Planar Cell Polarity Breaks the Symmetry of PAR Protein Distribution prior to Mitosis in *Drosophila* Sensory Organ Precursor Cells

**Highlights**

- PAR asymmetry in SOPs is set prior to mitosis
- Planar cell polarity breaks PAR symmetry

**In Brief**

Besson, Bernard, et al. combine live imaging with modeling to detect the onset of PAR planar polarization in *Drosophila* epithelial cells. PAR proteins become asymmetric in SOPs prior to mitosis independently of the mitotic kinase AuroraA. Planar cell polarity breaks the planar symmetry of PAR protein distribution.

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Planar Cell Polarity Breaks the Symmetry of PAR Protein Distribution prior to Mitosis in Drosophila Sensory Organ Precursor Cells

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SUMMARY

During development, cell-fate diversity can result from the unequal segregation of fate determinants at mitosis [1]. Polarization of the mother cell is essential for asymmetric cell division (ACD). It often involves the formation of a cortical domain containing the PAR complex proteins Par3, Par6, and atypical protein kinase C (aPKC) [1–5]. In the fly notum, sensory organ precursor cells (SOPs) divide asymmetrically within the plane of the epithelium and along the body axis to generate two distinct cells [6–12]. Fate asymmetry depends on the asymmetric localization of the PAR complex. In the absence of planar cell polarity (PCP), SOPs divide with a random planar orientation but still asymmetrically, showing that PCP is dispensable for PAR asymmetry at mitosis [6, 13–15]. To study when and how the PAR complex localizes asymmetrically, we have used a quantitative imaging approach to measure the planar polarization of the proteins Bazooka (Baz, fly Par3), Par6, and aPKC in living pupae. By using imaging of functional GFP-tagged proteins with image processing and computational modeling, we find that Baz, Par6, and aPKC become planar polarized prior to mitosis in a manner independent of the AuroraA kinase and that PCP is required for the planar polarization of Baz, Par6, and aPKC during interphase. This indicates that a “mitosis rescue” mechanism establishes asymmetry at mitosis in PCP mutants. This study therefore identifies PCP as the initial symmetry-breaking signal for the planar polarization of PAR proteins in asymmetrically dividing SOPs.

RESULTS AND DISCUSSION

A Quantitative Live-Imaging Approach

Asymmetric localization of the Baz-Par6-aPKC complex at the posterior pole of dividing SOPs is critical for the unequal segregation of the fate determinants Numb and Neur [1, 12, 16]. Thus, deciphering when and how this complex becomes asymmetric are essential to understand fate asymmetry. Since formation of the Baz-Par6-aPKC complex is regulated by the AuroraA (AurA) mitotic kinase in SOPs [17], it is usually assumed that the Baz-Par6-aPKC complex forms and localizes asymmetrically at mitosis. To examine the dynamics of Baz, Par6, and aPKC asymmetry, we have developed a quantitative live-imaging approach using functional GFP-tagged proteins (Figures 1A–1G). Specifically, we used a Par6-GFP genomic rescue construct [18] and a GFP-Baz protein-trap. Since GFP-Baz only reports on the distribution of two of the Baz isoforms [19], we also generated a BAC-encoded Baz-GFP protein that tags all isoforms. Last, we produced a BAC-encoded aPKC-GFP that also tags all aPKC isoforms. Genomic rescue assay showed that the BAC-encoded Baz-GFP and aPKC-GFP proteins are fully functional (see Supplemental Experimental Procedures). These four GFP-tagged proteins localized at the apical cell cortex in living pupae (Figures 1A–1D) and localized at the posterior pole of dividing SOPs (Figure S1) like the corresponding endogenous proteins.

To study the polar distribution of Baz, Par6, and aPKC in individual cells, we moved away from partly subjective, hence error-prone, categorization, such as scoring “polar” versus “non-polar” cases, and developed a quantitative approach. Live imaging of developing pupae produced 4D (x,y,z,t) movies. After z-maximal projection of the apical planes, the contour of individual cells was extracted and an intensity-normalized polarity vector was calculated for each time point (Figure 1H; Figure S1; see Supplemental Experimental Procedures). The magnitude of this polarity vector revealed planar asymmetry at the cell cortex while the direction (α) gave the orientation of this planar asymmetry relative to the anterior-posterior (a-p) axis (Figure 1). Thus, high-magnitude values and a posterior direction reflect planar polarization along the a-p axis. Using this method, the polarity vector was measured over time in both SOPs and epidermal (epi) cells (Figures 1H’ and 1H”). To compare polarization dynamics in different cells, a relative timescale was used with t = 0 corresponding to mitosis entry. This approach was used to measure the planar polarization of Baz, Par6, and aPKC.
Planar Polarization at Interphase

The planar polarization of Baz-GFP, GFP-Baz, Par6-GFP, and aPKC-GFP was measured from ~13.5 to ~17.5 hr after puparium formation (APF) in pupae mutant for baz, par6, and apkc, respectively (see Supplemental Experimental Procedures for detailed genotypes). The magnitude and direction were plotted over time (Figures 2A–2D, raw data; Figures 2A’–2D’, averaging over ten time points). At t = −225 (~13.5 APF), the Baz-GFP, GFP-Baz, Par6-GFP, and aPKC-GFP vectors had low-magnitude values and were randomly oriented in both SOPs and epi (SOPs were identified using Histone2B-RFP expressed under the control of a SOP-specific enhancer). This indicated that these PAR complex proteins were not planar polarized. Interestingly, all four vectors had increased magnitude and oriented posteriorly over time in SOPs (Figures 2A–2D) showing that Baz, Par6, and aPKC localized at the posterior apical cortex during late interphase consistent with the asymmetric and oriented mode of SOP division [6, 16]. By contrast, polarity vectors showed low magnitude and random direction in epi cells at all time points (Figures 2A–2D’). This suggested that Baz, Par6, and aPKC localized in a non-polar manner in epi cells, consistent with their symmetric mode of division [6]. Also, since Baz-GFP and GFP-Baz gave similar polarization profiles, they were used interchangeably afterward.

While low-magnitude values and random direction suggested a non-polar distribution, different magnitudes values were measured in epi cells for Baz, Par6, and aPKC. Higher values could result from a weak and randomly oriented polarity or from stochastic fluctuations in protein distribution at the cell cortex, i.e., random patches (Figure 2E). Since the GFP signal along the cell cortex could be described as patches of higher intensity on top of a more or less uniform background (Figure 2F), these two possibilities could be discriminated using synthetic cells that were generated in silico. These synthetic cells comprised randomly located patches with similar statistics in patch number and intensities relative to the actual cells (Figure 2F). For epi cells, the average magnitude of the polarity vectors computed from these synthetic cells was nearly identical to those measured for Baz, Par6, and aPKC (Figures 2A–2D’). Thus, random fluctuations in the GFP signal along the cell cortex alone could account for differences in magnitude values. Thus, our synthetic cell modeling approach clearly established that Baz, Par6, and aPKC are not planar polarized in epi cells. Similarly, the magnitude values measured in SOPs for Baz, Par6, and aPKC were also identical to those obtained from synthetic profiles at t = −225 (Figures 2A–2D’). This showed that Baz, Par6, and aPKC are not planar polarized at ~13.5 hr APF. We further found that the measured values deviated over time from the synthetic profiles in SOPs (Figures 2A–2D’), indicating that randomly located patches could not account for the high-magnitude values measured from t = −125 onward. This difference between measured magnitudes and those calculated from the synthetic cells revealed the progressive planar polarization of Baz, Par6, and aPKC in SOPs. Thus, our quantitative image analysis combined with modeling clearly demonstrated that Baz, Par6, and aPKC are planar polarized prior to mitosis in SOPs.

PAR Complex Formation and Polarization

Baz, Par6, and aPKC are thought to form complexes at the posterior cortex of SOPs. Consistent with this view, all three proteins become asymmetrically distributed at around the same time, between t = −175 and t = −125. Furthermore, the polarization of Par6-GFP was abolished upon silencing of baz, and the polarization of aPKC-GFP was decreased in baz heterozygous pupae (Figures 3A and 3B; aPKC-GFP did not correctly localize at the cortex in baz mutant cells, see Figure 3C). Thus, asymmetric localization of Par6-aPKC prior to mitosis appeared to depend on proper Baz levels. Reciprocally, the asymmetric localization of Baz and Par6 was also dependent on proper Par6 levels (Figures 3E and 3F; in the complete absence of par6, cells had shape defects with a reduced apical domain, see Figure 3D). Thus, Baz, Par6, and aPKC appeared to be dependent on each other for their asymmetric distribution prior to mitosis. These data therefore indicated that the formation of the
Baz-Par6-aPKC complex is a limiting parameter for its polarization. Consistent with this, reducing the levels of Lethal(2) giant larvae (Lgl), a protein that prevents Baz binding to Par6-aPKC and inhibits aPKC activity [17], led to the earlier planar polarization of Par6-GFP (Figure 3G).

Since planar polarization was observed ~2 hr prior to SOP division, we wondered whether the mitotic kinase AurA could be dispensable for this early polarization. Indeed, live imaging of aurA mutant pupae showed that the initial polarization of Par6-GFP during interphase did not require this mitotic kinase (Figure 3H). We propose that the PAR complex forms and localizes asymmetrically during interphase in an AurA-independent manner and that AurA acts later at mitosis to amplify this asymmetry [17].
PCP-Dependent Polarization and Mitosis Rescue

We next investigated how asymmetry is established in SOPs during interphase. The anterior Gαi-Pins complex is known to act redundantly with PCP to restrict the Baz-Par6-aPKC complex posteriorly in mitotic SOPs [13, 20, 21]. We therefore studied the potential role of Gαi, Pins, and PCP in the early polarization of SOPs. We first found that the complete loss of Gαi activity had a very minor effect on the polarization of Par6-GFP at late interphase (Figure 4A). Additionally, the silencing of pins did not affect the planar polarization of Par6-GFP and GFP-Baz (Figures 4B and 4C). Thus, the activities of Gαi and Pins were not essential for the initial polarization of Baz and/or Par6. We next investigated the role of the PCP gene frizzled (fz). In fz mutant pupae, the measured magnitude of the Par6-GFP and Baz-GFP vectors remained low and did not significantly differ from those calculated in synthetic cells (Figures 4D and 4E), showing that planar polarization was lost. A similar result was obtained for GFP-Baz upon silencing of fz (data not shown). Also, the silencing of the PCP genes Van Gogh (Vang) and dishevelled (dsh) similarly abolished the planar polarization of Par6-GFP.

Figure 3. Baz-, Par6-, and aPKC-Dependent but AurA-Independent Polarization

(A, B, and E–H) Measured and synthetic cell profiles of the Par6-GFP (A, F–H), aPKC-GFP (B), and Baz-GFP (E) polarity vectors in bazRNAi (A: 20 SOPs, 18 epis), baz heterozygous (B: 22 SOPs, 23 epis), par-6 heterozygous (E: 31 SOPs, 30 epis; F: 31 SOPs, 31 epis), lgl heterozygous (G: 26 SOPs, 25 epis), and aurA mutant (H: 17 SOPs, 11 epis) SOPs (blue) and epis (orange). The corresponding wild-type SOP profiles (red) are also shown. Early planar polarization depends on proper levels of Baz, Par6, and aPKC but does not depend on aurA.

(C) aPKC-GFP is largely cytoplasmic in baz mutant cells (that have two copies of aPKC-GFP) and baz heterozygous cells (that have one copy of aPKC-GFP; note that baz+/+ cells lack the aPKC-GFP transgene). Loss of cortical aPKC prevented us from extracting cell contours, hence studying planar polarization.

(D) par-6 mutant cells (that have two copies of Baz-GFP; heterozygous cells have one copy of Baz-GFP; clone border in blue) showed reduced apical area. The wild-type cells (border of the twin clone in red) do not carry the Baz-GFP transgene.

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(legend on next page)
and GFP-Baz (Figures 4F–4I). In all cases, the direction of the Baz and Par6 polarity vectors was randomized. These data clearly demonstrated that PCP is required for the initial planar polarization of Baz and Par6 in SOPs. We propose that the PAR complex reads the PCP information in SOPs to accumulate asymmetrically. How PCP regulates PAR complex distribution in SOPs remains to be determined [22, 23]. Also, since PCP operates across the notum to polarize all epithelial cells [14, 15], mechanisms restricting the interpretation of PCP to SOPs for SOP asymmetry at mitosis [6, 13]. Thus, a mitosis rescue determination must exist (Figure 4J).

Our finding that PCP provides a symmetry breaking input for the planar polarization of Baz, Par6, and aPKC was unexpected since PCP had previously been shown to be largely dispensable for SOP asymmetry at mitosis [6, 13]. Thus, a mitosis rescue mechanism must operate at mitosis to create asymmetry, albeit less efficiently and in a non-oriented manner, when it fails to be established by PCP prior to mitosis. A similar mitosis rescue phenomenon was observed in neuroblasts: while a microtubule-dependent orientation cue normally persists from mothers to daughters to position the division axis, asymmetry can still form de novo at mitosis upon loss of this cue but with a random orientation relative to the previous division axis [24]. Since pins acts redundantly with fz to localize Baz asymmetrically in dividing SOPs [16], this mitosis rescue likely involves Pins. Pins is phosphorylated at mitosis by AurA, and this phosphorylation is important for its recruitment at the cortex by Discs-large (Dig) to form an anterior Dig-Pins complex [16, 25]. AurA also phosphorylates Par6 and Lgl and may thereby relieve the inhibition exerted by Lgl on the aPKC activity [17, 26, 27]. These data suggest that AurA may amplify the initial asymmetry established by PCP prior to mitosis and/or reinforce the possible input of PCP on asymmetry at mitosis. Accordingly, AurA would trigger mitosis rescue when the initial PCP-dependent asymmetry fails to be set up (Figures 4J and 4K).

In summary, our study revealed that all three PAR proteins become asymmetrically localized in SOPs prior to mitosis in a mutually dependent manner and that PCP is required for their initial planar polarization. Future studies will address how planar polarization of the PAR complex is restricted to SOPs. We propose a model whereby first notum epithelial cells become planar polarized at larval stages; second, SOPs are selected within this epithelium at early pupal stages; this cell fate change allows Baz, Par6, and aPKC to read the PCP information and localize asymmetrically at interphase; third, at mitosis, mitotic kinases, possibly together with PCP, regulate the amplification of this initial asymmetry (Figures 4J and 4K). This model of cell-specific interpretation of PCP via the planar polarization of the PAR complex is likely conserved from flies to mammals [28].

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and one figure and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.02.073.

AUTHOR CONTRIBUTIONS

F.S., C.B., and F.B. designed the project. H.R. conceived the polarity vector. F.C. developed the synthetic cell approach. K.M. created the BAC transgenes. C.B. and F.B. performed together most of the experiments. E.R. and A.K. performed together the experiments shown in Figures 2D, 4D, 4E, 4H, and 4I. All authors participated in the writing of the manuscript.

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Figure 4. PCP Regulates the Early Planar Polarization of Baz and Par6
(A–I) Measured and synthetic cell profiles of the Par6-GFP (A, B, D–F, and H), GFP-Baz (C, G, and I), and Baz-GFP (E) polarity vectors in Gs3 mutant (A; 31 SOPs, 29 epmis), pinsRNAi (B; 33 SOPs, 36 epmis; C; 34 SOPs, 30 epmis), z mutant (D; 31 SOPs, 34 epmis; E; 24 SOPs, 20 epmis), vangRNAi (F; 25 SOPs, 24 epmis; G; 41 SOPs, 33 epmis), and dsyRNAi (H; 36 SOPs, 35 epmis; I; 20 SOPs, 17 epmis) SOPs (blue) are compared to the corresponding wild-type profiles (red) and mutant epmis (orange) as in Figure 3. The early planar polarization of Par6 and Baz was lost in the absence of PCP but remained unchanged upon loss and/or reduced Gs3/Pins. (J–K’) Model. Prior to SOP specification (J), all notum cells are planar polarized with Vang anterior (yellow) and Fz posterior (green). Par proteins (red) localize at the apical cortex and are not planar polarized. Following SOP specification (J’), PAR proteins become planar polarized only in SOPs, leading to asymmetric division (J’). In PCP mutants, PCP is lost (K), PAR proteins are not planar polarized prior to mitosis (K’) and mitosis rescue restores asymmetry but not orientation along the body axis (K’).


