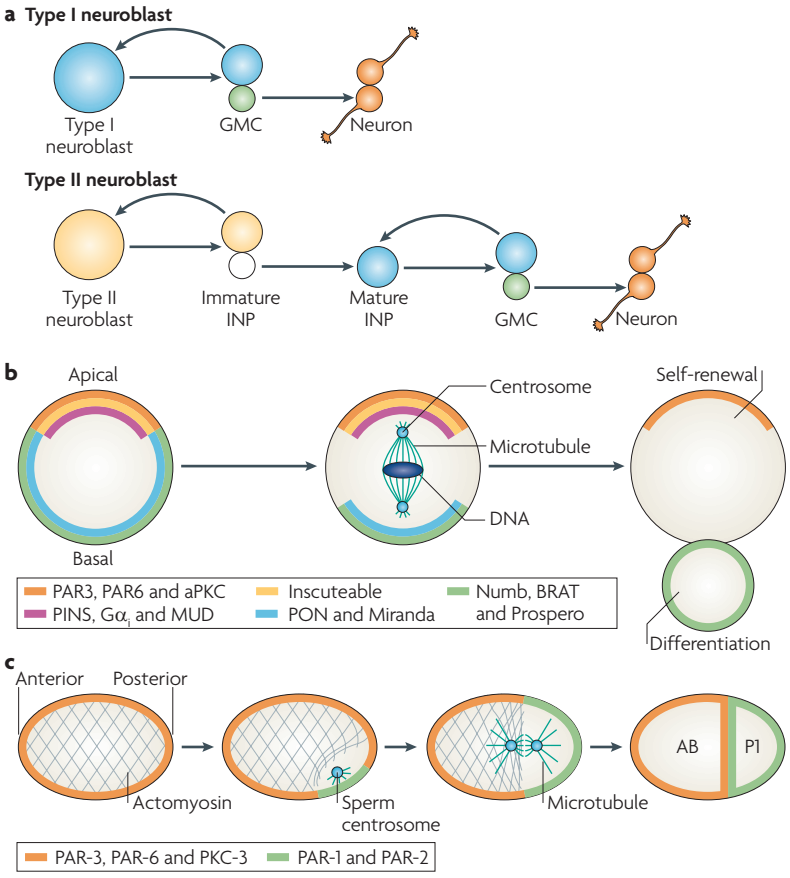

Figure 1 | **Models for asymmetric cell division. a** | *Drosophila melanogaster* type I neuroblasts divide asymmetrically into one neuroblast and one ganglion mother cell (GMC). The neuroblast self-renews, and the GMC divides terminally into two neurons. Type II neuroblasts divide into one self-renewing type II neuroblast and one immature intermediate neural precursor (INP). The INP starts expressing the neuroblast markers Asense and Deadpan to become a mature INP, which divides asymmetrically into one GMC and one mature INP. Differential expression of the markers Deadpan, Asense, Prospero and Embryonic lethal abnormal vision (ELAV) allows the unique identification of individual cell types in type I, type II and optic lobe (not shown) neuroblast lineages (see Supplementary information S2 (figure)). **b** | In *D. melanogaster* neuroblasts, the apically localized Partitioning defective 3 (PAR3)–PAR6–atypical protein kinase C (aPKC) complex is connected to partner of Inscuteable (PINS; also known as RAPS)–G protein α_i-subunit ($G\alpha_i$)–MUD by the adaptor protein Inscuteable. During mitosis, this apical complex directs the orientation of the mitotic spindle and the asymmetric localization of the adaptor proteins Partner of Numb (PON) and Miranda and, consequently, of the cell fate determinants Numb, Brain tumour (BRAT) and Prospero to the basal cell cortex. After mitosis, Numb, BRAT and Prospero act together to prevent self-renewal and induce cell cycle exit and differentiation. **c** | In the *Caenorhabditis elegans* zygote, the anterior Par proteins PAR-3, PAR-6 and PKC-like 3 (PKC-3) segregate into the anterior AB cell, and the posterior Par proteins PAR-1 and PAR-2 segregate into the posterior P1 cell. Polarization starts after fertilization, when interactions between the sperm centrosome and cortex allow PAR-2 to accumulate at the posterior cortex. This initiates an anterior contraction of the cortical actin cytoskeleton, which allows anterior movement of PAR-3, PAR-6 and PKC-3.

Neuroblast
A *D. melanogaster* neural progenitor cell that generates all of the neurons and glial cells in the brain.

***Asymmetric cell division in* D. melanogaster.** During the past 10 years, most of the progress in understanding asymmetric cell division in *D. melanogaster* has been made in neuroblasts, which are cells that delaminate from the ventral neuroectoderm during embryogenesis. In embryos, neuroblasts undergo up to 20 rounds of asymmetric cell division to generate the neurons of the larval nervous system, and they become quiescent at the end of embryogenesis. During the larval stages of development, neuroblasts re-enter the cell cycle and continue to divide asymmetrically to generate the neurons of the adult fly brain[19]. Several types of larval neuroblasts can be distinguished on the basis of lineage and location (FIG. 1a), and unique markers exist to allow their identification (Supplementary information S2 (figure)). Most prevalent are the type I neuroblasts, which divide into a large cell that remains a neuroblast and a smaller ganglion mother cell (GMC); the GMC subsequently divides into two terminally differentiated neurons. Type II neuroblasts are located in the dorsoposterior region of each central brain hemisphere and divide to give rise to a different cell lineage to type I neuroblasts[20–22]. The smaller daughter cell of type II neuroblasts becomes an intermediate neural precursor (INP), which continues to undergo self-renewing asymmetric divisions, each division generating one INP and one GMC. Furthermore, specialized kinds of type I neuroblasts exist in the mushroom bodies[19,23] and the optic lobes[24].

The basic mechanism of asymmetric cell division is common to all *D. melanogaster* neuroblasts[25–28] (FIG. 1b). The endocytic protein Numb[29] (which inhibits Notch–Delta signalling) and the translation inhibitor Brain tumour (BRAT)[30] transiently accumulate at the basal plasma membrane in late prometaphase[3,31–33]. Their asymmetric localization is facilitated by two adaptor proteins that localize asymmetrically at the same time as Numb and BRAT. BRAT localizes by binding Miranda[31,33], and Numb localization is facilitated by (but does not depend on) the adaptor protein Partner of Numb (PON)[34,35]. In type I neuroblasts and INPs, Miranda also transports the transcription factor Prospero into the GMC[36–40]. Slightly after the basal determinants localize, the mitotic spindle is set up in an apical–basal orientation so that these determinants are inherited by the basal daughter cell.

The asymmetric localization of basal determinants also requires another set of proteins that accumulate at the apical cell cortex before mitosis. These include the PDZ domain-containing proteins PAR3 and PAR6 and the protein kinase atypical PKC (aPKC[13–17]; the *D. melanogaster* homologue of *C. elegans* PKC-3). The group of proteins also includes the adaptor protein Inscuteable[41,42], which links PAR3–PAR6–aPKC to a second protein complex containing the heterotrimeric G protein α_i-subunit ($G\alpha_i$)[43] and the adaptor protein Partner of Inscuteable (PINS; also known as RAPS)[43–45]. PINS binds to the microtubule-associated dynein-binding protein MUD[46–48] and thereby provides a cortical attachment site for astral microtubules to ensure the apical–basal orientation of the mitotic spindle.

The initial apical localization of PAR3, PAR6 and aPKC is inherited from epithelial cells of the ventral neuroectoderm when the neuroblasts delaminate[13,14,16,17]. In these epithelial cells, Par proteins localize apically and are required for establishing and maintaining apicobasal polarity. In fact, PAR3, PAR6 and aPKC — and their homologues in other organisms — play a key part in almost all known cell polarity events, including

epithelial polarity, axon outgrowth, synapse formation and specification of the anteroposterior body axis[6,7]. How Par proteins direct the asymmetric localization of cell fate determinants during asymmetric cell division and how the apical localization of Par proteins is maintained during subsequent neuroblast cell cycles have become clear only recently and are discussed below.

***Asymmetric cell division in* C. elegans.** In *C. elegans,* the first cell division during development generates an anterior AB cell and a posterior P1 cell (for excellent reviews, see REFS 49,50). The size and fate of these two daughter cells are different, and the mechanisms that generate this asymmetry are similar to those that act in neuroblasts (FIG. 1c). Polarization of the zygote starts when the entire cortical actin cytoskeleton moves towards the anterior pole[51]. This movement is initiated by the sperm centrosome[52,53] and by the Rho guanine nucleotide exchange factor (RhoGEF) cytokinesis defect 4 (CYK-4)[54], which is contributed during fertilization and remains localized close to the posterior male pronucleus. As a result of anterior cortical movement, surface contractions that initially occur throughout the cell are progressively confined to the anterior half of the zygote, whereas the posterior side becomes smooth[50]. PAR-3, PAR-6 and PKC-3 are initially uniformly cortical but concentrate at the anterior side after fertilization[51], although a second, actomyosin-independent mechanism has been described[55]. PAR-1 and PAR-2 become enriched in the posterior, non-contracting cell cortex, and inhibitory interactions between the anterior and posterior Par proteins ensure that the groups maintain their localization to opposite cortical domains. PAR-2, for example, prevents the cortical localization of PKC-3 (REF. 56) and PKC-3 phosphorylates PAR-2; this removes it from the plasma membrane. Thus, in contrast to those in *D. melanogaster*, Par proteins in *C. elegans* are involved in regulating both asymmetric cell division and the symmetry-breaking events that establish the anteroposterior axis in the zygote.

The distinction between segregating determinants and proteins establishing polarity is not as clear in *C. elegans* as in *D. melanogaster*[57]. In addition to the effects of the Par proteins, the asymmetric division of the zygote is influenced by the CCCH-Zn finger proteins muscle excess 1 (MEX-1), MEX-5, MEX-6, posterior segregation protein 1 (POS-1) and pharynx and intestine in excess protein 1 (PIE-1), the RNA-binding proteins MEX-3 and spindle orientation defective protein 4 (SPN-4; also known as PIP-1) and the homeodomain protein posterior alae in males protein 1 (PAL-1)[57]. PIE-1 is inherited by the posterior P1 cell[58], where it blocks transcriptional elongation[59] and prevents the expression of genes that would promote somatic differentiation in the germline blastomeres[60]. MEX-5 and MEX-3 segregate into the anterior AB daughter cell and inhibit the specification of muscle cell fate in its progenitors[61,62]. Par proteins are essential for asymmetric segregation of PIE-1, MEX-5 and MEX-3. However, the accumulation of Par proteins themselves, as well as actomysin flow, is regulated redundantly by MEX-5 and the highly related MEX-6 (REF. 63). In fact, most of these proteins are also involved in the asymmetric segregation of other factors, with the notable exception of PIE-1 and PAL-1, and they are therefore considered to be polarity mediators rather than segregating determinants[57].

Asymmetric localization of determinants

The mechanisms that lead to the asymmetric localization of Numb, BRAT and Prospero in *D. melanogaster* neuroblasts had remained a mystery for many years. Similarly, it was unclear how cytoplasmic determinants are segregated into the AB or P1 cell in *C. elegans*. Initial experiments using chemical inhibitors in *D. melanogaster* showed that the process does not require microtubules but depends on actin and myosin[36,64–66]. This led to the formulation of a model in which an actomyosin-dependent process moves asymmetrically, segregating cell fate determinants along the cell cortex to concentrate them on the basal side[18,67]. Support for this model came from the demonstration that myosin VI is important for asymmetric cell division[68] and from the finding that the cytoskeletal protein Lethal (2) giant larvae (L(2)GL) is important for the basal localization of Numb but not for the apical localization of Par proteins[69,70]. L(2)GL binds and inhibits cytoplasmic non-muscle myosin[71,72], and this interaction is inhibited through phosphorylation by the apical protein aPKC[73,74]. As a result, L(2)GL is inhibited in the apical half but active in the basal half, where it could potentially inhibit myosin. Consistent with this hypothesis, myosin II is concentrated apically in neuroblasts[75] and, when it is inhibited by mutation or chemical inhibitors of Rho-associated protein kinase (ROCK), Numb and its interacting protein Miranda (see below) no longer concentrate on the basal side[75].

Although the cortical transport model is attractive, it has been challenged by several recent observations. The asymmetry in myosin localization is not observed in external sensory organs[76] and could not be confirmed in more recent reports, which actually describe myosin localization to the basal side of the neuroblast[77]. Furthermore, the ROCK inhibitor that was used to demonstrate the requirement of myosin for Numb and Miranda localization can also inhibit aPKC[78]. Finally, fluorescence recovery after photobleaching (FRAP) experiments did not reveal unidirectional cortical transport of the Numb adaptor PON[79]. Instead, FRAP recovery rates showed that PON and Numb rapidly exchange between cortex and cytoplasm and that local differences in cortical 'on' and 'off' rates, rather than in cortical transport, are responsible for the asymmetric localization of these proteins[80]. Therefore, the cortical transport model has been replaced by more dynamic models, in which the differential mobility or cortical attachment of protein determinants to the apical and basal plasma membranes regulates their asymmetric localization. Below, I discuss how those models explain asymmetric segregation of determinants in *D. melanogaster* and describe similar models that explain the asymmetric localization of cytoplasmic proteins in *C. elegans*.

Mushroom body
A mushroom-shaped paired-neuropil structure that is found in the *D. melanogaster* brain and functions in learning and memory.

Optic lobe
A morphologically distinct part of the developing *D. melanogaster* brain that forms the visual processing centres.

Blastomere
A cell that is generated during embryonic cleavage divisions.

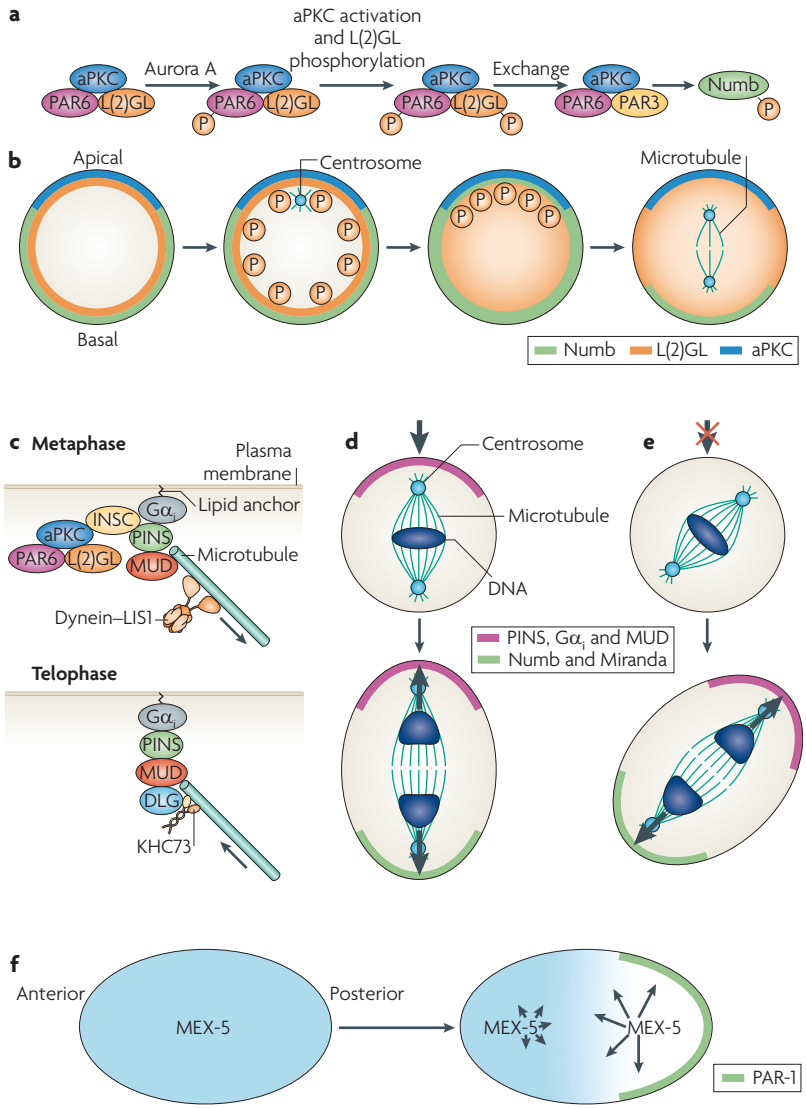

Figure 2 | **Asymmetric segregation of protein determinants. a** | In *Drosophila melanogaster* neuroblasts, activation of Aurora A results in the phosphorylation of Partitioning defective 6 (PAR6), which in turn activates atypical protein kinase C (aPKC), leading to Lethal (2) giant larvae (L(2)GL) phosphorylation and exit from the complex. L(2)GL is exchanged for PAR3, which acts as an adaptor that allows aPKC to phosphorylate Numb. Phosphorylated Numb is then released into the cytoplasm. As aPKC is restricted to the apical cortex, Numb is retained on the basal side and segregates into the basal daughter cell. **b** | Localization of Numb, L(2)GL and aPKC in the cell during mitosis. **c** | In metaphase, G protein α_i-subunit ($G\alpha_i$), Partner of Inscuteable (PINS; also known as RAPS) and MUD establish a cortical attachment site for astral microtubules to orient the mitotic spindle. In telophase, however, it is the mitotic spindle that influences cortical polarity of neuroblasts through a microtubule-dependent pathway. In this case, kinesin KHC73, which is transported on astral microtubules, binds Discs large (DLG). This, in turn, recruits $G\alpha_i$ and PINS, which then recruits MUD. This results in the accumulation of determinants over one spindle pole. **d**,**e** | Normally, the telophase pathway (**d**) is not essential. When components of the apical complex are missing, however, it rescues the formation of opposing cortical domains in anaphase and telophase. The new polarity axis aligns with the mitotic spindle and not necessarily with the apicobasal axis (**e**). **f** | In *Caenorhabditis elegans*, muscle excess 5 (MEX-5) and pharynx and intestine in excess protein 1 (PIE-1; not shown) exist as fast- and slow-diffusing forms. The fast-diffusing form of MEX-5 is more abundant posteriorly, and the fast-diffusing form of PIE-1 is concentrated anteriorly, resulting in the asymmetric distribution of these cytoplasmic proteins. For MEX-5, phosphorylation by posteriorly localized PAR-1 may be responsible for the faster diffusion rate. INSC, Inscuteable; LIS1, Lissencephaly 1.

The prophase pathway: asymmetric phosphorylation. In *D. melanogaster* neuroblasts, Numb is recruited to the plasma membrane through the phospholipid interactions of positively charged amino acids in its amino terminus[65]. Next to those residues are several phosphorylation sites for aPKC, mutation of which to Ala abolishes the asymmetric localization of Numb in mitosis[81]. These observations suggest that aPKC-mediated phosphorylation neutralizes positive charges and thereby inhibits the membrane association of Numb[76].

In interphase, aPKC forms a complex with PAR6 and L(2)GL (FIG. 2a,b); this complex cannot phosphorylate Numb, presumably because the substrate-binding site is blocked. On entry into mitosis, the kinase Aurora A phosphorylates PAR6 (REF. 76), leading to the activation of aPKC and consequent phosphorylation of L(2)GL. This reduces the affinity of L(2)GL for PAR6 and aPKC, thereby releasing it from the complex and allowing PAR3 to enter[82]. PAR3 can bind to both Numb and aPKC and might act as an adaptor between kinase and substrate. This subunit exchange initiates the phosphorylation of Numb because aPKC phosphorylates Numb only when it is bound to PAR3 and not when bound to L(2)GL. Therefore, the function of L(2)GL is not to recruit determinants to the cortex, as previously thought, but to regulate the substrate specificity and maybe also the activity of aPKC. In *l(2)gl* mutants, for example, it is premature aPKC phosphorylation, rather than myosin defects, that prevents Numb localization; moreover, the effects of overexpression of an L(2)GL that cannot be phosphorylated are due to inhibition of aPKC[78] rather than active recruitment of asymmetric determinants to the cortex. These new findings have converted Numb localization from a complete mystery to one of the best-understood mitotic events.

In fact, aPKC-dependent phosphorylation is a general mechanism for asymmetric protein localization during mitosis, at least in *D. melanogaster*. aPKC can also phosphorylate Miranda[76] and regulate its cortical localization, similarly to how it controls Numb localization[78]. Furthermore, the E3 ubiquitin ligase Neuralized, which segregates asymmetrically in sensory-organ precursor cells, contains aPKC consensus sites in its N-terminal phosphoinositide-binding domain[83], suggesting that it might also be regulated by aPKC. This new model of phosphorylation-dependent asymmetric cell division does not implicate actomyosin as a major player in asymmetric protein localization. Consistent with this, the weak actin inhibitor cytochalasin D does not inhibit the process, although it can prevent cytokinesis[36]. This model might also explain why asymmetric segregation of aPKC alone is sufficient to generate different fates, even when Numb and Miranda are inherited by both daughter cells in mutants with altered spindle orientation[84]. As both proteins need to be membrane bound to carry out their functions (Numb acts on endocytic vesicles and Miranda recruits other proteins to the cortex), they can be inhibited by aPKC phosphorylation in one of the two daughter cells. Therefore, it is the ratio between aPKC and basal determinants that ultimately determines the fate of each daughter cell[84].

The telophase pathway: microtubule–cortex interactions. Numb and Miranda still segregate asymmetrically in mutants in which asymmetric localization in prophase and metaphase is completely abolished[15,69,70]. This is due to a second pathway for asymmetric localization of determinants that acts in anaphase and telophase of the cell cycle (reviewed in REF. 85). In contrast to the prophase pathway, the telophase pathway is sensitive to microtubule-depolymerizing drugs or to mutations affecting astral microtubules[85,86] (FIG. 2c). In wild-type *D. melanogaster*, the pathway is not required for asymmetric protein localization in metaphase, as disruption of microtubules has no effect[36]. However, in *inscuteable* mutants — in which PAR3–PAR6–aPKC is delocalized in interphase[43,44] and mitotic spindles are no longer oriented along the apicobasal axis — the microtubule-dependent pathway is responsible for PINS and $G\alpha_i$ accumulation over one of the two spindle poles in mitosis and for asymmetric segregation of determinants (FIG. 2d,e) so that cell fate specification occurs normally in a large subset of neuroblasts[86]. The microtubule-dependent pathway for neuroblast polarization depends on the PINS-binding partner Discs large (DLG), which is a membrane-associated guanylate kinase that also plays a part in Numb and Miranda localization in wild-type embryos. The pathway also requires the kinesin KHC73, which localizes to microtubule plus ends and can bind DLG. These observations have suggested a model in which KHC73, transported on astral microtubules, is responsible for the accumulation of DLG and PINS over one spindle pole. PINS then recruits the microtubule-binding protein MUD, and this mutual microtubule–cortex interaction stabilizes spindle orientation (FIG. 2c).

Although this model is attractive and consistent with all of the available data, several key questions remain. For example, it is unknown how DLG and PINS establish the localization of basal determinants when the Par proteins are not asymmetrically localized. Furthermore, the phenotype of *dlg* mutants is not entirely consistent with the model: basal determinants do not localize correctly in metaphase in these mutants, but the rescue of asymmetric cell division in telophase still occurs[69,70]. Therefore, DLG is required for the telophase pathway when other regulators are missing, but its function can be replaced when the rest of the machinery is intact. The precise molecular function of the telophase pathway still needs to be defined.

***Asymmetric protein segregation in* C. elegans.** The mechanisms regulating asymmetric cell division in *C. elegans* are remarkably similar to those in *D. melanogaster*, even though the segregating determinants PIE-1 and MEX-5 localize asymmetrically in the cytoplasm and not at the cortex. Their asymmetric localization is mediated by regulated protein degradation and a reaction–diffusion mechanism in which asymmetry is established through different ratios of slowly and rapidly diffusing isoforms in the anterior and posterior halves.

Protein degradation contributes to PIE-1 asymmetry in late-stage embryos but not in the zygote[87–89]. PIE-1 degradation during these late cycles is mediated by the suppressor of cytokine signalling (SOCS) box protein Zn finger-interacting factor 1 (ZIF-1)[87], which interacts with the CCCH-Zn fingers of PIE-1 and also binds to a ubiquitin ligase complex containing elongin C, cullin 2 (CUL-2) and E2 ubiquitin-conjugating enzyme 5 (UBC-5). Together, these proteins degrade PIE-1 in somatic cells and thereby restrict its expression to the germ line. Interestingly, MEX-5 activates ZIF-1 and is also required for restricting PIE-1 to the germ line. This degradation mechanism explains the antagonistic expression of MEX-5 and PIE-1 in later embryos.

In the zygote, the asymmetric localization of PIE-1 and MEX-5 is thought to be established through a reaction–diffusion mechanism[88,90] (FIG. 2f). This mechanism is used to describe chemical reactions and involves two substances that can be converted into each other by a chemical reaction and that move in space with different kinetics. The mechanism was initially applied to biology by Turing[91], and it is now well established that reaction–diffusion mechanisms are responsible for pattern generation in many biological systems[92]. In this case, the two substances can be differentially modified forms of a protein or a free and a complex-associated form. FRAP and fluorescence correlation spectroscopy (FCS) experiments have shown that PIE-1 and MEX-5 exist as rapidly and slowly diffusing isoforms[88,90]. The ratio between these isoforms is different in the anterior and posterior parts of the zygote, with more slowly diffusing PIE-1 localized posteriorly and more slowly diffusing MEX-5 localized anteriorly (FIG. 2f). In both cases, mathematical modelling of the protein distributions that would result from the measured diffusion coefficients predicts the observed asymmetric protein distributions.

So how are the apparent differences in cytoplasmic mobility established? MEX-5 needs to be phosphorylated by PAR-1 to localize asymmetrically[89]. PAR-1 is concentrated posteriorly and can locally change the mobility of MEX-5 by modifying its association with the actin cytoskeleton. This explains the actin dependence of MEX-5 asymmetry, although the asymmetric movement of the actin meshwork itself adds an additional complication. For PIE-1, differential association with posterior P granules was proposed[88]. P granules segregate asymmetrically in a Par protein-dependent manner, so this would explain PIE-1 asymmetry, although biochemical evidence for this is still lacking.

Thus, differential association with membranes or other cellular components, rather than directional transport, establishes the asymmetric localization of cell fate determinants in both *D. melanogaster* and *C. elegans*.

A new role for the centrosome

Ten years ago, microtubules were thought not to have a role during asymmetric cell division in *D. melanogaster*[18,67]. Now, it is clear that microtubules play an important part in the telophase pathway. In addition, microtubule-dependent cortical interactions are integral to maintain polarity over many divisions.

D. melanogaster neuroblasts repeatedly divide along the apicobasal axis. Real-time analysis of spindle orientation has revealed that the mitotic spindle is established

P granule
A type of ribonucleoprotein particle that segregates with and marks all cells of the *C. elegans* germ line.

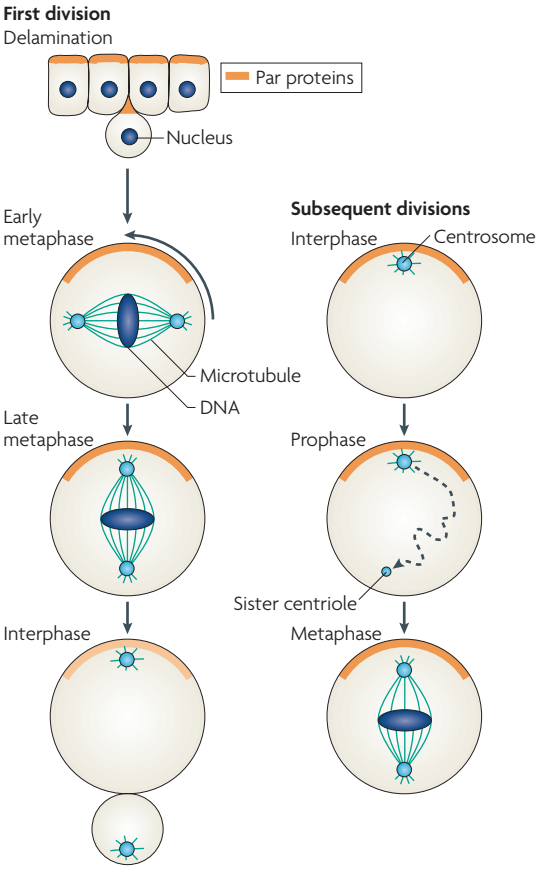

Figure 3 | **Par proteins and centrosomes establish cortical polarity in *Drosophila melanogaster* neuroblasts.** During the first neuroblast cycle, Partitioning defective (Par) proteins are inherited from the apical cortex of the overlying epithelium. Through a series of adaptor proteins, they recruit MUD, which forms cortical attachment sites for astral microtubules and thereby induces spindle rotation into an apicobasal orientation. During subsequent cell cycles, centrosomes are responsible for maintaining cortical polarity in interphase. Cortical Partner of Inscuteable (PINS; also known as RAPS), in turn, is required for maintaining the apical centrosome while the sister centriole migrates through the cytoplasm. On the basal side, this centriole recruits pericentriolar material to form a bipolar spindle in the proper apicobasal orientation.

parallel to the embryonic surface but then rotates by 90° into its final vertical position[93] (FIG. 3). It was thought that both centrosomes organize microtubule asters simultaneously at the onset of mitosis and set up a bipolar mitotic spindle in prophase. More recently, it became clear that this mechanism applies only to the first division of embryonic neuroblasts. During subsequent divisions, the apical position of the neuroblast centrosome that results from the previous cytokinesis is maintained throughout interphase[94,95]. After centriole duplication, the daughter centriole is devoid of pericentriolar material when it migrates to the basal side of the neuroblast. A second microtubule aster appears in prophase, shortly before breakdown of the nuclear envelope. As a result, the mitotic spindle is already set up in its final, vertical orientation and does not rotate substantially in metaphase. Thus, contrary to what was previously thought, the orientation of most neuroblast divisions is established early in the cell cycle.

The orientation of the spindle across several neuroblast divisions is maintained by crosstalk between the centrosome and apical proteins. In *pins* mutants, the apical aster loses its microtubule-nucleating activity and starts to migrate basally, resulting in two identical centrioles and random spindle orientation[95]. This suggests that apical proteins maintain the apical position of the centrosome in interphase. However, the positioning of apical proteins can also be instructed by the centrosome itself. When microtubules are transiently inactivated, the apical centrosome assumes a random position and induces the localized accumulation of Par proteins at its new position[96]. This symmetry-breaking property of the neuroblast centrosome is strikingly similar to what occurs in the *C. elegans* zygote, in which the sperm centrosome breaks symmetry and establishes the localization of Par proteins to the anterior and posterior domains. In contrast to *D. melanogaster*, however, in the *C. elegans* zygote the centrosome removes, rather than attracts, the PAR-3–PAR-6–PKC-3 complex. One important implication of these new findings is that the sister centrioles are not identical in neuroblasts and could therefore be involved in maintaining asymmetric cell division. In yeast, it has been shown that the newly born centriole (known as the spindle pole body in this case) is always inherited by the bud cell and never by the mother cell. During the asymmetric divisions of *D. melanogaster* testes, the mother centriole remains anchored at the stem cell niche and is always inherited by the daughter cell, which retains the self-renewal capacity. These observations have raised speculations about centrioles having fate-determining properties[97]. For example, during brain development in vertebrates, the mother centriole is preferentially inherited by the progenitor cell[98]. In this case, removal of ninein, a protein that ensures this inheritance pattern, causes randomization of centriole inheritance and a defect in progenitor cell maintenance. Although this is just a correlation, this finding indicates that centrosome asymmetry might contribute to asymmetric cell division in vertebrate cells.

Specifying daughter cell sizes

Besides having different cell fates, the daughter cells of both *D. melanogaster* neuroblasts and the *C. elegans* zygote are different in size. Identification of the mechanisms through which this asymmetry is established has revealed an exciting role for heterotrimeric G proteins in mediating microtubule–cortex interactions (FIG. 4). Although the involvement of G proteins was clear 10 years ago, how they interact with microtubules and establish cell asymmetry was discovered only recently.

In *C. elegans*, size asymmetry during the first division is due to an asymmetric displacement of the mitotic spindle towards the posterior end of the cell (FIG. 4a). This is thought to be due to increased pulling forces exerted on the spindle at the posterior end that are mediated by heterotrimeric G proteins and their binding partners, the *C. elegans* PINS homologues G protein regulator 1

Centriole
A small organelle (consisting of two short, barrel-like arrays of microtubules) that organizes the centrosome and contributes to cytokinesis and cell-cycle progression.

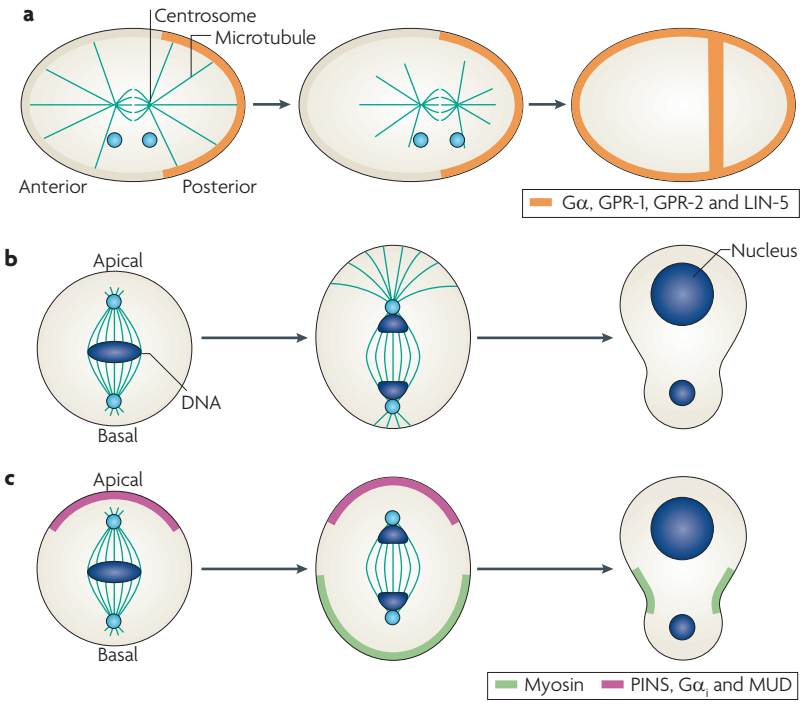

Figure 4 | **Three ways to generate different daughter cell sizes. a** | In the *Caenorhabditis elegans* zygote, heterotrimeric G protein α-subunit (Gα) and the GoLoco proteins G protein regulator 1 (GPR-1) and GPR-2 recruit the dynein-binding protein LIN-5 to the cortex to facilitate microtubule–cortex interactions. Higher concentrations of GPR-1 and GPR-2 on the posterior side result in a net pulling force and posterior spindle displacement. **b** | In *Drosophila melanogaster* neuroblasts, the apical microtubule aster is larger, resulting in spindle displacement towards the basal side and asymmetric cleavage. **c** | In neuroblasts, the apical Partner of Inscuteable (PINS; also known as RAPS)–Gα$_i$–MUD complex induces a basal shift of cortical myosin, resulting in basal displacement of the cleavage furrow. This pathway can generate asymmetric daughter cell sizes even in the absence of a mitotic spindle.

(GPR-1) and GPR-2 (REFS 99,100 and reviewed in REF. 49). GPR-1 and GPR-2 carry a GoLoco domain that binds to the GDP-bound form of Gα. Gα–GPR-1 and Gα–GPR-2 recruit LIN-5 (REF. 101) (the *C. elegans* homologue of the human microtubule-binding protein nuclear mitotic apparatus protein 1 (NuMA)), the microtubule minus-end-directed motor dynein and the dynein-binding protein lissencephaly 1 (LIS-1)[102]. LIN-5, dynein and LIS-1 form a complex in the cytoplasm and are recruited to the plasma membrane by binding to Gα. After it is recruited to the plasma membrane, the complex can form an attachment site for the plus ends of astral microtubules, thereby exerting pulling force on the mitotic spindle. As the concentration of GPR-1 and GPR-2 is higher at the posterior cortex, the mitotic spindle is pulled towards this end.

It is likely that the mechanism identified in *C. elegans* applies to *D. melanogaster* and vertebrates, as all components of the system are conserved and their subcellular localization and biochemical interactions are similar to those seen in *C. elegans*. In *D. melanogaster* neuroblasts, however, the mechanisms regulating daughter cell sizes are different from those in the *C. elegans* zygote. First, the mitotic spindle itself is asymmetric in shape, with a large apical and a much smaller basal microtubule aster (FIG. 4b). Second, recent experiments suggest that, in addition to the spindle-induced pathway, the site of cytokinesis is determined by a second, cortical pathway[77] (FIG. 4c). Evidence for this pathway comes from live-imaging experiments showing that the cleavage furrow proteins Anillin, Pavarotti (a *D. melanogaster* guanine nucleotide exchange factor for Rho) and myosin accumulate in the basal side of the cell before the mitotic spindle becomes asymmetric, and this is mediated by the apical PINS–Gα$_i$–MUD complex. Surprisingly, this cortical asymmetry and the resulting asymmetric cleavage furrow can even be established when spindle formation is blocked by microtubule-depolymerizing drugs, and the resulting checkpoint arrest is overcome by a mutation in a kinetochore protein. In mutants with abnormal spindle orientation but normal cortical polarity, the cortical polarity pathway and the classical spindle-induced pathway are both active, resulting in the formation of anucleate lobes of cytoplasm that are cleaved from the mother cell. These surprising recent findings that challenge the dogma for how cytokinesis is established will certainly spark new insights into this important process. As myosin asymmetry has recently also been described in *C. elegans*, the new mechanism seems to be conserved and might also exist in higher organisms[103].

Asymmetric division in tumour formation

The connection between asymmetric cell division and tumorigenesis has been one of the most surprising and important findings in the field in the past 10 years. Furthermore, studies in mammals have identified a link between tumorigenesis and dysregulated asymmetric cell division of stem cells.

***Tumorigenesis in* D. melanogaster.** Genetic screens carried out in the 1970s for brain tumour formation in *D. melanogaster* revealed an involvement for the genes *l(2)gl*, *dlg*, *lethal (2) giant discs* (*l(2)gd*), *brat* and *lethal (3) malignant brain tumour* (*l(3)mbt*)[104,105]. Neuroblasts fail to differentiate in *D. melanogaster* embryos that are mutated for any of these genes, leading to tumour-like overproliferation. After they have been transplanted into the abdomen of another fly, the tumours continue to grow, undergo metastasis and become aneuploid.

The identification of L(2)GL and DLG as key regulators of asymmetric cell division[69,70] and of BRAT as a segregating determinant[31,33] suggested that these tumours actually arise from defects in asymmetric cell division. Indeed, transplantable tumours also form in mitotic neuroblast clones that are mutated for *numb* or *prospero*[20,32] or on overexpression of activated aPKC[106]. Subsequent analysis showed that tumours can also occur in mutants for the mitotic Ser/Thr protein kinases Aurora A[107,108] and Polo[35], following overactivation of Notch[108], or in mutants with aberrant spindle orientation[46–48,84,109]. Tumours can even occur when neuroblasts divide with an excess of centrosomes[110,111]. In all these cases, defects in asymmetric cell division are the root cause of tumour formation. Notably, however, mutations in apical proteins such as aPKC or PINS have the opposite phenotype, resulting in fewer neuroblasts.

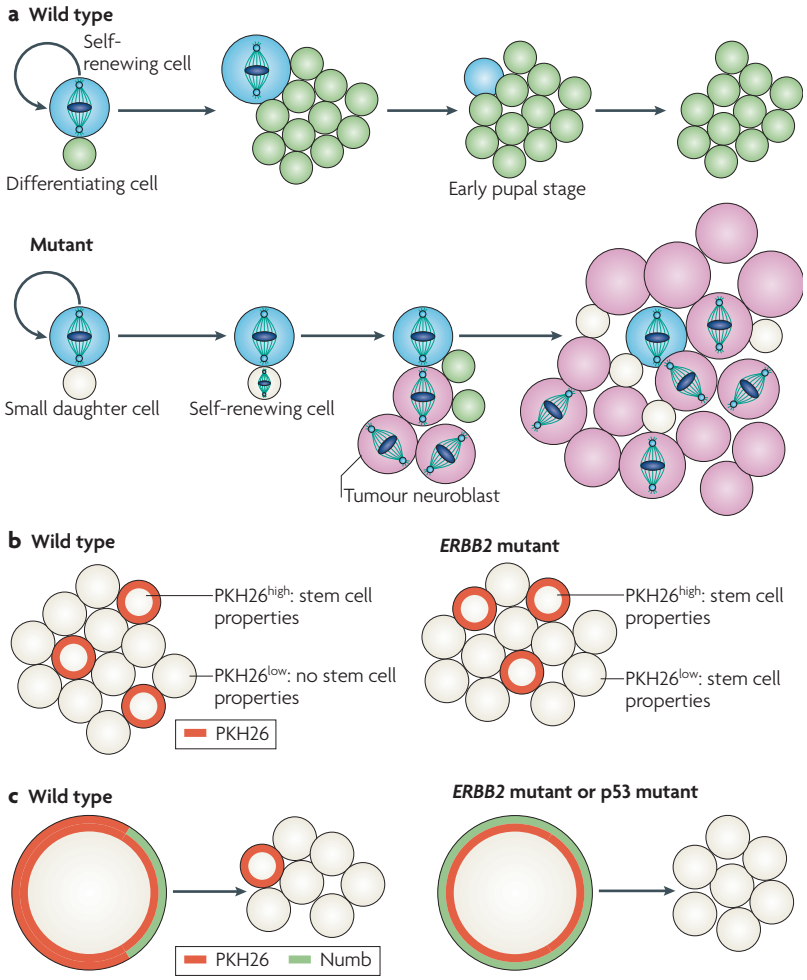

Figure 5 | **Asymmetric cell division and tumour formation. a** | Wild-type *Drosophila melanogaster* neuroblasts generate one large self-renewing daughter cell and one small differentiating daughter cell. The differentiating daughter cell exits the cell cycle after a terminal division (not shown). The neuroblast shrinks during pupal stages and undergoes apoptosis. In mutants that are defective in asymmetric cell division, the smaller daughter cell cannot differentiate. After some time, it undergoes mitosis and reverts to a tumour neuroblast. These tumour neuroblasts are abnormal because they do not exit the cell cycle during pupal stages. Whether the original neuroblast (blue) disappears or also continues to proliferate is unclear. **b** | Mammospheres that are grown from wild-type mammary gland tissue or erythroblastosis oncogene B2 (*ERBB2*)-mutant tumour tissue contain the same number of slowly proliferating (PKH26 dye-retaining) cells ($PKH26^{high}$). In wild-type tissue, only the cells retaining the dye can form secondary mammospheres, but in tumour tissue mammospheres can be grown from any cell. **c** | In wild-type tissue, $PKH26^{high}$ cells localize Numb asymmetrically. When cultured, one dye-retaining cell remains, indicating that the initial division was asymmetric. When isolated from an *ERBB2* tumour model or from p53-mutant mice, $PKH26^{high}$ cells do not divide asymmetrically and all daughter cells lose the dye, indicating that the initial division was symmetric.

The simplest explanation for tumour formation is that defects in segregating determinants result in symmetric divisions, giving rise to two neuroblasts (FIG. 5a). The resulting exponential increase in neuroblast number would explain certain aspects of tumour formation. However, it does not explain why tumour neuroblasts do not exit the cell cycle but continue to proliferate even in adult brains[32] or after transplantation[112]. In addition, a detailed analysis of *brat*-mutant clones showed that tumour formation does not simply involve a series of symmetric divisions[20]. After an initial delay phase in which the BRAT-inheriting cell fails to initiate correct marker expression and enters a prolonged cell cycle block, it divides and enters a second phase in which it proliferates rapidly and indefinitely. Thus, defects in asymmetric cell division cause the formation of tumour neuroblasts that lack the mechanisms responsible for cell cycle exit during pupal development.

The formation of tumour neuroblasts in mutants with aberrant asymmetric cell division can be explained by genetic or epigenetic defects or by the intrinsic properties of the growth control mechanism. In the genetic model, DNA mutations are responsible for immortalizing neuroblasts; however, although aneuploidy does occur in transplanted neuroblast tumours[104] and may be responsible for their metastatic behaviour[112], it has not been described in primary tumours. As mutations causing genome instability do not result in brain tumours[111], it is more likely that transcriptional and/or epigenetic changes alter the behaviour of the mutant neuroblasts. The transcriptional network governing self-renewal in neuroblasts needs to be reprogrammed towards a stable and irreversible differentiation state after asymmetric cell division. Defects in this process could create a new stable state, in which the self-renewal programme is active but the modules controlling exit from proliferation are missing. For example, neuroblasts serially express different transcription factors at different developmental stages[113], and a reset of this developmental timer during each defective asymmetric cell division could explain immortalization. Finally, it is possible that the growth control mechanism acting in pupae can deal with only a limited number of neuroblasts — for example, because a growth inhibitor is limiting or because neuroblasts secret an autocrine growth-promoting factor that competes with a systemic extrinsic factor.

Several redundant mechanisms have been proposed to stop neuroblast proliferation in wild-type flies. In the abdomen of the ventral nerve chord, transient expression of the homeotic gene *abdominal A* eliminates neuroblasts by inducing apoptotic cell death[114]. In the central brain, a decrease in insulin and phosphoinositide 3-kinase signalling causes a reduction in neuroblast size followed by caspase-mediated cell death during the pupal stages of development[115]. When caspase activation is prevented, neuroblast size is still reduced, and the cells are eliminated by a caspase-independent autophagic pathway that is regulated by the transcription factor Forkhead box O (FOXO). When both FOXO and caspases are inhibited, neuroblasts continue to proliferate and generate functional neurons, even in adult flies. Surprisingly, however, this does not result in a tumour, indicating that both an increase in neuroblast number and inhibition of the elimination pathways contribute to tumour formation.

Clearly, identifying the molecular events that connect asymmetric cell division to cell immortalization is one of the greatest new challenges in the field. This is particularly important because defects in asymmetric cell division are relevant for human tumorigenesis[116] (see below)[117,118] and may be part of the mechanisms that convert a normal mammalian stem cell into what is known as a cancer stem cell (BOX 1).

Box 1 | **Tumour stem cells**

The tumour stem cell hypothesis[116] states that tumours contain a rare population of cells that have stem cell properties and are the only tumour cells that can generate all other cell types in the tumour. The hypothesis is based on xenotransplantation experiments in which transplantation of human tumours into immunocompromized mice recapitulates the human tumour histology. It had long been known that only a few cells in a tumour could initiate tumour formation in those transplantation experiments. In the 1990s, it was shown that these few cells in leukaemia express stem cell markers and that tumour formation involves a cellular hierarchy that is similar to the one in normal haematopoiesis[132,133]. These findings formed the basis of the tumour stem cell hypothesis and, soon after, similar experiments identified tumour stem cells in brain and mammary tumours[134,135] and in almost all other types of human cancer[136].

Whether or not the formation of cancer stem cells is an intrinsic property of tumorigenesis is intensely debated[137]. Opponents of the theory argue that cancer stem cells are simply a subtype of human tumour cells that adapt more easily to the environment of the mouse host. Whether human tumours actually arise from stem cells is debated. In mouse models, intestinal cancer can be induced by mutating the adenomatous polyposis coli (APC) tumour suppressor in stem cells but not in non-stem-cell types[138]. In a mouse glioblastoma model, tumour formation coincides with the appearance of abnormal stem cell populations[139]. These results suggest that DNA mutations in stem cells might be the initial event in those tumours. Experiments in *Drosophila melanogaster*, in which this hypothesis can be stringently tested, might shed light on the mechanisms that cause stem cells to become malignant.

Tumorigenesis and mammalian stem cells. The ability to generate both self-renewing and differentiating daughter cells is a defining feature of any stem cell, and asymmetric cell division is one of the mechanisms used to establish this. Evidence for asymmetric cell division exists for stem cells in muscle[119], skin[120], the gut[121], mammary glands[117], the haematopoietic system[118] and the developing brain[98,122]. Nevertheless, the mechanisms that guide these asymmetric cell divisions are generally not well understood (BOX 2). In fact, transferring our detailed understanding of the process from *D. melanogaster* and *C. elegans* to vertebrates has been much more challenging than expected. Almost all of the molecular players are conserved in vertebrates, but they often act in distinct ways. Numb, for example, is polarized in vertebrate neural progenitors, but this is because it regulates trafficking of E-cadherin at adherens junctions[123]. Par proteins are apical but, unlike in neuroblasts, only a few progenitor divisions are aligned along the axis of Par protein polarity[124]. Nevertheless, building on the results from flies and worms, some exciting connections between asymmetric cell division and tumorigenesis have recently been identified.

Stem cells from mouse mammary glands can grow into spherical cultures known as mammospheres that recapitulate the mammary morphogenic programme[125]. The stem cells can be isolated because they retain a lipophilic vital dye following labelling, whereas dividing cells do not[117] (FIG. 5b). When purified from wild-type mammary glands, these stem cells divide asymmetrically and segregate Numb into one of their two daughter cells (FIG. 5c). In a mouse mammary tumour model, the number of stem cells is increased (FIG. 5b) and they divide symmetrically — Numb is no longer asymmetrically localized, and both daughter cells behave identically in terms of dye dilution (FIG. 5c). Similar observations have been made in p53-mutant mice. As p53 degradation is regulated by Numb[126], it is possible that the asymmetric inheritance of Numb regulates p53 levels and restricts stem cell fate to only one of the two daughter cells. Consistent with this, Numb is a major tumour suppressor in breast cancer[127]. Numb also acts in the haematopoietic system, where it can inhibit the progression of chronic myeloid leukaemia (CML). Cultured haematopoietic progenitors normally divide and segregate Numb asymmetrically, but their divisions become symmetric following the expression of the fusion protein NUP98–HOXA9 (REF. 118) (encoded by a fusion of two genes that occurs during tumour formation and is characteristic for a specific form of

Box 2 | **Asymmetric cell division in vertebrates**

Almost all of the molecules regulating asymmetric cell division in *Drosophila melanogaster* and *Caenorhabditis elegans* are conserved in vertebrates. Similarly to those in invertebrate model organisms, Partitioning defective 3 (PAR3), PAR6 and atypical protein kinase C (aPKC) homologues act together to establish cell polarity in vertebrates[7]. The vertebrate Partner of Inscuteable (PINS; also known as RAPS) homologue, G protein α_i-subunit ($G\alpha_i$), and nuclear mitotic apparatus protein 1 (NuMA; the LIN-5 and Mushroom body defect (MUD) homologue) regulate spindle orientation and microtubule–cortex association[140], and Numb controls endocytosis[141]. Nevertheless, the function of these proteins during asymmetric cell division in vertebrates is not clear.

Asymmetric cell division in neural progenitor cells is the best understood asymmetric cell division process in mammals[142]. After an initial expansion phase by symmetric division, progenitors undergo asymmetric divisions, giving rise to two daughter cells: one progenitor cell and one cell that either differentiates into a neuron or becomes an intermediate progenitor, which forms two neurons after a terminal symmetric division. Progenitors are located in the apical side of the neuroepithelium, where Par proteins accumulate in the apical cortex. Unlike in *D. melanogaster*, however, the apical membrane domain in dividing progenitors is very narrow, and even slight twists of the cleavage plane lead to asymmetric inheritance[124]. Numb is expressed by the progenitors and concentrates on apical adherens junctions and on the basolateral plasma membrane. This has led to a model in which the asymmetric inheritance of PAR3 during oblique divisions (divisions occuring at ~45° angle) inactivates Numb in one of the two daughter cells so that it no longer inhibits Notch, and two daughters with unequal Notch signalling levels are formed[143]. As PAR3 is a key factor promoting Numb phosphorylation by aPKC in *D. melanogaster*[76], it is possible that, in vertebrates, differential phosphorylation of Numb might be responsible for the different activity in the two daughter cells. In addition to this Par protein-mediated asymmetry, the asymmetric inheritance of apical and basal processes[144], the polarized localization of the vertebrate Brain tumour (BRAT) homologue E3 ubiquitin-protein ligase tripartite motif-containing protein 3 (TRIM3)[145] and extracellular signals might have a role in establishing asymmetry.

leukaemia). NUP98–HOXA9 induces the expression of Musashi 2, which in turn inhibits Numb, potentially triggering the enormous expansion of undifferentiated progenitors in advanced-stage CML[128]. Thus, the conserved connection with tumorigenesis establishes an unprecedented clinical relevance for research on asymmetric cell division.

Open questions and future challenges

The progress in our understanding of asymmetric cell division during the past 10 years has been enormous. We have learned that the phosphorylation of cell fate determinants by asymmetrically distributed kinases is the driving force for the asymmetric localization of these determinants, whereas polarized transport seems to have a minor role. It has become clear that microtubules, which were originally thought not to be involved in asymmetric cell division, mediate essential interactions between centrosomes and the cell cortex. These interactions maintain the polarity axis over multiple divisions and guide asymmetric protein localization during late mitosis. An exciting connection between asymmetric cell division and tumorigenesis has emerged in flies, mice and humans and has given rise to major challenges, in part because of our still incomplete understanding of asymmetric cell division in vertebrates. In addition, newly emerging technologies lay the groundwork for a systems-level understanding of the process.

Although we have learned the basic principles of asymmetric determinant segregation, our understanding of the cell fate choices that are influenced by those determinants is limited. We know that Numb acts on Notch, that Prospero is a transcription factor and that BRAT regulates post-transcriptional events, but how these factors cooperate to prevent self-renewal is unclear. In fact, we do not understand the transcriptional network that governs and maintains self-renewal in *D. melanogaster* neuroblasts. We also do not know how the initial bias in this network is stabilized over time and results in a daughter cell that terminally exits proliferation. And in particular, we do not know how defects in fate specification result in the formation of misguided tumour-initiating cells.

The solutions to these problems may come from the spectacular technological advances in the field. The establishment of genome-wide transgenic RNA interference libraries in flies[129] allows us to test gene functions at an unprecedented speed and on a near genome-wide level[130]. In addition, the development of new sequencing technologies has opened new dimensions for genome-wide profiling of transcription, RNA splicing and chromatin association[131]. It is likely that these technologies will establish *D. melanogaster* neuroblasts as one of the best model systems for the establishment and stabilization of cell fate choices and will shed light on the mechanisms of stem cell-derived tumour formation. The potential clinical relevance of those findings will be a strong motivation to embark on these difficult tasks.

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