Cell

Microtubule-Induced Pins/Gαi Cortical Polarity in *Drosophila* Neuroblasts

Sarah E. Siegrist[1] and Chris Q. Doe[1,*]
[1] Institutes of Neuroscience and Molecular Biology, Howard Hughes Medical Institute, University of Oregon, Eugene, Oregon 97403, USA
*Contact: cdoe@uoneuro.uoregon.edu
DOI 10.1016/j.cell.2005.09.043

SUMMARY

Cortical polarity regulates cell division, migration, and differentiation. Microtubules induce cortical polarity in yeast, but few examples are known in metazoans. We show that astral microtubules, kinesin Khc-73, and Discs large (Dlg) induce cortical polarization of Pins/Gαi in *Drosophila* neuroblasts; this cortical domain is functional for generating spindle asymmetry, daughter-cell-size asymmetry, and distinct sibling fates. Khc-73 localizes to astral microtubule plus ends, and Dlg/Khc-73 and Dlg/Pins coimmunoprecipitate, suggesting that microtubules induce Pins/Gαi cortical polarity through Dlg/Khc-73 interactions. The microtubule/Khc-73/Dlg pathway acts in parallel to the well-characterized Inscuteable/Par pathway, but each provides unique spatial and temporal information: The Inscuteable/Par pathway initiates at prophase to coordinate neuroblast cortical polarity with CNS tissue polarity, whereas the microtubule/Khc-73/Dlg pathway functions at metaphase to coordinate neuroblast cortical polarity with the mitotic spindle axis. These results identify a role for microtubules in polarizing the neuroblast cortex, a fundamental step for generating cell diversity through asymmetric cell division.

INTRODUCTION

Cell polarity is essential for asymmetric cell division, cell migration, and the proper function of many differentiated cell types. We are interested in how cell polarity is generated in *Drosophila* neuroblasts, a model system for studying cortical polarity and asymmetric cell division (reviewed in Betschinger and Knoblich, 2004). Embryonic neuroblasts delaminate from an apical/basal polarized epithelium and divide asymmetrically along the apical/basal axis. Mitotic neuroblasts form distinct apical/basal cortical domains and assemble a morphologically asymmetric mitotic spindle, which aligns with the apical/basal axis to generate daughter cells with different sizes and cell fates. The apical neuroblast is larger and proliferative, whereas the basal ganglion mother cell (GMC) is smaller and only divides once before differentiating into neurons or glia.

Neuroblast cortical polarity regulates all known aspects of neuroblast asymmetric cell division, and thus it is essential to understand how cortical polarity is established. Cortical polarity is first visible at late interphase, when a large number of proteins are targeted to the apical cortex. These include Bazooka (Baz; Par-3 in worms and mammals), aPKC (atypical protein kinase C), and Par-6 proteins (hereafter called the Par complex) as well as the Inscuteable (Insc), Partner of Inscuteable (Pins), and Gαi proteins (reviewed in Betschinger and Knoblich, 2004). In addition, during metaphase there is a transient apical enrichment of nonmuscle myosin II (Barros et al., 2003) and the cortical tumor suppressor proteins Discs large (Dlg), Scribble (Scrib), and Lethal giant larvae (Lgl) (Albertson and Doe, 2003).

Recent work has highlighted the central role played by the two apical cortical proteins, Pins and Gαi, in regulating asymmetric cell division and spindle-cortex interactions from worms to mammals (Cai et al., 2003; Colombo et al., 2003; Du and Macara, 2004; Du et al., 2001; Fuse et al., 2003; Gotta et al., 2003; Parmentier et al., 2000; Schaefer et al., 2000, 2001; Srinivasan et al., 2003; Yu et al., 2000, 2003). Gαi can form a heterotrimer with Gβ/Gγ proteins, while Pins has three Gαi binding GoLoco domains as well as seven tetratricopeptide repeats (TPRs) that directly bind Insc. In all animals studied to date, Pins is proposed to activate both Gαi and Gβ/Gγ in a receptor-independent fashion by binding Gαi and disrupting the inactive Gαi/Gβ/Gγ heterotrimer (reviewed in Willard et al., 2004).

Despite the importance of Pins/Gαi cortical polarity in regulating *Drosophila* asymmetric cell division, it remains unclear how Pins/Gαi cortical localization is regulated. In neuroblasts, Insc was initially reported as essential for Pins/Gαi cortical localization (Schaefer et al., 2000, 2001; Yu et al., 2000); however, several recent papers have suggested otherwise (Cai et al., 2003; Yu et al., 2003; Yu et al., 2002). In adult sensory organ precursors (SOPs), Insc is not expressed, yet SOPs undergo asymmetric cell division mediated by asymmetrically

polarized Pins/Gαi (Bellaiche et al., 2001; Schaefer et al., 2001).

Here we describe a novel astral microtubule-dependent pathway for inducing Pins/Gαi cortical polarity that includes the plus-end-directed microtubule motor protein, kinesin heavy chain 73 (Khc-73), and the membrane-associated guanylate kinase (MAGUK) protein, Dlg. We find that the microtubule-dependent Khc-73/Dlg pathway and the cortical Insc/Par pathway are partially redundant for inducing Pins/Gαi cortical polarity in neuroblasts yet have unique functions: The Insc/Par pathway is active at late interphase and coordinates neuroblast cortical polarity with CNS tissue polarity, whereas the Khc-73/Dlg pathway is active at metaphase and coordinates neuroblast cortical polarity with the mitotic spindle axis.

RESULTS

A current model for the establishment of neuroblast cortical polarity is that an unknown cue recruits Baz, aPKC, Par-6, and Insc to the apical cortex of the neuroblast just prior to prophase, which is closely followed by the apical recruitment of Pins/Gαi proteins, presumably via Insc-Pins direct interactions (reviewed in Betschinger and Knoblich, 2004). We term this the cortical ''Insc/Par pathway'' of Pins/Gαi localization to distinguish it from the Insc-independent ''microtubule-based pathway'' of Pins/Gαi localization that is the focus of this paper.

Astral Microtubules Can Induce Pins/Gαi/Dlg Cortical Polarity in Metaphase Neuroblasts

We confirmed that *insc*^{22} null mutant embryos (*insc* mutants) lack apical localization of the Insc/Par complex proteins (Insc, Baz, aPKC, and Par-6; Figures 1H–1J, data not shown) (Petronczki and Knoblich, 2001; Wodarz et al., 1999, 2000), but interestingly we found that Pins, Gαi, and Dlg still form robust crescents in the majority of *insc* mutant metaphase neuroblasts (Figures 1B–1E and 1O). Similar results were observed in mitotic neuroblasts from embryos homozygous for the *TE35* deficiency in which *insc* is not transcribed (Figure S1A) (Cai et al., 2001). Although Pins/Gαi/Dlg crescents form in *insc* mutants, the timing and position of crescent formation differed from wild-type. First, in wild-type neuroblasts Pins/Gαi/Dlg crescents always formed at the apical surface adjacent to the overlying ectoderm, whereas in *insc* mutant neuroblasts Pins/Gαi/Dlg crescents were found at all positions around the cortex (compare crescent position in Figure 1A with those in Figures 1B–1E and S1A). Second, in wild-type neuroblasts Pins/Gαi crescents formed by early prophase (94%, n = 50; data not shown), whereas in *insc* mutants Pins/Gαi crescents were not detected at prophase (0%, n = 54; data not shown) but only at metaphase (78%, n = 100; Figure 1O). These results suggest that there is an Insc/Par-independent pathway that is active at metaphase to induce formation of Pins/Gαi/Dlg cortical crescents.

A clue to the identity of the Insc/Par-independent pathway was the observation that Pins/Gαi/Dlg crescents were always colocalized over one spindle pole, which can be mispositioned relative to the overlying ectoderm in *insc* mutants (Figures 1D, 1E, and S1A). This observation suggested that either spindle microtubules induced cortical polarity, or cortical polarity formed spontaneously at a nonapical position and induced spindle alignment. To distinguish between these mechanisms, we depolymerized microtubules in *insc* mutant neuroblasts with Colcemid and scored for Pins/Gαi/Dlg cortical crescents. We found that Colcemid treatment of *insc* mutant neuroblasts resulted in a nearly complete loss of Pins/Gαi/Dlg crescents: Pins is mostly cytoplasmic and Gαi/Dlg are uniform cortical (Figures 1F and 1O; data not shown). In contrast, Colcemid treatment of wild-type neuroblasts had no effect on Pins/Gαi/Dlg crescent formation (Figures 1G and 1O; data not shown), likely due to the association of Pins/Gαi/Dlg with the apical Insc/Par complex. In fact, the Insc/Par pathway of Pins/Gαi/Dlg localization requires only Insc and Baz proteins, because *aPKC* mutants that lack aPKC/Par-6 protein localization but retain Baz/Insc localization still formed Pins/Gαi/Dlg crescents in the absence of microtubules (Figures 1O, S1B, and S1C). We conclude that spindle microtubules have the ability to induce Pins/Gαi/Dlg cortical crescents over one spindle pole in the absence of an Insc/Par pathway.

We next tested whether astral microtubules were required for inducing Pins/Gαi/Dlg cortical polarity using both pharmacological and genetic methods. First, we selectively abolished astral microtubules with low concentrations of nocodazole. Wild-type nocodazole-treated neuroblasts had an intact central spindle but lacked detectable astral microtubules as judged by tubulin staining (Figure S2B) and failed to align their spindle with cortical polarity markers (Figure 1K and Table S1), similar to *cnn* mutants lacking astral microtubules (Megraw et al., 2001). Despite loss of astral microtubules, wild-type nocodazole-treated neuroblasts progressed through mitosis and showed normal colocalization of Pins/Gαi/Dlg with Insc/Par complex proteins at the cortex (Figures 1K and 1O; data not shown). In contrast, *insc* mutant neuroblasts treated with nocodazole showed a dose-dependent loss of Pins/Gαi/Dlg crescent formation (Figures 1L and 1O, and Supplemental Figures 2C and 2D), suggesting that astral microtubules were required to induce Pins/Gαi/Dlg crescent formation. In addition, we abolished astral microtubules genetically using the *fizzy*^{1} mutation. *fizzy* encodes an essential component of the anaphase-promoting complex, and *fizzy* mutant neuroblasts show a delay in cell cycle as well as short barrel shaped spindles that lack astral microtubules (Dawson et al., 1995) (Supplemental Figure 2E). *fizzy* single mutant neuroblasts formed normal Pins/Gαi/Dlg crescents colocalized with Insc/Par, but *insc fizzy* double mutant neuroblasts showed a loss of Pins/Gαi/Dlg crescents: Pins was delocalized from the cortex, and Gαi/Dlg were uniform cortical (Figures 1M and 1N; data not shown). Thus, both microtubule inhibitor and genetic data allow us to conclude that astral microtubules are required to induce Pins/Gαi/Dlg crescents in the absence of apical Insc/Par complex proteins, revealing a ''microtubule-to-cortex'' signaling pathway that is sufficient to generate cortical cell polarity.

Importantly, the microtubule-induced Pins/Gαi/Dlg cortical crescents are functional because *insc* mutant neuroblasts

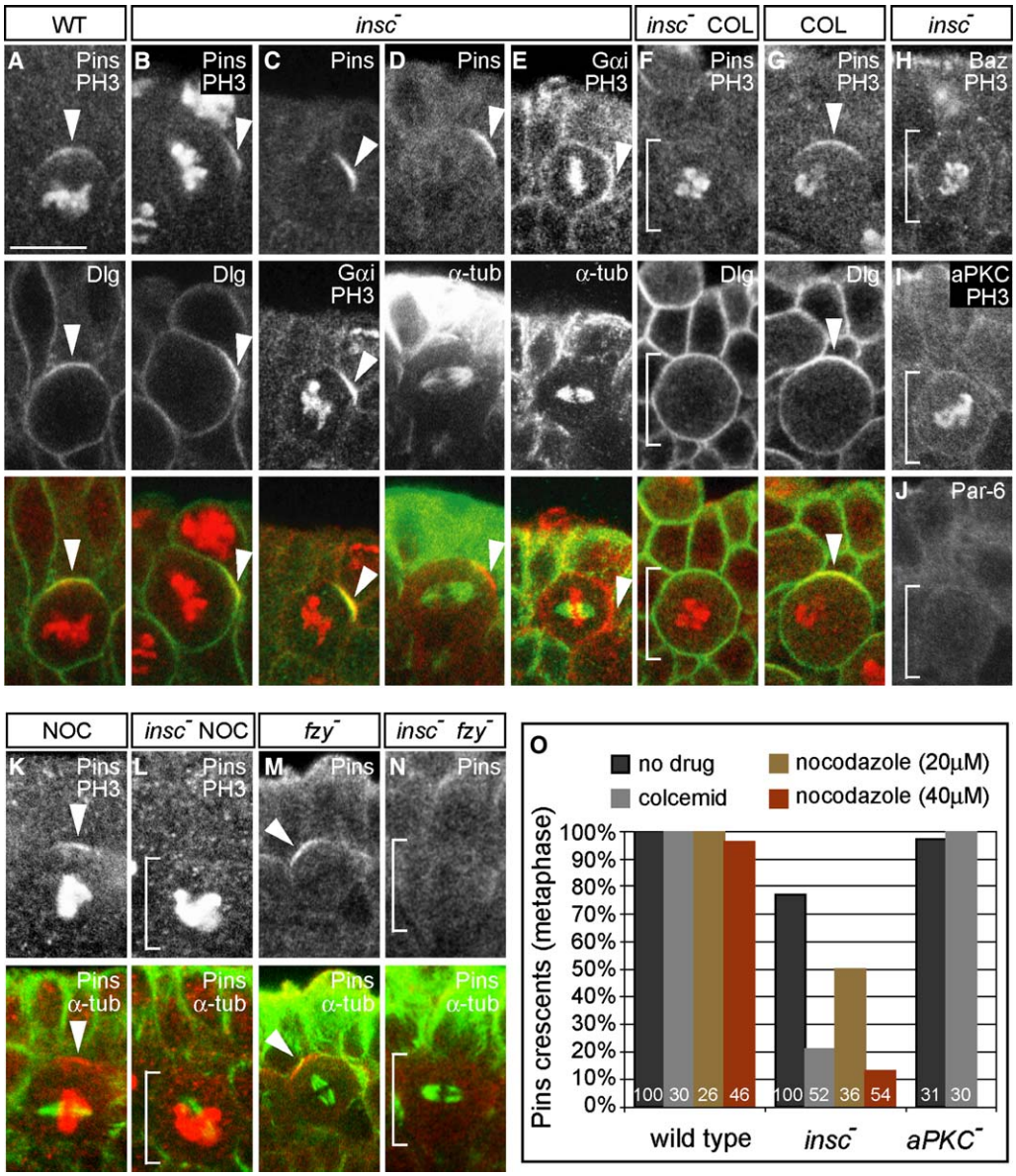

Figure 1. Astral Microtubules Induce Pins/Gαi/Dlg Cortical Polarity in Neuroblasts

(A–N) Lateral view of stage 10 metaphase neuroblasts. Genotypes and/or drug treatments are listed above panels.

(F and G) Colcemid (COL) and (K and L) 20 μM nocodazole (NOC). Markers are listed within each panel, and double-labeled neuroblasts are shown in the bottom row of (A)–(G) and (K)–(N); the mitotic marker phospho-histone H3, PH3. Crescents are indicated by arrowheads, and neuroblasts without crescents are in brackets in this and all subsequent figures.

(O) Quantitation of the Pins crescents formed in each genotype with or without drug; number of neuroblasts scored indicated within each bar. Apical is up and scale bar is 10 μm in this and subsequent figures unless noted.

have a normal asymmetric mitotic spindle morphology, cell size asymmetry, and neuroblast/GMC identity (Table S1; data not shown) (Kaltschmidt et al., 2000; Kraut et al., 1996).

Dlg Is Required for Microtubule-Induced Pins/Gαi Cortical Polarity

How do astral microtubules induce Pins/Gαi/Dlg cortical polarity? We first tested the role of Dlg because it is the best candidate to link microtubules to cortical Pins/Gαi proteins: Dlg and Pins are reported to directly interact (Bellaiche et al., 2001), and mammalian Dlg orthologs associate with the microtubule binding proteins GAKIN, MAP1a, CRIPT, APC, or KIF1B (Brenman et al., 1998; Hanada et al., 2000; Matsumine et al., 1996; Mok et al., 2002; Passafaro et al., 1999). We assayed dlg^{m52} *insc* double mutant embryos, where there is no zygotic Dlg protein (dlg^{m52} is a genetic null allele)

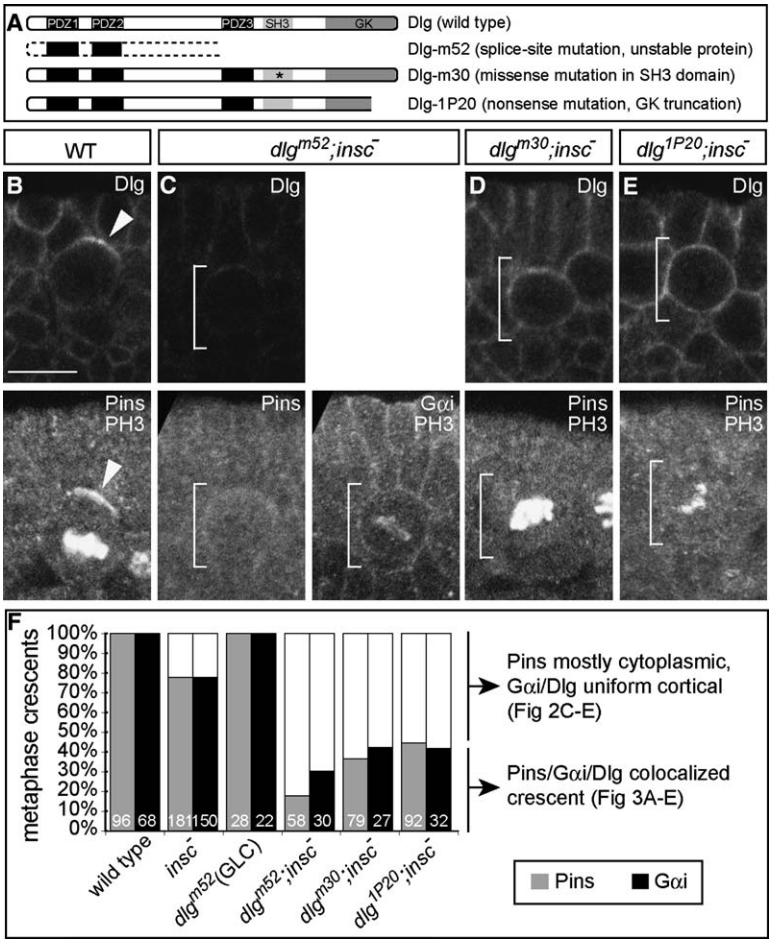

Figure 2. Dlg Is Required for Microtubule-Induced Pins/Gαi Cortical Polarity

(A) Dlg domain structure and mutant lesions.

(B–E) Lateral view of stage 10 metaphase neuroblasts. Genotypes are listed above panels; markers listed within each panel. Images in the top row were taken at the same confocal settings to allow comparison of Dlg protein levels.

(F) Quantitation of the Pins/Gαi crescents formed in each genotype; solid black/gray bars, normal to weaker cortical crescents; white bars, no cortical asymmetry (Gαi uniform cortical, Pins mostly cytoplasmic); number of neuroblasts scored indicated within each bar.

but maternal Dlg protein keeps embryonic Dlg levels at about 50% of wild-type (Figures 2B and 2C) (Woods et al., 1996). We found that *dlg*^{m52} *insc* double mutant embryos showed severe defects in Pins/Gαi cortical polarity: Pins was mostly cytoplasmic (80%, n = 58), and Gαi was uniformly distributed at the cortex (73%, n = 30) (Figure 2C; quantified in Figure 2F, white bars). This is not due to loss of astral microtubules (Figure S2F) but rather to an inability of astral microtubules to induce Pins/Gαi cortical polarity. In contrast, when the Insc/Par pathway is functional, Pins/Gαi crescents can form without Dlg or without both Dlg and microtubules (Figures 2F, S1D, S1E, S3D, and S3H; data not shown). We conclude that Dlg is required specifically for microtubule-induced Pins/Gαi cortical polarity.

To test whether Dlg is required for inducing basal cortical polarity, we assayed Miranda cortical localization. Wild-type neuroblasts show basal Miranda crescents at metaphase and telophase (Ikeshima-Kataoka et al., 1997; Shen et al., 1998). *insc* mutant neuroblasts often failed to localize Miranda at metaphase but exhibited an Insc/Par-independent "telophase rescue" of basal Miranda localization (Kraut et al., 1996) (Figure S4A). Likewise, neuroblasts from *dlg*^{m52} germline clone embryos also failed to form basal Miranda crescents at metaphase yet exhibited "telophase rescue" (Figure S4B). We found that *dlg*^{m52} *insc* double mutants lack "telophase rescue" of Miranda localization (Figure S4C), showing that the microtubule/Dlg pathway can directly or indirectly induce basal cortical polarity in the absence of the Insc/Par pathway.

Next we were interested in determining which Dlg domain is required for microtubule-induced Pins/Gαi polarity. Dlg contains multiple protein–protein interaction domains, including three PDZs, an atypical SH3, and an inactive guanylate kinase (GK) domain (Figure 2A) (reviewed in Funke et al., 2005). We scored for Pins/Gαi crescents in *dlg insc* mutants that selectively affect the SH3 domain (*dlg*^{m30}) or GK domain (*dlg*^{1P20}) (diagrammed in Figure 2A). Normal levels of Dlg protein are made in both mutants (Figures 2B–2E) (Woods et al., 1996). We found that the majority of mitotic neuroblasts from both mutant backgrounds (*dlg*^{m30} *insc* and *dlg*^{1P20} *insc)* showed uniform cortical mutant Dlg, mostly cytoplasmic Pins, and uniform cortical Gαi (Figures 2D and 2E; data not shown; quantified in Figure 2F, white bars). Thus, both the SH3 and GK domains are required for reliable microtubule-induced Dlg crescent formation and the subsequent establishment of Pins/Gαi cortical polarity.

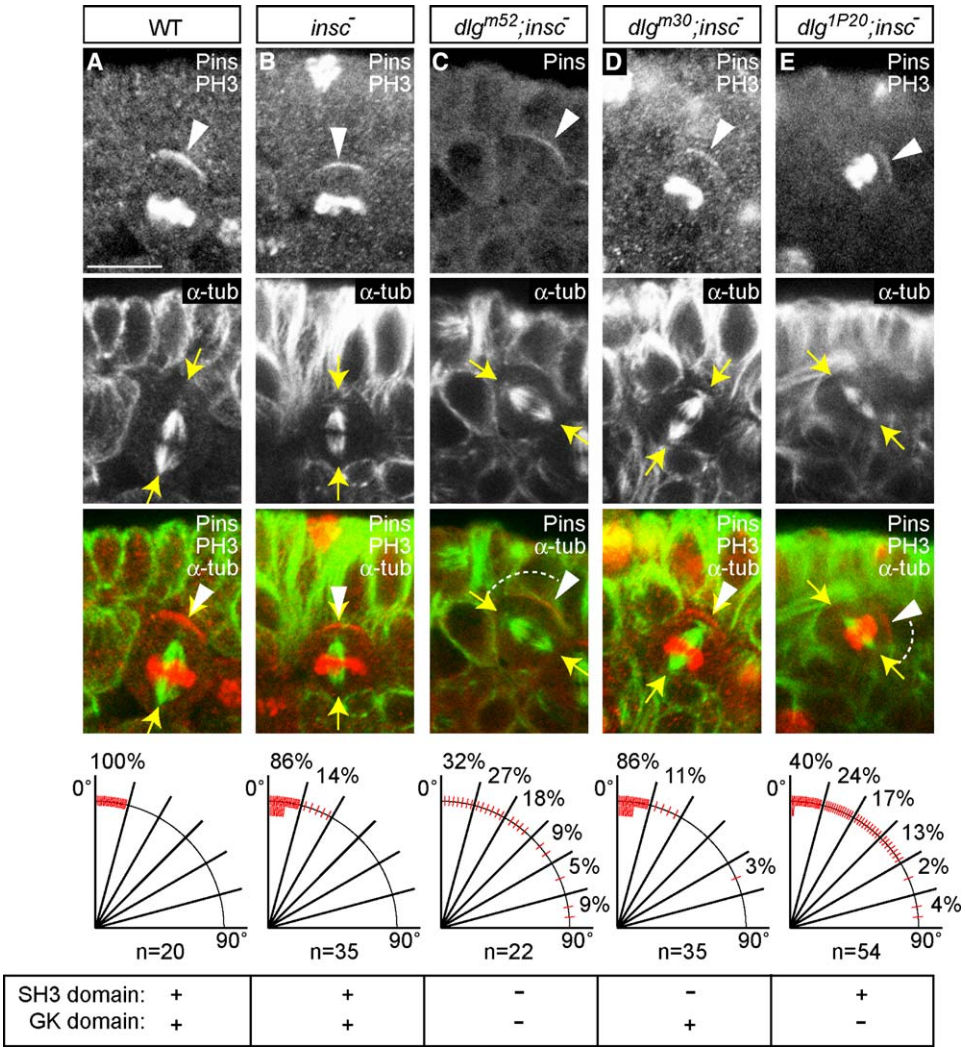

Figure 3. Dlg GK Domain Is Required to Maintain Dlg-Microtubule Alignment

(A–E) Lateral view of stage 10 metaphase neuroblasts. Genotypes are listed above panels. Top row, Pins staining with or without PH3. Neuroblasts in (B)–(E) are from the pool represented by solid gray/black bars in Figure 2F (i.e., the minority of *dlg insc* neuroblasts that form Pins/Gαi crescents). Second row, same neuroblast with α-tubulin staining to show spindle position. Third row, double-labeled neuroblasts to show position of the mitotic spindle relative to cortical polarity. White arrowheads indicate center of the crescent; yellow arrows point to spindle poles, along the long axis of the mitotic spindle. Bottom row, quantitation of spindle orientation relative to Pins/Gαi crescents. Each red line indicates the angle between the spindle axis and the center of the Pins/Gαi crescent for each metaphase neuroblast scored, represented as a dashed arc in (C) and (E).

The Dlg GK Domain Is Required to Maintain Pins/Gαi/Dlg-Spindle Alignment

Although the Dlg SH3 and GK domains are both required for fully penetrant Dlg crescent formation, when we analyzed the small pool of *dlg insc* neuroblasts that form weak Pins/Gαi crescents (10%–45%, gray/black bars in Figure 2F), we were able to assign the GK domain a distinct function in aligning the mitotic spindle with the Pins/Gαi/Dlg cortical crescent. As expected, the mitotic spindle was tightly aligned with the Pins/Gαi/Dlg crescent in both wild-type and *insc* mutant neuroblasts (Figures 3A and 3B). In contrast, the spindle showed poor alignment with the weak Pins/Gαi/Dlg crescent in the dlg^{m52} *insc* double mutant, indicating that the low levels of maternal Dlg were insufficient for spindle orientation (Figure 3C). Interestingly, the dlg^{m30} *insc* neuroblasts that had a Dlg SH3 domain mutation maintained excellent spindle alignment with the Pins/Gαi/Dlg crescent (Figure 3D), whereas the dlg^{1P20} *insc* mutant neuroblasts that had a premature Dlg GK domain truncation failed to maintain mitotic spindle alignment with the Pins/Gαi/Dlg crescent (Figure 3E). Thus, the ability of the mitotic spindle to tightly align with the Pins/Gαi/Dlg crescent requires the Dlg GK domain.

Khc-73 Coimmunoprecipitates with Dlg and Is Required for Microtubule-Induced Pins/Gαi/Dlg Crescent Formation

Because the Dlg GK domain regulates Dlg-spindle alignment, we searched the literature for proteins capable of binding both microtubules and the GK domain. Mammalian MAP1A and GAKIN/Kif13b have these properties (Brenman et al., 1998; Hanada et al., 2000), but only GAKIN has a clear ortholog in *Drosophila*. GAKIN has two microtubule binding domains (a canonical motor domain and a C-terminal CAP-Gly domain) and a central 224 aa "MAGUK binding stalk" (MBS) that binds the GK domain of human Dlg (Asaba et al., 2003; Hanada et al., 2000). *Drosophila* Kinesin heavy chain 73 (Khc-73) protein is 50% identical, has the same domain composition as mammalian GAKIN, and is a member of the Kinesin 3 family (formerly Unc-104/KIF1A family). In *Drosophila* embryos, *Khc-73* transcripts are maternally deposited and become enriched in the developing CNS (data not shown and Li et al., 1997). We generated *Khc-73* transgenes to drive embryonic expression of a hemagglutinin (HA) epitope-tagged full-length Khc-73 (HA:Khc-73FL) or a shorter form containing just the putative Dlg binding MBS domain (HA:Khc-73MBS). We found that both HA:Khc-73FL and HA:Khc-73MBS proteins were able to immunoprecipitate endogenous Dlg protein from embryonic lysates but not Pins protein, whereas HA antibodies failed to immunoprecipitate Dlg from control lysates lacking HA:Khc-73 protein (Figures 4A and 4B; data not shown). In addition, Dlg and Pins proteins can be coimmunoprecipitated specifically from embryonic CNS tissue (Figure 4C). We conclude that Khc-73/Dlg and Dlg/Pins associate within *Drosophila* embryos but find no evidence for a stable Khc-73/Dlg/Pins complex (see Discussion).

Next we tested whether Khc-73 is required for microtubule-induced formation of Pins/Gαi/Dlg cortical crescents. Because there are no existing mutations in *Khc-73*, we used RNA interference (RNAi) to reduce Khc-73 levels. *Khc-73* RNAi treatment alone gave the expected strong Pins crescents colocalized with apical Insc/Par proteins (Figure 4E; quantified in Figure 4I). However, when we induced the expression of a *Khc-73* hairpin dsRNA in *insc* mutant neuroblasts or performed *insc Khc-73* double RNAi, we observed delocalization of Pins into the cytoplasm and Dlg uniform around the cortex in metaphase neuroblasts (Figures 4D and 4F; quantified in Figure 4I). This phenotype is not due to loss of astral microtubules (Figure S2G) but rather to an inability of astral microtubules to induce Pins/Gαi/Dlg cortical polarity in the absence of Khc-73. We conclude that Khc-73 and Dlg can interact in vivo and that Khc-73 is required for microtubule-induced Pins/Gαi cortical polarity in neuroblasts.

To determine the importance of the Khc-73/Dlg interaction for Khc-73 function, we overexpressed the putative Dlg binding MBS domain of Khc-73. Overexpression of the Khc-73 MBS in *insc* RNAi-treated neuroblasts resulted in mostly cytoplasmic Pins and uniform Dlg in the majority of metaphase neuroblasts (Figure 4H; data not shown; quantified in Figure 4I). MBS overexpression alone did not alter Pins or Dlg apical enrichment (Figure 4G; quantified in Figure 4I). Thus, Khc-73/Dlg interactions appear to be important for Khc-73-induced cortical polarity.

Khc-73 Is Localized to Microtubule Plus Ends in Mitotic Neuroblasts

Khc-73 has two microtubule binding domains, a motor domain and a CAP-Gly domain, commonly found in microtubule plus-end binding proteins. To determine the subcellular localization of Khc-73, we expressed low levels of HA:Khc-73 in neuroblasts. We found HA:Khc-73 localized in puncta at microtubule plus ends, which were readily apparent in Taxol-stabilized mitotic asters (Figures 5A–5C and Movie S1). HA:Khc-73 was localized to plus ends near the cortex (presumptive astral microtubules) and plus ends associated with condensed DNA (presumptive kinetochore microtubules). We did not detect Dlg in the HA:Khc-73 puncta, suggesting that Khc-73 first contacts Dlg at the cell cortex. Taken together, Khc-73 localization and biochemical data are consistent with a model in which Khc-73 associates with astral microtubule plus ends, contacts cortical Dlg, and induces Dlg cortical clustering; this promotes the subsequent recruitment of Pins/Gαi (see Discussion).

The Microtubule/Khc-73/Dlg Pathway Is Required for Reliable Spindle Orientation

We have shown that a microtubule/Khc-73/Dlg pathway can polarize the neuroblast cortex in the absence of Insc/Par complex proteins. Here we test whether the microtubule/Khc-73/Dlg pathway has a function in the presence of Insc/Par protein function. We scored *Khc-73* RNAi neuroblasts and dlg^{m52} null mutant neuroblasts—which have normal Insc/Par apical protein crescents—for proper spindle alignment relative to the center of the apical Insc/Par protein crescent. In wild-type embryonic or larval neuroblasts, the metaphase spindle is tightly aligned with the center of the Insc/Par protein crescent (Figure 6A). In contrast, *Khc-73* RNAi-treated and dlg^{m52} larval mutants showed only 65%–70% neuroblasts with tightly aligned mitotic spindles (Figures 6B and 6C). Thus, the microtubule/Khc-73/Dlg pathway is required for reliable linkage between the mitotic spindle and Insc/Par cortical proteins. Similarly, *pins* or *Gαi* zygotic mutant larvae had neuroblasts with only 50%–55% spindles aligned with Insc/Par crescents (Figures 6D and 6E). We could not assay for a stronger phenotype in *pins* or *Gαi* maternal zygotic mutant embryos because they fail to form Insc/Par protein crescents (Yu et al., 2000, 2003). We conclude that Khc-73, Dlg, Pins, and Gαi are required for proper spindle orientation relative to cortical polarity (intrinsic spindle orientation). Thus, we have revealed evidence for bidirectional signaling between the mitotic spindle and the cell cortex: The astral microtubule/Khc-73 pathway can induce Pins/Gαi/Dlg cortical polarity, and cortical Pins/Gαi/Dlg proteins regulate spindle orientation (Figure 7A).

DISCUSSION

Recent work has shown that microtubules can directly regulate cortical polarity in yeast (Behrens and Nurse, 2002)

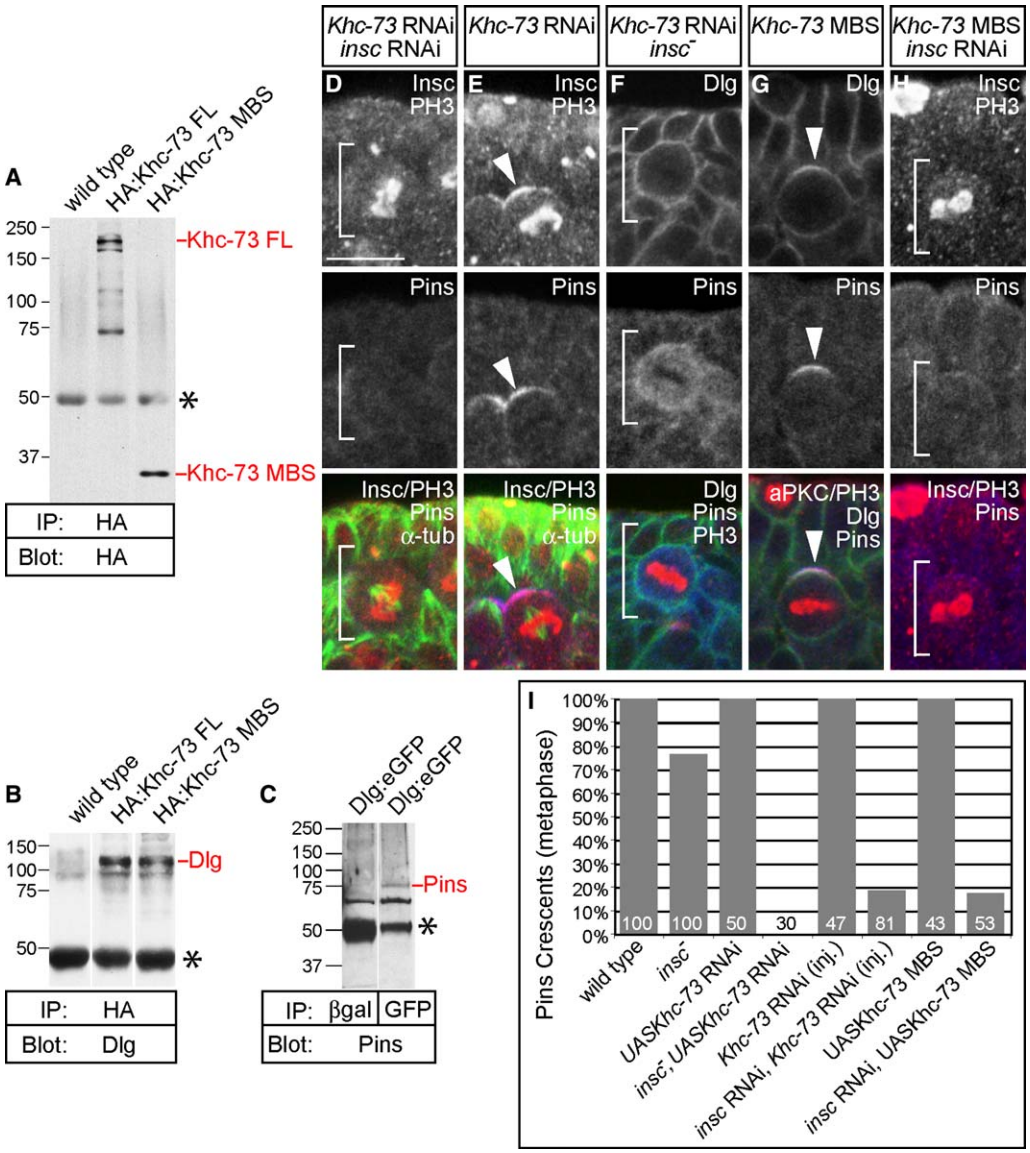

Figure 4. Khc-73 Coimmunoprecipitates with Dlg and Is Required for Microtubule-Induced Pins/Gαi/Dlg Crescent Formation

(A and B) Khc-73/Dlg coimmunoprecipitate from embryonic lysates. The maternal *V32a-GAL4* line drives ubiquitous expression of *UAS-HA:Khc-73FL* or *UAS-HA:Khc-73MBS* during embryogenesis. Protein complexes from these lysates were immunoprecipitated with HA antibodies and detected by Western blot using either α-HA (A) or α-Dlg (B). As a control, α-HA fails to coimmunoprecipitate Dlg from a wild-type lysate (A and B).

(C) Dlg/Pins coimmunoprecipitate from embryonic CNS tissue. *UAS-Dlg:eGFP* was specifically expressed in the embryonic CNS using *worniu-GAL4*. Dlg:eGFP protein was immunoprecipitated with GFP antibodies, and the immunoprecipitant was probed for Pins by Western blot. β-galactosidase antibody was used as a negative control. IgG heavy chain, asterisk.

(D–H) Khc-73 is required to induce Pins and Dlg cortical polarity in the absence of Insc. Lateral view of stage 10 metaphase neuroblasts. Genotypes or RNAi treatments are listed above panels and markers listed within panels.

(D) *insc Khc-73* double RNAi neuroblasts show loss of Pins localization while (E) *Khc-73* single RNAi neuroblasts show normal Pins crescents.

(F) The same phenotype is seen in *insc* mutant neuroblasts expressing a *Khc-73* hairpin dsRNA.

(G and H) Normal Pins and Dlg crescents in embryos overexpressing HA:KhcMBS requires Insc.

(I) Quantitation of Pins crescents formed in each genotype or RNAi treatment (inj, injected dsRNA).

during *C. elegans* meiosis (Cowan and Hyman, 2004; Wallenfang and Seydoux, 2000) and in migrating cells (Etienne-Manneville and Hall, 2001). An important question is the extent to which microtubules regulate cortical cell polarity in other contexts. Here we identify a microtubule/kinesin pathway for inducing cortical polarity in *Drosophila* neuroblasts. This pathway is sufficient to induce cortical polarization of the evolutionarily conserved Dlg, Pins, and Gαi proteins and is necessary for reliable spindle orientation relative to apical Insc/Par cortical proteins.

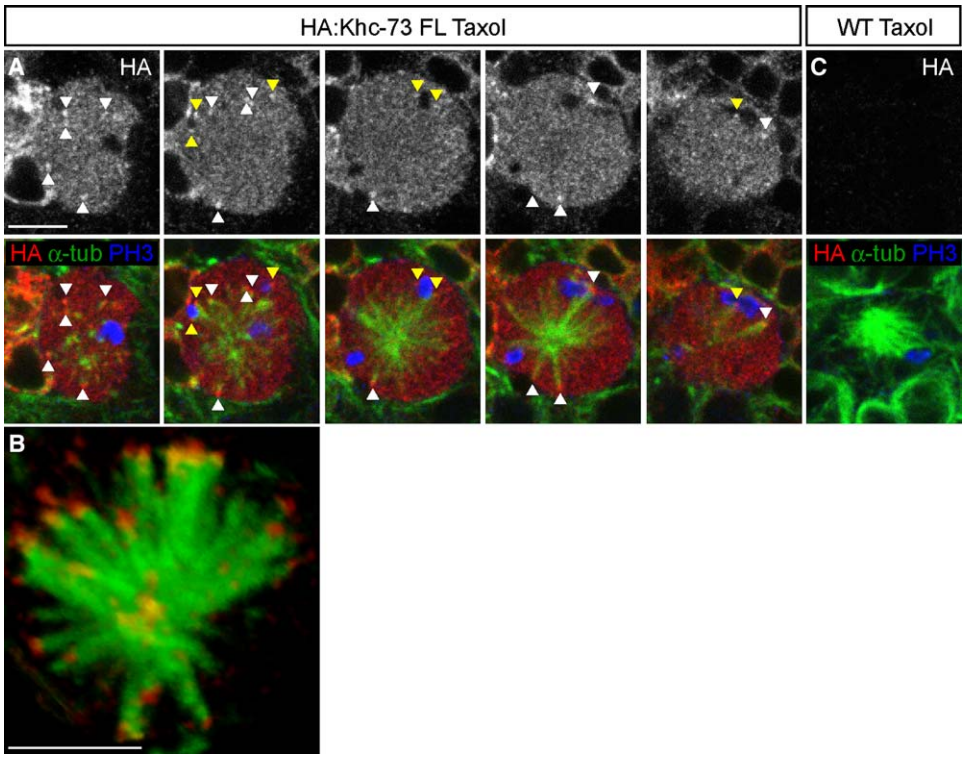

Figure 5. HA:Khc-73 Localizes to Microtubule Plus Ends in Mitotic Neuroblasts

(A) 1.0 to 1.2 μm serial optical sections from a Taxol-treated larval neuroblast from an animal expressing *UAS-HA:Khc-73FL* induced by *worniu-GAL4*. Top row, HA staining marking localization of HA:Khc-73FL. Bottom row, same neuroblast triple-labeled with HA, α-tubulin, and PH3. White arrowheads indicate localization of HA:Khc-73FL to microtubule plus ends. Yellow arrowheads indicate localization of HA:Khc-73FL to microtubule plus ends associated with DNA. Scale bar = 5 μm.

(B) Projection from the 3D reconstruction of the neuroblast shown in (A) and in Movie S1. The central red puncta is at the plus end of a microtubule projecting toward the reader. Scale bar = 5 μm.

(C) As a control, a single optical section from a wild-type Taxol-treated neuroblast showing no background HA immunostaining.

The Microtubule/Khc-73/Dlg Pathway

A model for the microtubule/Khc-73/Dlg pathway, in the absence of the Insc/Par function, is shown in Figure 7D and summarized below.

(1) At prophase, the microtubule/Khc-73/Dlg pathway is unable to polarize. Dlg/Gαi are uniform cortical, and Pins is predominantly cytoplasmic. It is not clear when Khc-73 is localized to microtubule plus ends because Taxol-treated neuroblasts metaphase arrest (Figure 5).

(2) At metaphase, Khc-73 is localized at microtubule plus ends where it can contact cortical Dlg protein. We propose that Khc-73 first contacts Dlg at the cortex because we do not detect Dlg or Dlg:eGFP on astral microtubules or colocalized with Khc-73 at microtubule plus ends. Association of Khc-73 with microtubule plus ends may be mediated by its CAP-Gly domain, similar to the Clip-170 and APC plus-end binding proteins (reviewed in Galjart and Perez, 2003). The Khc-73/Dlg interaction could occur between the Khc-73 MBS stalk domain and the Dlg GK domain because the related GAKIN/hDlg domains directly interact (Asaba et al., 2003; Hanada et al., 2000). While we can immunoprecipitate Dlg with the Khc-73 MBS domain from embryonic lysate, we are unable to detect these direct protein–protein interactions between Khc-73 and Dlg in vitro, suggesting that this interaction may be highly regulated (Figure 4; data not shown).

(3) Dlg clustering occurs over one spindle pole, although low levels persist around the cortex. An attractive model for Dlg clustering is that Khc-73/Dlg interaction blocks Dlg SH3-GK intramolecular interactions to favor Dlg intermolecular oligomerization (McGee and Bredt, 1999). In support of this model, we can express Dlg:eGFP specifically in neuroblasts and use an anti-GFP antibody to immunoprecipitate endogenous Dlg proteins (Figure S5). It is not clear why clustering occurs over just one spindle pole; perhaps the spindle poles are intrinsically different (Lambert and Nagy, 2002), or perhaps cortical heterogeneity (e.g., residual Par proteins) favors crescent formation over one spindle pole.

(4) Pins/Gαi cortical clustering occurs. Pins/Gαi clustering requires Dlg and may be mediated by direct Dlg/Pins interactions (Bellaiche et al., 2001) and Pins/Gαi interactions (Schaefer et al., 2001). However, the Dlg/Pins interaction must be highly regulated or indirect as we have been able

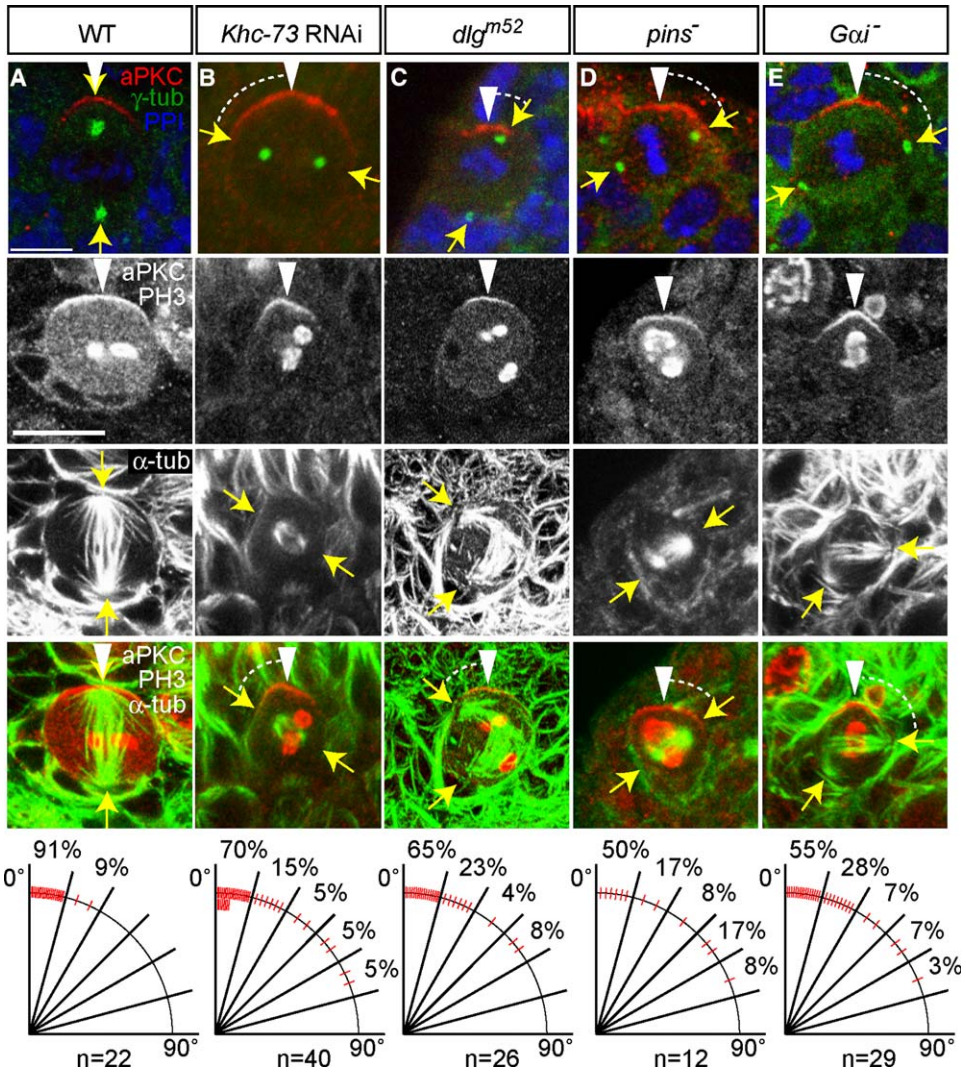

Figure 6. Khc-73, Dlg, Pins, and Gαi Are Required for Tight Coupling of the Apical Spindle Pole with Cortical Insc/Par Proteins

Metaphase neuroblasts scored for apical spindle pole alignment with the aPKC cortical crescent. Markers and genotypes indicated. First row, neuroblasts triple-labeled for aPKC (marked with arrowhead), γ-tubulin (marked with yellow arrows), and DNA. Dashed arc indicates distance between the two. Second row, neuroblasts labeled with aPKC (crescents marked with arrowhead) and the DNA marker PH3. Third row, same neuroblast with α-tubulin staining to show position of the mitotic spindle; spindle poles labeled with yellow arrows. Fourth row, same neuroblasts triple-labeled to show position of the mitotic spindle relative to cortical polarity. Bottom row, quantitation of spindle orientation defects. Each red line indicates the angle (represented by the dashed lines in the fourth row panels) between the spindle axis and the center of the Pins/Gαi crescent. Only α-tubulin-labeled neuroblasts with a clear apical spindle pole were scored; in some neuroblasts, spindle morphology defects precluded scoring. Neuroblasts are shown at the stage where the phenotype is most penetrant (this varies because there are different amounts of residual maternal protein present in each genotype) or at the stage where we have the strongest Gal4 driver.

(A, C, and E) Third larval instar.

(B) Embryonic stage 10.

(D) Second larval instar. Scale bar in top left panel = 5 μm, and scale bar in panel below = 10 μm.

to coimmunoprecipitate Dlg/Pins from in vivo lysates but see no binding by in vitro assays (Figure 4 and data not shown).

(5) Khc-73/Dlg/Pins/Gαi signals to the mitotic spindle. Loss of any of these proteins results in spindle orientation and varying morphology defects, even in the presence of the Insc/Par pathway (Figure 6). It is not clear which protein most directly mediates the cortex to microtubule signal, but it is intriguing to note that the Pins ortholog LGN can bind the microtubule-associated protein NuMA and regulate spindle biology in mammals (Du and Macara, 2004). Gα subunits can bind tubulin to regulate microtubule dynamics (Roychowdhury et al., 1999), and finally Khc-73/Dlg microtubule binding could also directly regulate spindle behavior.

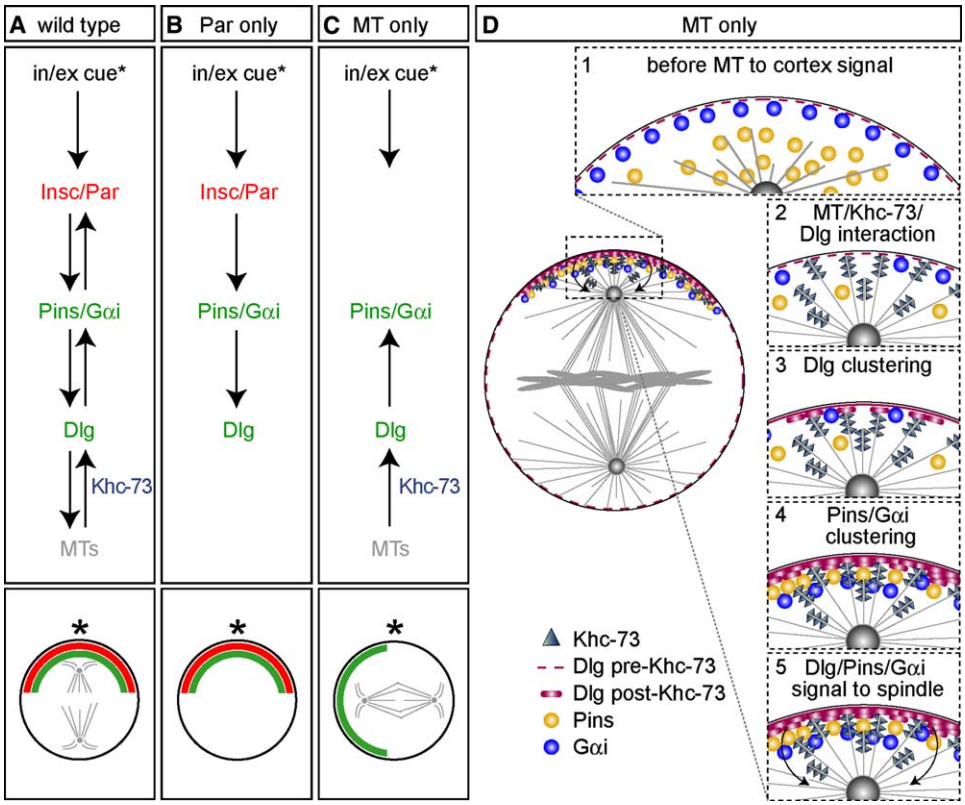

Figure 7. The Insc/Par and Microtubule/Khc-73/Dlg Pathways

(A) Both the Insc/Par and the microtubule/Khc-73/Dlg pathways are active in wild-type neuroblasts, which may provide a positive feedback loop to tightly link Insc/Par/Pins/Gαi/Dlg proteins to one spindle pole.

(B) The Insc/Par pathway is sufficient to polarize Pins/Gαi/Dlg to the apical cortex in the absence of all microtubules. An unknown intrinsic or extrinsic cue (in/ex cue, *) positions Insc/Par proteins at the apical side of the neuroblast. This pathway is active by prophase.

(C) The microtubule/Khc-73/Dlg pathway is sufficient to cluster Pins/Gαi/Dlg over one spindle pole in the absence of Insc/Par proteins; however, the mitotic spindle is not linked to the apical cue. This pathway is active at metaphase.

(D) Model for microtubule/Khc-73/Dlg polarization. See text for details.

Two Pathways for Generating Cortical Polarity in Neuroblasts

Asymmetric localization of Pins/Gαi proteins can be induced by two distinct pathways in embryonic neuroblasts: a well-characterized cortical pathway involving the Insc/Par proteins (reviewed in Betschinger and Knoblich, 2004) and a microtubule-dependent Khc-73/Dlg pathway (this work). Each pathway is regulated differently and has unique features that provide different temporal and spatial information for generating cortical polarity.

First, each pathway is initiated by a different mechanism and provides unique information for the timing of Pins/Gαi polarization. The Insc/Par pathway is initiated at late interphase in response to an unknown extrinsic cue (Siegrist and Doe, 2006) and is required for the early prophase cortical polarization of Pins/Gαi (Figure 7B). In contrast, the Khc-73/Dlg pathway is initiated later at prometaphase/metaphase by astral microtubules and is required for cortical polarization of Pins/Gαi only in the absence of Insc/Par complex proteins (Figure 7C). Consistent with this timeline, asymmetric enrichment of Dlg normally occurs well after polarization of Insc/Par/Pins/Gαi during the prometaphase/metaphase transition, and this temporal progression of Dlg cortical enrichment is not affected in *insc* mutants (Figure S6). The temporal polarization of Dlg coincides precisely with the onset of Pins/Gαi cortical polarity at prometaphase/metaphase that occurs in the absence of the Insc/Par pathway.

Next, each pathway provides different spatial information for the cortical polarization of Pins/Gαi. The Insc/Par pathway recruits Pins/Gαi to the apical cortex of the neuroblast at a position just below the overlaying epithelium, thus coordinating neuroblast cortical polarity with the neuroblast environment. In the absence of this pathway (e.g., *insc* mutant neuroblasts), cortical polarity can be generated but is not linked to tissue polarity, resulting in mispositioning of neuroblast progeny. In contrast, the microtubule/Khc-73/Dlg pathway induces Pins/Gαi crescent formation over one spindle pole, thus coordinating the neuroblast cortical polarity with the spindle axis. In the absence of this pathway (e.g., *dlg* mutant or *Khc-73* RNAi neuroblasts), Insc/Baz can still recruit Pins/Gαi to the apical cortex, yet the spindle is not always properly aligned with this cortical polarity. Together

these two pathways ensure the correct temporal and spatial positioning of apical complex proteins relative to extrinsic and intrinsic landmarks.

Microtubule-Induced Cortical Polarity in Other Cell Types

Drosophila sense organ precursors (SOPs) divide asymmetrically to generate an anterior pIIb cell and a posterior pIIa cell. During this division, Pins, Gαi, Dlg, and Numb form cortical crescents over the anterior spindle pole, and Baz localizes over the posterior spindle pole (reviewed in Betschinger and Knoblich, 2004). Cell division orientation is fixed along the anterior/posterior axis by planar polarity cues mediated by the seven pass transmembrane receptor Frizzled. However, Frizzled signaling is required only for the position of Dlg/Pins crescents, not for their formation (Bellaiche et al., 2001). When we remove both frizzled and microtubules together, we find about 10% of the mitotic SOPs lack Pins crescents (n = 50; Figure S7). This mild phenotype suggests that while astral microtubules may contribute to Dlg/Pins polarization in SOPs, there must be an additional mechanism involved. The best candidates for this third mechanism are the Par proteins because Par crescents still form in *frizzled* mutant SOPs at metaphase (Bellaiche et al., 2001).

There are many similarities between asymmetric division of fly neuroblasts and the *C. elegans* zygote (reviewed in Betschinger and Knoblich, 2004), but there are also striking differences. One of the most noteworthy differences is that *C. elegans par* mutants undergo symmetrically sized embryonic cell divisions, whereas in *Drosophila*, *par* or *insc* mutants maintain sibling cell size asymmetry. Our work provides an explanation for this discrepancy. We show that astral microtubules are capable of generating Pins/Gαi cortical polarity in the absence of localized Par proteins and that this microtubule-induced Pins/Gαi cortical polarity is fully functional for generating an asymmetric spindle, cell size, and unique daughter cell fates (data not shown and Kaltschmidt et al., 2000; Kraut et al., 1996). It is likely that *C. elegans* lacks this "microtubule-based pathway" for inducing GPR1/2 (Pins) and Gα cortical polarity, at least during the first embryonic cell division, because posterior cortical localization of GPR1/2 is absent in *par* mutants and the daughter cells are equal in size (Colombo et al., 2003; Gotta et al., 2003). Interestingly, we do observe an increase in symmetrically dividing neuroblasts in neuroblasts lacking both Insc/Par and microtubule pathways, compared to loss of single pathways alone (Figure S4, Table S1, and data not shown). It appears that either the Insc/Par or microtubule/Khc-73/Dlg pathway is sufficient to induce Pins/Gαi cortical polarity, which generates daughter cells of different sizes and fates.

The microtubule/kinesin-induced Dlg clustering pathway that we describe here may be evolutionarily conserved. In mammals, hDlg and the Khc-73 ortholog GAKIN are coexpressed in T cells and coimmunoprecipitate, and T cell activation leads to recruitment of hDlg to the immunological synapse (Hanada et al., 2000). Interestingly, GAKIN targets hDlg into ectopic cellular projections in MDCK cells, and this targeting depends on microtubules (Asaba et al., 2003). This has lead to the hypothesis that GAKIN may use a microtubule-based mechanism to target hDlg to the T cell immune synapse, similar to the microtubule/Khc-73 pathway described in this paper.

EXPERIMENTAL PROCEDURES

Fly Stocks and Genetics

Mutant phenotypes were analyzed using the following stocks: *pins*^{p62} and *pins*^{p89}; *Gαi*^{8}; *insc*^{22}; *Df (2L)TE35BC-3*; *aPKC*^{k06403}; *dlg*^{m52}, *dlg*^{m30}, and *dlg*^{1P20}; *fizzy*^{1}; *fz*^{k21} and *fz*^{kD4a}; *Df(3L)D21*, and *l(3)SG*^{11}*fz*^{1}. These alleles were used to generate double mutants. Mutations were rebalanced over either *ftz-lacZ* (for embryos), *actin-GFP* (for larvae), or *Tb* (for pupae) marked chromosomes to pick homozygous mutant animals. *dlg*^{m52} germline clones were generated using FLP-DFS methods (Chou and Perrimon, 1996) and crossed to y/*FM7c-ftz-lacZ* males.

We used *v32a-GAL4* for ubiquitous embryonic expression, *worniu-GAL4* (Albertson et al., 2004) for neuroblast-specific expression, and *neuralized-GAL4* for SOP expression. We used *UAS-Dlg:eGFP* (Koh et al., 1999), *UAS-HA:Khc-73 FL*, *UAS-HA:Khc-73 MBS*, and *UAS-Khc-73* hairpin dsRNA lines. The *UAS-Dlg:eGFP* and *worniu-GAL4* transgenes were separately recombined onto the *insc*^{22} chromosome for live imaging. Embryos and larvae were raised at 30ºC for GAL4-induced gene expression.

Khc-73 Subcloning and Constructs

A full-length *Khc-73* cDNA was made from joining the two partial yet overlapping ESTs LP11192 and GH09175, and it was subcloned in-frame following hemagglutinin (HA) sequence in *pUAST* to generate *UAS-HA:Khc73*. Sequence corresponding to 581–800 aa of Khc-73 was used to generate *UAS-HA:Khc-73 MBS*.

Antibody Staining and Drug Treatment

Embryos, larval brains, and pupal nota were fixed and processed by standard methods (Albertson and Doe, 2003; Bellaiche et al., 2001). Primary antibodies used for these studies include rabbit phospho-Histone H3 (1:1,000; Upstate), mouse α-tubulin (1:2,000; Sigma-Aldrich), rat α-tubulin (1:100; Serotec), mouse γ-tubulin (1:2,000; Sigma-Aldrich), rabbit Pins (Yu et al., 2000), rat Pins (1:500; F. Yu), rabbit Gαi (Schaefer et al., 2001), mouse Dlg 4F3E2 (Parnas et al., 2001), rabbit Insc (1:500; W. Chia), rabbit anti-Baz (1:500; A. Wodarz), rabbit aPKC (1:500; Santa Cruz), rat Par-6 (Rolls et al., 2003), and guinea pig Senseless (Nolo et al., 2000). In addition, rat antibodies were generated against His-tagged full-length Pins. Fluorescent-conjugated secondary antibodies (Jackson) were used. Images were collected using a Biorad Radiance confocal using a 60 × /1.4NA objective. SOP images were collected on a Leica TCS SP2 confocal using a 63 × /1.4NA objective. Biorad LaserSharp, Metamorph, Volocity, Photoshop, and Illustrator software were used for data analysis, movies, or figures.

For drug studies, 0–1 hr embryos were aged for 4–6 hr and drug treated for 1 hr by standard methods (Albertson and Doe, 2003). Larval brains and pupal nota were placed in Schneider's medium with or without drug for 2 hr or 45 min, respectively. Drug concentrations used: 5 μg/ml Colcemid (Sigma-Aldrich) or 20 μM or 40 μM nocodazole (Sigma-Aldrich) for embryos and nota; and 10 μg/ml Colcemid or 5 μM Paclitaxel (Sigma-Aldrich) for larvae.

Live Imaging

Dlg:eGFP localization in wild-type neuroblasts was imaged in embryos collected from a cross between *UAS-Dlg:eGFP* and *worniu-GAL4* flies. Dlg:eGFP localization in *insc* homozygous mutant neuroblasts was imaged in embryos collected from a cross between *UAS-Dlg:eGFP*, *insc*^{22} / *CyO* and *worniu-GAL4*, *insc*^{22} / *CyO* flies (only *insc*^{22} homozygous embryos are GFP positive). Embryos were mounted in halocarbon oil on a Teflon membrane fixed within a stainless steel slide (Kiehart

et al., 1994). Three to five 1–1.5 μm Z steps were collected at 15 s intervals on a BioRad confocal as described above.

RNAi Experiments

For *insc* RNAi, T7 sites were created on both 5′ and 3′ ends of a SacI fragment (bp 1597–2249, from the start ATG) from an *insc* cDNA by PCR. For *Khc-73* RNAi, a 562 bp fragment (bp 4185-4747 from the start ATG) was PCR-amplified from the EST GH09175 (BDGP) with T7 sites on both ends. dsRNA was synthesized using the MEGAscript kit (Ambion). dsRNA was injected for either *Khc-73* alone or for both *Khc-73* and *insc* at a final concentration of 1 mg/ml. The *UAS-Khc-73* hairpin dsRNA construct was made using pWIZ (Lee and Carthew, 2003) with 621 nucleotides from exon 9.

Biochemistry

Five hundred microliters of 0–12 hr embryos were homogenized in an equal volume of Cytoskeletal Buffer (50 mM Hepes, pH 7.5, 150 mM KCL, 8% Glycerol, .1% Triton, .5 mM DTT, and Protease inhibitors). The lysate was centrifuged and the soluble fraction was precleared against Protein A or Protein G agarose (Roche); either α-GFP (Torrey Pines) or α-HA (Sigma-Aldrich) antibodies were added for 2 hr at 4ºC. Protein complexes were pulled down using either Protein A or G agarose. The agarose was washed 4–6 times in Cytoskeletal Buffer, boiled in Sample Buffer, separated by SDS-PAGE gel electrophoresis, transferred to nitrocellulose, and probed with antibodies to Pins (1:2000), Dlg (1:500), GFP (1:1000), or HA (1:1000) using standard methods (Harlow and Lane, 1999). We used HRP-conjugated secondary antibodies (Jackson) and enhanced chemiluminescence.

Supplemental Data

Supplemental Data include seven figures, one table, and one movie and can be found with this article online at http://www.cell.com/cgi/content/full/123/7/1323/DC1/.

ACKNOWLEDGMENTS

We thank P. Adler, H. Bellen, K. Bhat, V. Budnik, P. Bryant, W. Chia, X. Yang, F. Matsuzaki, D. St. Johnston, J. Roote, M. Simon, A. Wodarz, J.E. Gomes, Bloomington Stock center, the Berkley *Drosophila* Genome Project, and especially Jürgen Knoblich for providing fly, DNA, or antibody reagents. We thank Amy Sheehan, Amanda Marusich, Victoria Robinson, Kristen Robinson, and especially Keiko Hirono for help in cloning, antibody production/stainings, and RNAi injections; and B. Hampölz and Ken Prehoda for protein biochemistry assistance. Karsten Siller, Stephan Schneider, Morgan Goulding, and Bruce Bowerman provided helpful comments on the manuscript. This work was funded by an NIH training grant (S.E.S.) and HHMI (C.Q.D.).

Received: June 5, 2005
Revised: August 26, 2005
Accepted: September 22, 2005
Published: December 28, 2005

REFERENCES

Albertson, R., and Doe, C.Q. (2003). Dlg, Scrib and Lgl regulate neuroblast cell size and mitotic spindle asymmetry. Nat. Cell Biol. *5*, 166–170.

Albertson, R., Chabu, C., Sheehan, A., and Doe, C.Q. (2004). Scribble protein domain mapping reveals a multistep localization mechanism and domains necessary for establishing cortical polarity. J. Cell Sci. *117*, 6061–6070.

Asaba, N., Hanada, T., Takeuchi, A., and Chishti, A.H. (2003). Direct interaction with a kinesin-related motor mediates transport of mammalian discs large tumor suppressor homologue in epithelial cells. J. Biol. Chem. *278*, 8395–8400.

Barros, C.S., Phelps, C.B., and Brand, A.H. (2003). Drosophila nonmuscle myosin II promotes the asymmetric segregation of cell fate determinants by cortical exclusion rather than active transport. Dev. Cell *5*, 829–840.

Behrens, R., and Nurse, P. (2002). Roles of fission yeast tea1p in the localization of polarity factors and in organizing the microtubular cytoskeleton. J. Cell Biol. *157*, 783–793.

Bellaiche, Y., Radovic, A., Woods, D.F., Hough, C.D., Parmentier, M.L., O'Kane, C.J., Bryant, P.J., and Schweisguth, F. (2001). The Partner of Inscuteable/Discs large complex is required to establish planar polarity during asymmetric cell division in Drosophila. Cell *106*, 355–366.

Betschinger, J., and Knoblich, J.A. (2004). Dare to be different: asymmetric cell division in *Drosophila,* C. elegans and vertebrates. Curr. Biol. *14*, R674–R685.

Brenman, J.E., Topinka, J.R., Cooper, E.C., McGee, A.W., Rosen, J., Milroy, T., Ralston, H.J., and Bredt, D.S. (1998). Localization of postsynaptic density-93 to dendritic microtubules and interaction with microtubule-associated protein 1A. J. Neurosci. *18*, 8805–8813.

Cai, Y., Chia, W., and Yang, X. (2001). 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.

Cai, Y., Yu, F., Lin, S., Chia, W., and Yang, X. (2003). Apical complex genes control mitotic spindle geometry and relative size of daughter cells in *Drosophila* neuroblast and pI asymmetric divisions. Cell *112*, 51–62.

Chou, T.B., and Perrimon, N. (1996). The autosomal FLP-DFS technique for generating germline mosaics in Drosophila melanogaster. Genetics *144*, 1673–1679.

Colombo, K., Grill, S.W., Kimple, R.J., Willard, F.S., Siderovski, D.P., and Gonczy, P. (2003). Translation of polarity cues into asymmetric spindle positioning in Caenorhabditis elegans embryos. Science *300*, 1957–1961.

Cowan, C.R., and Hyman, A.A. (2004). Centrosomes direct cell polarity independently of microtubule assembly in C. elegans embryos. Nature *431*, 92–96.

Dawson, I.A., Roth, S., and Artavanis-Tsakonas, S. (1995). The Drosophila cell cycle gene fizzy is required for normal degradation of cyclins A and B during mitosis and has homology to the CDC20 gene of Saccharomyces cerevisiae. J. Cell Biol. *129*, 725–737.

Du, Q., and Macara, I.G. (2004). Mammalian Pins is a conformational switch that links NuMA to heterotrimeric G proteins. Cell *119*, 503–516.

Du, Q., Stukenberg, P.T., and Macara, I.G. (2001). A mammalian Partner of inscuteable binds NuMA and regulates mitotic spindle organization. Nat. Cell Biol. *3*, 1069–1075.

Etienne-Manneville, S., and Hall, A. (2001). Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKCzeta. Cell *106*, 489–498.

Funke, L., Dakoji, S., and Bredt, D.S. (2005). Membrane-associated guanylate kinases regulate adhesion and plasticity at cell junctions. Annu. Rev. Biochem. *74*, 219–245.

Fuse, N., Hisata, K., Katzen, A.L., and Matsuzaki, F. (2003). Heterotrimeric G proteins regulate daughter cell size asymmetry in *Drosophila* neuroblast divisions. Curr. Biol. *13*, 947–954.

Galjart, N., and Perez, F. (2003). A plus-end raft to control microtubule dynamics and function. Curr. Opin. Cell Biol. *15*, 48–53.

Gotta, M., Dong, Y., Peterson, Y.K., Lanier, S.M., and Ahringer, J. (2003). Asymmetrically distributed *C. elegans* homologs of AGS3/PINS control spindle position in the early embryo. Curr. Biol. *13*, 1029–1037.

Hanada, T., Lin, L., Tibaldi, E.V., Reinherz, E.L., and Chishti, A.H. (2000). 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.

Harlow, E., and Lane, D. (1999). Using Antibodies: A Laboratory Manual (Cold Springs Harbor, NY: Cold Springs Harbor Laboratory Press).

Ikeshima-Kataoka, H., Skeath, J.B., Nabeshima, Y., Doe, C.Q., and Matsuzaki, F. (1997). Miranda directs Prospero to a daughter cell during Drosophila asymmetric divisions. Nature *390*, 625–629.

Kaltschmidt, J.A., Davidson, C.M., Brown, N.H., and Brand, A.H. (2000). Rotation and asymmetry of the mitotic spindle direct asymmetric cell division in the developing central nervous system. Nat. Cell Biol. *2*, 7–12.

Kiehart, D.P., Montague, R.A., Rickoll, W.L., Foard, D., and Thomas, G.H. (1994). High-resolution microscopic methods for the analysis of cellular movements in Drosophila embryos. Methods Cell Biol. *44*, 507–532.

Koh, Y.H., Popova, E., Thomas, U., Griffith, L.C., and Budnik, V. (1999). Regulation of DLG localization at synapses by CaMKII-dependent phosphorylation. Cell *98*, 353–363.

Kraut, R., Chia, W., Jan, L.Y., Jan, Y.N., and Knoblich, J.A. (1996). Role of inscuteable in orienting asymmetric cell divisions in Drosophila. Nature *383*, 50–55.

Lambert, J.D., and Nagy, L.M. (2002). Asymmetric inheritance of centrosomally localized mRNAs during embryonic cleavages. Nature *420*, 682–686.

Lee, Y.S., and Carthew, R.W. (2003). Making a better RNAi vector for Drosophila: use of intron spacers. Methods *30*, 322–329.

Li, H.P., Liu, Z.M., and Nirenberg, M. (1997). Kinesin-73 in the nervous system of Drosophila embryos. Proc. Natl. Acad. Sci. USA *94*, 1086–1091.

Matsumine, A., Ogai, A., Senda, T., Okumura, N., Satoh, K., Baeg, G.H., Kawahara, T., Kobayashi, S., Okada, M., Toyoshima, K., and Akiyama, T. (1996). Binding of APC to the human homolog of the Drosophila discs large tumor suppressor protein. Science *272*, 1020–1023.

McGee, A.W., and Bredt, D.S. (1999). Identification of an intramolecular interaction between the SH3 and guanylate kinase domains of PSD-95. J. Biol. Chem. *274*, 17431–17436.

Megraw, T.L., Kao, L.R., and Kaufman, T.C. (2001). Zygotic development without functional mitotic centrosomes. Curr. Biol. *11*, 116–120.

Mok, H., Shin, H., Kim, S., Lee, J.R., Yoon, J., and Kim, E. (2002). Association of the kinesin superfamily motor protein KIF1Balpha with postsynaptic density-95 (PSD-95), synapse-associated protein-97, and synaptic scaffolding molecule PSD-95/discs large/zona occludens-1 proteins. J. Neurosci. *22*, 5253–5258.

Nolo, R., Abbott, L.A., and Bellen, H.J. (2000). Senseless, a Zn finger transcription factor, is necessary and sufficient for sensory organ development in *Drosophila*. Cell *102*, 349–362.

Parmentier, M.L., Woods, D., Greig, S., Phan, P.G., Radovic, A., Bryant, P., and O'Kane, C.J. (2000). Rapsynoid/partner of inscuteable controls asymmetric division of larval neuroblasts in Drosophila. J. Neurosci. *20*, RC84.

Parnas, D., Haghighi, A.P., Fetter, R.D., Kim, S.W., and Goodman, C.S. (2001). Regulation of postsynaptic structure and protein localization by the Rho-type guanine nucleotide exchange factor dPix. Neuron *32*, 415–424.

Passafaro, M., Sala, C., Niethammer, M., and Sheng, M. (1999). Microtubule binding by CRIPT and its potential role in the synaptic clustering of PSD-95. Nat. Neurosci. *2*, 1063–1069.

Petronczki, M., and Knoblich, J.A. (2001). DmPAR-6 directs epithelial polarity and asymmetric cell division of neuroblasts in Drosophila. Nat. Cell Biol. *3*, 43–49.

Rolls, M.M., Albertson, R., Shih, H.P., Lee, C.Y., and Doe, C.Q. (2003). Drosophila aPKC regulates cell polarity and cell proliferation in neuroblasts and epithelia. J. Cell Biol. *163*, 1089–1098.

Roychowdhury, S., Panda, D., Wilson, L., and Rasenick, M.M. (1999). G protein alpha subunits activate tubulin GTPase and modulate microtubule polymerization dynamics. J. Biol. Chem. *274*, 13485–13490.

Schaefer, M., Shevchenko, A., and Knoblich, J.A. (2000). A protein complex containing Inscuteable and the Galpha-binding protein Pins orients asymmetric cell divisions in *Drosophila*. Curr. Biol. *10*, 353–362.

Schaefer, M., Petronczki, M., Dorner, D., Forte, M., and Knoblich, J.A. (2001). Heterotrimeric G proteins direct two modes of asymmetric cell division in the *Drosophila* nervous system. Cell *107*, 183–194.

Shen, C.P., Knoblich, J.A., Chan, Y.M., Jiang, M.M., Jan, L.Y., and Jan, Y.N. (1998). Miranda as a multidomain adapter linking apically localized Inscuteable and basally localized Staufen and Prospero during asymmetric cell division in Drosophila. Genes Dev. *12*, 1837–1846.

Siegrist, S.E., and Doe, C.Q. (2006). Extrinsic cues orient the cell division axis in *Drosophila* embryonic neuroblasts. Development, In press.

Srinivasan, D.G., Fisk, R.M., Xu, H., and van den Heuvel, S. (2003). A complex of LIN-5 and GPR proteins regulates G protein signaling and spindle function in C elegans. Genes Dev. *17*, 1225–1239.

Wallenfang, M.R., and Seydoux, G. (2000). Polarization of the anterior-posterior axis of C. elegans is a microtubule-directed process. Nature *408*, 89–92.

Willard, F.S., Kimple, R.J., and Siderovski, D.P. (2004). Return of the GDI: the GoLoco motif in cell division. Annu. Rev. Biochem. *73*, 925–951.

Wodarz, A., Ramrath, A., Kuchinke, U., and Knust, E. (1999). Bazooka provides an apical cue for Inscuteable localization in Drosophila neuroblasts. Nature *402*, 544–547.

Wodarz, A., Ramrath, A., Grimm, A., and Knust, E. (2000). Drosophila atypical protein kinase C associates with Bazooka and controls polarity of epithelia and neuroblasts. J. Cell Biol. *150*, 1361–1374.

Woods, D.F., Hough, C., Peel, D., Callaini, G., and Bryant, P.J. (1996). Dlg protein is required for junction structure, cell polarity, and proliferation control in Drosophila epithelia. J. Cell Biol. *134*, 1469–1482.

Yu, F., Morin, X., Cai, Y., Yang, X., and Chia, W. (2000). Analysis of partner of inscuteable, a novel player of *Drosophila* asymmetric divisions, reveals two distinct steps in inscuteable apical localization. Cell *100*, 399–409.

Yu, F., Ong, C.T., Chia, W., and Yang, X. (2002). Membrane targeting and asymmetric localization of Drosophila partner of inscuteable are discrete steps controlled by distinct regions of the protein. Mol. Cell. Biol. *22*, 4230–4240.

Yu, F., Cai, Y., Kaushik, R., Yang, X., and Chia, W. (2003). Distinct roles of Galphai and Gbeta13F subunits of the heterotrimeric G protein complex in the mediation of Drosophila neuroblast asymmetric divisions. J. Cell Biol. *162*, 623–633.