

Asymmetric cell division: microtubule dynamics and spindle asymmetry

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Summary

Asymmetric cell division can produce daughter cells with different developmental fates and is often accompanied by a difference in cell size. A number of recent genetic and *in vivo* imaging studies in *Drosophila* and *Caenorhabditis elegans* have begun to elucidate the mechanisms underlying the rearrangements of the cytoskeleton that result in eccentrically positioned cleavage planes. As a result, we are starting to gain an insight into the complex nature of the

signals controlling cytoskeletal dynamics in the dividing cell. In this commentary we discuss recent findings on how the mitotic spindle is positioned and on cleavage site induction and place them in the context of cell size asymmetry in different model organisms.

Key words: Asymmetric cell division, Microtubules, Spindle, Par proteins, G-protein signalling, Dynein, Dynactin

Asymmetry generates diversity

Asymmetric partitioning of cell fate determinants is employed to generate cell type diversity in a number of different organisms[†] (Horvitz and Herskowitz, 1992). However, asymmetric division does not necessarily result in unequal cell size. While asymmetrically segregated determinants can give cells a distinct developmental potential[§], cell size can correlate with how often a cell divides and can thereby fulfil an important function during cell lineage determination. For example, the *C. elegans* zygote divides to give two differently sized daughter cells: AB is about 25% larger than P1 and most of the cells of the hatching larva, 389 of 558, are derived from the AB lineage (Sulston et al., 1983). During neurogenesis in *Drosophila*, neural precursor cells (neuroblasts) divide asymmetrically. The newly born neuroblast is approximately 75% larger than its sister ganglion mother cell (GMC) and divides between 1 and 30 times further (Bossing et al., 1996). In contrast, the GMC divides only once to give rise to two glia or neurons. As a result, tissues are generated in the correct position and at the correct time in the developing embryo.

The direction of division and the ability of a cell to divide symmetrically or asymmetrically in size is brought about by rearrangement of the cytoskeleton. We know little about the molecular mechanisms that regulate cell size asymmetry;

however, in the last few years much has been learned about the targets of these controls. One such substrate is the mitotic spindle and there is good evidence that its orientation and position in the cell determines the site of cell cleavage (reviewed by Strome and Wood, 1983). Asymmetric division can be likened to slicing a piece of cake (Fig. 1) A vertical slice divides the cake into two pieces of equal size and content, both with the same amount of chocolate cake and strawberry icing (Fig. 1A). However, a horizontal slice gives two pieces of unequal size and content, a large piece of chocolate cake and a small piece of cake with all of the icing (Fig. 1B). If instead of icing we consider cell fate determinants, it then becomes clear how the orientation and position of the mitotic spindle and the cleavage furrow direct symmetric or asymmetric cell division. For example, *Drosophila* epithelial cells divide symmetrically along the planar axis of the embryo to produce two daughters of equal size and mitotic potential. Factors localised at the basolateral cortex are segregated equally to both daughter cells (Fig. 1C) (Matsuzaki et al., 1998). In contrast, during neuroblast division localised cell fate determinants such as Prospero, a homeodomain-containing transcription factor that contributes to the identity of the GMC, are segregated asymmetrically into the basal GMC (Fig. 1D) (Doe et al., 1991; Matsuzaki et al., 1992; Matsuzaki et al., 1998; Vaessin et al., 1991). Therefore, despite having the same ectodermal origin as epithelial cells, *Drosophila* neuroblasts divide asymmetrically and the resulting daughter cells have distinct cell sizes, mitotic potential and cell fate (reviewed by Lu et al., 2000).

Changing from a symmetric to an asymmetric division requires a reorientation of the division axis. In *Drosophila* embryonic neuroblasts, this involves a 90° rotation of the pro/metaphase mitotic spindle (Kaltschmidt et al., 2000). In the early *C. elegans* embryo a 90° rotation of the centrosome-nucleus complex positions the cleavage plane such that localised P-granules, which are thought to play a role in germ line determination in the later embryo, are asymmetrically partitioned to the germ line precursor

[†]The idea of asymmetric distribution of determinants is not new. Studies by Whitman on the leech cell lineage showed that distinct cytoplasmic domains are partitioned to the descendant cells (Whitman, 1878). Subsequent observations by Conklin supported this idea; he identified five distinct pigmented areas of egg cytoplasm that segregate to cells giving rise to five distinct cell types (Conklin, 1905).

[§]Cell fate determination demands a stable change in the interior of the cell. A cell is characterised by fate determining factors (also called 'cell fate determinants'), such as proteins and mRNAs. Presence and absence in the cell can result in daughter cells having properties different from each other. For example, the presence or absence of transcription factors can result in gene expression being turned on or off. In yeast, the ability to switch mating type is determined by HO endonuclease, whose transcription is repressed by Ash1p (asymmetric synthesis of HO endonuclease) (Bobola et al., 1996; Sil and Herskowitz, 1996). Ash1p is present in the daughter but not in the mother, resulting in HO endonuclease expression and mating type switching in the mother, but not in the daughter (Nasmyth et al., 1987).

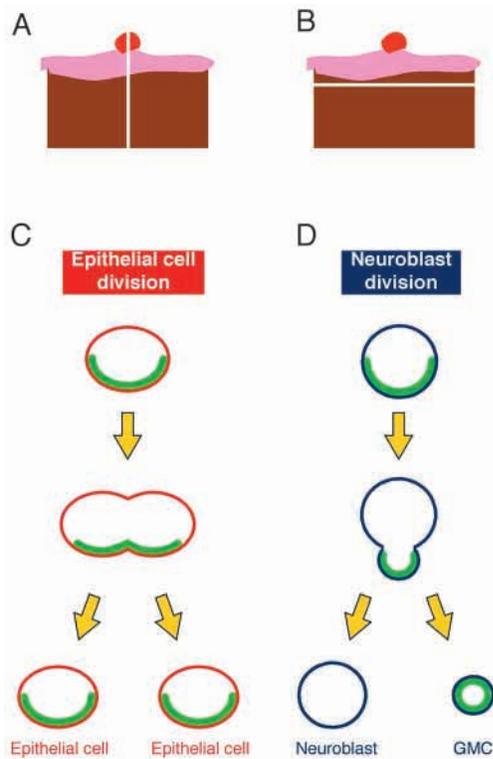


Fig. 1. Chocolate cake with strawberry icing (A,B) and epithelial cell versus neuroblast division (C,D). (A) Symmetric division: a vertical slice divides the cake into two pieces of equal size and content, both containing the same amount of chocolate cake and strawberry icing. (B) Asymmetric division: a horizontal slice yields two pieces of unequal size and content, a large piece of chocolate cake and a small piece of cake with all of the icing. (C) Epithelial cells (red) divide symmetrically, giving rise to two equal cells. Cell fate determinants (green) localised at the basolateral cortex are partitioned equally to both daughter cells. (D) Neuroblast (blue) divisions are intrinsically asymmetric: the two daughter cells differ in cell size, mitotic potential and cell fate. Asymmetrically localised cell fate determinants, such as proteins or mRNAs (green), are segregated asymmetrically into the basal GMC. Apical is up and basal is down.

daughter (Hyman and White, 1987; Kemphues and Strome, 1997; Strome and Wood, 1982). Additional control of spindle dynamics is necessary when, as in both *Drosophila* neuroblasts and the *C. elegans* zygote, the generation of daughter cells of distinct cell fates is accompanied by a difference in cell size. The mitotic spindle plays a key role in setting up the eccentrically placed cleavage furrow. Until recently the cleavage furrow in animal cells was thought to form equidistant between the two spindle poles; however, a growing number of observations show that asymmetry can be achieved in different ways, both between organisms and within a single organism. Here we present a short survey of asymmetric cell division, highlighting the recent findings on the control of spindle positioning and specification of the cleavage plane in the two-cell nematode *C. elegans* embryo and comparing them with results that have been obtained in other systems, especially the fruit fly, *Drosophila melanogaster*.

Cleavage plane induction

The first evidence to suggest a role for the mitotic spindle in

positioning the cleavage plane came from experiments carried out over 80 years ago (Conklin, 1917). By exposing *Crepidula* eggs to centrifugal force and thereby moving the mitotic spindle, Conklin showed that its position determines the cleavage plane and that different regions of the cell cortex can respond to form a cleavage furrow. Conklin's work, together with later experiments by Hiramoto, in which he aspirated the spindle from sea urchin embryos (Hiramoto, 1956), further established the timing of cleavage site determination by the spindle and showed that it is complete by mid-anaphase[†].

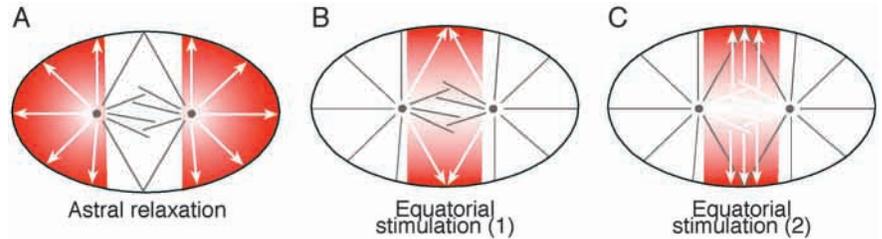
To initiate the cleavage furrow the mitotic spindle must dictate local changes of the cell surface in the form of either relaxation or contraction of the cell membrane. Which element of the mitotic spindle, the asters or the midzone microtubules[‡], provides that stimulus, remains an important question that has yet to be fully resolved. Nor is it necessary that all organisms, or even all cells within, use the same mechanism. Three mechanisms have been proposed by which different elements of the mitotic spindle could signal to the cell cortex to position the cleavage furrow (reviewed by Field et al., 1999; Gatti et al., 2000; Oegema and Mitchison, 1997). According to the astral relaxation model (White and Borisy, 1983), the asters signal to the cell cortex near the poles, inducing it to relax (Fig. 2A). Alternatively, the equatorial region of the cell could be induced to contract, either by a signal from the asters (Fig. 2B) (Devore et al., 1989; Rappaport, 1986) or from the overlapping microtubules of the spindle midzone (Fig. 2C). It is also possible that both signals act together.

Several landmark experiments have addressed whether the spindle asters or the midzone produce the signal that positions the division plane. Historical evidence that the spindle asters determine the site of cleavage comes from micro-manipulation studies on sea urchin eggs (Rappaport, 1961). Microsurgical removal of the centre of the egg during the first division results in a horseshoe-shaped cell with two nuclei. At the next division, the two spindles produce three cleavage planes: two that bisect each of the spindles and one extra plane between the two adjacent spindle poles. As a result, four daughter cells are formed. The outcome of this experiment suggests that interacting spindle asters control the position of the cleavage plane, possibly by where they touch the cell cortex. However, this hypothesis is difficult to verify since there is, as yet, no direct experimental evidence for such causality. Nevertheless, one possible explanation in support of this model has been put forward (Foe et al., 2000). Foe and co-workers have studied the interactions of spindle microtubules and the actomyosin cytoskeleton in syncytial *Drosophila* blastoderm embryos and find that filamentous actin and cytoplasmic myosin II are

[†]Anaphase is characterised by a shortening of the kinetochore microtubules resulting in the poleward movement of sister chromatids (anaphase A) followed by the elongation of polar microtubules (anaphase B) leading to separation of the two spindle poles (see also [‡]).

[‡]Microtubules are the primary structural component of the mitotic spindle. The polarity of the spindle microtubules is such that the minus ends are at the centrosomes and the plus ends directed towards the cell cortex (or chromosomes). There are three different kinds of spindle microtubule, each named after the position of the plus end. The first class, kinetochore microtubules, extend from the centrosome to the kinetochores and are important for the segregation of the chromosomes to the spindle poles during anaphase. The second class, astral microtubules, stretch from the centrosome towards the periphery, with their plus ends contacting the cell cortex. This interaction is important for spindle positioning and cleavage plane localisation during cytokinesis. The third kind are midzone microtubules, which reach from one centrosome into the spindle midzone towards the other centrosome. While midzone microtubules from opposite centrosomes interact, they generate an outward force through antiparallel sliding, which counteracts the inward forces generated by the kinetochores.

Fig. 2. Three models explaining cleavage plane induction. (A) According to the astral relaxation model the asters signal to the cell cortex near the poles, inducing it to relax. The additional two models, equatorial stimulation through the asters (B) and equatorial stimulation through the spindle midzone microtubules (C), are based on induction of the equatorial region of the cell, either by signals from the asters (B) or from the overlapping microtubules of the spindle midzone (C). White arrows indicate stimuli and are pointed towards the induced area of the cell cortex (red). Microtubules are indicated as grey lines and centrosomes as filled grey circles (see also footnote on p. 2258).



transported towards microtubule plus ends (Foe et al., 2000). From these findings they suggest that cleavage plane induction occurs at sites where actin filaments attach both to the cortex and to microtubules.

A number of studies in grasshopper neuroblasts (Kawamura, 1977), newt kidney epithelial cells and echinoderm eggs (Rappaport and Rappaport, 1974) support the model that the midzone microtubules specify the site of cleavage furrow formation. For example, inserting a small block between the midzone microtubules and the cell cortex in flattened echinoderm eggs results in inhibition of cell division (Rappaport, 1986; Rappaport and Rappaport, 1983). By comparing cleavage activity with the position of midzone microtubules in cultured epithelial cells (Cao and Wang, 1996; Wheatley and Wang, 1996), it was concluded that the signal triggering furrow formation is emitted by the midzone microtubules. A more exacting experiment would be to eliminate the astral microtubules specifically and observe whether the midzone on its own is able to induce a cleavage plane. Bonaccorsi et al. did just this and showed that spermatocytes and larval neuroblasts from a *Drosophila asterless (asl)* mutant could still undergo anaphase and telophase, thus implying that astral microtubules are not necessary to induce cytokinesis (Bonaccorsi et al., 2000; Bonaccorsi et al., 1998). It is difficult in these experiments to be certain that all astral microtubules are lacking, and it is possible that only a few astral microtubules are sufficient to induce cytokinesis. Furthermore, it has been shown that cells with acentrosomal spindles (which resemble anastral spindles in that they also lack astral microtubules) form bipolar spindles and enter anaphase, but cytokinesis often fails (Khodjakov and Rieder, 2001).

Asymmetric cell division requires an eccentrically placed cleavage plane

The best-studied example of asymmetric cell division is the *C. elegans* embryo, which is characterised by a cascade of asymmetric divisions throughout its early development. The large cell size, simplicity and transparency of the animal, together with the strictly defined hierarchy of cell divisions, makes it well suited to study the mechanics of asymmetric cell division and cytokinesis (reviewed by Plasterk, 1999). Forward and reverse genetic approaches, together with mechanical manipulation and sophisticated imaging techniques, have been used to address spindle positioning and specification of the cleavage plane during early *C. elegans* development.

The anterior-posterior polarity of the one-cell *C. elegans* embryo is established between the time of fertilisation and the

first mitotic division in response to an external cue provided by the sperm (Wallenfang and Seydoux, 2000). The next phase in generating polarity is marked by a number of characteristic changes, which result in the production of two cells distinct in cell fate and cell size. After the oocyte and sperm pronuclei meet in the posterior hemisphere, they move to the centre of the embryo (Albertson, 1984; Hyman and White, 1987). Following pronuclear migration, the mitotic spindle in the *C. elegans* zygote is initially positioned symmetrically along the anterior-posterior axis. As the spindle grows, one centrosome moves towards the posterior cell cortex while the other remains relatively stationary, generating a spindle that is off-centre. Cleavage occurs midway between the two spindle poles giving rise to a large anterior AB cell and a smaller posterior P1 cell (Albertson, 1984).

What are the molecular forces that act on the mitotic spindle to cause this asymmetry? Two types of force that are dependent on microtubules play a role in spindle positioning and spindle pole separation in different model organisms. First a sliding force generated between the overlapping midzone microtubules, possibly mediated by plus-end-directed motor proteins of the kinesin family, could separate spindle poles (Fig. 3A). Second, the astral microtubules on each pole could act, perhaps via the minus-end directed microtubule motor dynein, to drag the spindle poles to opposite sides of the cell (Fig. 3B). In addition, cortical cues could cause microtubules to be selectively destabilised (Fig. 3C) or stabilised (Fig. 3D) in one region of the cell, which would result in an overall imbalance of the microtubule polymerisation forces.

A possible mechanism by which such cell polarity cues translate to the asymmetric spindle positioning in *C. elegans* has been proposed (Grill et al., 2001). Time-lapse analysis of the one-cell *C. elegans* embryo had previously shown that the anterior centrosome remains fixed in position, while the posterior centrosome oscillates and becomes smaller as it moves closer to the cell cortex (Albertson, 1984; Hyman and White, 1987; Keating and White, 1998). The consequence of such unequal centrosomal movement is an asymmetrically positioned spindle with the posterior centrosome closer to the cell wall. To reveal the forces that act on each spindle pole, Grill et al. removed the central spindle by laser ablation while leaving both spindle poles intact (Grill et al., 2001). In irradiated wild-type embryos, the posterior spindle pole moved faster and further than the anterior pole. This elegant experiment reveals that pulling forces act on the spindle poles, and that the posterior shift of the spindle in wild-type *C. elegans* zygotes results from a larger pulling force acting on the posterior pole than on the anterior pole.

The asymmetry of the net forces acting on the two spindle

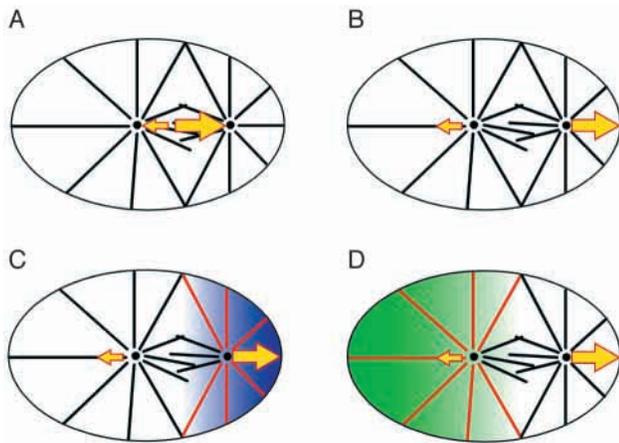


Fig. 3. Possible models by which an unequal pulling force could be generated. (A) The force generated by the pushing apart of the overlapping midzone microtubules is unequal. (B) The force of the pulling astral microtubules is unequal. The microtubules on one side of the cell cortex are either destabilised (red lines in blue area in C) or stabilised (red lines in green area in D). Microtubules are indicated as black lines and centrosomes as filled black circles (see also footnote on p. 2258).

poles is under control of the *par* genes (Grill et al., 2001). In wild-type one-cell *C. elegans* embryos, PAR-3 localises to the anterior cortex (Etemad-Moghadam et al., 1995), while PAR-2 localises to the posterior (Boyd et al., 1996). The spindle in both, *par-2* and *par-3*, is centrally positioned (Kemphues et al., 1988). After removal of the central spindle in *par-3* mutants, the velocity of both spindle poles resembles that of the posterior spindle pole in wild-type zygotes. In contrast, after removal of the central spindle of *par-2* mutants both spindle poles move apart with a velocity equal to that of the anterior spindle pole in wild-type zygotes. This shows that the microtubule dynamics in the *C. elegans* zygote are under the control of polarity factors asymmetrically localised in the cell (Grill et al., 2001).

PAR-3 may stabilise or anchor microtubules (reviewed by Rose and Kemphues, 1998; Cheng et al., 1995; Etemad-Moghadam et al., 1995) and there is good reason to think that asymmetrically localised PAR-3 could act to regulate aster movement by controlling microtubule stability. Only microtubules on the anterior side of the zygote are stabilised, leaving those of the posterior aster free to depolymerise. It is intriguing that the mammalian homologues of the serine-threonine kinase PAR-1, which is localised to the posterior cortex of the *C. elegans* zygote, have been shown to destabilise microtubules (Drewes et al., 1997; Ebnet et al., 1999). Although no direct effect of PAR-1 on microtubule dynamics has yet been observed, these findings would support the working model proposed by Grill et al.: all microtubules of both asters generate equal forces, but the interactions of the microtubules and the posterior cortex are weaker than those of the anterior cortex (Grill et al., 2001).

Eccentrically placed cleavage planes

Until recently the cleavage furrow in animal cells was thought to form equidistant between the two poles of the mitotic spindle. If so, then repositioning of the mitotic spindle or migration of the nucleus would be sufficient to give rise to an eccentrically

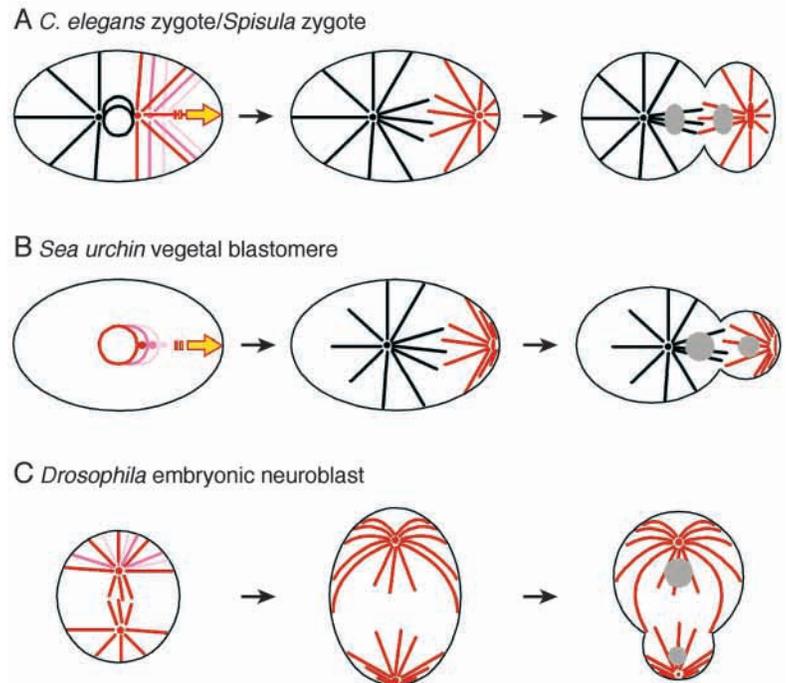
placed cleavage plane. As described above, the asymmetric division of the *C. elegans* zygote provides an example for repositioning the mitotic spindle (Fig. 4A). Similarly, in the clam *Spisula* zygote, one aster is stationary while the other oscillates to a position closer to the cell cortex (Fig. 4A) (Dan and Inoué, 1987). In vegetal cells of sea urchin embryos, lateral migration of the nucleus led by a centrosome prior to spindle formation has been shown to give rise to an asymmetrically placed spindle (Fig. 4B) (Dan, 1979; Schroeder, 1987). However, the generation of asymmetry during *Drosophila* neuroblast division follows a different scheme altogether, since the cleavage furrow does not form equidistant between the two spindle poles (Fig. 4C).

During neurogenesis in *Drosophila*, neuroblasts delaminate from the neuroectoderm and undergo asymmetric stem-cell like divisions, generating another neuroblast and a GMC. In vivo imaging of *Drosophila* embryos expressing a GFP (green fluorescent protein) fusion to the microtubule binding protein tau (Brand, 1995) reveals that, in embryonic neuroblasts, the mitotic spindle is symmetric and centrally placed through metaphase. However, at the onset of anaphase, the microtubules appear to shorten on the basal side of the cell and elongate on the apical side (Kaltschmidt et al., 2000). The overlapping apical and basal astral microtubules, which are distinctly different in length in *Drosophila* embryonic neuroblasts, could specify the eccentric position of the cleavage furrow. The elongation of the apical astral microtubules towards the emerging GMC occurs before the cell membrane starts to pucker, and membrane invagination occurs before the midbody moves towards the cleavage furrow (Kaltschmidt et al., 2000). The eccentric placement of the cleavage plane in *Drosophila* embryonic neuroblasts might, therefore, support the postulated role of astral microtubules in specifying the site of the cleavage furrow as described above (reviewed by Oegema and Mitchison, 1997). In contrast, in *Drosophila* *asl* and *centrosomin* (*cnn*) mutants, larval neuroblast divisions are still asymmetric, in spite of partially defective mitotic centrosomes and the absence of detectable astral microtubules (Bonaccorsi et al., 2000; Megraw et al., 2001; Megraw et al., 1999; Vaizel-Ohayon and Schejter, 1999). Giansanti et al. postulate that the eccentric position of the cleavage plane in *Drosophila* neuroblasts is defined by signals originating from the midbody, and it is the asymmetric position of the midbody that leads to the overall spindle asymmetry (Giansanti et al., 2001). It is interesting to note that these two types of asymmetric division, an asymmetric division reminiscent of that seen in the *C. elegans* zygote and an asymmetric spindle similar to that described for *Drosophila* neuroblasts, can exist within the same lineage, such as that of the *Drosophila* sensory organ precursor (SOP) cells (Roegers et al., 2001).

Possible controls of cleavage plane positioning

The source of information to position an eccentric cleavage plane is unclear. One possibility is a mechanical connection between the mitotic spindle and the cell cortex (Dan and Ito, 1984). Indeed, a specific mechanical linkage between the spindle and the plasma membrane has been demonstrated in a number of unequally dividing cell types (Conklin, 1917; Harvey, 1935). The most convincing evidence for a specific site comes from micro-manipulation studies in *Chaeopterus* oocytes: after being displaced by a needle, the spindle returns to its original position (Lutz et al., 1988).

Fig. 4. Generation of eccentrically placed cleavage plane in different model organisms. (A) In the *C. elegans* zygote, the mitotic spindle is initially positioned symmetrically along the anterior-posterior axis. Anterior is left and posterior is right. The centrosome-pronucleus complex (pronuclei are represented as empty circles, centrosomes as small filled circles) is oriented along the anterior-posterior axis. During anaphase the anterior aster is stationary (black lines) while the posterior aster oscillates (red lines changing to pink) to a position closer to the cell cortex. At telophase (grey ovals represent telophase DNA) the spindle is asymmetrically positioned with the posterior elongated centrosome closer to the cell wall. Similarly, in the clam *Spisula* zygote, one aster is stationary while the other oscillates to a position closer to the cell cortex. (B) In vegetal cells of sea urchin embryos, lateral migration of the nucleus (empty circle changing from red to pink) led by a centrosome (small filled circle changing from red to pink) prior to spindle formation gives rise to an asymmetrically placed spindle. At telophase (grey ovals represent telophase DNA) the micromere aster (red) is fattened while the macromere aster (black) is spherical. (C) In the *Drosophila* embryonic neuroblast, the spindle forms symmetrically between the two spindle poles. Apical is up and basal is down. At the onset of anaphase, the microtubules appear to shorten on the basal side of the cell and elongate/enrich on the apical side. At telophase (grey ovals represent telophase DNA) the centrosome of the basal aster is smaller than that of the apical aster.



Giansanti et al. suggest that in *Drosophila* neuroblasts the shift of the midbody towards the GMC occurs via a mechanical link between the cortex, nucleus and midbody (Giansanti et al., 2001). Alternatively, one could imagine a mechanism whereby astral microtubules on the basal spindle pole of *Drosophila* neuroblasts are induced to depolymerise while those of the apical aster are stabilised, resulting in a larger apical and smaller basal aster that together constitute an asymmetric spindle. This model agrees with a role of astral microtubules in the placement of the cleavage plane as previously suggested (Rappaport, 1961). Certainly, both this model and that of mechanical linkage predict a specialised site on the GMC cortex to facilitate local interaction between the spindle microtubules and the cell cortex. If such an interaction actively destabilises microtubules locally, then polymerisation forces would become unbalanced, resulting in either asymmetrically positioned asters and/or differently sized asters.

Bearing in mind the requirement for asymmetrically stabilised spindle poles during asymmetric cell division, it is intriguing that all the above mentioned examples of eccentrically placed spindles share an asymmetry in the morphology of their centrosomes and the microtubules they produce. For example, the anterior aster of the asymmetrically-positioned spindle of the *C. elegans* zygote is large and has many microtubules, while the posterior aster appears flattened and smaller and has fewer astral microtubules (Keating and White, 1998). A difference in aster size and morphology has also been described for the divisions of the four-cell sea urchin embryo. The micromere centrosomes contain less centrosomal material than the macromere poles (Holy and Schatten, 1991) and the macromere aster is spherical, whereas the micromere aster undergoes elongation during late anaphase and telophase and is flattened perpendicular to the spindle axis

(Holy and Schatten, 1991). Note, however, that it is possible that micromere centrosomes contain the same material but are merely more condensed than the macromere centrosomes. The unique morphology of the micromere aster in sea urchin embryos has been suggested to be due to proximity to the plasma membrane (Dan and Nakajima, 1956). As the two centrosomes have already begun to become distinct in metaphase (Holy and Schatten, 1991), this must also reflect an intrinsic difference between the two microtubule organising centres. In *Drosophila* embryonic neuroblasts, as the astral microtubules become longer and more abundant at the beginning of anaphase, the apical aster enlarges. The basal aster is concomitantly reduced in size and the basal centrosome has reduced levels of the centrosomal proteins γ -tubulin, CP60 and CP190 (Kaltschmidt et al., 2000). This was found also to be the case for larval *Drosophila* neuroblasts (Bonaccorsi et al., 2000; Ceron et al., 2001).

Microfilaments may be part of the spindle-positioning machinery in several organisms. First, actin has been reported to localise temporarily to the anterior region of the *C. elegans* zygote (Hill and Strome, 1988; Strome, 1986) and asymmetric positioning of the mitotic spindle is inhibited by disrupting microfilaments with cytochalasin D during a narrow time interval in the first cell cycle (Strome and Wood, 1983). Second, an enrichment of actin has also been postulated to play a role in establishing cortical polarity in the mouse egg (Longo and Chen, 1985). In immature mouse oocytes actin is cortical, while in mature eggs it is asymmetrically localised. When induced to undergo maturation, the meiotic spindle forms in the centre of the oocyte and then moves towards the actin-rich periphery, where it becomes anchored to the plasma membrane (Chambers, 1917; Conklin, 1917; Longo and Chen, 1984). Disruption of microfilaments with cytochalasin B inhibits this

movement. Third, actin has also been shown to localise asymmetrically in *Drosophila* larval neuroblasts (McCartney et al., 1999) and it is possible that localised actin functions (possibly only briefly) to set up or respond to the spatial cues that are needed to establish the asymmetry in the spindle of *Drosophila* larval neuroblasts.

What are the factors known to be necessary for induction of spindle asymmetry in embryonic *Drosophila* neuroblasts, and how are they regulated during the embryonic neuroblast cell cycle? First, Inscuteable [a protein of 859 amino acids encoding a putative SH3 target site, ankyrin repeats and a PDZ-binding domain (Kraut and Campos-Ortega, 1996)] localises as an apical crescent in neuroblasts from late interphase until anaphase (Kraut et al., 1996) and is both necessary and sufficient to direct apical-basal cell division in neuroblasts (Knoblich et al., 1999; Kraut et al., 1996; Tio et al., 1999). In *inscuteable*^{P72} null embryos, the mitotic spindle fails to rotate and the direction of neuroblast division is no longer strictly apical-basal (Kaltschmidt et al., 2000; Kraut et al., 1996). Several proteins are required to localise Inscuteable. Pins (Partner of Inscuteable), a tetratricopeptide (TPR) repeat protein, binds to Inscuteable and shows an almost identical subcellular localisation (Parmentier et al., 2000; Schaefer et al., 2000; Yu et al., 2000). Inscuteable localisation is established but not maintained in *pins* mutants and as a consequence the mitotic spindle in embryonic neuroblasts is misoriented.

Pins encodes three 'GoLoco' motifs, which are present in proteins that bind the α subunits of heterotrimeric G-proteins, $G\alpha_0$ and $G\alpha_i$ (Schaefer et al., 2000). $G\alpha_0/G\alpha_i$, together with $G\beta\gamma$, comprise the G-protein complex and are involved in the organisation of the actin cytoskeleton and asymmetric localisation of cortical proteins in several different organisms (reviewed by Chant, 1999; Jin et al., 2000). Schaefer et al. have recently shown that, in *Drosophila* embryonic neuroblasts, Inscuteable functions via Pins as an apical adaptor for $G\alpha_i$, which in turn sets up a polarity cue at the apical neuroblast cortex (Schaefer et al., 2001). In addition, overexpression of $G\alpha_i$ in neuroblasts produces two equal-sized daughter cells (Schaefer et al., 2001). The heterotrimeric G-protein cascade, which is confined to the apical cell cortex, thereby mediates asymmetric neuroblast division, possibly via reorganisation of the actin cytoskeleton (reviewed by Schweisguth, 2000). $G\alpha$ is also required for correct positioning and morphology of the mitotic spindle in the *C. elegans* zygote (reviewed by Gotta and Ahringer, 2001a; Gotta and Ahringer, 2001b). Thus, G-protein signalling during spindle orientation may be a process conserved between *Drosophila* and *C. elegans*.

Gotta and Ahringer suggest that G-protein signalling may function to connect spindle position and polarity in *C. elegans* (Gotta and Ahringer, 2001a). The mammalian homologue of Pins, Ags-3, functions as a receptor-independent activator of G-protein signalling (Takesono et al., 1999). In *C. elegans* simultaneous inhibition of two genes with weak homology to Pins (*ags-3.2* and *ags-3.3*) recapitulates the phenotype of embryos lacking $G\alpha$ activity: asymmetric spindle positioning is affected as is the generation of different-sized daughter cells [M. Gotta and J. Ahringer, personal communication; S. Grill, P. Gonczy and A. Hyman, personal communication (Gotta and Ahringer, 2001a)]. In *Drosophila pins* mutants a large number of larval neuroblasts divide symmetrically (Parmentier et al., 2000), although this has not been observed in embryonic neuroblasts

(Schaefer et al., 2000; Yu et al., 2000), possibly due to maternal Pins protein being present and sufficient for embryonic divisions.

Lu et al. have shown that adherens junctions are responsible for the default, planar orientation of the mitotic spindle in epithelial cell (Lu et al., 2001). RNAi against the epithelial-cell-enriched (E)-adenomatous polyposis coli (APC) tumor suppressor protein and microtubule-associated protein EB1 (both of which are adherens-junction-associated proteins), causes epithelial cells to switch from a symmetric to an asymmetric division pattern (Lu et al., 2001). This implies a possible function of adherens junctions in preventing asymmetric cell division. It is noteworthy, however, that cells in the procephalic neurogenic region (PNR) have adherens junctions but nonetheless divide asymmetrically.

A role for the dynein-dynactin complex in generating asymmetry?

The minus-end directed microtubule motors and their activators may also play a role in generating asymmetry. Several models have been proposed for the function of dynein and dynactin during nuclear migration/spindle positioning in *S. cerevisiae*. For example, dynein may alter microtubule dynamics (Carminati and Stearns, 1997). In the absence of dynein, the rates of microtubule polymerisation and depolymerisation are significantly slower, and catastrophe frequencies are reduced by half. One explanation could be the presence of dynein at the spindle pole and on cytoplasmic microtubules (Yeh et al., 1995). Dynein may induce conformational changes in microtubules and thereby affect microtubule growth dynamics (e.g. reduce growth and shrinking rates as well as the frequency of catastrophe). Alternatively, the presence of dynein on a microtubule might exclude binding of microtubule-associated proteins that would normally act to stabilise the microtubules.

In the *C. elegans* zygote, cytoplasmic dynein and dynactin are required during pronuclear migration, centrosome positioning and pronuclear rotation (Gönczy et al., 1999; Skop and White, 1998). Cytoplasmic dynein is further involved in maintaining the tight association between the centrosomes and the male pronucleus. Gönczy et al. suggest a mechanism by which cytoplasmic dynein, anchored to the pronucleus, drives centrosome separation. This model predicts that the pulling forces required during centrosome separation are provided by interactions between cytoplasmic dynein anchored on the nuclear membrane and astral microtubules (Gönczy et al., 1999).

The dynein-dynactin complex has also been implicated in the rotation of the centrosome-nucleus-complex in the *C. elegans* P1 cell (Skop and White, 1998; Waddle et al., 1994). The original model suggested a cortical capture mechanism (Hyman and White, 1987). Laser microsurgery experiments identified a cortical site rich in actin, actin-capping proteins and dynactin (Hyman, 1989; Waddle et al., 1994). Reducing the levels of two orthologues of the *C. elegans* dynactin complex results in misalignment of the spindle in the P1 cell (Skop and White, 1998). By localising to the cell cortex, dynactin may both tether microtubule ends and bind to the minus-end directed, microtubule-associated dynein, thereby activating its motor activity. While tethered to the cell cortex, dynein could reel in one aster by moving along astral microtubules, depolymerising and shortening them (Skop

and White, 1998; Waddle et al., 1994). An alternative interpretation of P1 spindle misalignment is that the spindle is displaced to an eccentrically localised cortical site as a result of asynchronous ingression of the first cleavage furrow (Gönczy et al., 1999). This would imply that the dynein-dynactin complex controls the position of spindle attachment.

In *Drosophila* embryonic neuroblasts, a subunit of dynactin, p150^{Glued}, is localised in a basal cortical crescent before it is asymmetrically segregated to the GMC cortex (J.A.K. and A.H.B., unpublished). It is possible that, by binding to dynactin at the GMC cortex, dynein mediates both the rotation of the pro/metaphase spindle and the difference in length of astral microtubules in the neuroblast.

Common themes in generating cell diversity

Asymmetric cell division relies on the position of the mitotic spindle, which is regulated by several different mechanisms, including the Par proteins, G-protein signalling and the dynein-dynactin complex. It remains to be seen how these mechanisms are coordinated and regulated. Technical advances, such as laser ablation (reviewed by Khodjakov et al., 1997) and speckle imaging analysis of microtubule dynamics (Waterman-Storer and Salmon, 1999) should help to further our understanding of these questions.

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