Dlg, Scrib and Lgl regulate neuroblast cell size and mitotic spindle asymmetry

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Asymmetric cell division is important in generating cell diversity from bacteria to mammals. *Drosophila melanogaster* neuroblasts are a useful model system for investigating asymmetric cell division because they establish distinct apical–basal cortical domains, have an asymmetric mitotic spindle aligned along the apical–basal axis, and divide unequally to produce a large apical neuroblast and a small basal daughter cell (GMC)[1,2]. Here we show that Discs large (Dlg), Scribble (Scrib) and Lethal giant larvae (Lgl) tumour suppressor proteins regulate multiple aspects of neuroblast asymmetric cell division. Dlg/Scrib/Lgl proteins show apical cortical enrichment at prophase/metaphase, and then have a uniform cortical distribution. Mutants have defects in basal protein targeting, a reduced apical cortical domain and reduced apical spindle size. Defects in apical cell and spindle pole size result in symmetric or inverted neuroblast cell divisions. Inverted divisions correlate with the appearance of abnormally small neuroblasts and large GMCs, showing that neuroblast/GMC identity is more tightly linked to cortical determinants than cell size. We conclude that Dlg/Scrib/Lgl are important in regulating cortical polarity, cell size asymmetry and mitotic spindle asymmetry in *Drosophila* neuroblasts.

The subcellular localization of Dlg and Lgl in neuroblasts is unclear, having been described as apically enriched and/or uniform cortical[3,4]. In addition, the localization of Scrib in neuroblasts has not been reported. Here, we show that the localization of Dlg/Scrib/Lgl proteins is cortical, with apical enrichment from late interphase to metaphase (Fig. 1a, f, j, o–r, t), but with a uniform cortical localization during anaphase and telophase in both larval and embryonic neuroblasts (Fig. 1s). In addition, a small fraction of neuroblasts showed bipolar enrichment of Dlg and Scrib at telophase (data not shown). Dlg and Scrib precisely colocalized, consistent with the ability of both proteins to bind to Gukholder[5] (Gukh), which was also apically enriched in neuroblasts (Fig. 1q). We confirmed the localization of Dlg by imaging a functional Dlg–green fluorescent protein (GFP) protein during neuroblast cell division *in vivo* (see Supplementary Information, Movie 1). The localization of Lgl differed slightly from Dlg/Scrib and was extremely fixation-sensitive (see Methods), so it will be necessary to image Lgl–GFP subcellular localization in live neuroblasts to confirm the apical enrichment of Lgl protein described here. Although previously unreported, apical enrichment of Lgl is not surprising, because Lgl has physical and genetic interactions with Myosin-II[3,4,6], which is also apically enriched in metaphase neuroblasts (K. Siller and C.Q.D., unpublished observations). Dlg/Scrib/Lgl are interdependent for localization in epithelia[7]; however, in neuroblasts, we found that Dlg was required for localization of Scrib and Lgl, but that localization of Dlg was essentially normal in *scrib* and *lgl* mutants (Fig. 1c–e, g, k). We conclude that Dlg/Scrib/Lgl proteins show cortical localization with apical enrichment during early mitosis, and that Dlg is required for the cortical recruitment of both Scrib and Lgl (Fig. 1n).

Next, we examined if apical enrichment of Dlg/Scrib is dependent on previously identified apical cortical proteins. All known apical proteins — Bazooka (Baz), Par-6, atypical protein kinase C (aPKC), Inscuteable (Insc), Partner of Inscuteable (Pins) and Gαi — are undetectable or delocalized in *insc* mutants; in addition, there are defects in mitotic spindle orientation[8–14]. *insc* mutant neuroblasts had normal apical enrichment of Dlg/Scrib at prophase, but by metaphase the Dlg/Scrib crescents were often misoriented (Fig. 1u–w; data not shown). We conclude that Insc or Insc-dependent apical proteins are required for the maintenance of Dlg/Scrib enrichment at the apical neuroblast cortex. Interestingly, *insc* mutant neuroblasts with abnormal metaphase spindle orientation (orthogonal to the apical–basal axis) always showed cortical enrichment opposite one or both spindle poles (Fig. 1v, w; data not shown). Microtubules were not required for formation of Dlg/Scrib crescents (Fig. 1x, y; data not shown), raising the possibility that Dlg/Scrib cortical enrichment provides a cue for spindle orientation in the absence of other apical proteins.

We began our analysis of *dlg*, *scrib* and *lgl* neuroblast phenotypes by assaying apical/basal protein targeting in metaphase neuroblasts. We assayed the apical localization of aPKC, Insc, Pins and Gαi, and the basal localization of the coiled-coil protein Miranda and the transcription factor Prospero. Apical protein localization was essentially normal in all dlg^{m52}, lgl^{4}, and $scrib^{1}$ single, double and triple mutants (Figs 2a–d, 4d, 4e and data not shown)[3,4]. Displaced aPKC or Insc crescents were observed at low frequency, but all crescents remained aligned with the mitotic spindle (Fig. 2e, data not shown). In contrast, basal protein targeting was abnormal in all dlg^{m52}, lgl^{4}, and $scrib^{1}$ single, double and triple mutants. Miranda and Prospero were detected on the spindle microtubules, centrosomes and in the cytoplasm (Fig. 2f–o and data not shown). However, it is important to note that all *dlg*, *scrib*, *lgl* mutant genotypes successfully targeted some Miranda and Prospero protein to the basal cortex by telophase (Fig. 2n, arrowhead; also see below). In addition to having similar neuroblast phenotypes, *dlg*, *scrib* and *lgl* also showed similar positive and negative genetic interactions, suggesting that they act in a common pathway to regulate basal protein targeting. Single zygotic mutants showed a relatively weak phenotype, because of persistence of the maternal gene product; however, all double and triple mutant combinations had stronger-than-additive phenotypes (Fig. 2o). In addition, we found that reduced levels of Myosin-II (in embryos homozygous for a null *zipper* mutation) rescued basal localization of Miranda in both *lgl* and *scrib* single-mutant neuroblasts (Fig. 2l, o)[3,4]. We conclude that Dlg, Scrib and Lgl are required for basal protein targeting in metaphase neuroblasts (but not telophase neuroblasts), that they regulate a common process through similar genetic interactions and that

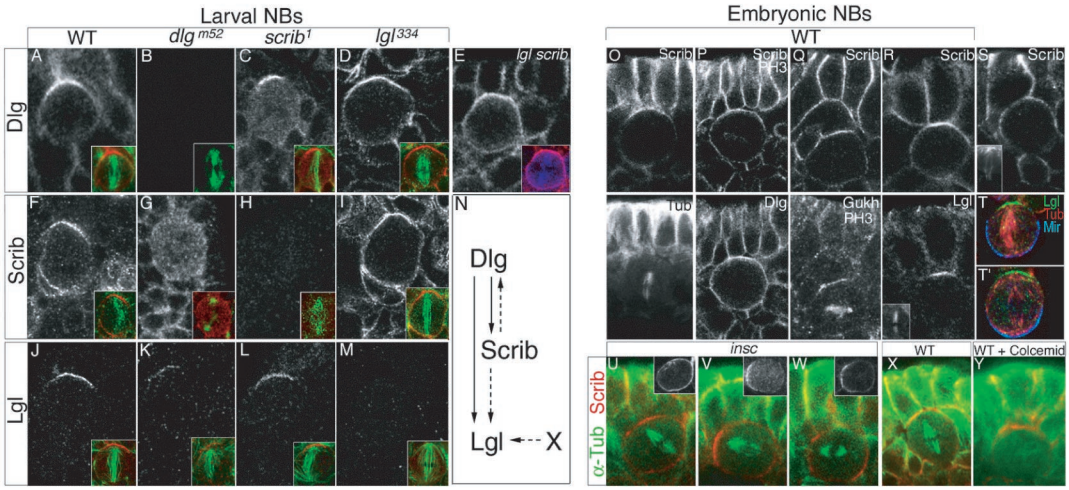


Figure 1 **Localization of Dlg/Scrib/Lgl and regulation in larval and embryonic neuroblasts. a**–**n**, Larval metaphase neuroblasts labelled for Dlg, Scrib, or Lgl (protein at left, zygotic genotype at top). All three proteins are apically enriched in wild-type neuroblasts (**a**, **f**, **j**) and are partially interdependent for localization (summarized in **n**). In *dlg*^{m52} null mutant neuroblasts, Scrib and Lgl are delocalized, although a weak Lgl crescent can persist (**g**, **k**); in *scrib*^{1} null mutant neuroblasts, Dlg and Lgl show slightly reduced cortical localization (**c**, **l**); in *lgl*^{334} mutant neuroblasts, Dlg and Scrib are normal (**d**, **i**). Insets show the same neuroblast labelled for Dlg/Scrib/Lgl localization (red) and α-tubulin (green) to document metaphase cell cycle stage, except **e**, which shows spindle-associated Miranda (blue). Apical is defined by aPKC staining (data not shown) and is up in all panels. Wild type, *dlg*^{M52}, *scrib*^{1} mutants are shown at second-larval instar, *lgl*^{334} is shown at third-larval instar and *lgl*^{4}*; scrib*^{1} is shown at embryonic stage 10. **o**–**s**, Embryonic stage-10 metaphase (**o**–**r**) and telophase (**s**) neuroblasts labelled for the indicated. PH3, phosphohistone H3. **t**, Mitotic neuroblasts from *in vitro* primary cultures showing apical Lgl (green), α-tubulin (red) and basal Miranda (blue). **u**–**y**, Embryonic stage-10 neuroblasts double-labelled for Scrib (red) and α-tubulin (green) in *insc*^{22} mutants (**u**–**w**), wild-type mock-treated (**x**) or wild-type treated with the microtubule-inhibitor Colcemid (**y**). Insets show localization of Miranda in the same neuroblast.

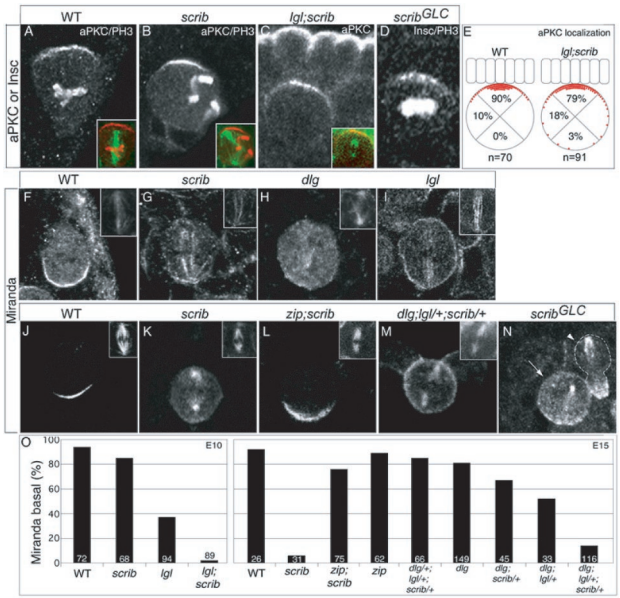
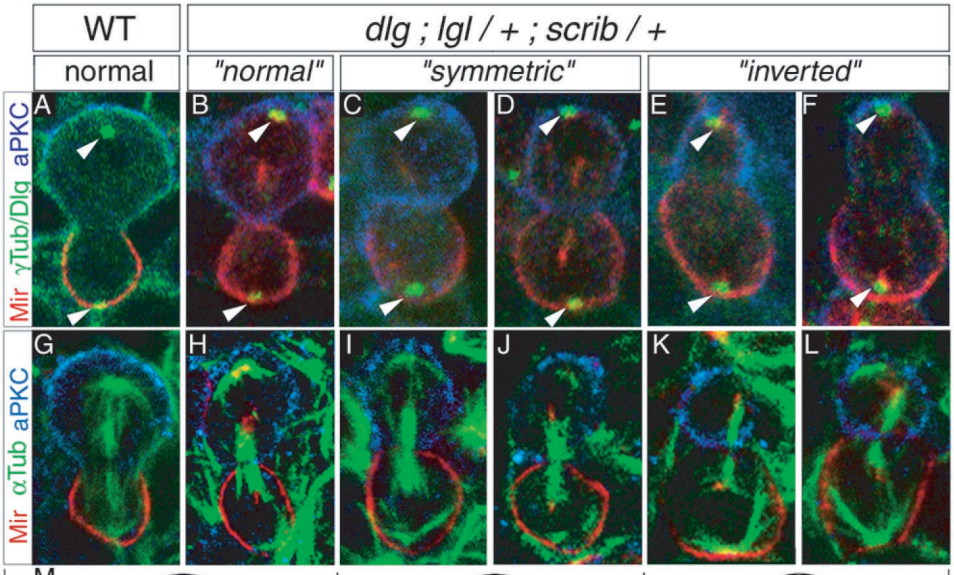


Figure 2 **Dlg/Scrib/Lgl specifically regulates basal targeting in metaphase neuroblasts. a**–**n**, Apical (**a**–**e**) or basal (**f**–**n**) proteins assayed are indicated at the left. Genotypes are indicated above each panel (*dlg* = *dlg*^{m52}; *scrib* = *scrib*^{1}; *lgl* = *lgl*^{4} (**c**, **l**, **m**, **o**) or *lgl*^{334} (**i**); *zip* = *zip*^{1}; *scrib*^{GLC} = *scrib*^{1} homozygous embryos derived from maternal *scrib*^{1} germline clones). Neuroblasts are from stage-10 embryos (**c**–**e**, **n**), second-instar larvae (**a**, **b**, **f**–**h**), third-instar larva (**i**) and stage-15 embryos (**j**–**m**). All neuroblasts are in metaphase except one at telophase (**n**, arrowhead). Insets show the mitotic spindle. Red dots (**e**) show the centre of aPKC crescent. **o**, Quantification of Miranda basal localization defects in metaphase neuroblasts. Neuroblasts scored in embryonic stage-10 or -15 embryos (E10, E15; upper right corner of box). Number in bars represent the number of neuroblasts scored.

Myosin-II functions antagonistically in this process.

The fact that *dlg*, *scrib*, and *lgl* mutants had normal apical–basal cortical polarity at telophase allowed us to score for defects in cell size and mitotic spindle asymmetry (Figs 3 and 4f). We assayed cell size asymmetry by measuring the relative size of the apical (aPKC-positive) and basal (Miranda-positive) cortex at telophase in embryonic neuroblasts (Fig. 3; quantified in Supplementary Information, Fig. S1). In wild-type neuroblasts, the apical aPKC-positive cortex was always larger than the basal Miranda-positive cortex, resulting in the production of a large neuroblast and small GMC. In *dlg*, *scrib* or *lgl* single mutants, the apical cortical domain was smaller than normal, resulting in symmetric or inverted cell divisions (Fig. 3m). In the more severe *dlg*, *scrib*, *lgl* double or triple mutants we observed an even higher frequency of inverted divisions, although normal divisions were still observed (Fig. 3b–f, m). Thus, Dlg/Scrib/Lgl activity promotes the formation of a large apical cortical domain and subsequently a large neuroblast:GMC cell size ratio.

Next, we assayed mitotic spindle asymmetry by examining α- and γ-tubulin staining in embryonic telophase neuroblasts (Fig. 3; quantified in Supplementary Information, Fig. S2). In wild-type neuroblasts, the apical (aPKC-associated) spindle pole had a larger centrosome, longer centrosome-to-cortex distance, longer spindle-pole length and longer astral microtubules; the basal (Miranda-associated) spindle pole was reduced for all of these features (Fig. 3g). In all *dlg*, *scrib* and *lgl* mutant genotypes, we observed 20–30% of the neuroblasts with 'symmetrical' mitotic spindles that were equalized for centrosome size and position, spindle pole length and astral microtubule length (Fig. 3i, j). Strikingly, up to 22% of the neuroblasts had 'inverted' mitotic spindles, in which the apical spindle pole is reduced for astral microtubule length, spindle pole length and centrosome–cortex distance; whereas the basal spindle pole is expanded for all these features (Fig. 3k, l). We conclude that Dlg/Scrib/Lgl activity promotes apical spindle pole growth or suppresses basal spindle pole growth and that



genotype				n
w.t.	95%	5%	0%	41
dlg (zyg)	80%	20%	0%	46
dlg (GLC)	68%	22%	10%	49
scrib (zyg)	65%	31%	4%	45
scrib (GLC)	74%	22%	4%	27
lgl (zyg)	57%	31%	12%	73
dlg;lgl/+ (zyg)	59%	26%	15%	47
dlg;scrib/+ (zyg)	52%	32%	16%	50
dlg;lgl/+;scrib/+ (zyg)	56%	22%	22%	94

Figure 3 **Dlg/Scrib/Lgl regulate cell size and mitotic spindle asymmetry.** Embryonic stage-15 telophase neuroblasts stained for proteins, as indicated at left (Mir, Miranda; α-Tub, α-Tubulin; γ-Tub, γ-Tubulin); genotypes at top, apical is up. Wild type and mutant neuroblasts show normal cortical polarity by telophase: aPKC and Miranda are targeted to opposite membrane domains, although some Miranda is associated with microtubules and centrosomes in mutant neuroblasts. **a**–**f**, Centrosome asymmetry. In wild type (**a**) or 'normal' mutant neuroblasts (**b**), the apical centrosome is larger and positioned off the cortex, whereas the basal centrosome is smaller and tightly associated with the cortex. In neuroblasts undergoing symmetrical divisions (**c**, **d**), both apical and basal centrosomes are similar in size and position. In neuroblasts undergoing inverted divisions (**e**, **f**), the centrosomes can be similar in size and position (**e**), or unequal in size and position (**f** and data not shown). See Supplementary Information, Fig. S3 for quantification. **g**–**l**, Spindle asymmetry. In wild type (**g**) or 'normal' mutant (**h**) neuroblasts, the apical spindle pole is more developed than the basal spindle pole. In symmetrical neuroblast divisions, both spindle poles have a similar morphology (**i**, **j**). Symmetrical spindles can have long (**i**) or short (**j**) astral microtubules, but they are always similar in length. In inverted neuroblast divisions (**k**, **l**), the mitotic spindle is asymmetric but is reversed relative to cortical polarity. See Supplementary Fig. 3 for quantification. **m**, Schematics and quantification of normal, symmetrical and inverted divisions in wild type and mutant embryonic stage-15 telophase neuroblasts. GLC, homozygous embryos derived from maternal germline clones; zyg, zygotic mutant embryos; aPKC, blue; Miranda, red; mitotic spindle and centrosomes, green.

apical–basal spindle pole size is correlated with apical–basal cortical domain size, rather than with known cortical protein markers.

Wild-type neuroblasts and GMCs showed a tight correlation between cortical polarity, cell size and cell fate. The inverted neuroblast divisions in *dlg; scrib/+; lgl/+* embryos allowed us to test the role of cell size or cortical polarity in specifying neuroblast/GMC identity. Wild-type neuroblasts had relatively large nuclei that were Worniu- and Asense-positive, but Prospero-negative; conversely, GMCs had relatively small nuclei that were Worniu- and Asense-negative, but Prospero-positive, soon after their birth (Fig. 5a–d). In *dlg/Y; lgl/+; scrib/+* embryos, neuroblasts were generally smaller than in wild type and included a novel population of 'small neuroblasts', whereas GMC size in mutants was generally larger than in wild type and included a novel population of 'large GMCs' (Fig. 5a, b). Importantly, the 'large GMCs' expressed GMC-specific markers, despite their large size, and the 'small neuroblasts' expressed neuroblast-specific markers despite their small size (Fig. 5e, f). These large GMCs and small neuroblasts were often adjacent and were represented in about the same frequency that we observed inverted divisions (15–25%), consistent with these cells being the progeny of an inverted neuroblast division. We conclude that neuroblast/GMC identity is more tightly correlated with cortical markers than cell size.

We now consider how Dlg/Scrib/Lgl regulate basal protein targeting in metaphase neuroblasts. Both positively and negatively acting myosins are involved, as pharmacological inhibition of myosins mimics the *dlg*, *scrib* and *lgl* phenotype[3,4], and reduction of the apically

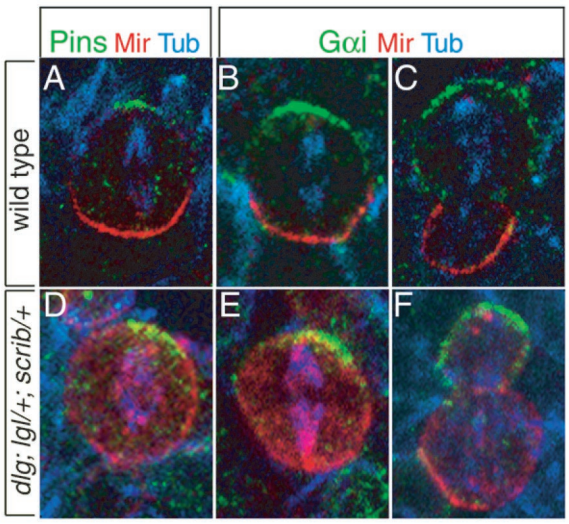


Figure 4 **Pins and Gαi are apically localized in *dlg; lgl/+; scrib/+* embryos.** Embryonic stage-15 metaphase (**a**, **b**, **d**, **e**) or telophase (**c**, **f**) neuroblasts stained for proteins indicated at top (Mir, Miranda; Tub, α-Tubulin); genotypes at left, apical is up. Neuroblasts were identified as large Miranda-positive cells within the superficial layer of the central nervous system. **a**–**c**, Wild-type neuroblasts show mutually exclusive cortical crescents of Pins/Miranda (**a**) or Gαi/Miranda (**b**, **c**). **d**–**f**, *dlg*^{m52}*; lgl*^{4}*/+; scrib*^{1}*/+* triple mutants also show Pins and Gαi crescents at metaphase (**d**, **e**), and mutually exclusive cortical crescents of Gαi/Miranda at telophase, even during an inverted cell division (**f**).

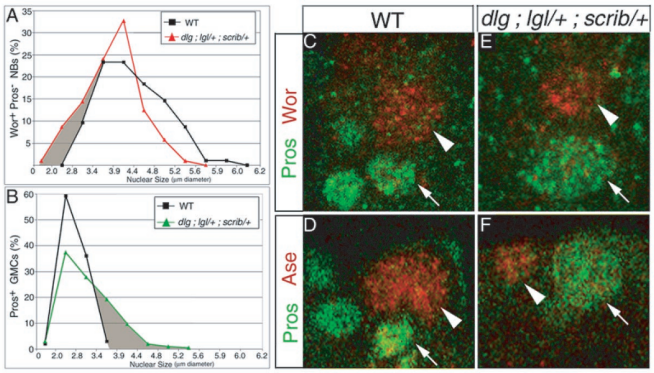


Figure 5 **The role of cell size and cortical polarity in specifying neuroblast/GMC identity.** Antibody probes indicated at left (Wor, Worniu; Ase, Asense; Pros, Prospero). Genotypes indicated at top; embryonic stage 15; apical, up. **a**, **b**, Size distribution of neuroblasts and GMCs in wild type (black lines) and *dlg*^{m52}*; lgl*^{4}*/+; scrib*^{1}*/+* mutants (coloured lines). Each data point shows the percentage of cells in the size category shown below. Size distribution of Worniu-positive, Prospero-negative neuroblast nuclei (**a**): the average size of neuroblast nuclei is larger in wild type (4.35 ± 2.41 μm; $n = 104$) than in mutants (3.90 ± 2.27 μm; $n = 105$), and some mutant 'small neuroblasts' are outside the wild type range. Size distribution of Prospero-positive GMC nuclei (**b**): the average size of GMC nuclei is smaller in wild-type (2.62 ± 1.32 μm; $n = 106$) than in mutants (3.16 ± 2.05 μm; $n = 232$), and some mutant 'large GMCs' are outside the wild type range. **c**, **d**, In wild type, neuroblasts have large Worniu-positive Asense-positive Prospero-negative nuclei (arrowheads) and GMCs have small Worniu-negative Asense-negative Prospero-positive nuclei (arrows). **e**, **f**, *dlg*^{m52}*; lgl*^{4}*/+; scrib*^{1}*/+* triple mutants have abnormally small Worniu-positive Asense-positive Prospero-negative neuroblasts (arrowheads) adjacent to abnormally large Worniu-negative Asense-negative Prospero-positive GMCs (arrows).

enriched Myosin-II (K. Siller and C.Q.D., unpublished observations) suppresses the phenotype[3,4]. One attractive model is that apical Myosin-II inhibits Lgl, thereby limiting active Lgl to the basal cortex. The formation of a stable, inactive Lgl–MyosinII complex at the apical cortex may explain the observed apical enrichment of both Lgl and Myosin-II in neuroblasts. An alternative model is that Lgl inhibits apical activity of Myosin-II, thus preventing Myosin-II from competing with a positively acting myosin that transports Miranda away from the apical cortex.

Another question concerns how Dlg, Scrib and Lgl positively regulate the size of the neuroblast cell cortex and mitotic spindle pole. Apical Dlg/Scrib/Lgl may directly promote apical spindle pole growth; this is consistent with the observation that vertebrate Dlg orthologues physically interact with known microtubule-binding proteins[15–18]. Alternatively, Dlg/Scrib/Lgl may promote the basal localization of a spindle inhibitory factor, perhaps by the same mechanism used to target Miranda to the basal cortex. Both of these models postulate that the primary function of Dlg/Scrib/Lgl is to regulate spindle morphology, with spindle morphology specifying cortical properties, such as the size of apical/basal domains and the position of the cleavage furrow. This is consistent with the known role of the mitotic spindle in establishing cortical properties in *Caenorhabditis elegans*[19] and cleavage furrow position in many cell types[20]. An alternative model that we cannot exclude is that Dlg/Scrib/Lgl activity establishes a large apical cortical domain, perhaps by functioning as cortical scaffolding proteins, that indirectly permits the formation of a large apical spindle pole.

Finally, we consider whether Dlg, Scrib and Lgl function in a common pathway. All three proteins have similar subcellular localization in neuroblasts, although they have not yet been detected in a single protein complex. *dlg*, *scrib* and *lgl* mutants show strong positive genetic interactions for regulating basal protein targeting in metaphase neuroblasts (Fig. 2o), and each null single mutant shows a fully penetrant phenotype, suggesting that Dlg/Scrib/Lgl function in a common pathway for this process. In contrast, much weaker genetic interactions are observed for spindle or cell size asymmetry phenotypes (Fig. 3m), and each null single mutant shows only a partially penetrant phenotype, suggesting that parallel or partially redundant pathways regulate this process. Good candidates for contributing to cell size and spindle asymmetry are the apical proteins aPKC, Pins and Gαi. We have found that *lgl; aPKC* double mutants have a higher penetrance of spindle asymmetry defects than *lgl* single mutants (H. Shih, R.A., C.-Y. Lee, and C.Q.D., unpublished observations). In addition, neuroblasts with reduced levels of Pins or uniform cortical Gαi have been observed to divide symmetrically[13,21]. aPKC, Pins and Gαi are localized apically in *dlg*; *lgl/+*; *scrib/+* triple-mutant neuroblasts, and Dlg/Scrib are apically enriched in neuroblasts with reduced levels of aPKC/Pins/Gαi. Thus, Dlg/Scrib/Lgl and aPKC/Pins/Gαi could provide partially redundant functions in promoting apical cell size and spindle asymmetry. □

Methods

Fly strains

According to Flybase, *scrib*^{1}, *dlg*^{M52}, *lgl*^{4}, *insc*^{22}, and *zip*^{1} are all null alleles and *lgl*^{334} is a hypomorphic allele. Homozygous embryos or larvae were identified by lack of *ftz-lacZ* balancer chromosome (embryos), lack of *actin-GFP* balancer chromosome (larvae) and/or presence of Msl-1 staining (for *dlg/Y* hemizygotes). *dlg*^{M52} mutants were raised at 18 °C to allow survival to second-instar larval stages on standard media; all other stocks were raised at 25 °C. Stable stocks were made for *zip*^{1}*; scrib*^{1} and *lgl*^{4}*; scrib*^{1} genotypes; other double or triple mutants were generated from the following crosses: *dlg*^{M52}*/Y; lgl*^{4}*/+* were derived from *dlg*^{M52}*/FM7* females crossed to *lgl*^{4}*/CyO* males. *dlg*^{M52}*/Y; scrib*^{1}*/+* were derived from *dlg*^{M52}*/FM7* females crossed to *scrib*^{1}*/TM3* males. *dlg*^{M52}*/Y; lgl*^{4}*/+; scrib*^{1}*/+* were derived from *dlg*^{M52}*/FM7* females crossed to *lgl*^{4}*/CyO; scrib*^{1}*/TM3* males. Males containing *UAS-Dlg-GFP*^{22} and *Zeus-GFP*^{23} were crossed to *V32a-GAL4* virgins to produce embryos for live neuroblast imaging.

Antibodies

Rabbits and mice were immunized with a GST fusion protein containing the Scrib carboxy-terminal

431 amino acids; this antibody recognizes the expected Scrib band[24] (with a relative molecular mass (M_r) of 210,000) on a western blot. Lgl antisera were raised in rats immunized with the Lgl C-terminal 21-amino-acid peptide conjugated to Keyhole Limpet Haemocyanin. Dlg, Scrib and Lgl staining was specific, as no staining was observed in mutant neuroblasts. Larval brains and embryos were fixed for 20 min in 6% and 9% formaldehyde, respectively. Lgl staining requires ethanol- and methanol-free fixation: whole embryos were hand-dissected from vitelline membranes in a 3.7% formaldehyde bath and left for 20 min to fix; *in vitro*-cultured neuroblasts were fixed for 20 min in 4% paraformaldehyde and processed for immunostaining using published protocols[25]. Larval brains were dissected in Schneiders Medium (Invitrogen, Carlsbad, CA) and fixed for precisely 20 min in 4% formaldehyde. Deviations from these procedures result in cytoplasmic or weakly uniform cortical Lgl distribution. Drug treatments were performed as previously described[4]. Primary antibodies were used at 1:1000, except where indicated: rabbit and mouse anti-Scrib (1:2500), rat anti-Lgl (1:150), rabbit anti-phosphohistone H3 (Upstate Biotechnology, Waltham, MA), rat anti-α-Tubulin and mouse anti-γ-Tubulin (Sigma, St Louis, MO), rabbit anti-Insc[8], rabbit anti-aPKC (Santa Cruz Biotechnology, Santa Cruz, CA), mouse anti-Dlg (1:100)[26], rabbit anti-Gukh (1:400)[5], rabbit anti-Miranda (1:500)[4], mouse anti-Prospero (1:4)[27], rabbit anti-Prospero[28], rabbit anti-β-galactosidase (1:3000; Cappel, Costa Mesa, CA), rabbit anti-asense (1:3000)[28], mouse anti-Worniu[29] and guinea pig anti-Msl-1 (1:400; M. Kuroda).

Microscopy

x-y-z three-dimensional image stacks and *x-y-z*-time 4D movies were collected on a Biorad Radiance confocal microscope. Movies were made from data sets containing three images 1.5 μm apart in the *z*-axis that were collected every 30 s, assembled from single *z* images at each time using Metamorph software, converted into a stack of TIFF images using NIH Image and saved as a Quicktime movie. Cell size quantification was performed by averaging the longest axis and the orthogonal axis of the apical or basal cortical domain in telophase neuroblasts.

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1. Knoblich, J. A. Asymmetric cell division during animal development. *Nature Rev. Mol. Cell Biol.* **2,** 11–20 (2001).
2. Doe, C. Q. & Bowerman, B. Asymmetric cell division: fly neuroblast meets worm zygote. *Curr. Opin. Cell Biol.* **13,** 68–75 (2001).
3. Ohshiro, T., Yagami, T., Zhang, C. & Matsuzaki, F. Role of cortical tumour-suppressor proteins in asymmetric division of Drosophila neuroblast. *Nature* **408,** 593–596 (2000).
4. Peng, C. Y., Manning, L., Albertson, R. & Doe, C. Q. The tumour-suppressor genes *lgl* and *dlg* regulate basal protein targeting in *Drosophila* neuroblasts. *Nature* **408,** 596–600 (2000).
5. Mathew, D. *et al.* Recruitment of Scribble to the synaptic scaffolding complex requires GUK-holder, a novel DLG binding protein. *Curr. Biol.* **12,** 531–539 (2002).
6. Strand, D. *et al.* A human homologue of the *Drosophila* tumour suppressor gene *l(2)gl* maps to 17p11.2–12 and codes for a cytoskeletal protein that associates with nonmuscle myosin II heavy chain. *Oncogene* **11,** 291–301 (1995).
7. Bilder, D., Li, M. & Perrimon, N. Cooperative regulation of cell polarity and growth by *Drosophila* tumor suppressors. *Science* **289,** 113–116 (2000).
8. Kraut, R., Chia, W., Jan, L. Y., Jan, Y. N. & Knoblich, J. A. Role of inscuteable in orienting asymmetric cell divisions in *Drosophila*. *Nature* **383,** 50–55 (1996).
9. Schober, M., Schaefer, M. & Knoblich, J. A. Bazooka recruits Inscuteable to orient asymmetric cell divisions in *Drosophila* neuroblasts. *Nature* **402,** 548–551 (1999).
10. Petronczki, M. & Knoblich, J. A. DmPAR-6 directs epithelial polarity and asymmetric cell division of neuroblasts in *Drosophila*. *Nature Cell Biol.* **3,** 43–49 (2001).
11. Wodarz, A., Ramrath, A., Grimm, A. & Knust, E. *Drosophila* atypical protein kinase C associates with Bazooka and controls polarity of epithelia and neuroblasts. *J. Cell Biol.* **150,** 1361–1374 (2000).
12. Schaefer, M., Shevchenko, A. & Knoblich, J. A. A protein complex containing Inscuteable and the Gα-binding protein Pins orients asymmetric cell divisions in *Drosophila*. *Curr. Biol.* **10,** 353–362 (2000).
13. Schaefer, M., Petronczki, M., Dorner, D., Forte, M. & Knoblich, J. A. Heterotrimeric G proteins direct two modes of asymmetric cell division in the *Drosophila* nervous system. *Cell* **107,** 183–194 (2001).
14. Wodarz, A., Ramrath, A., Kuchinke, U. & Knust, E. Bazooka provides an apical cue for Inscuteable localization in *Drosophila* neuroblasts. *Nature* **402,** 544–547 (1999).
15. Brenman, J. E. *et al.* Localization of postsynaptic density-93 to dendritic microtubules and interaction with microtubule-associated protein 1A. *J. Neurosci.* **18,** 8805–8813 (1998).
16. Niethammer, M. *et al.* CRIPT, a novel postsynaptic protein that binds to the third PDZ domain of PSD-95/SAP90. *Neuron* **20,** 693–707 (1998).
17. Matsumine, A. *et al.* Binding of APC to the human homolog of the *Drosophila* discs large tumor suppressor protein. *Science* **272,** 1020–1023 (1996).
18. Hanada, T., Lin, L., Tibaldi, E. V., Reinherz, E. L. & Chishti, A. H. GAKIN, a novel kinesin-like protein associates with the human homologue of the *Drosophila* discs large tumor suppressor in T lymphocytes. *J. Biol. Chem.* **275,** 28774–28784 (2000).
19. Gomes, J. E. *et al.* The maternal gene *spn-4* encodes a predicted RRM protein required for mitotic spindle orientation and cell fate patterning in early *C. elegans* embryos. *Development* **128,** 4301–4314 (2001).
20. Guertin, D. A., Trautmann, S. & McCollum, D. Cytokinesis in eukaryotes. *Microbiol. Mol. Biol. Rev.* **66,** 155–178 (2002).
21. Parmentier, M. L. *et al.* Rapsynoid/partner of inscuteable controls asymmetric division of larval neuroblasts in *Drosophila*. *J. Neurosci. (Online)* **20,** RC84 (2000).
22. Koh, Y. H., Popova, E., Thomas, U., Griffith, L. C. & Budnik, V. Regulation of DLG localization at synapses by CaMKII-dependent phosphorylation. *Cell* **98,** 353–363 (1999).
23. Morin, X., Daneman, R., Zavortink, M. & Chia, W. A protein trap strategy to detect GFP-tagged proteins expressed from their endogenous loci in *Drosophila*. *Proc. Natl Acad. Sci. USA* **98,** 15050–15055 (2001).
24. Bilder, D. & Perrimon, N. Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. *Nature* **403,** 676–680 (2000).
25. Broadus, J. & Doe, C. Q. Extrinsic cues, intrinsic cues and microfilaments regulate asymmetric protein localization in *Drosophila* neuroblasts. *Curr. Biol.* **7,** 827–835 (1997).
26. Zito, K., Fetter, R. D., Goodman, C. S. & Isacoff, E. Y. Synaptic clustering of Fascilin II and Shaker: essential targeting sequences and role of Dlg. *Neuron* **19,** 1007–1016 (1997).
27. Spana, E. P. & Doe, C. Q. The prospero transcription factor is asymmetrically localized to the cell cortex during neuroblast mitosis in *Drosophila*. *Development* **121,** 3187–3195 (1995).
28. Vaessin, H. *et al. prospero* is expressed in neuronal precursors and encodes a nuclear protein that is involved in the control of axonal outgrowth in *Drosophila*. *Cell* **67,** 941–953 (1991).
29. Cai, Y., Chia, W. & Yang, X. A family of snail-related zinc finger proteins regulates two distinct and parallel mechanisms that mediate *Drosophila* neuroblast asymmetric divisions. *EMBO J.* **20,** 1704–1714 (2001).

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COMPETING FINANCIAL INTERESTS

The authors declare that they have no competing financial interests.

Movie 1. Dlg::GFP shows dynamic apical enrichment adjacent to the apical spindle pole during metaphase in living embryonic neuroblasts. Dlg::GFP and the microtubule-binding protein Zeus::GFP are imaged in a lateral view of a stage 11 embryo. The apical neuroblast cortex is indicated with white arrowheads during mitosis. 180X accelerated.

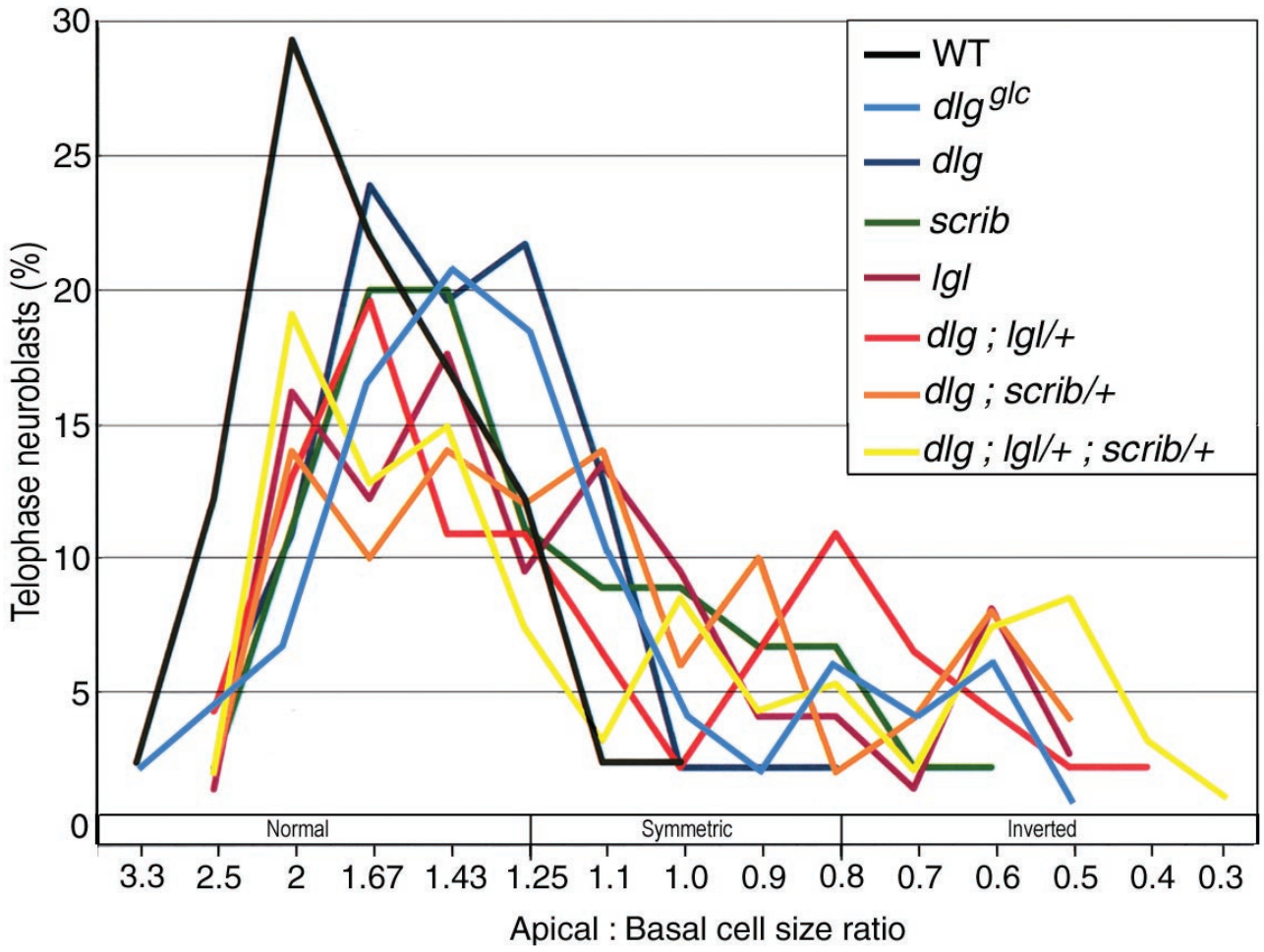
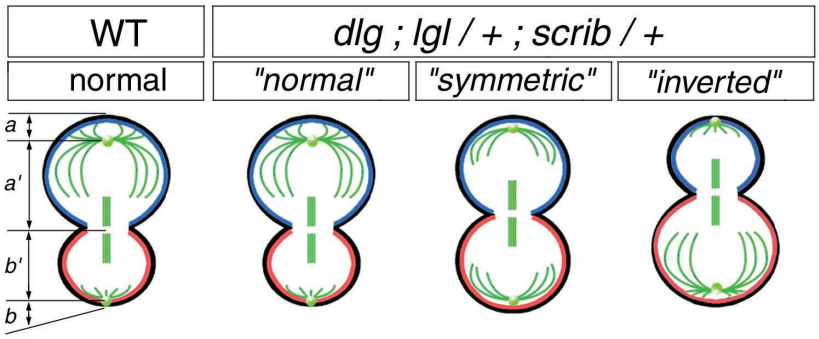


Figure S1. Quantitation of apical/basal cell size ratios in wild type and mutant neuroblasts. Upper right: color code for each zygotic genotype (dlg^{M52}, $scrib^{1}$, lgl^{4} alleles used) or maternal zygotic genotype (dlg^{M52} germline clones, dlg^{GLC}). Ratios of apical:basal cell size are given in a linear scale, with numbers to each side of 1.0 being reciprocals. Apical and basal cells are defined by aPKC and Miranda staining, respectively. Wild type neuroblasts always have a larger neuroblast and smaller GMC, although some fall into the "symmetric" division category (e.g. those with apical:basal cell size ratios of 1.1-1.25).



Centrosome Size

Apical > Basal	95%	75%	58%	17%
Apical = Basal	5%	25%	42%	61%
Apical < Basal	0%	0%	0%	22%
	(38)	(36)	(19)	(18)

Centrosome - Cortex distance (um)

Apical (a)				
0.9 - 1.6	87%	81%	37%	6%
0.4 - 0.9	13%	14%	32%	44%
0.1 - 0.4	0%	5%	32%	50%
Basal (b)				
0.9 - 1.6	0%	17%	16%	39%
0.4 - 0.9	4%	25%	42%	55%
0.1 - 0.4	96%	58%	42%	6%
	(38)	(36)	(19)	(18)

Spindle pole length (a'/b' ratio)

Average	1.27	1.30	1.03	0.77
Range	1.19-1.41	1.11-1.55	0.90-1.09	0.71-0.83
	(11)	(14)	(11)	(10)

Figure S2. Quantitation of centrosome and spindle asymmetry in wild type and mutant neuroblasts. Genotypes and cell division categories are indicated at top; embryonic stage 15 telophase neuroblasts; apical, up. Apical/basal cell size ratios for each cell division category: normal, > 1.25; symmetrical, 0.8-1.25; inverted, < 0.8. Cortex-centrosome distance for the apical and basal centrosomes is a,b respectively; spindle pole length for the apical and basal spindle poles is a', b', respectively. (n) = number of neuroblasts scored. Centrosome-to-cortex distance was measured from the centrosome center to the outer edge of cortex.