Discs Large Links Spindle Orientation to Apical-Basal Polarity in *Drosophila* Epithelia

Dan T. Bergstralh,1 Holly E. Lovegrove,1 and Daniel St Johnston1,*

1The Gurdon Institute and the Department of Genetics, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QN, UK

Summary

Mitotic spindles in epithelial cells are oriented in the plane of the epithelium so that both daughter cells remain within the monolayer, and defects in spindle orientation have been proposed to promote tumorigenesis by causing epithelial disorganization and hyperplasia [1]. Previous work has implicated the apical polarity factor aPKC, the junctional protein APC2, and basal integrins in epithelial spindle orientation, but the underlying mechanisms remain unclear. We show that these factors are not required for spindle orientation in the *Drosophila* follicular epithelium. Furthermore, aPKC and other apical polarity factors disappear from the apical membrane in mitosis. Instead, spindle orientation requires the lateral factor Discs large (Dlg), a function that is separable from its role in epithelial polarity. In neuroblasts, Pins recruits Dlg and Mud to form an apical complex that orients spindles along the apical-basal axis. We show that Pins and Mud are also necessary for spindle orientation in follicle cells, as is the interaction between Dlg and Pins. Dlg localizes independently of Pins, however, suggesting that its lateral localization determines the planar orientation of the spindle in epithelial cells. Thus, different mechanisms recruit the conserved Dlg/Pins/Mud complex to orient the spindle in opposite directions in distinct cell types.

Results and Discussion

Mitotic Spindle Orientation in the Follicle Cell Epithelium Is Independent of Integrins and Adherens Junctions

The *Drosophila* follicular epithelium is a well-established model for the study of epithelial cell polarity, but mitotic spindle orientation in this tissue has received less attention. Previous work has demonstrated that the follicle cells behave like a typical epithelium and tend to orient their mitotic spindles parallel to the plane of the monolayer [2]. Consistent with this, we observed that all spindles lie within ~30° of the plane of the epithelium once the spindle and metaphase plate are clearly visible, and the spindle retains this orientation through anaphase (Figures 1A and 1C). The position of the spindle is much more variable earlier in mitosis, however, and the spindle often assembles perpendicular to the plane of the epithelium (Figures 1A and 1B and Figures S1A and S1B available online). Thus, the spindle is positioned after it has assembled and is not oriented by the prepositioning of the centrosomes.

The original model for spindle orientation in *Drosophila* epithelia based on studies in the embryonic ectoderm proposed that the spindles align toward the adherens junctions (AJ) through interactions mediated by APC2 (a homolog of mammalian adenomatous polyposis coli) [3]. We observed that mitotic follicle cells maintain adherens junctions with their neighboring cells during metaphase (Figure 1D). However, the metaphase spindle is always positioned below the level of the AJ marker Armadillo (*Drosophila* β-cat) (Figure 1D). APC-2-GFP is slightly enriched at adherens junctions in mitotic cells but also localizes around the rest of the cortex (Figure S1B).

We further investigated the role of adherens junctions in spindle orientation by removing them completely using a null allele of arm. As reported previously, we saw some flattening and loss of epithelial cells in arm5 homozygous mutant clones [5], but spindle orientation was wild-type (Figures 1E and 1H), consistent with a previous report that APC2 is not required for spindle orientation in the embryonic ectoderm [4].

Spindle Orientation in Follicle Cells Does Not Require aPKC

The apical polarity factor atypical protein kinase C (aPKC) has been implicated in spindle orientation in Madin-Darby canine kidney (MDCK) cells and the *Drosophila* imaginal wing disc epithelium, although it does not appear to play a role in chick neuroepithelial cells [6–8]. We therefore analyzed the role of aPKC during mitosis in the follicular epithelium.

Surprisingly, we observed a loss of apical cortical identity during mitosis. aPKC disappears from the apical cortex, as do other apical polarity factors, such as Crumbs and Bazooka (Figures 2A–2C) [9]. Thus, aPKC is not in the right place to control the positioning of the spindle during metaphase. Consistent with this, follicle cells homozygous for the “kinase-dead” allele *apkc<sup>dsu141</sup>* [10], show normal spindle orientation (Figures 2E and 2F). This allele does not disrupt the follicle epithelium (Figure 2D), suggesting that the kinase activity of aPKC is not required for epithelial polarity in this tissue.

*Correspondence: d.stjohnston@gurdon.cam.ac.uk

This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike License, which permits non-commercial use, distribution, and reproduction in any medium, provided the original author and source are credited.
Egg chambers transheterozygous for apkc<sup>2</sup> and the loss-of-function allele apkc<sup>k06403</sup>, have cyst encapsulation defects at 25°C that preclude the accurate measurement of mitotic spindle angles [8]. At 18°C, large regions of organized epithelium persist in most egg chambers, although 68% (n = 28) show some encapsulation defects (Figure 2G). The follicle cells in the regions with normal epithelial organization show no spindle orientation defects (Figures 2H and 2I). The loss of apical aPKC in mitosis and the normal distribution of mitotic spindles in aPKC mutants indicate that aPKC is dispensable for spindle orientation in the follicular epithelium.

**Pins and Mud Are Involved in Spindle Orientation**

Much of our understanding of spindle orientation derives from studies of asymmetric divisions in the nematode and the fly (recently reviewed in [11, 12]). In *Drosophila*, spindle orientation has been studied primarily in neuroblasts, which divide asymmetrically along their apical-basal axis, and in the sensory organ precursor cell, which divides along a planar axis [12]. Additional studies have been carried out using a cultured *Drosophila* cell system with artificially induced cell polarity [13]. This work has identified a number of factors that are required to orient the spindle, including Partner of Inscurable (Pins) and its binding partner Mushroom body defective (Mud) (reviewed in [12]). Work in symmetrically dividing vertebrate cells, including cultured MDCK cells, the chick neuroepithelium, and asymmetrically dividing skin cells has revealed that spindle orientation also depends on the Pins and Mud homologs LGN and NuMA [12, 14–18].

Pins and Mud orient the spindle by exerting a pulling force on astral microtubules through dynein/dynactin [19, 20]. If they are playing a role in spindle orientation in the follicular epithelium, then metaphase plates should misorient when astral microtubules are lost. We treated ovaries with the microtubule-depolymerizing drug colcemid for 1 hr and observed that metaphase plates and centrosomes were often misoriented with respect to the plane of the epithelium (Figures S2A and S2B and Figure 3A), indicating that microtubules are required for spindle orientation.

We next examined the expression of Pins and Mud in follicle cells. In the adult fly, Pins transcript expression is highest in the ovary (Figure S2C). Immunostaining reveals that Mud is expressed in the follicle cell epithelium up until approximately stage 6 of egg chamber development, which is when the follicle cells cease dividing (Figure S2D). In the chick neuroepithelium and in other models, Pins (LGN) and Mud (NuMA) localize along the lateral cortex in dividing cells [12, 17]. Pins-YFP localizes along the apical cortex in interphase follicle cells, but it largely relocates to the lateral cortex during metaphase, where it colocalizes with the lateral polarity factor Dlg (Figures S2A and S2B and Figure 3A), indicating that microtubules are required for spindle orientation.

In mammalian cells, knockdown of the Pins homolog LGN causes spindle disorganization [14]. These defects were not observed in pins<sup>Δ</sup> clones, as revealed by staining for tubulin, the centrosomal protein Centrosomin (Figures 3F and 3F), and the microtubule-nucleating factor γ-tubulin (Figure S2C).
Metaphase spindle angles were also examined in follicle cells from mud²/mud³ transheterozygous egg chambers (Figure 3G). In agreement with previous work, spindle orientation was disrupted in these cells (Figure 3H) [23]. We note that the spindle angles were not randomized, as in pins mutant cells, as the line of best fit has a slope of 1.5. These data are statistically consistent with a normal (Gaussian) distribution around a mean of 40°. This reflects the fact that many spindles orient toward an apical corner, as shown in Figure 3G. This result is consistent with a previous study in S2 cells that showed that Pins can attract a spindle pole in the absence of Mud but cannot center it through pulling [13]. We suggest that this mechanism is also at work in mud mutant follicle cells; the developing spindle may be caught at the edge of a Pins basolateral crescent in the absence of Mud function.

Dlg Is Required for Spindle Orientation in Follicle Cells

Dlg is recruited by Pins to the cortex of asymmetrically dividing cells, such as neuroblasts and SOPs, and is required to orient the spindle toward the Pins crescent [13, 24, 25]. Since Dlg colocalizes with Pins and Mud at the lateral cortex of the follicle cells (Figures 3D and 3E), we investigated whether it is also necessary for spindle orientation in this epithelium. Dlg is essential for apical-basal polarity in epithelia, however. This complicates the analysis of its role in spindle orientation, because cells homozygous mutant for a strong loss-of-function allele, dlg¹⁴ (also called dlg¹⁴PSO), round up and lose their epithelial organization (Figure S3A) [26]. We therefore restricted our analysis to those dlg¹⁴ mutant clones in which the cells remained in a monolayer and observed that the spindles are randomly oriented (Figures S3B and S3C).

Dlg interacts with Pins through its C-terminal guanylate kinase (GUK) domain, which is disrupted in cells homozygous for the mutant allele dlg¹⁸ (also called dlg¹⁸PSO), a premature stop mutation that removes the last 43 amino acids of the protein [27, 28]. Importantly, dlg¹⁸ does not disrupt the lateral localization of Dlg, and apical-basal polarity is unaffected in early-stage mutant clones, which form a normal epithelial monolayer (Figure 4A). Despite this wild-type epithelial organization, dlg¹⁸ randomizes the orientation of the mitotic spindles to give a cumulative distribution with a slope of 1.1 (R² = 0.93) (Figures 4B and 4C).

Spindles are oriented normally in dlg⁰⁶⁴⁰³, which removes the last 14 amino acids of Dlg, leaving the GUK domain intact (Figures S3D–S3F) [24]. Thus, Dlg is required for spindle orientation in the follicle cells, and this function is separable from its role in epithelial polarity. The role of Dlg in spindle orientation depends on the presence of an intact GUK domain and therefore presumably requires its interaction with Pins, strongly suggesting that the Dlg/Pins/Mud complex orients the spindle in epithelia, as it does in asymmetrically dividing cells.

In neuroblasts, Pins is required for the apical localization of Dlg during mitosis, whereas Dlg reinforces the apical localization of Pins through a pathway that depends on astral microtubules [25]. The situation in epithelia appears to be different, however, as Dlg localizes normally along the lateral cortex in clones of the pins null mutant, pins⁰⁶⁵² (Figure 4D). Since Dlg localizes laterally throughout the cell cycle, it is presumably localized by the same polarity-related mechanisms in interphase and mitotic cells. We also examined whether Dlg is required for the localization of Pins and observed that Pins still localizes around the cortex during mitosis in the absence of Dlg (dlg¹⁴) but is not enriched laterally (Figure 4E). The lateral enrichment of Pins also appears reduced in cells homozygous for the GUK domain mutant dlg¹⁸, suggesting...
that its interaction with Dlg contributes to its recruitment to the lateral cortex, although this phenotype is more variable than in the null (Figure 4F).

It has previously been proposed that the aPKC excludes Pins from the apical domain during mitosis in MDCK cells and the Drosophila wing imaginal disc, although not in chick...
neuroepithelial cells [7, 8, 17]. In agreement with the latter finding, Pins-YFP shows a wild-type lateral localization during mitosis in apkc02/apkc026403 transheterozygous flies maintained at 18° (Figure 4G). Thus, the lateral enrichment of Pins in mitotic follicle cells is independent of aPKC.

In conclusion, we have demonstrated that the planar orientation of the mitotic spindle in the follicular epithelium is independent of apical, junctional, or basal cues and depends instead on Dlg, Pins, and Mud. It therefore seems likely that the spindle is aligned within the plane of the epithelium by the same mechanisms that orient the spindle along the apical-basal axis in neuroblasts and that the key determinant of spindle orientation in both cell types is the location of the Dlg/Pins/Mud complex. The restriction of this complex to the lateral cortex in epithelial cells depends on Dlg, and its dual role in apical-basal polarity and spindle positioning therefore provides a mechanism to couple spindle orientation with the overall polarity of the tissue.

Experimental Procedures

Drug treatment

Ovaries were suspended in Schneider’s medium (Sigma) containing 5 μg/ml insulin (Sigma) with or without 100 μg/ml colcemid (Sigma) for 1 h before fixation.

Imaging

Somatic clone induction, immunofluorescence, and fixed-cell imaging were performed as previously described [29].

Spindle Angle Measurements

Spindle angles were calculated with Image J. The angle of the spindle was determined relative to a line drawn connecting the adherens junctions at the two apical corners of the mitotic cell. These corners are shared by this cell and its neighbors.

Supplemental Information

Supplemental information includes Supplemental Experimental Procedures and three figures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.07.017.

Acknowledgments

We thank Jordan Raff, Howard Nash, Chris Doe, Yohanns Bellaïche, Andreas Wodarz, and Jürgen Knoblich, and their labs for fly stocks. We are grateful to Erico de Sa and other members of the St Johnston lab, the Pid Jin lab, and The Gurdon Institute for helpful criticism and comments made over the course of this study. This work was supported by a Wellcome Trust Principal Fellowship to D.S.J. (049818 and 080007) and core funding from the Wellcome Trust (092096) and Cancer Research UK (A14192). D.T.B. was supported by a Marie Curie Fellowship. H.E.L. was supported by a Herchel Smith Studentship.

Received: June 13, 2013
Revised: July 3, 2013
Accepted: July 4, 2013
Published: July 25, 2013

References

1. McCaffrey, L.M., and Macara, I.G. (2011). Epithelial organization, cell polarity and tumorigenesis. Trends Cell Biol. 21, 727–735.
2. Fernández-Mirán, A., Martín-Bermudo, M.D., and González-Reyes, A. (2007). Integrin signaling regulates spindle orientation in Drosophila to preserve the follicular-epithelium monolayer. Curr. Biol. 17, 683–688.
3. Lu, B., Roegiers, F., Jan, L.Y., and Jan, Y.N. (2001). Adherens junctions inhibit asymmetric division in the Drosophila epithelium. Nature 409, 522–525.
4. McCartney, B.M., Price, M.H., Webb, R.L., Hayden, M.A., Holot, L.M., Zhou, M., Bejsovec, A., and Peifer, M. (2006). Testing hypotheses for the functions of APC family proteins using null and truncation alleles in Drosophila. Development 133, 2407–2418.
5. Tanentzapf, G., Smith, C., McGlade, J., and Tepass, U. (2000). Apical, lateral, and basal polarization cues contribute to the development of the follicular epithelium during Drosophila oogenesis. J. Cell Biol. 151, 891–904.
6. Cox, D.N., Seyfried, S.A., Jan, L.Y., and Jan, Y.N. (2001). Bazooka and atypical protein kinase C are required to regulate oocyte differentiation in the Drosophila ovary. Proc. Natl. Acad. Sci. USA 98, 14475–14480.
7. Hao, Y., Du, Q., Chen, X., Zheng, Z., Balsbaugh, J.L., Malra, S., Shalgi, R., and Macara, I.G. (2010). Parc controls epithelial spindle orientation by aPKC-mediated phosphorylation of apical Pins. Curr. Biol. 20, 1809–1818.
8. Guilgur, L.G., Prudêncio, P., Ferreira, T., Pimenta-Marques, A.R., and Martinho, R.G. (2012). Drosophila aPKC is required for mitotic spindle orientation during symmetric division of epithelial cells. Development 139, 503–513.
9. St Johnston, D., and Ahringer, J. (2010). Cell polarity in eggs and epithelia: parallels and diversity. Cell 141, 757–774.
10. Kim, S., Gall, I., Mousianis, B., Luschnig, S., Goette, M., Fricke, K., Homemann-Capito, M., Grubmüller, H., and Wodarz, A. (2009). Kinase-activity-independent functions of atypical protein kinase C in Drosophila. J. Cell Sci. 122, 3759–3771.
11. Siller, K.H., and Doe, C.Q. (2009). Spindle orientation during asymmetric cell division. Nat. Cell Biol. 11, 365–374.
12. Morin, X., and Bellaïche, Y. (2011). Mitotic spindle orientation in asymmetric and symmetric cell divisions during animal development. Dev. Cell 21, 102–119.
13. Johnston, C.A., Hirono, K., Prehoda, K.E., and Doe, C.Q. (2009). Identification of an Aurora-A/PinsLINKER/Dlg spindle orientation pathway using induced cell polarity in S2 cells. Cell 138, 1150–1163.
14. 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.
15. Kaushik, R., Yu, F., Chia, W., Yang, X., and Bahri, S. (2003). Subcellular localization of LGN during mitosis: evidence for its cortical localization in mitotic cell culture systems and its requirement for normal cell cycle progression. Mol. Biol. Cell 14, 3144–3155.
16. Zheng, Z., Zhu, H., Wan, Q., Liu, J., Xiao, Z., Siderovski, D.P., and Du, Q. (2010). LGN regulates mitotic spindle orientation during epithelial morphogenesis. J. Cell Biol. 189, 275–286.
17. Peyre, E., Jaouen, F., Saadaoui, M., Haren, L., Merdes, A., Durbec, P., and Morin, X. (2011). A lateral belt of cortical LGN and NuMA guides mitotic spindle movements and planar division in neuroepithelial cells. J. Cell Biol. 193, 141–154.
18. Williams, S.E., Beronja, S., Pasolli, H.A., and Fuchs, E. (2011). Asymmetric cell divisions promote Notch-dependent epidermal differentiation. Nature 470, 353–358.
19. Pecreaux, J., Röper, J.-C., Kruse, K., Jülicher, F., Hyman, A.A., Grill, S.W., and Howard, J. (2006). Spindle oscillations during asymmetric cell division require a threshold number of active cortical force generators. Curr. Biol. 16, 2111–2122.
20. Kotak, S., Busso, C., and Gönçzy, P. (2012). Cortical dynein is critical for proper spindle positioning in human cells. J. Cell Biol. 199, 97–110.
21. 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.
22. Le Borgne, R., and Schweiguth, F. (2003). Unequal segregation of Neuralized biases Notch activation during asymmetric cell division. Dev. Cell 5, 139–148.
23. Yu, J.X., Guan, Z., and Nash, H.A. (2006). The mushroom body defect gene product is an essential component of the meiosis II spindle apparatus in Drosophila oocytes. Genetics 173, 243–253.
24. Bellaïche, Y., Radovic, A., Woods, D.F., Hough, C.D., Parmentier, M.L., O‘Kane, C.J., Bryant, P.J., and Schweiguth, F. (2001). The Partner of Insuteable/Disco-large complex is required to establish planar polarity during asymmetric cell division in Drosophila. Cell 106, 355–366.
25. Siegrist, S.E., and Doe, C.Q. (2005). Microtubule-induced Pins/Galphai cortical polarity in Drosophila neuroblasts. Cell 123, 1323–1335.
26. Bilder, D. (2004). Epithelial polarity and proliferation control: links from the Drosophila neoplastic tumor suppressors. Genes Dev. 18, 1909–1925.
27. 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.

28. Johnston, C.A., Doe, C.Q., and Prehoda, K.E. (2012). Structure of an enzyme-derived phosphoprotein recognition domain. PLoS ONE 7, e36014.

29. Morais-de-Sá, E., Mirouse, V., and St Johnston, D. (2010). aPKC phosphorylation of Bazooka defines the apical/lateral border in Drosophila epithelial cells. Cell 141, 509–523.