Where does asymmetry come from?

Loyer, Nicolas; Januschke, Jens

Published in:
Current Opinion in Cell Biology

DOI:
10.1016/j.ceb.2019.07.018

Publication date:
2020

Document Version
Publisher's PDF, also known as Version of record

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Cell Biology

Where does asymmetry come from? Illustrating principles of polarity and asymmetry establishment in *Drosophila* neuroblasts

Nicolas Loyer and Jens Januschke

Abstract

Asymmetric cell division (ACD) is the fundamental process through which one cell divides into two cells with different fates. In animals, it is crucial for the generation of cell-type diversity and for stem cells, which use ACD both to self-renew and produce one differentiating daughter cell. One of the most prominent model systems of ACD, *Drosophila* neuroblasts, relies on the PAR complex, a conserved set of proteins governing cell polarity in animals. Here, we focus on recent advances in our understanding of the mechanisms that control the orientation of the neuroblast polarity axis, how the PAR complex is positioned, and how its activity may regulate division orientation and cell fate determinant localization and discuss how important findings about the composition polarity complexes in other models may apply to neuroblasts.

Addresses

Cell & Developmental Biology, School of Life Sciences, University of Dundee, Dundee, UK

Corresponding author: Januschke, Jens (j.januschke@dundee.ac.uk)

Current Opinion in Cell Biology 2020, **62**:70–77

This review comes from a themed issue on **Cell Architecture**

Edited by **Sandrine Etienne-Manneville** and **Robert Arkowitz**

For a complete overview see the Issue and the Editorial

https://doi.org/10.1016/j.ceb.2019.07.018

Keywords

Asymmetric cell division, Cell polarity, Drosophila, Neuroblasts.

Introduction

The exact molecular and cellular mechanisms regulating asymmetric cell division (ACD) vary dramatically across different animal models and cell types [1,2]. ACD can be characterized as extrinsically or intrinsically controlled. In extrinsic ACD, different daughter cell fates are controlled by exposure to different external signals (Figure 1a). In intrinsic ACD, differences in intracellular localization of cell fate–determining molecules result in their asymmetrical segregation into the resulting daughter cells, conferring them different identities (Figure 1b). The focus of this review is on the role of cell polarity in a well-established intrinsic ACD model, the *Drosophila* neuroblasts.

Neuroblasts are neural stem cells that, during development, divide asymmetrically to self-renew and simultaneously produce daughter cells that will differentiate into neurons or glial cells forming the central nervous system. Neuroblast divisions are not only asymmetric in fate but also asymmetric in size, resulting in the formation of a large neuroblast and a smaller daughter cell destined for differentiation [3,4].

ACD in neuroblasts is achieved through the following steps [3,4]: Apical–basal polarity is established at the onset of mitosis when the conserved PAR complex proteins, PAR3 (Bazooka in the fly), atypical protein kinase C (aPKC), and PAR6, form an apical cortical cap. The activity of the PAR complex then drives the basal localization of molecules involved in cell-fate determination including Miranda and Numb. To achieve asymmetric segregation of fate determinants to the daughter cells that will differentiate, spindle orientation is aligned with the apical–basal polarity axis (Figure 1c). Studies using neuroblasts have contributed to our understanding of cell fate generation [5], the link between stem cell ACD and cancer [6,7], the connection between cell polarity and spindle orientation [8–10], and cell size asymmetry [3].

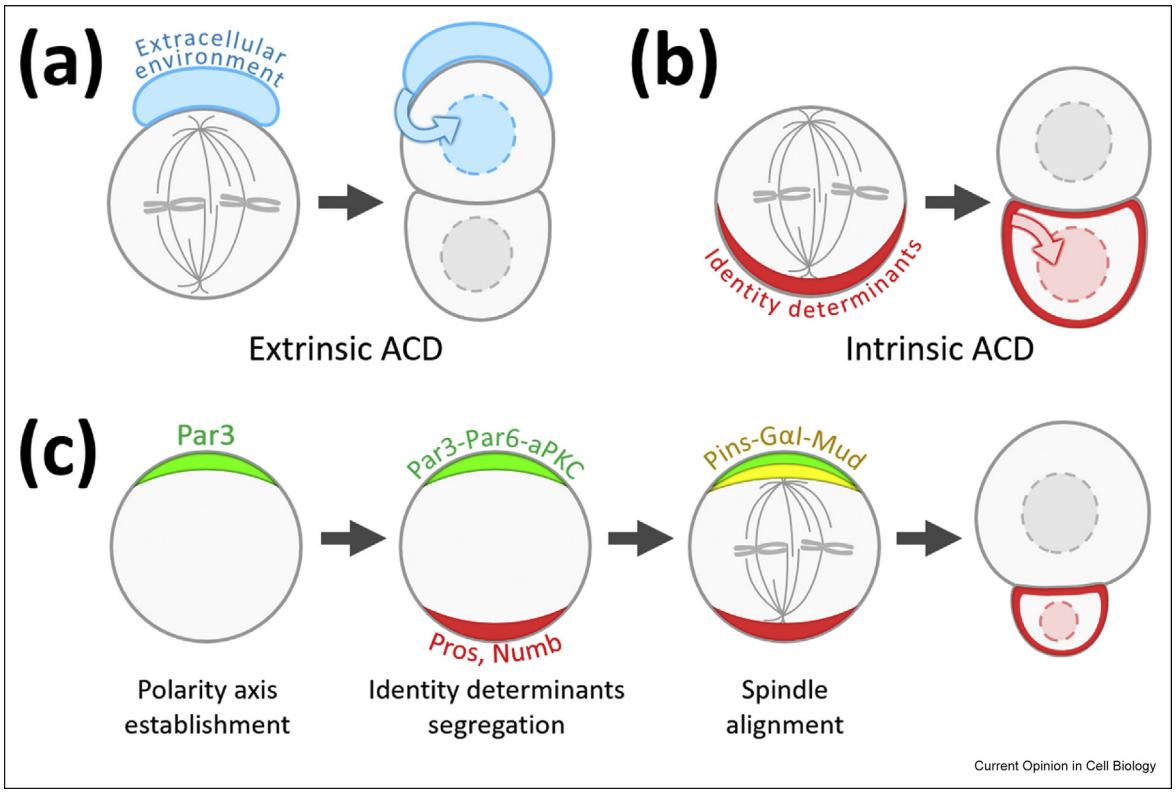
Here, we discuss recent advances in our understanding of how the apical–basal polarity axis is oriented, that is, how PAR3 is positioned in neuroblasts. We further discuss recent findings of how the PAR complex engages the spindle orientation machinery based on structural biology work and how it gets activated from studies using *Caenorhabditis elegans*. Finally, we discuss how aPKC activation, an important effector of PAR polarity, and other processes may provide spatial information for asymmetric cell-fate determinant localization in neuroblasts.

Discussion

How is PAR3 positioned in neuroblasts?

Neuroblasts are highly proliferative cells with very short cell cycle times, which facilitates the study of how cell

Figure 1



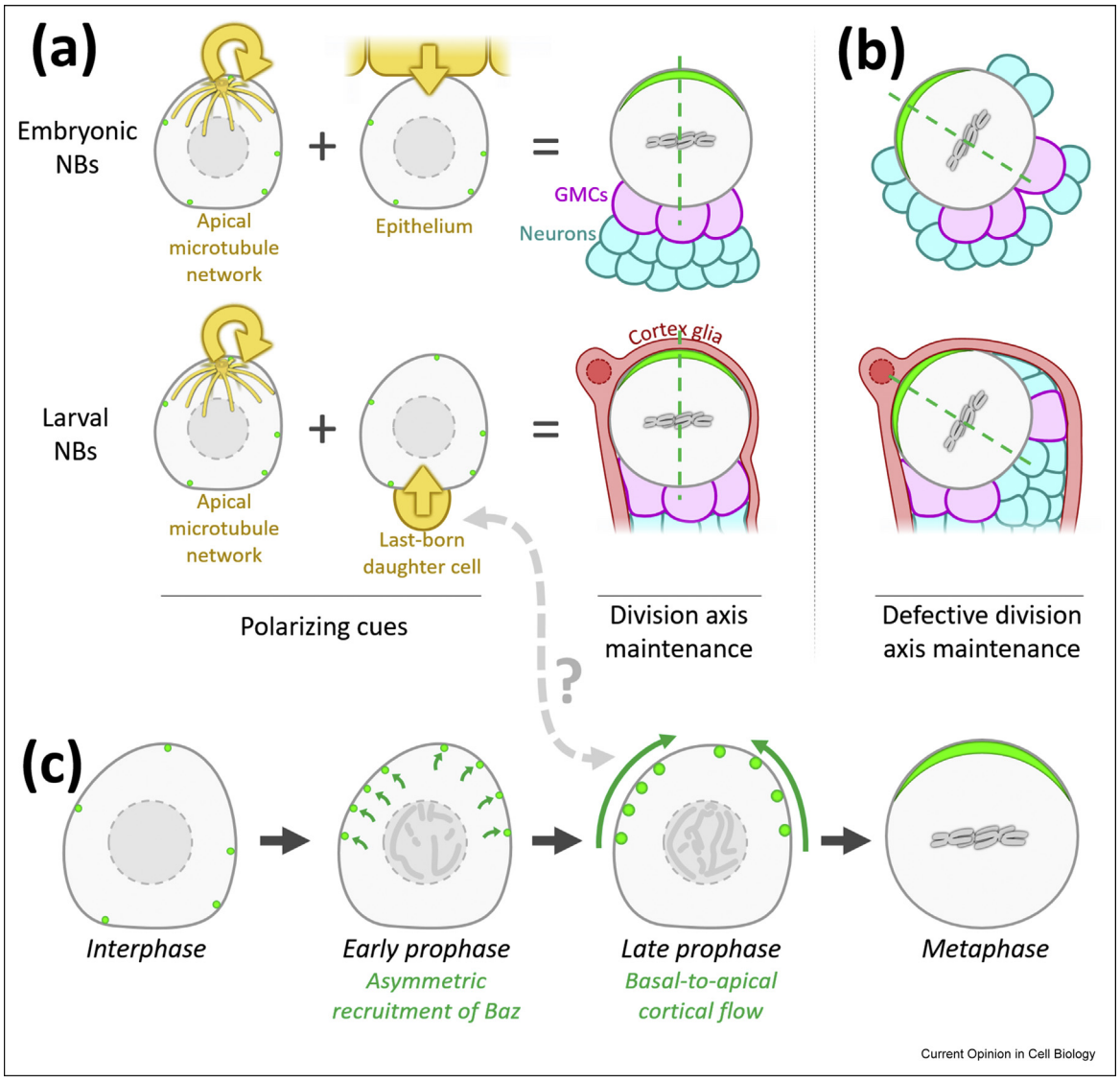
Modes of ACD. **(a)** In extrinsic ACD, the orientation of the division is regulated according to the cellular environment so that the two daughter cells are exposed to different extrinsic factors determining their fates. **(b)** In intrinsic ACD, alignment of the spindle with asymmetrically segregated intracellular determinants results in the daughter cells inheriting different determinants, conferring them different identities. **(c)** Steps of intrinsic ACD in *Drosophila* neuroblasts. Asymmetric cortical localization of Par3 defines the apical pole, establishing a polarity axis. Par3 recruits Par6 and aPKC (green), whose activity restricts identity determinant (Pros and Numb, red) localization to the basal cortex. Par3 also recruits the Pins−Galphai−Mud spindle orientation machinery (yellow), aligning the spindle with the polarity axis. ACD, asymmetric cell division.

polarity is established in consecutive cell cycles. This system has proven ideal to investigate how the apical−basal polarity axis is positioned. PAR3 forms a cortical cap defining the apical pole of neuroblasts at the onset of each mitosis. After each division, this cortical cap is lost as PAR3 relocalizes into the cytoplasm and uniformly distributed cortical clusters. Nonetheless, the same cortical region is defined as apical in the subsequent mitosis.

Thereby, the division orientation of individual neuroblasts is maintained from one cell cycle to the next, suggesting strict regulation and conserved function. Cell-intrinsic and cell-extrinsic cues clearly guide this process. In larval and likely in embryonic neuroblasts, the apically localized centrosome and microtubules [11−13] act as an intrinsic cue, orienting the neuroblast polarity axis and its division [14].

The nature of the extrinsic polarizing cues depends on the developmental context. In embryos, neuroblasts initially receive a signal of unknown origin from the overlaying, contacting epithelium, which they interpret using an orphan G protein−coupled receptor. This results in the cortical recruitment of the spindle orientation machinery to the neuroblast/epithelium contact site, which in turn recruits PAR3 [15,16]. It was recently revealed that in larvae, division axis maintenance of neuroblasts, that are no longer in contact with an overlaying epithelium, also relies on cell−cell contact, but this time between neuroblasts and their own daughter cells [17] (Figure 2a). Disrupting these orienting cues in both contexts results in the functional mislocalization of PAR-driven polarity, which results in apparently normal ACD, but mispositions daughter cells. Misaligned neuroblast divisions in the larvae cause neuroblasts to bud off daughter cells between themselves and the glial cell that wraps around each neuroblast and its offspring [18]. These glial cells normally offer protection against oxidative stress and starvation [19]. Misaligned neuroblast divisions result in a reduced neuroblast−glia contact area and a measurable reduction in neuroblast proliferation upon stress [17] (Figure 2b). Therefore, the control of division orientation of larval neuroblasts

Figure 2



Regulation and dynamics of Par3 polarization. **(a)** Various polarizing cues (yellow) provide spatial information (yellow arrow) to neuroblasts (NBs), allowing Par3 (green) always to be recruited at the same cortical region, resulting in neuroblasts always dividing in the same direction. Dashed line: division axis. **(b)** Defective division axis maintenance of NBs causes them to bud off daughter cells (ganglion mother cells [GMCs], magenta) in other directions, resulting in larval NBs reducing their contacts with cortex glial cells (pink) protecting them against oxidative stress and starvation. **(c)** Polarization of Par3 (green) occurs stepwise. In interphase, Par3 is not polarized. In early prophase, it is asymmetrically recruited to growing cortical foci (small green arrows), which are later concentrated at late prophase at the apical pole by an actin-driven basal-to-apical cortical flow (large green arrows). Dashed double arrow: the polarizing cue provided by the last-born daughter cell in the larval neuroblast **(a)** might be transduced from one end of the NB to the other end by this basal-to-apical flow **(c)**.

may function to optimize neuroblast glial cell communication for effective protection against environmental stresses.

Cell–cell contacts have been known to be able to polarize cells [20], but the molecular mechanisms are unclear. The question arises as to how the last-born daughter cell, contacting in fact the future basal pole of neuroblasts, can instruct apical polarization of PAR3 at the opposite end of the cell during a subsequent mitosis. Hints may come from the establishment of polarity in the *C. elegans* zygote, in which the sperm centrosome acts as a polarizing cue that initiates an actomyosin cortical flow at one end of the cell, resulting in PAR3 polarization at the other end [21]. Indeed, PAR3 polarization seems to occur in several steps in larval neuroblasts and is actin dependent [22]. A recent study looked at this in higher resolution: first, from early to late prophase, PAR3 is recruited to cortical foci broadly distributed over the apical half of the cortex;

second, shortly before metaphase, a basal-to-apical actin-dependent cortical flow concentrates these foci around the apical pole [23]. Thus, the spatial cue provided by the basal position of the last-born daughter cell could be transduced to the opposite apical end of the neuroblast by controlling the origin and direction of such cortical actin flows (Figure 2c). Interestingly, cell adhesion molecules and actin regulators were found among the proteins that potentially mediate this PAR3-positioning event in neuroblasts [17].

Subcomplex formation and regulation of spindle orientation in ACD

Once PAR3 is localized to the apical pole of neuroblasts, it triggers the recruitment of the machinery that aligns the mitotic spindle with the apical–basal polarity axis. Conserved key molecules involved in cell polarity establishment and those involved in coupling cell polarity to spindle orientation are often colocalized or directly interact. Therefore, it seemed plausible that the alignment of the mitotic spindle with the polarity axis of a cell is the result of interconnected macromolecular assemblies bridging the cortex with the spindle. A key player in this process is the ternary spindle orientation complex composed of Pins, GαI, and Mud (LGN, GαI, and Numa, respectively, in mammals, with the latter able to bind spindle microtubules). Because both PAR3 and Pins were observed to physically interact with Inscuteable (Insc) individually, a potential explanation is that Insc is a direct physical bridge between the PAR complex at the cortex and the spindle orientation machinery, linking these two processes [24–26].

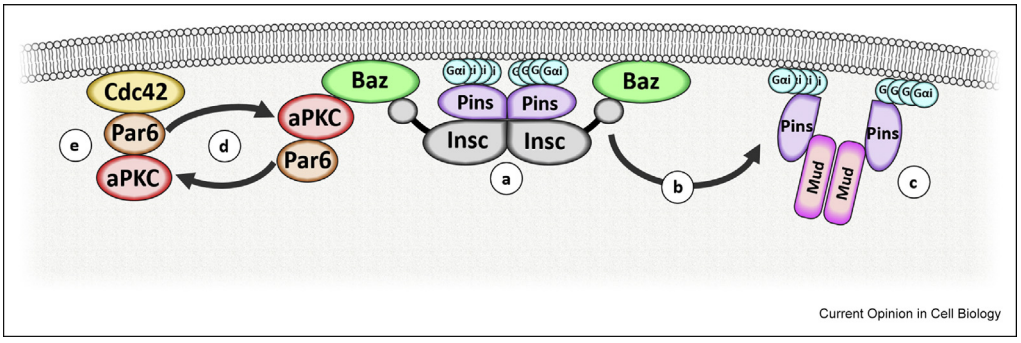
Structural biology studies have challenged this view, however, revealing that Insc and Numa bind competitively to LGN [27,28]. Thus, Insc cannot function as a physical linker between the two processes. Indeed, the system is much more sophisticated as demonstrated by recent work carried out on asymmetrically dividing mammary stem cells, which require Insc for ACD [29]. Critically, LGN can be associated with different subcomplexes at the apical cortex during mitosis [30]. As mammary stem cells set up for division, stable PAR3-containing assemblies form initially at the apical membrane that include Insc, LGN, and GαI in its GDP-bound form. This configuration does not yet engage spindle microtubules, but allows the system to position GαI. A subsequent step requiring the conversion of GαI into its GTP-bound from triggers its release from the Insc/PAR3/LGN cluster upon which GTP is presumably hydrolyzed to GDP. This step frees correctly positioned GDP-bound GαI to engage with LGN–NUMA–DYNEIN complexes that then can tether microtubules to engage the spindle [30] (Figure 3a–c). Thus, the interplay between cell polarity and spindle orientation is a highly dynamic process, requiring subcomplex formation offering regulation at multiple levels — all influenced by spatial information provided by PAR3.

The PAR3-dependent sequential formation of apical subcomplexes to position the spindle orientation machinery is conceptually very reminiscent to the sequential formation of subcomplexes leading to the activation of aPKC in the PAR polarity system in the *C. elegans* zygote [31]. In addition, here, immobile PAR3-containing complexes form initially and serve as a spatially restricted platform to recruit PAR6 and aPKC. In a subsequent step, which is not completely clear yet, CDC42 engages with PAR6 and aPKC, which becomes activated, and concomitantly, the complex is released from the immobile PAR3-containing fraction and becomes mobile [32–34] (Figure 3d and e). Both observations highlight the role that PAR3 serves as a platform to position specific, yet context-dependent, functions that direct ACD.

Once aPKC is active, what happens? A neuroblast perspective

A consequence of sequential recruitment and activation of aPKC in *C. elegans* is the generation of an aPKC activity gradient across the anterior–posterior axis of the zygote

Figure 3



Separate apical subcomplexes. The Baz–Insc–Pins–Gαi complex **(a)** promotes the assembly **(b)** of a separate Gαi–Pins–Mud complex **(c)** in mammals. aPKC and Par-6 cycles between Baz and Cdc42 **(d)**, forming a separate Cdc42–Par6–aPKC complex **(e)** in *C. elegans*. aPKC, atypical protein kinase C.

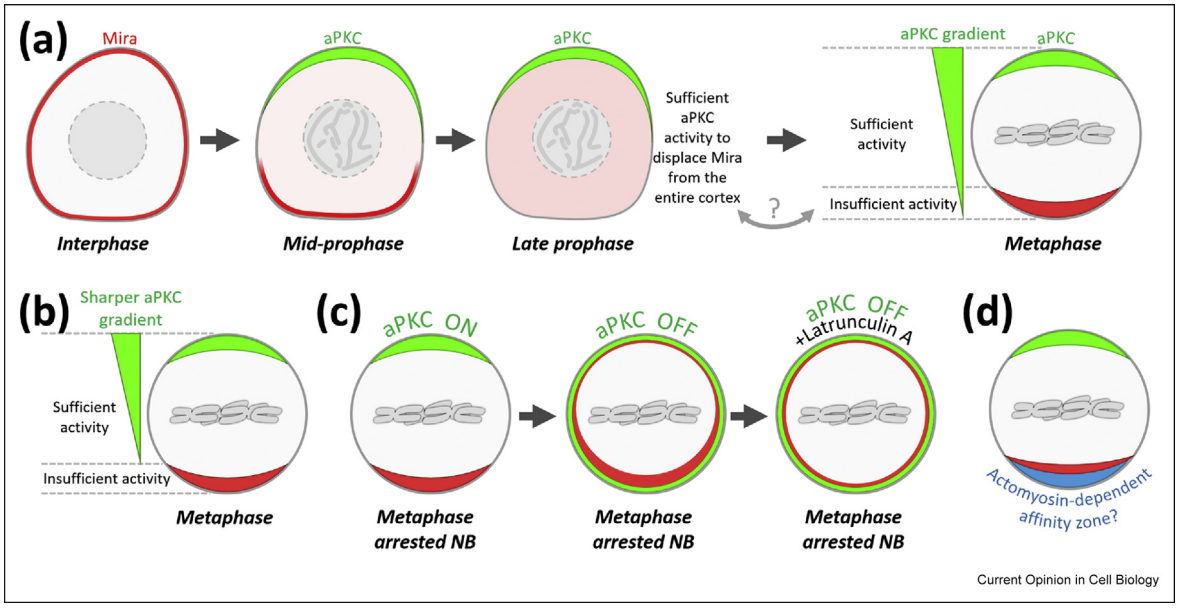
[31]. aPKC is also a regulator of neuroblast ACD [35,36], and an aPKC activity gradient declining along the apical–basal axis would be a tempting model to explain basal fate determinant localization in neuroblasts because an established function of aPKC in neuroblasts is to negatively regulate the ability of its substrates such as Miranda [37,38] to interact with the plasma membrane (PM), by phosphorylating the PM interaction motif of the substrates [22,39,40]. However, the picture is not so clear.

Live-cell imaging revealed that in interphase, when aPKC is presumably inactive, Miranda localizes uniformly to the PM (Figure 4a, interphase), but is removed from the apical pole at the onset of mitosis immediately after aPKC recruitment and its removal continues in an apical-to-basal direction [22] (Figure 4a, mid-prophase). This observation is compatible with the idea that, similar to the *C. elegans* zygote, aPKC is recruited to the apical pole, activated, upon which its activity spreads away from the initial site of activation which establishes an apical-to-basal activity gradient. After nuclear envelope breakdown (NEB), Mira localizes in a crescent at the basal pole [22,41,42]. At this stage, a gradient of aPKC activity appears as a plausible explanation for the basal localization of Mira: aPKC activity is high enough throughout the cell to prevent Miranda's ability to bind the PM and, toward the basal pole, reaches a threshold under which it is too low to prevent Miranda localization (Figure 4a, metaphase).

However, Miranda is removed from the PM just before NEB (Figure 4a, late prophase), after which it rapidly forms a crescent and even redecorates regions of the basal cortex, from which it was just removed. This could mean that the aPKC activity gradient is under temporal control and becomes steeper at this stage (Figure 4b). This could be driven by cytoskeletal changes accompanying entry into mitosis, which may slow diffusion along the cortex [22] limiting the spread of aPKC activity toward the basal pole, or it is the rapid reduction of the apical area covered by aPKC just before NEB [22,23], potentially driving the source of the gradient away from the basal pole, or indeed a combination of both.

An alternative interpretation is that spatial information may be also encoded elsewhere. For instance, acute inhibition of aPKC in metaphase results in slow and only partial loss of Miranda asymmetry, and Miranda continues to localize with a basal bias even after extended periods of aPKC inhibition [43] (Figure 4c). Furthermore, a phosphomimetic mutant of Miranda carrying a serine-to-aspartic acid substitution of the aPKC phosphorylation site within Miranda's PM interaction

Figure 4



Identity determinant segregation. **(a)** Mira (red) localizes uniformly to the cortex in interphase, is cleared from the cortex in an apical-to-basal direction during prophase, and relocalizes to a basal crescent during metaphase. An apical-to-basal aPKC gradient (green triangle) could drive basal Mira localization in metaphase by generating sufficient aPKC activity to remove Mira from most of the cortex and insufficient activity toward the basal pole, allowing Mira recruitment to the plasma membrane. However, aPKC activity is sufficient to remove Mira from the entire cortex in late prophase. **(b)** This difference in aPKC effect between late prophase and metaphase (gray double arrow) might be explained by a sharpening of the aPKC gradient in metaphase. **(c)** aPKC inhibition results in redistribution of aPKC and Mira to the entire cortex, but Mira localization retains a basal bias. This basal bias is lost upon actin cytoskeleton disruption by latrunculin A treatment. NB: neuroblast. **(d)** The existence of an actomyosin-dependent basal affinity zone may be another mechanism involved in identity determinant segregation in neuroblasts. aPKC, atypical protein kinase C.

domain is disruptive to Miranda PM interaction in interphase, but strikingly allows Miranda to localize in a basal crescent in mitosis [22]. These results suggest that, despite aPKC activity contributing to sharpen basal Miranda crescents in polarized neuroblasts, mechanisms other than direct phosphoregulation through aPKC may be involved in Miranda asymmetry (Figure 4d).

If it is not primarily an aPKC activity gradient, where then does the spatial information for basal Miranda localization originate? This may be the result of an underlying actomyosin-dependent patterning mechanism. Accordingly, the persistent basal localization of Miranda is lost after disruption of the actomyosin network [22],[43] (Figure 4c), and Miranda can directly bind actin and myosin II and VI [44–46]. During mitosis, specific spatiotemporal regulation of actomyosin occurs involving actin flows, myosin II, and Rho kinase. These processes have been studied with respect to the regulation of daughter cell size asymmetry [23,47–49]. An interesting observation in this context was that treating cycling neuroblasts with the Rho kinase inhibitor Y-27632 leads to enlarged Miranda crescents in mitosis. The normally apparent equatorial 'gap' between the PAR3/aPKC/PAR6 apical crescent and basally localized Miranda/Numb disappears and, intriguingly, results in larger daughter cells [22]. This does not appear to be a consequence of altering aPKC activity by Y-27632 [37] because acute aPKC inhibition (Figure 4c) but not Y-27632 treatment affects aPKC localization in mitotic neuroblasts [[43],[22]]. This effect of Y-27632 hints at the possibility that the spatial information for basal fate determinant localization and the regulation of daughter cell size asymmetry are coupled. Furthermore, understanding actomyosin-dependent patterning of neuroblasts will likely require taking into account the phosphoinositide composition in the PM [50]. Phosphatidylinositol-4,5-bisphosphate modulates actin organization and cell polarity in the *C. elegans* zygote [51], and the phosphatidylinositol transfer protein Vibrator/Giotto together with PI4KIIIα regulates levels of phosphatidylinositol-4-phosphate that binds and anchors myosin to the neuroblast cortex [52].

Concluding remarks

Understanding aPKC and actomyosin-dependent mechanisms will be necessary but probably not sufficient to draw a complete picture of asymmetric determinant segregation. Puzzling observations reveal that in some situations, that is, *aurora A* and *polo* mutants [53,54] or knockdown of moesin by RNA interference [55], aPKC mislocalization does not lead inevitably to Miranda mislocalization. Miranda protein further requires the localization of its own mRNA to maintain its basal localization [56]; anaphase-promoting complex/cyclosome activity and ubiquitylation of Miranda are required for its asymmetric localization [57] as well as dephosphorylation of a tyrosine residue, phosphorylated by an as-of-yet unidentified kinase [58] lying with the dimerization domain of Miranda [59]. These factors suggest the existence of additional layers of Miranda regulation, and how exactly they contribute to its localization remains to be determined. An interesting conceptual angle to understand fate determinant localization in this system comes from studies showing that another basally localized fate determinant Numb and its binding partner PON form phase-separated basal condensates as a result of multivalent interactions between them [60]. As multimerization and post-translational modifications are known drivers of protein condensate formation, it will be interesting to explore if this concept applies also to Miranda localization and if and how it offers better understanding to explain the process.

Conflict of interest statement

Nothing declared.

Acknowledgements

The authors would like to thank David Murray for critical comments on the manuscript. Work in JJ's laboratory is supported by the Wellcome Trust and the Royal Society (United Kingdom; grant reference: 100031/Z/12 and 100031/Z/12/A).

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Venkei ZG, Yamashita YM: **Emerging mechanisms of asymmetric stem cell division**. *J Cell Biol* 2018, **217**:3785–3795.
2. Vertii A, Kaufman PD, Hehnly H, Doxsey S: **New dimensions of asymmetric division in vertebrates**. *Cytoskeleton* 2018, **75**: 87–102.
3. Gallaud E, Pham T, Cabernard C: **Drosophila melanogaster neuroblasts: a model for asymmetric stem cell divisions**. *Results Probl Cell Differ* 2017, **61**:183–210.
4. Homem CCF, Knoblich JA: **Drosophila neuroblasts: a model for stem cell biology**. *Development* 2012, **139**:4297–4310.
5. Harding K, White K: **Drosophila as a model for developmental biology: stem cell-fate decisions in the developing nervous system**. *J Dev Biol* 2018, **6**:25. 2015, Vol. 3, Pages 129-157.
6. Gonzalez C: **Drosophila melanogaster: a model and a tool to investigate malignancy and identify new therapeutics**. *Nat Rev Cancer* 2013, **3**:172–183.
7. Gonzalez C: **Spindle orientation, asymmetric division and tumour suppression in Drosophila stem cells**. *Nat Rev Genet* 2007, **8**:462–472.
8. Allam AH, Charnley M, Russell SM: **Context-specific mechanisms of cell polarity regulation**. *J Mol Biol* 2018, **430**: 3457–3471.
9. Wen W, Zhang M: **Protein complex assemblies in epithelial cell polarity and asymmetric cell division**. *J Mol Biol* 2018, **430**: 3504–3520.
10. Hong Y: **aPKC: the kinase that phosphorylates cell polarity**. *F1000Res* 2018, **7**:903.
11. Rusan NM, Peifer M: **A role for a novel centrosome cycle in asymmetric cell division**. *J Cell Biol* 2007, **177**:13–20.

12. Rebollo E, Sampaio P, Januschke J, Llamazares S, Varmark H, Gonzalez C: **Functionally unequal centrosomes drive spindle orientation in asymmetrically dividing Drosophila neural stem cells**. *Dev Cell* 2007, **12**:467–474.

13. Rebollo E, Roldán M, Gonzalez C: **Spindle alignment is achieved without rotation after the first cell cycle in Drosophila embryonic neuroblasts**. *Development* 2009, **136**: 3393–3397.

14. Januschke J, Gonzalez C: **The interphase microtubule aster is a determinant of asymmetric division orientation in Drosophila neuroblasts**. *J Cell Biol* 2010, **188**:693–706.

15. Siegrist SE, Doe CQ: **Extrinsic cues orient the cell division axis in Drosophila embryonic neuroblasts**. *Development* 2006, **133**:529–536.

16. Yoshiura S, Ohta N, Matsuzaki F: **Tre1 GPCR signaling orients stem cell divisions in the Drosophila central nervous system**. *Dev Cell* 2012, **22**:79–91.

17. * Loyer N, Januschke J: **The last-born daughter cell contributes to division orientation of Drosophila larval neuroblasts**. *Nat Commun* 2018, **9**:3745.
Using a combination of laser ablation and RNAi candidate screen this study reveals that the neuroblast polarity axis is oriented by contact of the neuroblast with its own daughter cells. This study also puts forward a testable hypothesis for the physiological importance of division orientation of individual neuroblasts.

18. Dumstrei K, Wang F, Hartenstein V: **Role of DE-cadherin in neuroblast proliferation, neural morphogenesis, and axon tract formation in Drosophila larval brain development**. *J Neurosci* 2003, **23**:3325–3335.

19. Bailey AP, Koster G, Guillermier C, Hirst EMA, MacRae JI, Lechene CP, Postle AD, Gould AP: **Antioxidant role for lipid droplets in a stem cell niche of Drosophila**. *Cell* 2015, **163**: 340–353.

20. Ziomek CA, Johnson MH: **Cell surface interaction induces polarization of mouse 8-cell blastomeres at compaction**. *Cell* 1980, **21**:935–942.

21. Pacquelet A: **Asymmetric cell division in the one-cell C. elegans embryo: multiple steps to generate cell size asymmetry**. *Results Probl Cell Differ* 2017, **61**:115–140.

22. Hannaford MR, Ramat A, Loyer N, Januschke J: **aPKC-mediated displacement and actomyosin-mediated retention polarize Miranda in Drosophila neuroblasts**. *eLife* 2018, **7**:166.

23. Oon CH, Prehoda KE: **Asymmetric recruitment and actin-dependent cortical flows drive the neuroblast polarity cycle**. *Elife* 2019, **8**:723.

24. Lu MS, Johnston CA: **Molecular pathways regulating mitotic spindle orientation in animal cells**. *Development* 2013, **140**: 1843–1856.

25. Knoblich JA: **Asymmetric cell division: recent developments and their implications for tumour biology**. *Nat Rev Mol Cell Biol* 2010, **11**:849–860.

26. Morin X, Bellaïche Y: **Mitotic spindle orientation in asymmetric and symmetric cell divisions during animal development**. *Dev Cell* 2011, **21**:102–119.

27. Culurgioni S, Alfieri A, Pendolino V, Laddomada F, Mapelli M: **Inscuteable and NuMA proteins bind competitively to Leu-Gly-Asn repeat-enriched protein (LGN) during asymmetric cell divisions**. *Proc Natl Acad Sci USA* 2011, **108**:20998–21003.

28. Zhu J, Wen W, Zheng Z, Shang Y, Wei Z, Xiao Z, Pan Z, Du Q, Wang W, Zhang M: **LGN/mInsc and LGN/NuMA complex structures suggest distinct functions in asymmetric cell division for the Par3/mInsc/LGN and Gαi/LGN/NuMA pathways**. *Mol Cell* 2011, **43**:418–431.

29. Ballard MS, Zhu A, Iwai N, Stensrud M, Mapps A, Postiglione MP, Knoblich JA, Hinck L: **Mammary stem cell self-renewal is regulated by slit2/robo1 signaling through SNAI1 and mINSC**. *Cell Rep* 2015, **13**:290–301.

30. ** Culurgioni S, Mari S, Bonetti P, Gallini S, Bonetto G, Brennich M, Round A, Nicassio F, Mapelli M: **Insc:LGN tetramers promote asymmetric divisions of mammary stem cells**. *Nat Commun* 2018, **9**:1025.
This study uses structural biology approaches to understand how the spindle orientation machinery of asymmetrically dividing cells is activated and tethered to the cortex. The study explores homologs of key molecules from different species proposing a model how the machinery is positioned and subsequently able to engage the mitotic spindle.

31. Munro E: **Protein clustering shapes polarity protein gradients**. *Dev Cell* 2017, **42**:309–311.

32. * Rodriguez J, Peglion F, Martin J, Hubatsch L, Reich J, Hirani N, Gubieda AG, Roffey J, Fernandes AR, St Johnston D, *et al.*: **aPKC cycles between functionally distinct PAR protein assemblies to drive cell polarity**. *Dev Cell* 2017, **42**:400–415.
aPKC localization and activity are tightly coupled, making it difficult to understand how each is regulated. This study manipulates both independently in *C. elegans* by using a combination of chemical and genetic approaches and reveals the relative contributions of two different PAR subcomplexes to localization and activity of PKC-3.

33. * Dickinson DJ, Schwager F, Pintard L, Gotta M, Goldstein B: **A single-cell biochemistry approach reveals PAR complex dynamics during cell polarization**. *Dev Cell* 2017, **42**:416–434.
This study uses a single-cell protein interaction biochemical assay to probe the dynamics of PAR complex formation and finds that PAR complex formation is dynamic and under the control of the cell cycle.

34. * Wang S-C, Low TYF, Nishimura Y, Gole L, Yu W, Motegi F: **Cortical forces and CDC-42 control clustering of PAR proteins for Caenorhabditis elegans embryonic polarization**. *Nat Cell Biol* 2017, **19**:988–995.
This study reports that PAR complex is under the influence of actomyosin contractility in the C.elegans zygote. The authors further propose that PAR3 is recruited in response to mechanical forces to the cell cortex as the zygote polarizes.

35. 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* 2000, **150**: 1361–1374.

36. Rolls MM, Albertson R, Shih H-P, Lee C-Y, Doe CQ: **Drosophila aPKC regulates cell polarity and cell proliferation in neuroblasts and epithelia**. *J Cell Biol* 2003, **163**:1089–1098.

37. Atwood SX, Prehoda KE: **aPKC phosphorylates Miranda to polarize fate determinants during neuroblast asymmetric cell division**. *Curr Biol* 2009, **19**:723–729.

38. Wirtz-Peitz F, Nishimura T, Knoblich JA: **Linking cell cycle to asymmetric division: aurora-A phosphorylates the Par complex to regulate Numb localization**. *Cell* 2008, **135**:161–173.

39. Bailey MJ, Prehoda KE: **Establishment of par-polarized cortical domains via phosphoregulated membrane motifs**. *Dev Cell* 2015, **35**:199–210.

40. Dong W, Zhang X, Liu W, Chen Y-J, Huang J, Austin E, Celotto AM, Jiang WZ, Palladino MJ, Jiang Y, *et al.*: **A conserved polybasic domain mediates plasma membrane targeting of Lgl and its regulation by hypoxia**. *J Cell Biol* 2015, **211**: 273–286.

41. Ikeshima-Kataoka H, Skeath JB, Nabeshima Y, Doe CQ, Matsuzaki F: **Miranda directs Prospero to a daughter cell during Drosophila asymmetric divisions**. *Nature* 1997, **390**: 625–629.

42. Shen CP, Jan LY, Jan YN: **Miranda is required for the asymmetric localization of Prospero during mitosis in Drosophila**. *Cell* 1997, **90**:449–458.

43. ** Hannaford M, Loyer N, Tonelli F, Zoltner M, Januschke J: **A chemical-genetics approach to study the role of atypical Protein Kinase C in Drosophila**. *Development* 2019, **146**. dev170589.
This study reports the first analog sensitive kinase allele in Drosophila and uses this chemical genetics approach to interrogate the temporal requirement of aPKC activity in the process of asymmetric cell fate determinant localization.

44. Sousa-Nunes R, Chia W, Somers WG: **Protein phosphatase 4 mediates localization of the Miranda complex during Drosophila neuroblast asymmetric divisions**. *Genes Dev* 2009, **23**:359–372.

45. Barros CS, Phelps CB, Brand AH: **Drosophila nonmuscle myosin II promotes the asymmetric segregation of cell fate determinants by cortical exclusion rather than active transport**. *Dev Cell* 2003, **5**:829–840.

46. Petritsch C, Tavosanis G, Turck CW, Jan LY, Jan YN: **The Drosophila myosin VI Jaguar is required for basal protein targeting and correct spindle orientation in mitotic neuroblasts**. *Dev Cell* 2003, **4**:273–281.

47. Tsankova A, Pham TT, Garcia DS, Otte F, Cabernard C: **Cell polarity regulates biased myosin activity and dynamics during asymmetric cell division via Drosophila Rho kinase and protein kinase N**. *Dev Cell* 2017, **42**:143–155.

48. Roubinet C, Tsankova A, Pham TT, Monnard A, Caussinus E, Affolter M, Cabernard C: **Spatio-temporally separated cortical flows and spindle geometry establish physical asymmetry in fly neural stem cells**. *Nat Commun* 2017, **8**:237.

49. * Pham TT, Monnard A, Helenius J, Lund E, Lee N, Muller DJ, Cabernard C: **Spatiotemporally controlled myosin relocalization and internal pressure generate sibling cell size asymmetry**. *iScience* 2019, **13**:9–19.
This study uses sophisticated biophysical measurements and live cell manipulation and image analysis to provide a conceptual framework of how mechanical forces and the actomyosin network regulate daughter cell size asymmetry.

50. Krahn MP, Wodarz A: **Phosphoinositide lipids and cell polarity: linking the plasma membrane to the cytocortex**. *Essays Biochem* 2012, **53**:15–27.

51. Scholze MJ, Barbieux KS, De Simone A, Boumasmoud M, Süess CCN, Wang R, Gönczy P: **PI(4,5)P2 forms dynamic cortical structures and directs actin distribution as well as polarity in Caenorhabditis elegans embryos**. *Development* 2018, **145**. dev164988.

52. ** Koe CT, Tan YS, Lönnfors M, Hur SK, Low CSL, Zhang Y, Kanchanawong P, Bankaitis VA, Wang H: **Vibrator and PI4KIIIα govern neuroblast polarity by anchoring non-muscle myosin II**. *Elife* 2018, **7**:33501.
This study describes the link between phosphoinositide composition in the plasma membrane and regulation of the actomyosin network in Drosophila neuroblasts and proposes that phosphoinosites can anchor myosin, which is required for the proper execution of asymmetric neuroblast division and neuroblast homeostasis.

53. Lee CY, Lee C-Y, Andersen RO, Andersen RO, Cabernard C, Cabernard C, Manning L, Manning L, Tran KD, Tran KD, *et al.*: **Drosophila Aurora-A kinase inhibits neuroblast self-renewal by regulating aPKC/Numb cortical polarity and spindle orientation**. *Genes Dev* 2006, **20**:3464–3474.

54. Wang H, Ouyang Y, Somers WG, Chia W, Lu B: **Polo inhibits progenitor self-renewal and regulates Numb asymmetry by phosphorylating Pon**. *Nature* 2007, **449**:96–100.

55. Abeysundara N, Simmonds AJ, Hughes SC: **Moesin is involved in polarity maintenance and cortical remodeling during asymmetric cell division**. *Mol Biol Cell* 2018, **29**:419–434.

56. Ramat A, Hannaford M, Januschke J: **Maintenance of Miranda localization in Drosophila neuroblasts involves interaction with the cognate mRNA**. *Curr Biol* 2017, **27**:2101–2111. e5.

57. Slack C, Overton PM, Tuxworth RI, Chia W: **Asymmetric localisation of Miranda and its cargo proteins during neuroblast division requires the anaphase-promoting complex/cyclosome**. *Development* 2007, **134**:3781–3787.

58. Zhang F, Huang Z-X, Bao H, Cong F, Wang H, Chai PC, Xi Y, Ge W, Somers WG, Yang Y, *et al.*: **Phosphotyrosyl phosphatase activator facilitates localization of Miranda through dephosphorylation in dividing neuroblasts**. *Development* 2016, **143**:35–44.

59. Jia M, Shan Z, Yang Y, Liu C, Li J, Luo Z-G, Zhang M, Cai Y, Wen W, Wang W: **The structural basis of Miranda-mediated Staufen localization during Drosophila neuroblast asymmetric division**. *Nat Commun* 2015, **6**:8381.

60. Shan Z, Tu Y, Yang Y, Liu Z, Zeng M, Xu H, Long J, Zhang M, Cai Y, Wen W: **Basal condensation of Numb and Pon complex via phase transition during Drosophila neuroblast asymmetric division**. *Nat Commun* 2018, **9**:737.