

# Rotation and asymmetry of the mitotic spindle direct asymmetric cell division in the developing central nervous system

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focus on  
CYTOSKELETON

The asymmetric segregation of cell-fate determinants and the generation of daughter cells of different sizes rely on the correct orientation and position of the mitotic spindle. In the *Drosophila* embryo, the determinant Prospero is localized basally and is segregated equally to daughters of similar cell size during epidermal cell division. In contrast, during neuroblast division Prospero is segregated asymmetrically to the smaller daughter cell. This simple switch between symmetric and asymmetric segregation is achieved by changing the orientation of cell division: neural cells divide in a plane perpendicular to that of epidermoblast division. Here, by labelling mitotic spindles in living *Drosophila* embryos, we show that neuroblast spindles are initially formed in the same axis as epidermal cells, but rotate before cell division. We find that daughter cells of different sizes arise because the spindle itself becomes asymmetric at anaphase: apical microtubules elongate, basal microtubules shorten, and the midbody moves basally until it is positioned asymmetrically between the two spindle poles. This observation contradicts the widely held hypothesis that the cleavage furrow is always placed midway between the two centrosomes.

During the development of multicellular organisms, cellular diversity is generated, in part, by asymmetric cell division<sup>1</sup>. Asymmetry can manifest itself in two ways, namely by the unequal partitioning of cell-fate determinants and by the generation of daughter cells of different sizes. The mitotic spindle is a key regulator of both of these events. First, its orientation controls the axis of cell division and can determine whether localized cell-fate determinants are segregated symmetrically or asymmetrically<sup>2,3</sup>. Second, the position of the spindle within the dividing cell is thought to determine the relative size of the two daughter cells<sup>4-6</sup>. The developing nervous system of *Drosophila melanogaster* is a model system for studying spindle dynamics during asymmetric cell division. During neurogenesis, neuronal precursors, or neuroblasts, delaminate from a layer of ectodermal cells. They divide asymmetrically in a stem-cell lineage, generating a neuroblast and a smaller ganglion mother cell (GMC) at each division. Most of the cells that remain in the neuroectoderm are epidermoblasts; they divide symmetrically and give rise to epidermal cells.

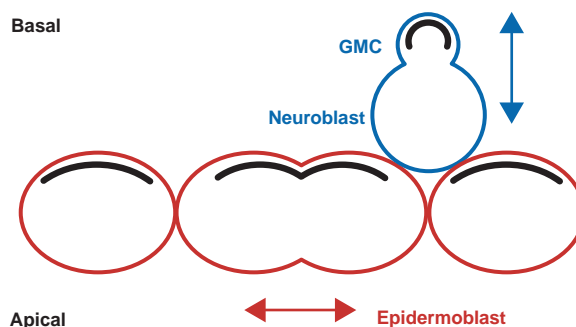
Epidermoblasts divide in a plane perpendicular to that of neuroblasts, which divide along the apical–basal axis of the embryo (Fig. 1). At prophase, cell-fate determinants such as Prospero are localized in a crescent on the basal side of both epidermoblasts and neuroblasts<sup>7-9</sup>. During epidermoblast division, Prospero segregates equally to both daughter cells (ref. 7 and J.A.K. and A.H.B., unpublished observations). In neuroblasts, the apical–basal orientation of the spindle at cytokinesis causes Prospero to be segregated to the basal daughter cell, the GMC<sup>7-10</sup>.

How does the spindle in neuroblasts come to be set up perpendicular to that in epidermal cells? It has been proposed that the reorientation of the spindle in neuroblasts starts at prophase, with the duplication of the centrosome on the apical side of the cell<sup>11</sup>. In this model, one centrosome migrates from the apical to the basal side of the cell, and the mitotic spindle then forms in an apical–basal orientation. By comparison, in the P blastomeres of the early *Caenorhabditis elegans* embryo, the centrosomes duplicate and migrate opposite to each other, and the centrosome–nucleus complex then rotates by 90° before the mitotic spindle forms<sup>12</sup>.

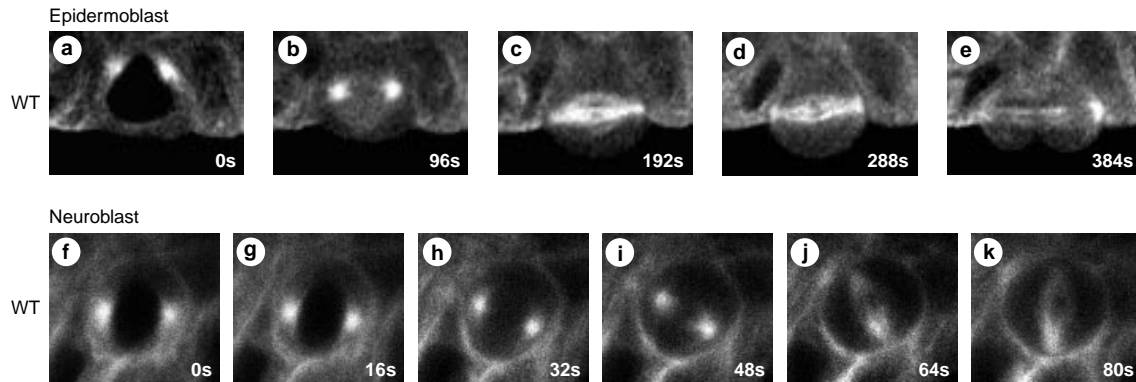
*C. elegans* also provides an example of how daughter cells of different sizes can be generated. During the first embryonic division,

one centrosome shifts posteriorly towards the cell cortex. As a result the metaphase plate, which forms equidistant between the two centrosomes, moves off-centre. The cleavage plane then bisects the mitotic spindle<sup>13</sup>, and gives rise to two differently sized cells<sup>5</sup>. In *Drosophila*, neuroblast divisions produce one large and one small daughter cell, unlike epidermoblast divisions, which give rise to daughters of equal size. Whether or not neuroblast asymmetry is generated by a mechanism similar to that described for *C. elegans* has not previously been investigated.

Earlier studies of spindle reorientation and asymmetric cell division in the *Drosophila* nervous system have been limited primarily to the observation of individual neuroblasts at a single time point in fixed embryos. To better understand the regulation of spindle orientation and asymmetric cell division, we have developed an assay with which to study neuroblast division in living embryos. We labelled microtubules by expression of the microtubule-binding protein tau–GFP (green fluorescent protein)<sup>14,15</sup>. In this way we can

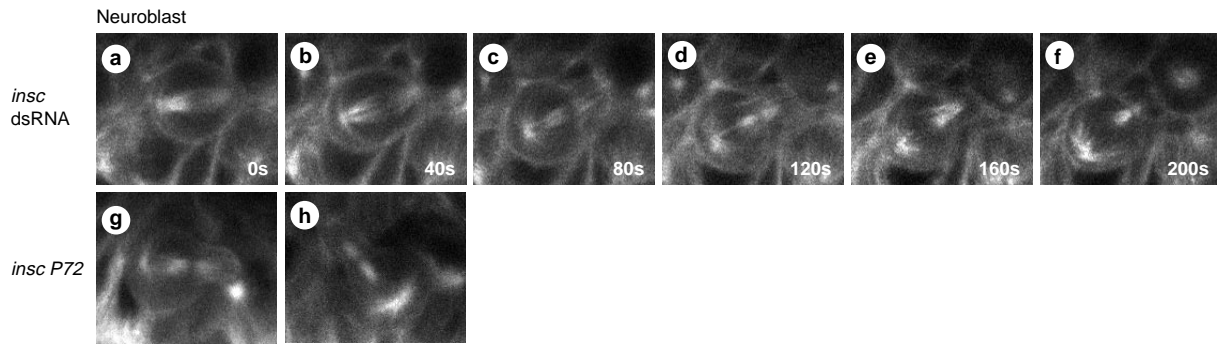


**Figure 1** *Drosophila* neuroblasts divide in an axis perpendicular to that of epidermoblast division. Epidermoblasts (red) divide parallel to the surface of the embryo while neuroblasts (blue) divide perpendicular to it, along the apical–basal axis. As a result, during epidermoblast division, basally localized proteins and mRNA (shown in black) segregate equally to both daughters. During neuroblast division, basally localized determinants segregate to the basal daughter.



**Figure 2 The neuroblast spindle rotates by 90° at metaphase.** Basal is at the top and apical at the bottom. These are lateral views of stage-10 and -11 embryos. **a–e**, In epidermoblasts, the centrosome duplicates on the basal side of the nucleus (**a**), and the two centrosomes migrate laterally to opposite sides of the cell (**b**). The spindle forms parallel to the surface of the embryo (**c**) and remains in this orientation throughout mitosis (**d**, **e**). Microtubules were labelled with tau-GFP, and confocal images of living embryos were recorded every 12s. **f–k**, In neuroblasts, the

centrosome duplicates on either the apical or the basal side of the nucleus (see Supplementary Information, movies 2,3). The two centrosomes migrate laterally to opposite sides of the cell (**f**). In most cases the spindle is formed before it rotates. Here, the bipolar spindle forms as it rotates by 90° to a position along the apical–basal axis of the cell (**g–k**; see Supplementary Information, movie 4). Microtubules were labelled with tau-GFP, and cell membranes were marked with Src-GFP to identify individual neuroblasts more easily. Images were taken every 4s.



**Figure 3 Loss of Inscuteable impairs spindle rotation.** Microtubules were labelled with tau-GFP and cell membranes with Src-GFP. Images were taken every 10s. Basal is at the top and apical at the bottom. These are lateral views of stage-10 embryos. **a–f**, In embryos injected with *inscuteable* double-stranded RNA (dsRNA), spindle rotation fails to occur in 10% of neuroblasts. At first the spindle forms

normally, perpendicular to the apical–basal axis (**a**). The spindle then seesaws but does not rotate (see Supplementary Information, movie 5). In this example, cell division occurs at a 45° angle to the apical–basal axis (**b–f**). **g, h**, A similar phenotype is seen in live *inscuteable*<sup>P72</sup> embryos. Two different neuroblasts are shown.

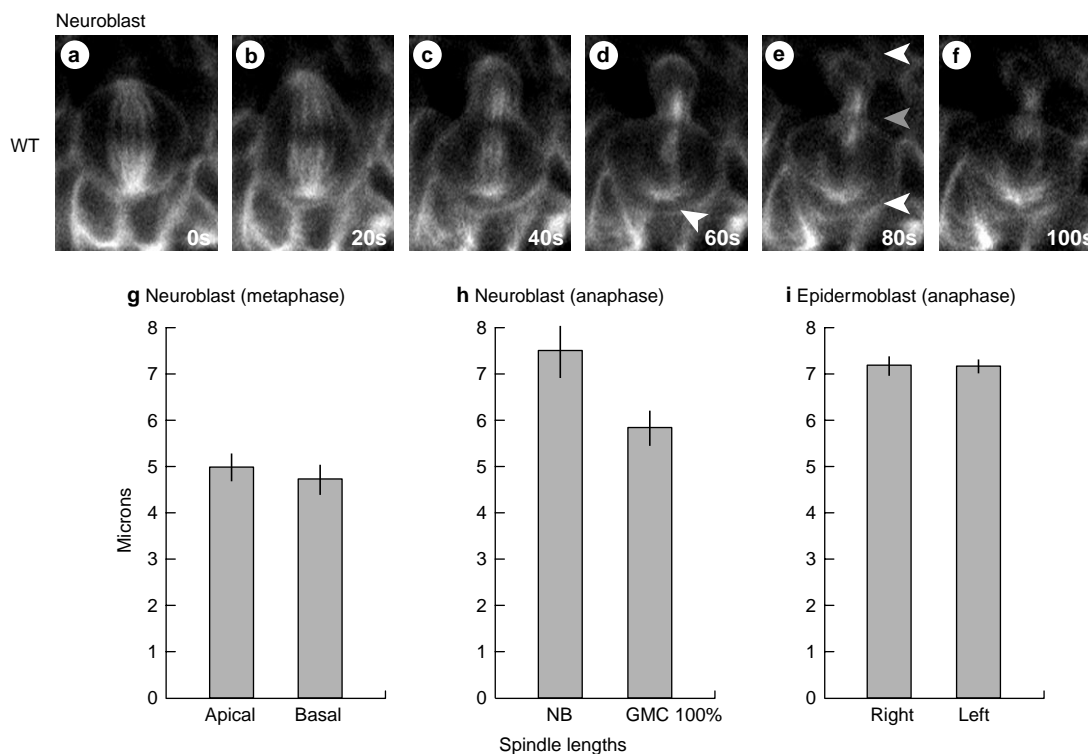
follow single neuroblasts at high resolution throughout the cell cycle in intact living embryos, avoiding potential artefacts associated with fixation.

Such *in vivo* imaging reveals, first, that the neuroblast spindle is formed in the same plane as epidermoblasts, but rotates before cell division. Interestingly, the direction of rotation usually correlates with the position of the centrosome at interphase: the spindle rotates in an anticlockwise direction when the centrosome is basal, and clockwise when it is apical. Second, the cleavage furrow is not positioned equidistant between the spindle poles. Daughter cells of different sizes arise because the spindle itself becomes asymmetric at anaphase. As apical microtubules elongate and basal microtubules shorten, the midbody moves basally until it is positioned asymmetrically between the two spindle poles, at the site of the cleavage furrow.

## Results

**Spindle dynamics in epidermoblasts and neuroblasts.** We studied division of epidermoblasts and neuroblasts by time-lapse confocal microscopy of embryos expressing tau-GFP, which labels microtubules. The results obtained in this way are supported by observa-

tions of fixed embryos (see  $\gamma$ -tubulin staining below). In epidermoblasts, the interphase centrosome duplicates on the basal side of the cell, directly over the nucleus (Fig. 2a; see Supplementary Information, movie 1). This result contrasts with observations of embryos at the syncytial and cellular blastoderm stages (3 h earlier), in which the centrosome duplicates on the apical side of the cell<sup>16,17</sup>. At prophase in epidermoblast division, each centrosome migrates laterally to opposite sides of the cell (Fig. 2a, b). The mitotic spindle then forms along the anterior–posterior axis of the embryo, and it remains in this orientation throughout cytokinesis (Fig. 2c–e). In contrast to epidermoblasts, the centrosome in neuroblasts is found on either the apical ( $n=16$ ) or the basal ( $n=9$ ) side of the cell, where it duplicates during interphase (see Supplementary Information, movies 2, 3). Next, the two centrosomes migrate laterally to opposite sides of the cell (Fig. 2f). The spindle forms and then rotates by 90° to an apical–basal orientation (Fig. 2g–k; see Supplementary Information, movie 4). In some cases, rotation begins before the spindle is fully formed. Rotation is complete within 60s. The direction of spindle rotation usually correlates with the position of the interphase centrosome. A basally positioned interphase centrosome leads to an anticlockwise spindle rotation (9 out of 9 cells in which the centrosome is basally located), while an apically positioned cen-



**Figure 4 The mitotic spindle becomes asymmetric during neuroblast cell division.** **a–f**, Microtubules were labelled with tau–GFP and cell membranes with Src–GFP. Confocal images were taken every 5 s. Basal is at the top and apical at the bottom. These are lateral views of a stage-10 embryo. **a**, The neuroblast mitotic spindle is symmetric and centrally placed at metaphase; see also Supplementary Information, movie 6. **g**, The bar graph shows the average length of the neuroblast spindle, measured from the apical or basal centrosome to the metaphase plate ( $n = 16$ ). **b–f**, At the start of anaphase in neuroblasts, the cell membrane begins to invaginate (**b**) and the spindle becomes asymmetric. The midbody gradually moves basally until it is positioned at the site of the cleavage furrow (grey arrowhead in **e**),

while the centrosomes move simultaneously and equally to opposite sides of the cell (white arrowheads in **e**). The apical, neuroblast, aster (white arrowhead in **d**) enlarges from anaphase onwards, and the GMC aster is reduced (**b–f**), while the neuroblast half of the spindle elongates and the GMC half becomes shorter (**c, d**). **h**, The bar graph measures the distance from the midbody to the apical centrosome (NB) and from the midbody to the basal-most cell membrane (GMC 100%) at anaphase (**d**). Measurements were taken from 14 dividing neuroblasts. **i**, The anaphase spindle is symmetric in epidermoblasts. The bar graph shows the average length of the epidermoblast spindle, measured from each centrosome to the midbody ( $n = 8$ ). **g–i**, Error bars indicate 1 s.d. in each direction.

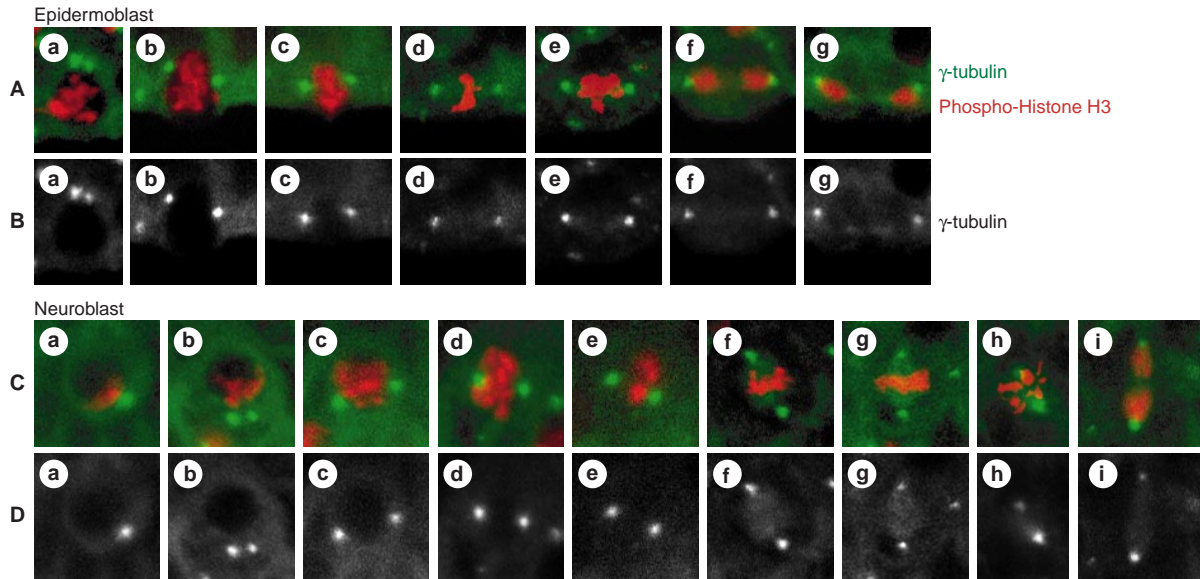
tosome leads to a clockwise spindle rotation (11 out of 16) (see Supplementary Information, movies 2, 3, and  $\gamma$ -tubulin staining below).

**Loss of Inscuteable impairs spindle rotation.** Inscuteable, a novel protein encoding a putative SH3 (Src homology 3) target site and a PDZ-binding domain, is both necessary and sufficient to direct apical–basal cell division in neuroblasts<sup>18–21</sup>. Inscuteable also directs the axis of cell division in epithelial cells of the procephalic neurogenic region (PNR)<sup>20</sup>. In *inscuteable* mutant embryos, the orientation of the neuroblast spindles becomes random, as is the site of formation of a crescent of Prospero; in contrast, spindle reorientation simply does not occur in epithelial cells of the PNR<sup>20</sup>.

To investigate how the loss of Inscuteable interferes with normal apical–basal cell division, we followed neuroblast spindle rotation in living *inscuteable*<sup>P72</sup> embryos. In these embryos (Fig. 3g, h), 20% of neuroblasts have spindles that fail to rotate ( $n = 34$ ). This percentage is in agreement with the number of misorientated neuroblast cell divisions we counted in fixed *inscuteable*<sup>P72</sup> embryos stained for DNA and neurotactin, which labels the cell membrane ( $n = 42$ ; data not shown). As Inscuteable is expressed in ovaries<sup>21</sup>, we also inhibited Inscuteable activity by RNA interference (RNAi)<sup>22</sup>. In principle, RNAi should block both maternal and zygotic messenger RNA and would be expected to produce a more severe phenotype than does the loss of zygotic Inscuteable alone. In embryos injected with *inscuteable* double-stranded RNA, the mitotic spindle initially forms normally, perpendicular to the apical–basal axis. In 10% of neuro-

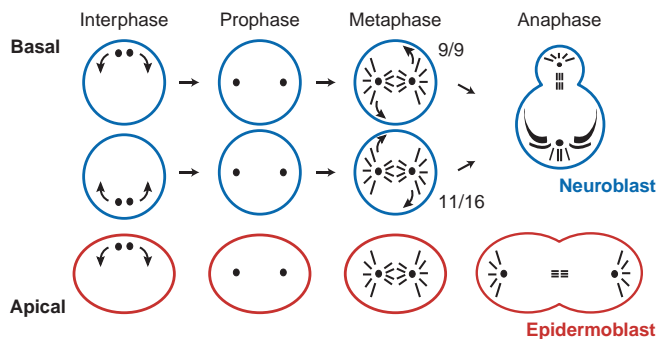
blasts ( $n = 30$ ), the spindles seesaw but fail to rotate (Fig. 3a–f; see Supplementary Information, movie 5), a phenotype also seen in *inscuteable*<sup>P72</sup> embryos. Seesawing is characteristic of epidermoblasts before cell division (see Supplementary Information, movie 7). The neuroblast spindle shown in Fig. 3a–f fails to rotate during metaphase. However, the cell turns slowly during anaphase and ultimately divides at a 45° angle to its original position. Although the spindle fails to rotate, the cell still divides asymmetrically, as has been described previously in *inscuteable* mutants<sup>20</sup>. We observed low levels of residual Inscuteable protein after RNAi (data not shown), which may explain the low penetrance of the spindle phenotype (the spindles in the remaining 90% of neuroblasts rotate normally). Spindle orientation in epidermoblasts is not affected after injection of *inscuteable* double-stranded RNA or in *inscuteable*<sup>P72</sup> embryos (data not shown).

**Neuroblast spindle asymmetry drives asymmetric cell division.** The first embryonic division in *C. elegans* generates two cells of different sizes<sup>5</sup>. To achieve this asymmetry, one centrosome moves towards the posterior cell cortex, and the cleavage furrow is then placed midway between the two spindle poles<sup>5</sup>. *Drosophila* neuroblasts also divide asymmetrically, generating a large neuroblast and a smaller GMC. In neuroblasts, the mitotic spindle is symmetric and centrally placed throughout metaphase (Fig. 4a, g; see Supplementary Information, movie 6), as is the epidermoblast spindle throughout cell division (Fig. 4i, anaphase). However, at the onset of anaphase in neuroblasts, the microtubules appear to become shorter on the



**Figure 5 Neuroblast centrosomes become asymmetric at anaphase.** Lateral views of stage-10 embryos. Basal is at the top and apical at the bottom. All images are projections of sections in the Z-axis. **A–D**,  $\gamma$ -Tubulin (green in **A, C**; staining for phosphorylated histone H3 is in red; see below) is localized at the centrosomes of epidermoblasts (**A, B**) and neuroblasts (**C, D**). Each panel in **B, D** corresponds to the panel above in **A, C**, respectively, but shows only  $\gamma$ -tubulin staining. **A, B**, The intensity

of  $\gamma$ -tubulin staining at both epidermoblast centrosomes is similar from prophase (**a**) through to late anaphase (**g**). **C, D**, In neuroblasts, the centrosomes stain equally until metaphase (**a–g**), but from the onset of anaphase immunoreactivity is greater on the neuroblast centrosome than on the GMC centrosome (**h, i**). Anti-phospho-histone-H3 antibody stains phosphorylated histone H3 from prophase until late anaphase.



**Figure 6 Spindle dynamics during neuroblast and epidermoblast cell division.** In epidermoblasts (red), the centrosome (black circle) duplicates on the basal side of the nucleus. The mitotic spindle forms parallel to the surface of the embryo and remains in this orientation throughout mitosis. In neuroblasts (blue), the centrosome duplicates either basally or apically. The mitotic spindle in neuroblasts is initially orientated parallel to that of epidermoblasts. At metaphase, the neuroblast spindle rotates by  $\sim 90^\circ$  and comes to rest perpendicular to the epidermoblast spindle axis. A basally positioned interphase centrosome results predominantly in an apical movement of the anterior centrosome, and anticlockwise rotation, while an apically positioned centrosome leads to a basal movement of the anterior centrosome, and clockwise rotation. The spindle maintains this orientation throughout the rest of mitosis. At anaphase, the neuroblast spindle becomes strikingly asymmetric. Apical polar microtubules and astral microtubules elongate, while basal microtubules shrink. The midbody moves basally towards the site of the cleavage furrow, and the neuroblast divides asymmetrically.

basal side of the cell and elongate on the apical side (Fig. 4b–f; see arrowhead in Fig. 4d).

To describe the extent of the spindle asymmetry more accurately, we measured the distance from each spindle pole to the centre of the midbody (the region of overlapping microtubules) at metaphase and anaphase (Fig. 4). However, although the apical

(neuroblast) centrosome is visible at both of these stages, the basal (GMC) centrosome is no longer visible at anaphase. Therefore, we measured from the most distal GMC membrane to the midbody, which slightly overestimates the length of the GMC half of the spindle. At anaphase the apical (neuroblast) half of the spindle is 30% longer than the basal (GMC) half (Fig. 4d, h). This is the most conservative estimate of the difference between the two halves of the spindle. However, the neuroblast spindle consistently spans 80% of the distance from the midbody to the apical membrane. If we assume that the GMC spindle also spans only 80% of the total GMC length, then the neuroblast segment of the spindle is more than 50% longer than that in the GMC.

As the spindle becomes asymmetric, the midbody moves toward the nascent GMC. At the same time, the apical aster enlarges as the number and length of the astral microtubules increases dramatically, forming a cap-like structure (arrowhead in Fig. 4d). By comparison, very few astral microtubules are visible at the basal centrosome. As astral microtubules are nucleated at the centrosomes by  $\gamma$ -tubulin<sup>23,24</sup>, we determined whether or not the amount of  $\gamma$ -tubulin differs at each spindle pole. At metaphase, both neuroblast centrosomes stain equally well (Fig. 5C, e–g, D, e–g). However, from the onset of anaphase the apical centrosome stains more strongly for  $\gamma$ -tubulin than the basal centrosome (Fig. 5C, h, i, D, h, i). We obtained the same result when using antibodies against the centrosomal proteins CP60 and CP190 (refs 25–27 and data not shown). This contrasts with epidermoblasts, where the intensity of  $\gamma$ -tubulin, CP60 and CP190 staining is similar on both centrosomes throughout mitosis (Fig. 5A, a–g, B, a–g; data not shown).

### Discussion

We have described two mechanisms by which the mitotic spindle generates asymmetry during neuroblast division (Fig. 6): first, the neuroblast spindle rotates to a plane perpendicular to that in epidermoblasts; and, second, at anaphase the spindle becomes asymmetrical between the spindle poles. As a result, and contrary to the dogma that the cleavage furrow always forms equidistant from the

spindle poles, the cleavage furrow is placed asymmetrically between the spindle poles, thereby generating two daughter cells of different sizes.

The orientation of neuroblast cell division is dependent on the proteins Inscuteable and Bazooka<sup>18–21,28,29</sup>. Inscuteable is expressed only in neuroblasts, where it localizes apically. An earlier model for spindle reorientation, based on antibody staining, indicated that the centrosomes duplicate on the apical side of the neuroblast; this is followed by the basal movement of one centrosome and spindle formation along the apical–basal axis<sup>11</sup>. In this case, it would be possible to view Inscuteable as an anchor that restrains one of the centrosomes on the apical side of the cell at prophase.

By following cell division in living embryos, however, we have shown that the neuroblast centrosome can duplicate on either the apical or the basal side of the cell, and that both new centrosomes then migrate laterally. The mitotic spindle forms along the same axis as in epidermoblasts and subsequently rotates to line up along the apical–basal axis. These results indicate that Inscuteable might play a part in attracting one of the centrosomes to the apical side of the cell during metaphase. In epidermoblasts, where Inscuteable is not expressed, the epidermoblast centrosome duplicates on the basal side of the nucleus before the centrosomes migrate laterally. The spindle then forms parallel to the surface of the embryo but does not rotate. However, spindle rotation can be induced in epidermoblasts by ectopic expression of Inscuteable<sup>20</sup>.

The loss of Inscuteable has been reported to result in randomly orientated neuroblast spindles<sup>18–21,29</sup>. We find, however, that the final orientation of the spindle is not due to random rotation. Instead, as in epidermoblasts (see Supplementary Information, movie 7), the spindle seesaws but is unable to rotate (see Supplementary Information, movie 5). The GMC buds off in a lateral, rather than basal, position. Neighbouring cells appear to jostle the neuroblast as it divides, leading to a more random distribution of GMCs after cytokinesis.

Inscuteable also directs the axis of cell division in epithelial cells of the procephalic neurogenic region<sup>20</sup>. In the absence of Inscuteable, the cells divide parallel, rather than perpendicular, to the embryo surface. The loss of *inscuteable* was thought to have different effects on PNR epithelial cells and neuroblasts: the epithelial cells fail to reorientate their axis of division, whereas the neuroblast axis becomes randomized<sup>20</sup>. We suggest, however, that the *inscuteable* phenotype is actually the same in both types of cell: the mitotic spindle fails to rotate. The penetrance of the spindle phenotype in *inscuteable*<sup>72</sup> embryos is only 20%. Although at first this seems surprising, it may indicate that Inscuteable is only one of the proteins involved in spindle reorientation, and that others remain to be identified.

Although the neuroblast spindle can rotate in either direction, the direction of rotation usually correlates with the centrosome position at interphase (see Supplementary Information, movies 2–4; for stainings in fixed embryos see Fig. 5). This result indicates that the two centrosomes may become different from one another during or shortly after duplication, with one primed to move apically after the metaphase spindle is set up. When the centrosome duplicates basally, the anterior centrosome is primed and moves apically at metaphase, resulting in anticlockwise spindle rotation (in 9 out of 9 neuroblasts with basal centrosome duplication); when the centrosome duplicates apically, the posterior centrosome is primed and moves apically at metaphase, resulting in clockwise spindle rotation (in 11 out of 16 neuroblasts with apical centrosome duplication; Fig. 6). The apical movement of the primed centrosome may be mediated by an interaction with Inscuteable. Whether the interaction is direct or indirect remains to be seen.

It is not yet known how the position of the interphase centrosome is specified. If the newly delaminated neuroblast maintains the polarity of the cells in the neuroectoderm, then the interphase centrosome would be on the basal side of the cell. However, after cell division, the final position of the neuroblast centrosome is apical.

Therefore, in all subsequent neuroblast divisions, the interphase centrosome might be apical. This hypothesis can be tested by following a single neuroblast through several cell divisions, which so far has not been possible.

We have shown here that the asymmetry of neuroblast cell division is dictated by the spindle itself becoming asymmetric at anaphase. Microtubules on the apical side of the cell elongate, while those on the basal side become shorter. As the astral microtubules become longer, and seemingly more abundant, the apical aster enlarges (Fig. 4b–f). The basal aster is concomitantly reduced in size. This process is independent of Inscuteable, as the spindle remains asymmetric even when it fails to rotate in an *inscuteable* mutant (Fig. 3). Earlier reports have highlighted the existence of asymmetric spindle poles/centrosomes in yeast<sup>30,31</sup> and *C. elegans*<sup>32</sup>. However, in none of these cases were asymmetric midzone microtubules observed.

Astral microtubules have been proposed to be involved in specifying the site of the cleavage furrow at cytokinesis<sup>13,33</sup>. Our results are consistent with this model. We find that, during neuroblast cell division, the apical astral microtubules elongate dramatically and grow towards the emerging GMC before the cell membrane invaginates. The overlapping apical and basal astral microtubules, which are distinctly different in length, may specify the asymmetric site of the cleavage furrow. Bonaccorsi *et al.*<sup>34</sup> have suggested that astral microtubules are not required for cytokinesis. They showed that spermatocytes from an *asterless* mutant are still able to undergo cytokinesis, and they suggest instead that the midbody specifies the site of the cleavage furrow. Our results do not distinguish between these two hypotheses; however, it is interesting that in neuroblasts the midbody moves basally towards the cleavage site only after the cell membrane has started to invaginate (Fig. 4b–e; see Supplementary Information, movie 6).

It will be interesting to discover what, in addition to Inscuteable, regulates the rotation of the mitotic spindle. In *C. elegans*, rotation of the nucleus–centrosome complex depends upon dynein and dynactin<sup>35,36</sup>. Dynactin is proposed to tether the dynein motor to the cortex of the cell, where it reels in one centrosome by movement along the astral microtubules<sup>37</sup>. The speed of spindle rotation in *Drosophila* neuroblasts (less than 60 s) indicates that this process could also be mediated by dynein<sup>38</sup>.

To our knowledge, there is no precedent for a cleavage furrow being placed asymmetrically between the spindle poles. The asymmetric position of the midbody may be characteristic of cells in the nervous system, or may in the future be detected in other cell types as imaging of live cells becomes more prevalent. Spindle asymmetry is cell-cycle regulated, but does not depend on previous spindle rotation. It will be interesting to identify the factors that induce spindle asymmetry in neuroblasts, and to discover how they are regulated during the cell cycle. □

## Methods

### GFP fusion proteins.

Complementary DNA encoding tau was isolated as a 1.2-kilobase *EcoRI*–*BamHI* fragment from UAS-tau-GFP5 (ref. 14; A.H.B. and J.P. Haseloff, unpublished). The GFP variant<sup>15,39</sup> was amplified by the polymerase chain reaction (PCR) as a *BamHI*–*XhoI* fragment. DNAs encoding tau and mGFP6 were subcloned by three-way ligation into *EcoRI*–*XhoI*-digested pUAST<sup>40</sup> to give UAS-tau-mGFP6. The DNA construct was injected into  $\Delta 2$ -3Sb/TM6 embryos<sup>41</sup> as described<sup>40</sup>.

DNA encoding the first 90 amino acids of Src64B was isolated as a *Clal*–*NcoI* fragment from pBSSrc<sup>42</sup> and ligated to GFP, isolated as a *BamHI*–*XhoI* fragment from UAS-tau-GFP(S65T/I167T) (refs 15, 43). This introduces a short peptide, PMDP, between Src64B and the initiator methionine of GFP. The *Src-GFP* fusion was subcloned downstream of the *GAL4* upstream activation sequence (a *SphI*–*Cell* fragment from pUAST<sup>40</sup>) and upstream of the *hsp70* polyA site (a *Sall*–*SpeI* fragment from pGaTN<sup>40</sup>) in *SphI*–*SpeI*-cut pWhiteRabbit (N.H.B., unpublished) to give UAS-Src-GFP(S65T/I167T). Several independent transformant lines were generated. The line used in this study contains two or more insertions on the second chromosome and is homozygous viable.

Expression of tau-GFP and Src-GFP was driven by  $\alpha 4$ -tubulin-GAL4-VP16(V37) (ref. 44), a gift from D. St Johnston.

### Imaging of live embryos.

Embryos were prepared as described<sup>15</sup>. Briefly, embryos were dechorionated in 50% clorox for 3 min,

washed thoroughly with water, transferred to a drop of Voltalet or Halocarbon oil on an air-permeable teflon membrane, and covered with a coverslip. Two further coverslips were used as bridges to prevent the embryos from being squashed. The coverslips were not permanently fixed, enabling the embryos to be rolled. Neuroblasts were identified on the basis of the following criteria: first, the cells delaminate from the ectoderm to lie immediately above the neuroectodermal cells; second, they undergo asymmetric divisions, giving rise to two differently sized cells; and third, during their division, proteins such as Staufen are asymmetrically segregated to the smaller daughter cell (A.H.B., unpublished observations).

To follow mitotic spindles in *inscuteable* mutants, we used the line *inscuteable<sup>272</sup>/Kr-GAL4, UAS-GFP, CyO (Kr-GAL4, UAS-GFP, CyO flies were a gift from T. Kornberg).*

**Confocal microscopy.**

Time-lapse series and single images were collected by confocal microscopy using a BioRad MRC1024 scan head on a Nikon E800 microscope. Laser power was normally set at 10%, the iris between 6 and 8, and gain between 1,200 and 1,500. Images were collected at a size of 512x512 pixels (Fig. 2) or 1,024x1,024 pixels (Fig. 3, 4) with Kalman averaging of two to three frames. Images were imported into Adobe Photoshop 5.0, and assembled in Adobe Illustrator 7.0. Time-lapse series were imported as TIFF files into Adobe Premiere 5.0.

**Synthesis and injection of double-stranded RNA.**

The DNA template for transcription of double-stranded RNA covering the first 578 nucleotides of the *inscuteable* coding region<sup>18</sup> was generated by PCR as described<sup>45</sup>, except that a T3 promoter was engineered at the 5' end in place of a T7 promoter. *inscuteable* double-stranded RNA was dissolved in injection buffer<sup>46</sup> and injected as described<sup>45</sup>.

**Immunohistochemistry.**

Antibody staining was done as described<sup>47</sup> with the following modifications: to preserve the cytoskeleton, embryos were fixed for 3–5 min in a 1:1 mix of *n*-heptane and 40% formaldehyde. PBT (PBS plus 0.1% Triton X-100) replaces PEM throughout. After RNA interference, embryos were washed with PBT and fixed for 30 min in a 1:1 mix of *n*-heptane and 4% formaldehyde in PBT. Vitelline membranes were removed by hand and the embryos were washed with PBT and 100% methanol.

Mouse anti- $\gamma$ -tubulin antibody (Sigma) was used at a dilution of 1:100 in PBT; rabbit antibody against phosphorylated histone H3 (Upstate Biotechnology) was used at a dilution of 1:100 in PBT. Secondary antibodies, directly conjugated to fluorescein isothiocyanate (FITC) or Cy5 (Jackson ImmunoResearch), were used at a dilution of 1:200. Embryos were mounted in Vectashield (Vector Laboratories) and visualized by confocal microscopy.

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