Actin dependent membrane polarization reveals the mechanical nature of the neuroblast polarity cycle

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Abstract

The Par complex directs fate determinant segregation from the apical membrane of asymmetrically dividing *Drosophila* neuroblasts. While the physical interactions that recruit the Par complex have been extensively studied, little is known about how the membrane itself behaves during polarization. We examined the membrane dynamics of neuroblasts and surrounding cells with super-resolution imaging, revealing cellular-scale movements of diverse membrane features during asymmetric division cycles. Membrane domains that are distributed across the neuroblast membrane in interphase become polarized in early mitosis, where they mediate formation of cortical patches of the Par protein aPKC. Membrane and protein polarity cycles are precisely synchronized, and are generated by extensive actin dependent forces that deform the surrounding tissue. In addition to suggesting a role for the membrane in asymmetric division, our results reveal the mechanical nature of the neuroblast polarity cycle.

Introduction

The Par complex forms polarized membrane domains in animal cells that mediate functions such as directional transport and fate determinant segregation (Lang and Munro, 2017; Riga et al., 2020; Venkei and Yamashita, 2018). Formation of the Par domain, a continuous region along the cell membrane containing the Par complex proteins Par-6 and atypical Protein Kinase C (aPKC), is a highly dynamic process involving recruitment from the cytoplasm and movement along the membrane. In asymmetrically dividing *Drosophila* neuroblasts, for example, initially cytoplasmic Par-6 and aPKC accumulate in the membrane's apical hemisphere early in mitosis (Wodarz et al., 2000; Petronczki and Knoblich, 2001; Rolls et al., 2003; Homem and Knoblich, 2012). Membrane recruitment is followed by coalescence of the proteins into a cortical region concentrated around the apical pole. Extensive effort has been directed towards deciphering the physical interactions that recruit Par-6 and aPKC from the cytoplasm to the membrane. Less is known, however, about the neuroblast membrane itself and whether cellular processes besides regulated protein targeting might participate in the neuroblast polarization mechanism.

While current models for neuroblast polarity focus on the protein-protein interactions that recruit the Par complex to the membrane (Knoblich, 2010; Lang and Munro, 2017; Venkei and Yamashita, 2018), accumulating evidence indicates that additional mechanisms may contribute to polarity. Polarization is a stepwise process in which membrane targeting of the Par protein aPKC to the apical hemisphere is followed by coordinated movements towards the apical pole

(Oon and Prehoda, 2019). Coalescence of aPKC and maintenance of the polarized state require the actin cytoskeleton (Hannaford et al., 2018; Oon and Prehoda, 2019). Thus, the dynamics of polarity proteins and their dependence on the actin cytoskeleton indicate that, while regulated membrane targeting is an essential component of the neuroblast polarity mechanism, it is not sufficient to explain the multi-step process that leads to formation of the continuous Par domain that controls asymmetric cell division.

Although the membrane plays a central role in Par-mediated polarity by establishing the scaffold for formation of the Par domain, its precise role in polarity remains unclear. In polarized cells such as epithelia, localized accumulations of specific phospholipids are thought to be important for formation of the Par domain, although the existence and function of these microdomains is controversial (van IJzendoorn et al., 2020; Riga et al., 2020; Stone et al., 2017). In the worm zygote, a clear membrane asymmetry emerges during formation of the Par domain as filopodia-like structures preferentially form in the anterior membrane domain (Scholze et al., 2018; Hirani et al., 2019). Very little has been reported about the neuroblast membrane during its polarity cycle, although several phosphoinositide sensors have been reported to localize uniformly to the membrane (Doyle et al., 2017; Koe et al., 2018; Loyer and Januschke, 2018). Here we investigate whether the neuroblast membrane contains any heterogeneities and whether any such features might provide insight into the polarity cycle.

Results

The neuroblast membrane is heterogeneous and forms extensive contacts with progeny cells

We examined the neuroblast membrane using three markers, each of which interacts with the membrane via a distinct mechanism: a farnesyl-modified peptide that integrates into the bilayer (FP-farnesyl) (Zhou et al., 2015), the pleckstrin homology domain from PLCδ that interacts with the headgroup of the phosphoinositide PI(4,5)P_2 (FP-PH) (Verstreken et al., 2009), and the integral membrane protein CD8 that traverses the bilayer (FP-CD8) (Lee and Luo, 1999). We expressed fluorescent protein fusions of the markers specifically in the neuroblast lineage using the UAS promoter and worniu-GAL4 driver, and imaged actively dividing central nervous system neuroblasts from third instar larval brain explants. To image neuroblast membranes at super-resolution, we used spinning-disk confocal microscopy with optical photon reassignment (Azuma and Kei, 2015). Each of the membrane markers outlined the plasma membrane of neuroblasts and their smaller progeny cells, with little background from other

cells (Figure 1 and Video S1). For FP-farnesyl and FP-PH, the plasma membrane was marked nearly exclusively (small vesicle-like signals were occasionally seen with each) whereas FP-CD8 also marked some internal membranes.

Individual optical sections and maximum intensity projections (MIPs) of the full cell volume revealed a rich landscape of neuroblast membrane features, including blebs and filopodial-like extensions, smaller domains, and extensive contacts with progeny cells (Figure 1A-E and Videos S1 and S2). Extensions were typically greater than 0.5 μm in length whereas membrane domains were smaller areas of marker enrichment that were closer to the cell body but also protruded to some extent. Besides these three features, the membrane markers also revealed that progeny cells form extensive contacts with their associated neuroblast (Figure 1 and Video S1). These contacts were large in surface area and also typically included filopodial extensions that emanated from the progeny cell towards the opposite side of the neuroblast. In many instances, the progeny filopodia encircled the neuroblast to an extent that the progeny appeared to engulf part of the neuroblast. We observed the same membrane features when simultaneously imaging distinct membrane markers (Figure 1B-E), indicating that the markers report on the same set of features. Membrane features were associated with cortical F-actin, as detected by the Lifeact sensor (Figure 1F).

The smaller membrane domains dissipated when methyl-β-cyclodextrin was included in the surrounding media (Figure 1G; n = 25 neuroblasts). Cyclodextrin sensitivity and CD8 enrichment are characteristics of membrane microdomains – localized areas where specific phospholipids and proteins are concentrated (Barman and Nayak, 2007; Pang et al., 2007), although the increased membrane marker signal may be due to increased amounts of plasma membrane, consistent with their slight protrusion above the cell surface (Figure 1A-E) and similar observations in the worm zygote (Hirani et al., 2019).

The neuroblast membrane undergoes an actin cytoskeleton-dependent polarity cycle

To examine neuroblast membrane dynamics, we acquired time series of optical sections across the cell volume approximately once per five minutes, a frequency which did not cause significant photobleaching using the optical reassignment imaging method. Even at this temporal resolution, maximum intensity projections of sections through the cell's full volume revealed changes in the membrane over the course of the cell cycle (Figure 2A). Membrane dynamics were not constant, with relatively little change until sometime in mitosis when large

movements appeared to begin before cleavage furrow ingression and end shortly after the completion of division.

To follow the neuroblast membrane at higher temporal resolution, we used standard resolution spinning disk confocal microscopy, imaging the full cell volume every 20 seconds. Additionally, we simultaneously imaged the chromosomal marker RFP-His2A to more precisely identify the cell cycle stages at which transitions in membrane dynamics occur. From the resulting movies we identified four distinct temporal phases of neuroblast membrane movements (Figure 2B,C and Video S3). In interphase, membrane features exhibited only small movements that were uncoordinated and lacked any clear directionality. The relative calm of the interphase membrane gave way to a period of highly coordinated dynamics in early prophase in which features across the cell membrane moved rapidly towards the apical pole. We detected apical movement not only in features such as localized marker enrichments, but in progeny cell contacts that are typically near the basal pole, indicating that substantial force accompanies these movements (Figure 2B and Video S3). Continued apical movement during prophase ultimately led to accumulation of membrane features in the apical hemisphere of the cell (i.e. polarization) until this phase ends near metaphase (Figure 2B,C).

The polarized membrane state was maintained for a short period until anaphase began and membrane movements reversed direction, leading to their dispersal across the membrane surface (i.e. depolarization) by the completion of cytokinesis. The basally-directed movements that occur in anaphase also caused the progeny cell contacts at the basal region of the neuroblast membrane to relax to their pre-polarization state (Figure 2B,C and Video S3). Thus, there are four phases of membrane dynamics during neuroblast asymmetric cell division, a relatively static interphase with features evenly distributed across the cell surface, apically-directed movement during prophase that generates an apically polarized state that is maintained through metaphase, and a depolarization phase during anaphase. The process that drives membrane dynamics operates at the cellular scale, altering both the basal and apical membranes, and the force generated during the process is large enough to significantly deform the surrounding tissue.

To gain insight into the underlying process that drives membrane movements, we examined membrane dynamics in neuroblasts treated with the actin depolymerizing drug Latrunculin A (LatA). As shown in Figure 2D and Video S4, membrane domains halt apically-directed

movements during prophase immediately following LatA exposure and become nearly completely static (n = 15 neuroblasts). However, while the dynamics of membrane domains were dependent on the actin cytoskeleton, the domains themselves were unaffected by LatA treatment. Simultaneous imaging of GFP-PH and mRuby-Lifeact confirmed that membrane movements ceased when cortical actin dissipated (Figure 2E, Video S4). Thus, an intact actin cytoskeleton is required for the complex dynamics of membrane domains during asymmetric cell division, but the domains persist in the absence of F-actin.

The Par protein aPKC localizes to membrane domains and follows their dynamics

The phases of neuroblast membrane dynamics we observed, rapid polarization and then depolarization, resemble those of Par polarity proteins such as aPKC (Oon and Prehoda, 2019), with the key difference that membrane domains are present throughout the cell cycle whereas aPKC is cytoplasmic in interphase and does not begin accumulating on the membrane until early prophase (Hannaford et al., 2018; Oon and Prehoda, 2019). We examined whether membrane and protein dynamics are correlated by imaging larval brain neuroblasts expressing GFP-aPKC from its endogenous promoter and *worniu-GAL4* driven mCherry-PH. We observed accumulation of aPKC early in mitosis, with localized enrichments (i.e. patches) forming at apically localized membrane domains and aPKC that was more diffusely distributed elsewhere in the apical hemisphere (Figure 3 and Video S5). Both pools of cortical aPKC, membrane domain-localized patches and diffuse, moved towards the apical pole simultaneously, with protein and membrane movement beginning simultaneously (i.e. within one frame; n = 8 neuroblasts). Membrane domains that were initially in the basal hemisphere did not recruit aPKC until they passed the equator into the apical hemisphere (Figure 3E and Video S6). Upon completion of the polarization process, membrane domains and aPKC remained tightly localized around the apical pole for a short period before disassembling via basally directed movements. Disassembly of the polarized membrane domains and aPKC began simultaneously (within one frame; n = 8 neuroblasts), as both moved rapidly towards the emerging cleavage furrow. Following disassembly, aPKC signal rapidly dissipated from the membrane, whereas the depolarized membrane domains persisted into the following interphase. Thus, aPKC patches form at apically localized membrane domains and both populations of cortical aPKC, patches and diffuse, precisely follow membrane dynamics with the same coordinated apically- and basally-directed movements during prophase and anaphase, respectively.

Neuroblast membrane domains mediate aPKC cortical patch formation

The targeting of aPKC cortical patches to membrane domains and the correlated movement of membrane and polarity protein suggest that the two may be functionally related. We tested the effect of removing membrane domains on the cortical localization of aPKC by examining aPKC localization in neuroblasts that lacked membrane domains owing to cyclodextrin treatment. These neuroblasts failed to form patches of aPKC, with the remaining cortical aPKC signal diffusely distributed over the apical cortex (n = 12; Figure 4A and Video S7). The diffuse aPKC continued to polarize at the apical pole in the absence of membrane domains as it did in untreated neuroblasts (Figure 3 and Video S5). Thus, while membrane domains are required for localized cortical enrichment of aPKC, neither membrane domains nor the patches associated with them are strictly required for polarity. We also tested whether aPKC is required for membrane domains to form and noticed no effect on membrane structure in neuroblasts expressing an RNAi directed against aPKC (Video S8).

To determine if the loss of cortical patches influenced aPKC's ability to regulate polarity, we examined the localization of the basal factor Miranda in cyclodextrin-treated neuroblasts. Miranda is normally polarized to the basal cortex due to the activity of aPKC, leading to its segregation into the basal daughter cell during cytokinesis (Rolls et al., 2003). Although cortical Miranda was somewhat reduced in cyclodextrin-treated neuroblasts, segregation into the basal daughter cell continued to occur (Video S9). Taken together, the effects of cyclodextrin on aPKC and Miranda indicate that membrane domains are required for cortical aPKC patches, but that patches are not required for aPKC polarity or function. The diffusely cortical aPKC that remains in the absence of aPKC patches is polarized similarly regardless of the presence of membrane domains, and is sufficient for specifying basal polarity.

The actin cytoskeleton is required to maintain the diffusely localized pool of cortical aPKC in the apical hemisphere (Hannaford et al., 2018; Oon and Prehoda, 2019). We examined whether the membrane domain-localized cortical aPKC patches also require F-actin. In LatA-treated neuroblasts expressing GFP-aPKC and mCherry-PH as a membrane marker, we observed rapid dissipation of cortical aPKC that was not colocalized with a membrane domain, as previously described (Figure 4B and Video S10). However, aPKC patches remained localized to membrane domains in all neuroblasts examined (n = 23), indicating that their retention in the apical hemisphere does not require the actin cytoskeleton. Thus, the two pools of cortical

aPKC are maintained in the apical hemisphere via an actin-dependent mechanism for diffusely localized aPKC, and a membrane structure-dependent one for cortical aPKC patches.

Discussion

We examined the neuroblast membrane using super resolution imaging and found that it is very heterogenous, with features such as small cyclodextrin-sensitive domains, filopodial extensions, and extensive contacts with progeny cells (Figure 1 and Videos S1,S2). Membrane domains are initially dispersed across the cell surface in interphase, with progeny contacts typically near the basal pole. The membrane undergoes several phases of coordinated movements during mitosis (Figure 4C), first polarizing by enriching features in the apical hemisphere with coordinated, apically-directed movements that are dependent on the actin cytoskeleton. The forces that generate the polarized neuroblast membrane deform the surrounding tissue, indicating that they are significant in magnitude and scale. After a brief phase where the polarized state is maintained, basally-directed movements depolarize the membrane by redistributing the features across the cell surface. We found that these complex dynamics, including the timing of the transitions between phases and the characteristics of the movements, are precisely correlated with those of the Par polarity protein aPKC (Figure 3 and Video S5). Furthermore, membrane domains participate in the recruitment and retention of aPKC, specifically recruiting aPKC when they are in the apical hemisphere (Figure 3E and Video S6). The behavior of the neuroblast membrane challenges current models for neuroblast polarity, which focus entirely on regulated protein-protein interactions (Knoblich, 2010; Lang and Munro, 2017; Venkei and Yamashita, 2018).

The behavior of the membrane indicates that the neuroblast polarity cycle is a mechanical process that generates cellular-scale forces. Par proteins were recently found to undergo apically-directed movements that lead to neuroblast polarization (Oon and Prehoda, 2019), but whether the underlying cellular process that drives these movements acts locally or at a larger scale has not been known. We observed movement of membrane features in the apical hemisphere but also near the basal pole, including the extension of filopodial-like arms from progeny cells that wrap around the neuroblast. The deformation of the surrounding tissue is so extensive that the neuroblast polarity cycle can be readily followed without directly monitoring the neuroblast itself. Thus, the neuroblast polarity cycle is a cellular scale, actin-based mechanical process in which the membrane is remodeled in addition to the protein components of the cell cortex.

The membrane also plays a role in polarity initiation and maintenance. We observed recruitment of aPKC to apical membrane domains and retention of aPKC at these domains even in the absence of the actin cytoskeleton (Figures 3 and 4). While aPKC is also recruited to apical sites outside of membrane domains, this “diffuse” aPKC requires the actin cytoskeleton and rapidly depolarizes in the presence of LatA. Thus, we propose that the two pools of membrane-bound aPKC, diffuse and membrane-domain associated, act redundantly to initiate and maintain apical neuroblast polarity (Figure 4C). Future work will be directed at understanding differences in the physical nature of these two pools and how they are sculpted by the actin cytoskeleton during coalescence.

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources and reagents should be directed to the Lead Contact, Kenneth Prehoda (prehoda@uoregon.edu).

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

The raw data supporting the current study have not been deposited in a public repository because of their large file size but are available from the corresponding author on request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Fly Strains

For live imaging of membrane dynamics in neuroblasts, a Worniu-Gal4 driver line was used to express fluorescent membrane-bound fusion proteins under UAS control. Three classes of membrane-bound fusion proteins were used. FP-farnesyl expresses the C-terminal region of human K-Ras tagged with GFP which becomes farnesylated and membrane-anchored in cells. FP-PH expresses the pleckstrin homology domain of human PLCδ tagged with GFP or mCherry, which binds to the plasma membrane lipid phosphoinositide PI(4,5)P2. FP-CD8 (a gift from the Chris Doe Lab) expresses a single-pass transmembrane protein tagged with GFP. RFP-His2A expresses RFP-tagged His2A under the control of native promoters. The BAC-encoded GFP-aPKC (Besson et al., 2015) was used to track aPKC localization and dynamics.

METHOD DETAILS

Live Imaging

Intact *Drosophila* central nervous systems were dissected from third instar larvae in a bath of Schneider's Insect Media. These larval brain explants were then mounted dorsal side down on sterile poly-D-lysine coated 35mm glass bottom dishes (ibidi Cat#81156) containing modified minimal hemolymph-like solution (HL3.1). Explants were imaged on a Nikon Eclipse Ti-2 (60x H_2O objective) equipped with a Yokogawa CSU-W1 SoRa spinning disk head and dual Photometrics Prime BSI sCMOS cameras. GFP tagged proteins were illuminated with 488nm laser light. RFP and mCherry tagged proteins were illuminated with 561nm laser light. For time-lapse imaging 41-61 optical sections with a step size of 0.5 μm were acquired every 20 seconds. For super resolution imaging, individual neuroblasts were imaged with a step size of 0.3 μm using SoRa optics which achieve super resolution through optical photon reassignment. To examine the role of F-actin in membrane and aPKC dynamics, explants were treated with 50 μM latrunculin A (0.5% DMSO) during imaging. To examine the role of cholesterol in membrane and aPKC dynamics, explants were treated with 15 mM methyl-B-cyclodextrin (solubilized in HL3.1) during imaging.

Image Processing and Visualization

Images were analyzed using ImageJ (FIJI package) and Imaris (Bitplane) software. Neuroblasts were identified through their location in the brain and large size, as well as the presence of tissue-specifically expressed transgenes. For rotating movies, maximum intensity projections (MIPs) were assembled from optical slices through the entirety of the neuroblast volume. Photobleaching during time-lapse imaging was corrected for using ImageJ bleach correction tool in histogram matching mode. For aPKC images, a guassian blur of 0.5 pixels was used to improve signal to noise. For imaging of RFP and mCherry tagged transgenes, ImageJ despeckle and/or smooth tools were applied. Kymographs were created from time-lapse movies using a line along the basal-apical axis using ImageJ multi kymograph tool.

QUANTIFICATION AND STATISTICAL ANALYSIS

Not applicable

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, Peptides, and Recombinant Proteins		
Latrunculin A	Enzo Life Sciences	Cat#BML-T119-0100, CAS# 76343-93-6
Methyl-β-cyclodextrin	Caymen Chemical	Cat#21633, CAS# 128446-36-6
Experimental Models: Organisms/Strains		
UAS-aPKC RNAi	Vienna *Drosophila* Resource Center	v105624
FP-Farnesyl	Bloomington *Drosophila* Stock Center	BDSC Cat#80052, RRID:BDSC_80052
FP-PH (GFP)	Bloomington *Drosophila* Stock Center	BDSC Cat#39693, RRID:BDSC_39693
FP-CD8	Bloomington *Drosophila* Stock Center	
Worniu-Gal4	Chris Q. Doe Lab	
RFP-His2a (H2A)	Bloomington *Drosophila* Stock Center	BDSC Cat#23650, RRID:BDSC_23650
GFP-aPKC	François Schweisguth Lab; Besson et al., 2015	
FP-PH (mCherry)	Bloomington *Drosophila* Stock Center	BDSC Cat#51658, RRID:BDSC_51658
GFP-Miranda	Bloomington *Drosophila* Stock Center	BDSC Cat#56555, RRID:BDSC_56555
mRuby-Lifeact	Bloomington *Drosophila* Stock Center	BDSC Cat#35545, RRID:BDSC_35545
Software and Algorithms		
ImageJ (FIJI package)	National Institutes of Health	
Imaris	Bitplane	

Video legends

Video S1: Maximum intensity projections of neuroblasts and progeny cells expressing membrane markers.

Super resolution images of neuroblasts and their progeny from *Drosophila* third instar larvae. Neuroblasts expressed the indicated membrane marker under the control of the UAS promoter and driven by worniu-GAL4. Maximum intensity projections (MIP) made from optical sections through the neuroblast's entire volume are shown. Rotations of each cell's full volume MIP are shown in the following order: GFP-farnesyl, GFP-PH, GFP-CD8.

Video S2: The neuroblast membrane is a rich landscape of features.

Super resolution images of neuroblasts and their progeny from Drosophila third instar larvae. Neuroblasts expressed GFP-farnesyl under control of the UAS promoter and driven by worniu-GAL4. Rotating of maximum intensity projections (MIP) made from optical sections through the neuroblast's entire volume are shown.

Video S3: Neuroblast membrane dynamics through a full division cycle.

Time lapse imaging of neuroblasts and their progeny from Drosophila third instar larvae through a complete division cycle. Neuroblasts expressed GFP-PH under control of the UAS promoter driven by worniu-GAL4, and RFP-H2A under control of a constitutive promoter. Left: maximum intensity projection of the front hemisphere GFP-PH signal (”membrane”). Right: medial section with both GFP-PH in green and RFP-H2A (”chromatin”) in magenta. Time relative to anaphase onset (mm:ss) is shown.

Video S4: Membrane dynamics in neuroblasts treated with the actin depolymerizing drug Latrunculin A.

Time lapse imaging of neuroblasts and their progeny from *Drosophila* third instar larvae through a complete division cycle in the presence of LatA. The first two movies are of neuroblasts which expressed GFP-PH under control of the UAS promoter driven by worniu-GAL4 and RFP-H2A under control of a constitutive promoter. Movie A begins with the neuroblast in an untreated state before LatA was added to the media. Caption denotes when LatA was added. Left: maximum intensity projection of the front hemisphere GFP-PH signal (”membrane”). Right: medial section with GFP-PH in green and RFP-H2A (”chromatin”) in magenta. Movie B shows the same genotype and imaging conditions as the first movie, but was treated DMSO, serving as the vehicle control. Movie C shows a neuroblast which

expressed GFP-PH and mRuby-Lifeact both under control of the UAS promoter driven by worniu-GAL4. The movie begins with the neuroblast in a untreated state before LatA was added to the media. Caption denotes when LatA was added. Left: maximum intensity projection of the front hemisphere GFP-PH signal. Middle: maximum intensity projection of the front hemisphere mRuby-Lifeact signal. Right: merge of both channels with GFP-PH in green and mRuby-Lifeact in magenta. All movie timestamps are relative to anaphase onset (mm:ss).

Video S5: Simultaneous imaging of membrane and aPKC dynamics during neuroblast asymmetric division.
Time lapse imaging of Drosophila third instar larvae neuroblast expressing GFP-aPKC from its endogenous promoter and mCherry-PH via worniu-GAL4. Time relative (mm:ss) to nuclear envelope breakdown (NEB) is shown with a 20 seconds/frame time resolution. Left: GFP-aPKC. Middle: mCherry-PH. Right: merge of both channels with GFP-aPKC in green and mCherry-PH in magenta. Top row is maximum intensity projection (MIP) of the front hemisphere of the neuroblast. Bottom row is a medial section. As described previously, aPKC accumulates on the apical membrane beginning in early prophase followed by a coalescence phase shortly before NEB. Membrane features are present throughout the cell cycle, and their movements occur simultaneously with aPKC.

Video S6: aPKC is recruited to apical membrane patches
Time lapse imaging of Drosophila third instar larvae neuroblast expressing GFP-aPKC from its endogenous promoter and mCherry-PH via worniu-GAL4. Shown are maximum intensity projections (MIP) of the front hemisphere of the neuroblast. Left: GFP-aPKC. Middle: mCherry-PH. Right: merge of both channels with GFP-aPKC in green and mCherry-PH in magenta. Time relative (mm:ss) to nuclear envelope breakdown (NEB) is shown 20 seconds/frame time resolution. Depicted is an example of aPKC recruitment to a membrane patch as it moves from the basal to the apical hemisphere. Yellow arrowheads mark the membrane patch and the corresponding aPKC signal.

Video S7: Membrane and aPKC dynamics in a cyclodextrin-treated neuroblast
Time lapse imaging of membrane and aPKC dynamics in a Drosophila third instar larvae neuroblast in which membrane domains were removed via cyclodextrin treatment. Neuroblast expressed GFP-aPKC from its endogenous promoter and mCherry-PH via worniu-GAL4. Shown are maximum intensity projections (MIP) of the front hemisphere of the neuroblast. Left:

GFP-aPKC. Middle: mCherry-PH. Right: merge of both channels with GFP-aPKC in green and mCherry-PH in magenta. Time relative (mm:ss) to nuclear envelope breakdown (NEB) is shown with a 20 seconds/frame time resolution.

Video S8: Membrane dynamics in a neuroblast expressing RNAi directed against aPKC
Time lapse imaging of a *Drosophila* third instar larval neuroblast expressing GFP-PH under conditions of RNAi mediated knockdown of aPKC via worniu-GAL4. Shown is a maximum intensity projection (MIP) of the front hemisphere of the neuroblast. 20 seconds/frame time resolution.

Video S9: Miranda dynamics in a cyclodextrin-treated neuroblast
Time lapse imaging a *Drosophila* third instar larval neuroblast expressing GFP-Miranda via worniu-GAL4 and RFP-H2A via a constitutive promoter. Two different imaging conditions were employed: untreated control (first movie) and cyclodextrin treatment (second movie). Shown is a maximum intensity projection (MIP) of the front hemisphere of the neuroblast. Left: GFP-Miranda. Middle: RFP-H2A. Right: merge of both channels with GFP-Miranda in green and RFP-H2A in magenta. Time relative (mm:ss) to anaphase onset is shown.

Video S10: Membrane and aPKC dynamics in a Latrunculin A treated neuroblast
Time lapse imaging a Drosophila third instar larvae neuroblast expressing GFP-aPKC via endogenous promoter and mCherry-PH via worniu-GAL4. Shown is a maximum intensity projection (MIP) of the front hemisphere of the neuroblast. Left: GFP-aPKC. Middle: mCherry-PH. Right: merge of both channels with GFP-aPKC in green and mCherry-PH in magenta. Movie A begins with the neuroblast being untreated before LatA was added to the media. Caption denotes when LatA was added. Time relative (mm:ss) to anaphase onset is shown 20 seconds/frame time resolution.

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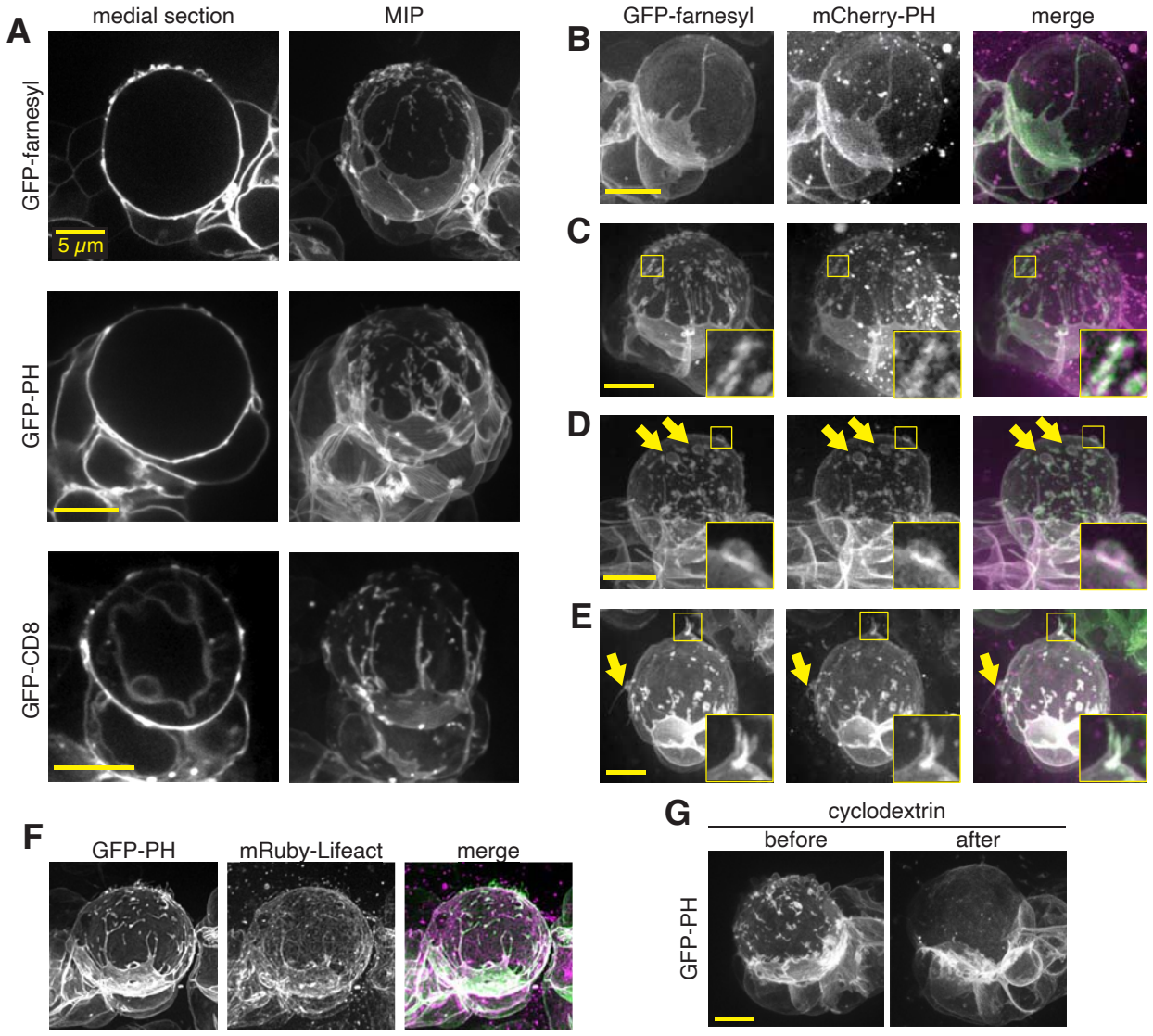

Figure 1 The neuroblast membrane is heterogenous (**A**) Super resolution images of neuroblasts and their progeny from *Drosophila* third instar larvae. Neuroblasts expressed the indicated membrane marker under the control of the UAS promoter and driven by worniu-GAL4. A single medial optical section is shown along with a maximum intensity projection made from optical sections through the neuroblast's front hemisphere. Rotations of each cell's full volume MIP are shown in Video S1 (**B-E**) Direct comparison of neuroblast features using simultaneous imaging of multiple membrane markers. Images are maximum intensity projections as in (A). Selected features are highlighted with arrows and boxes with magnified inset. Rotations of each cell's full volume MIP are shown in Video S2.(**F**) Colocalization of neuroblast membrane features (GFP-PH) and actin (mRuby-Lifeact). (**G**) Effect of cyclodextrin treatment on neuroblast membrane heterogeneity. Maximum intensity projections constructed from optical sections taken from the same neuroblast before and after treatment with cyclodextrin are shown.

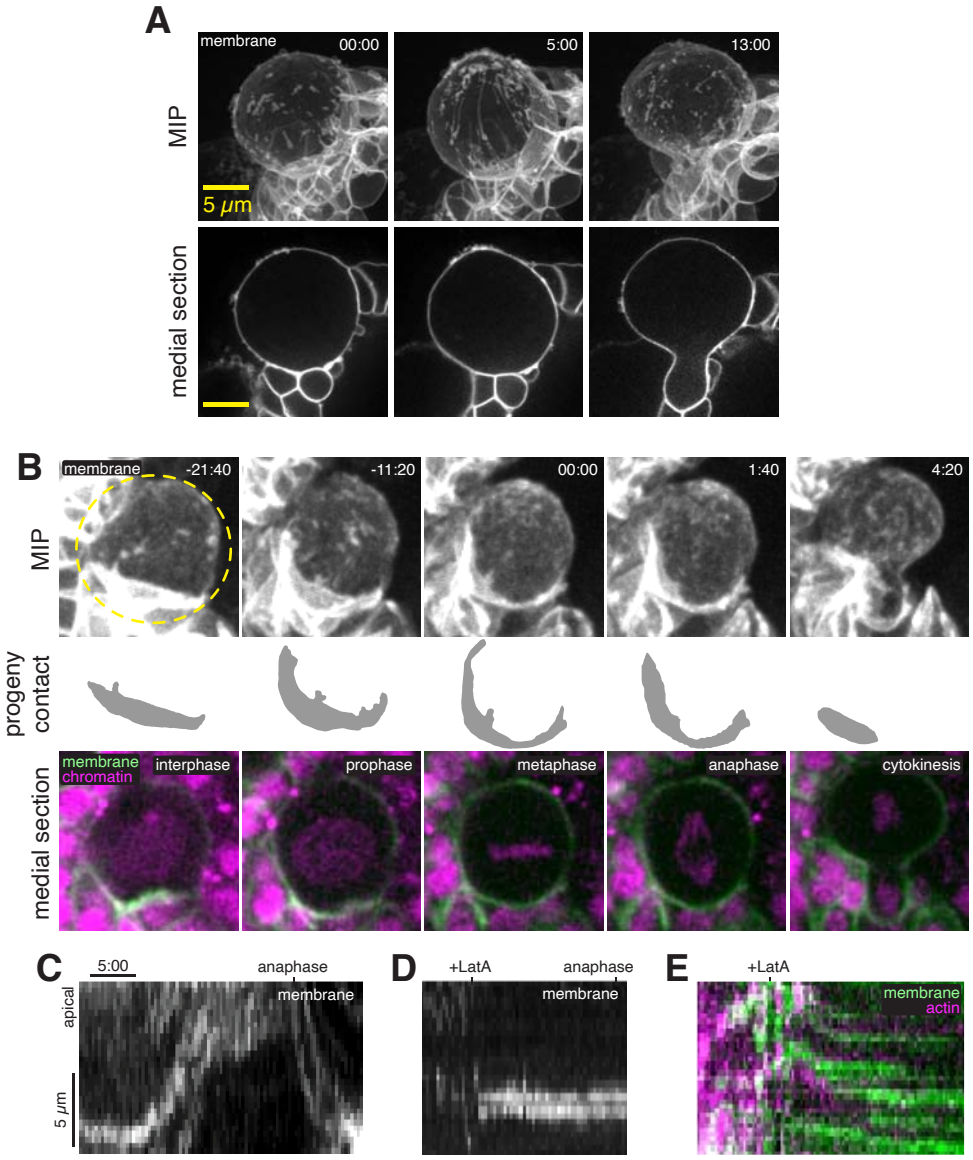

Figure 2 The neuroblast membrane undergoes a polarity cycle during asymmetric divisions (**A**) Time course of super resolution images of a dividing neuroblast and progeny cells expressing GFP-farnesyl (”membrane”). A single medial optical section is shown along with a maximum intensity projection (MIP) of optical sections through the front hemisphere. Time in minutes and seconds relative to when the first frame was collected is shown in each frame. (**B**) Neuroblast membrane dynamics through a full division cycle. Selected frames from Video S3 are shown in maximum intensity projection of the front hemisphere GFP-PH signal (”membrane”), with a manual segmentation of the basal daughter cell membrane that contacts the neuroblast (progeny contact), and a medial section with both GFP-PH and RFP-H2A (”chromatin”) signals. Time in minutes and seconds relative to anaphase onset is indicated in the upper right corner of each frame. (**C,D,E**) Kymographs along the apical-basal axis following the progression of the GFP-PH (”membrane”) signal over the course of mitosis for an untreated (C; Video S3) and a LatA treated (D,E; Video S4) neuroblast. The signal from mRuby-Lifeact (”actin”) is also shown in panel (E).

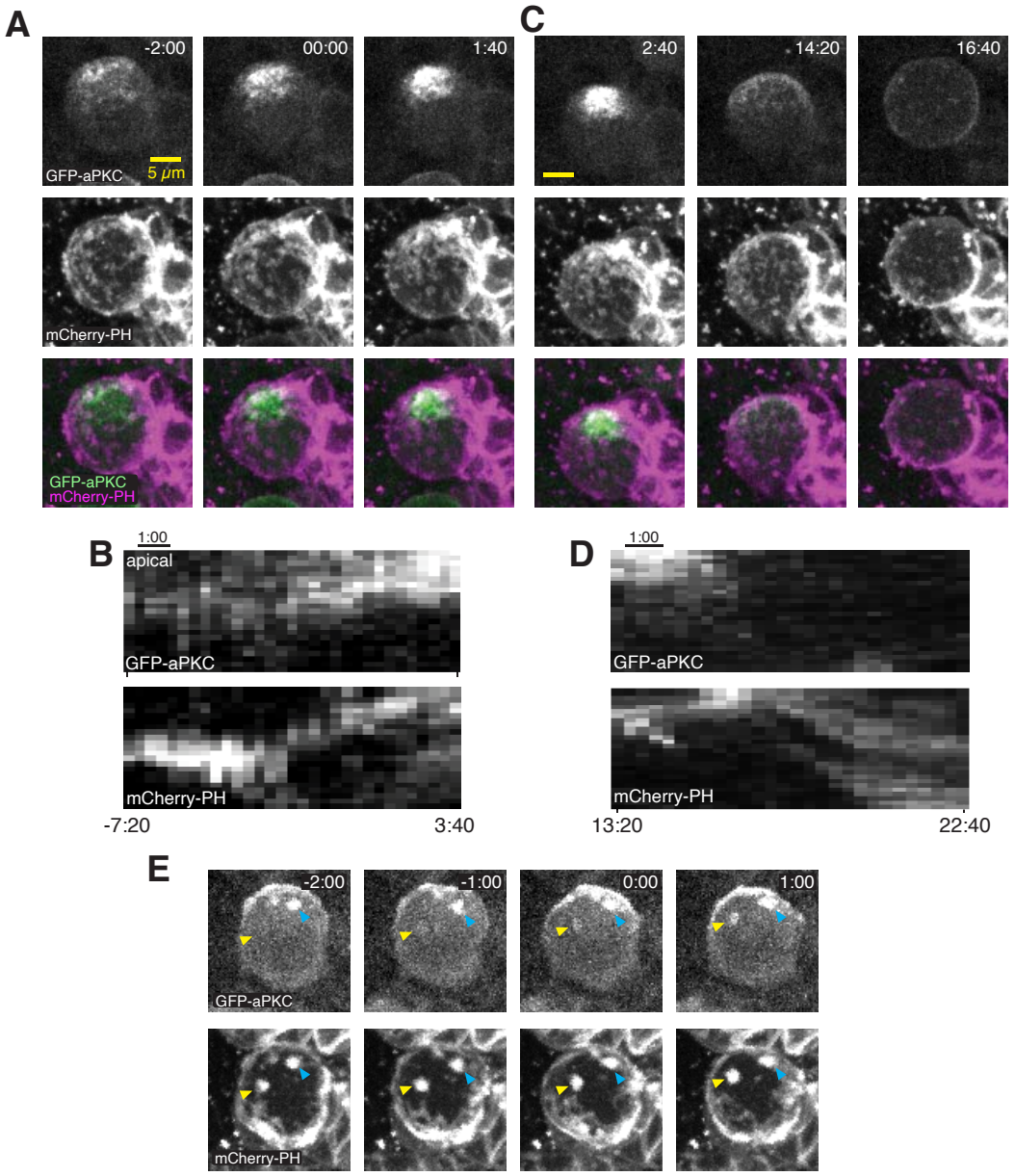

Figure 3 aPKC is recruited to apical membrane domains and protein and membrane dynamics are correlated (**A**) Simultaneous imaging of membrane and aPKC dynamics during neuroblast asymmetric division. Selected frames from Video S5 of a neuroblast expressing GFP-aPKC from its endogenous promoter and mCherry-PH via worniu-GAL4. Time relative to nuclear envelope breakdown (NEB) is shown. As described previously, aPKC accumulates on the apical membrane beginning in early prophase followed by a coalescence phase shortly before NEB. Membrane features are present throughout the cell cycle, and their movements occur simultaneously with aPKC. (**B**) Kymograph along the apical-basal axis of Video S5 showing the correlated dynamics of aPKC and the neuroblast membrane during polarization. (**C**) Correlated membrane and aPKC dynamics during neuroblast depolarization. Selected frames from Video S5 as in panel A. (**D**) Kymograph showing the correlated dynamics of aPKC and the neuroblast membrane during depolarization. The kymograph was constructed as in panel B. (**E**) Selected frames from Video S6 showing an example of aPKC recruitment to a membrane patch as it moves from the basal to the apical hemisphere. Yellow arrowheads mark the membrane patch and the corresponding aPKC signal. Cyan arrowheads mark a membrane patch and corresponding aPKC signal that starts and ends in the apical hemisphere.

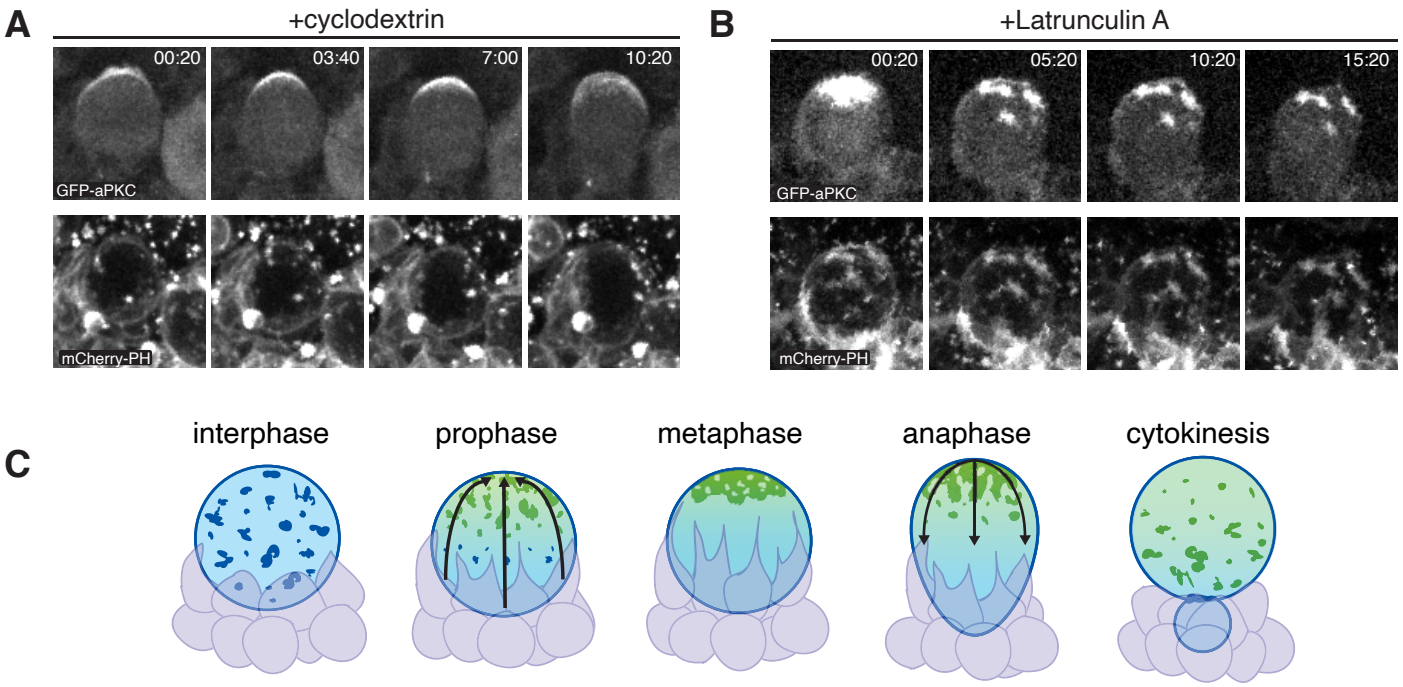

Figure 4 Membrane domains mediate aPKC polarity maintenance (**A**) Membrane and aPKC dynamics in a neuroblast in which membrane domains were removed via cyclodextrin treatment. Time is shown in minutes and seconds relative to NEB. (**B**) Membrane and aPKC dynamics in a neuroblast in which the actin cytoskeleton was disrupted by treatment with LatA. Frames following LatA addition are shown. (**C**) Model for neuroblast membrane dynamics. Membrane features are dispersed over the entire cell surface during interphase and progeny cell contacts are typically restricted to the basal pole. Apically-directed movements begin in prophase, when aPKC is recruited to the apical hemisphere of the membrane (enriched at membrane domains and diffuse elsewhere), and movements lead to metaphase polarization and deformation of progeny contacts. Basally-directed movements begin in anaphase and depolarize membrane features and return progeny cell contacts to the basal hemisphere.