Dynein-mediated transport and membrane trafficking control PAR3 polarised distribution

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ABSTRACT

The scaffold protein PAR3 and the kinase PAR1 are essential proteins that control cell polarity. Their precise opposite localisations define plasma membrane domains with specific functions. PAR3 and PAR1 are mutually inhibited by direct or indirect phosphorylations, but their fates once phosphorylated are poorly known. Through precise spatiotemporal quantification of PAR3 localisation in the *Drosophila* oocyte, we identify several mechanisms responsible for its anterior cortex accumulation and its posterior exclusion. We show that PAR3 posterior plasma membrane exclusion depends on PAR1 and an endocytic mechanisms relying on RAB5 and PI(4,5)P2. In a second phase, microtubules and the dynein motor, in connection with vesicular trafficking involving RAB11 and IKK-related kinase, IKKe, are required for PAR3 transport towards the anterior cortex. Altogether our results point to a connection between membrane trafficking and dynein-mediated transport to sustain PAR3 asymmetry.
Cell polarity is a fundamental process involved in diverse processes crucial for cell functions and development. The establishment and maintenance of cell polarity are under the control of a set of polarity proteins highly conserved through metazoans. This network of proteins, some of which PAR proteins, is set up through regulations that involve recruitments or repulsions (Assemat et al., 2008; Goldstein and Macara, 2007; Laprise and Tepass, 2011; Nelson, 2003; Rodriguez-Boulan and Macara, 2014; St Johnston and Ahringer, 2010). The precise localisation of these polarity complexes defines plasma membrane domains with specific functions (Rodriguez-Boulan and Macara, 2014; St Johnston and Ahringer, 2010). Accordingly, the polarity complexes are essential for the establishment of neural growth cone polarity (Yu et al., 2006), the apico-basal epithelial polarity (Kuchinke et al., 1998; Nance et al., 2003) or the antero-posterior polarity in Drosophila oocytes (Cox et al., 2001b; Tomancak et al., 2000) and C. elegans embryos (Kemphues, 2000).

Two major polarity modules control the establishment and maintenance of cell polarity: one module composed of the PAR3 (also named Bazooka [BAZ] in Drosophila), PAR6 and aPKC proteins, and a second module constituted of the PAR1, LGL and SLMB proteins (Goldstein and Macara, 2007; Rodriguez-Boulan and Macara, 2014; St Johnston and Ahringer, 2010). The localisation of these two modules is mutually exclusive and mainly involves interplays between physical interactions and phosphorylations (Coopman and Djiane, 2016). aPKC phosphorylates PAR1 to exclude it from the PAR3 cortical domain. Conversely, PAR3 is phosphorylated by the kinase PAR1, recognized by the 14.3.3 proteins and excluded from the PAR1 cortical domain (Benton and St Johnston, 2003; Morais-de-Sa et al., 2010). PAR3 is also maintained at the plasma membrane through physical interactions with membrane lipids, phosphoinositides (Krahn et al., 2010a). We reported in particular that the PI(4,5)P2 (phosphatidylinositol 4,5 bisphosphate) controls PAR3 apical targeting, in epithelial cells (Claret et al., 2014). In addition, the cytoskeleton seems to be important for a proper localisation of PAR3 and it is noteworthy that, the apical actin network is required to position PAR3 apically as well as microtubules (MTs) via a dynein-dependent transport in Drosophila embryos (Harris and Peifer, 2005; McKinley and Harris, 2012).

In Drosophila oocyte, PAR3, at the anterior cortical domain, and PAR1, at the posterior, specify the polarity axes by controlling the MT organisation (Cox et al., 2001a; Doerflinger et al., 2003), and thus the localisation of determinants such as bicaudal, oskar and gurken mRNAs, crucial for the subsequent future embryo development (St Johnston, 2005).
Generating mutually exclusive cortical domains is especially important for the polarisation of this large single-cell system. However, throughout oogenesis, the asymmetric localisation of PAR proteins is dynamic and PAR1 and PAR3 domains are not always mutually exclusive. During early oogenesis, the localisation of PAR3 and PAR1 is independent (Huynh et al., 2001), while at mid oogenesis (stage 7-8), PAR3 and PAR1 overlap at the posterior plasma membrane. In contrast, they both show mutually exclusive localisations at stage 9 (Doerflinger et al., 2010).

During stages 8 to 10, which correspond to the critical period for the localisation of the polarity axes determinants (Steinhauer and Kalderon, 2006), the oocyte undergoes a rapid threefold size increase while the subcellular localisation of PAR modules has to remain strictly conserved. Although the mutual antagonism between the PAR3/aPKC/PAR6 and PAR1/LGL/SLMB modules plays an important role to set up PAR3 restriction (Morais-de-Sa et al., 2014; Tian and Deng, 2008), the molecular mechanisms involve in these processes remain elusive and may not be sufficient to sustain PAR3 asymmetry. Indeed computer modelling have pointed out that mutual antagonism between the two complexes is not enough to maintain the asymmetry between the polarity determinants (Fletcher et al., 2012). Moreover, the fate of PAR3, once phosphorylated by PAR1, and how it is redirected from posterior to the anterior cortex is unknown. PAR3 could diffuse laterally in contact with plasma membrane until it reaches the anterior, or it could be dispersed from the posterior membrane in the cytoplasm as it has been suggested for the Drosophila embryo (McKinley and Harris, 2012) and then recycled. Our previous findings have highlighted a role of the PIP5Kinase Skittles (SKTL) and its product PI(4,5)P2 in PAR3 localisation in oocyte and in epithelial cells (Claret et al., 2014; Gervais et al., 2008). PI(4,5)P2, among others functions (Tan et al., 2015; Zimmermann et al., 2005), is crucial to recruit the endocytic machinery at the plasma membrane during the first step of endocytosis (Compagnon et al., 2009; Posor et al., 2015). We hypothesize that SKTL, by controlling endocytosis and/or vesicular trafficking could regulate indirectly PAR3 localisation.

Here, by using a quantitative analysis, we have examined the precise evolution of PAR3 distribution during stages 8 to 10 of oogenesis. We show that PAR3 is excluded from the posterior cortex much later than the establishment of PAR1 to the posterior and that actin cytoskeleton and endocytosis are important for this process. Subsequently, MTs and dynein motor are required for PAR3 transport to the anterior plasma membrane. This transport is connected with vesicular trafficking, cytoplasmic PAR3 being associated with PI(4,5)P2 enriched endosomes. We evidence physical interactions between PAR3, SKTL and the dynein...
light intermediate chain DLIC, which could explain the transport of PAR3 by dynein directly on vesicles. Finally, we found that IKK-related kinase, IKKε in the correct localisation of PAR3. The knockdown of IKKε leads to an accumulation of PAR3 near the minus ends of MTs. Altogether our results point to a connection between membrane trafficking and dynein mediated transport to sustain PAR3 asymmetry in the Drosophila oocyte.
RESULTS

Fine tuning of PAR3 distribution along the anterior posterior axis.

In order to characterize the evolution of PAR3 distribution during oocyte development, we developed a quantification method to monitor the precise variation of PAR3 distribution along the plasma membrane during late oogenesis (Figure 1A). The oocyte plasma membrane was therefore subdivided in three regions: the anterior plasma membrane (APM) that corresponds to the membrane in contact with the nurse cells, the posterior plasma membrane (PPM) that corresponds to the posterior domain of the oocyte where PAR1 and Staufen are localized (Figure 1-Figure supplement 3A-B), and the lateral plasma membrane (LPM) that corresponds to the plasma membrane between the two previous regions (Figure 1A). The APM domain corresponds to the juxtaposition of the oocyte and nurse cells plasma membranes. Therefore, for each oocyte individually, we quantified the mean fluorescent intensity of oocyte adjacent nurse cells. We then removed, from the anterior signal, the mean fluorescent intensity of the nurse cells plasma membrane to precisely quantify the signal coming only from the APM of this oocyte. Afterwards the intensity profile along the plasma membranes as well as the global intensity by plasma membrane and cytoplasm domains were compiled and the signal density was analysed (see Materials and methods for details).

To identify and to characterize PAR3 regulation mechanisms, we first used a Drosophila strain expressing a Bac-encoded PAR3-GFP under its endogenous regulatory regions, in absence of endogenous PAR3 (Figure 1-Figure supplement 1A, (Besson et al., 2015)). However, the accumulation of PAR3 at the apex of somatic follicular cells around the oocyte (Figure 1-Figure supplement 1B) masks the potential localisation of PAR3 at the plasma membrane of oocyte, the two membranes being very close. To circumvent this difficulty, we chose to follow PAR3 with a GFP tag, expressed only in the oocyte and its associated nurse cells (Benton and St Johnston, 2003). In this context, as previously reported (Doerflinger et al., 2010), PAR3 mostly accumulates at the anterior plasma membrane (Figure 1B-C). With our quantitative tool, we measured PAR3 signals coming from the different plasma membrane subdomains during late oogenesis (Figure 1D). We observe a clear increase of PAR3 quantity at the APM (in red) from stage 8 to stage 10 and in parallel an exclusion of PAR3 on the PPM (in blue). Thereafter, to avoid the fluctuations associated to the membrane growth during oogenesis or to experimental procedures, we have normalised the raw data to
the total amount of PAR3 signal in each oocyte and related to the membrane length (cf. materiel and methods, and Figure 1-Figure supplement 2). With this density results, although we cannot compare the quantity between different stages, we can follow the evolution of asymmetry in a stage. As expected, PAR3 density is the highest at the oocyte APM (Figure 1E). However, PAR3 density repartition presents a striking dynamic in both PPM and LPM.

At stage 8 and early stage 9 (stage 9A, Figure 1-Figure supplement 1C), PAR3 is denser at the PPM than at the LPM (Figure 1C, green arrows). Then at late stage 9 (stage 9B, Figure 1-Figure supplement 1C), PAR3 is progressively excluded from the PPM (Figure 1C, red arrows). These changes in PAR3 distribution reflect the establishment of two distinct plasma membrane domains and are highlighted by the asymmetric ratio of PAR3 measured by anterior to posterior density ratio (Figure 1F). The beginning of PAR3 posterior exclusion in the middle of stage 9 correlates with the localisation switch of other factors like Staufen (Figure 1-Figure supplement 3B) toward the posterior pole of the oocyte and correlated to the MT network reorganisation (Januschke et al., 2006). This result is surprising as PAR1 is assumed to exclude PAR3 from the plasma membrane yet PAR1 is already localised at the posterior pole since at least stage 7, long before PAR3 exclusion (Doerflinger et al., 2010). This may indicate that other processes participate to the disappearance of PAR3 from the PPM and the LPM. As the LPM appeared to follow the PPM comportment, thereafter we focused on the APM accumulation and on the PPM exclusion, two mechanisms important for the establishment of the antero-posterior polarity.

**Posterior exclusion and anterior accumulation are two PAR3 localisation processes that can be decoupled/separated**

In order to understand how PAR3 is excluded from the posterior domain and enriched at the anterior domain in the oocyte, we investigate further cytoskeleton involvement in this process. Upon latrunculin drug treatment, PAR3 is still predominantly at the APM like in control condition (Figure 2A, 2C, Figure 2-Figure supplement 1). However its posterior exclusion, which normally occurs at stage 9B, is not observed (Figure 2B) indicating that the actin network is required for PAR3 posterior exclusion. We next addressed the potential MT requirement for PAR3 antero/posterior distribution in the oocyte. In the presence of colchicin, a drug that depolymerises MT, PAR3 polarized distribution along the antero/posterior axis is lost and tends toward isotropy (Figure 2A, 2C, Figure 2-Figure supplement 1). Compared to control, PAR3 APM localisation is strongly reduced (Figure 2C). However, PAR3 is still
excluded from the PPM even if it is to a lesser extent than the control (Figure 2A-B). Thus, it confirms the importance of MTs in PAR3 polarised localisation except to the posterior region where exclusion is still present. Hence it appears that in the oocyte, posterior exclusion and anterior accumulation can be uncoupled. We also notice that both MT and actin disassembly increases the cytoplasmic accumulation of PAR3 in dotted structures (Figure 2D). We verified that these dotted structures are also detected in absence of PAR3 overexpression with a PAR3-Protein trap strain (Januschke and Gonzalez, 2010). In this condition, we can observe some dotted structure in the oocyte cytoplasm (Figure 2 - Figure supplement 2A). Furthermore, when microtubules are depolymerized by colchicin, PAR3 accumulates in numerous dotted structures in the cytoplasm (Figure 2 – Figure supplement 2B-D).

**SKTL by producing PI(4,5)P2 controls anterior accumulation and posterior exclusion of PAR3**

PAR3 is a cytoplasmic protein that can interact with membranes by direct interaction with the phospholipids in particular PI(4,5)P2 (Claret et al., 2014; McKinley et al., 2012; Wu et al., 2007) and/or by interaction with membrane associated proteins like those of adherens junction (Coopman and Djiane, 2016).

We previously shown that the PIP5Kinase SKTL, by providing the phosphoinositide PI(4,5)P2, is crucial to maintain PAR3 at the adherens junctions in epithelial cells (Claret et al., 2014). In oocytes, PAR3 is associated with the plasma membrane (Gervais et al., 2008) but also forms some particles in the cytoplasm. These particles are also associated in part with PI(4,5)P2 containing membranes (Figure 3G) and the lipid kinase SKTL, that produces PI(4,5)P2 (Figure 3H). By immunostaining and by colocalisation using Mander’s overlap coefficient, we identified that a half of PAR3 vesicles (50.05% ± 0.08 SEM, n=8) are PI(4,5)P2-positive (Figure 3G).

We then investigated SKTL requirement upon PAR3 polarized distribution along the oocyte anterior posterior axis focusing both on the amount of SKTL and on its kinase activity. In absence of SKTL (sktl<sup>2.3</sup>/sktl<sup>Δ5</sup>), PAR3 is more accumulated at the PPM than the APM (Figure 3A, 3D and 3E). Conversely, when SKTL is overexpressed (OE), PAR3 is more excluded from the PPM than in the control condition (Figure 3B, 3D and 3E). Thus SKTL seems to have preponderant function to exclude PAR3 from the PPM and to increase PAR3
density at the APM. To monitor whether SKTL kinase activity is required for this process, we performed the same experiment with a kinase dead form, SKTL\textsuperscript{DNRQ} whose mutation in mammalian homolog leads to a dominant negative effect (Coppolino et al., 2002). In such case, SKTL\textsuperscript{DNRQ} overexpression does not enhance PAR3 exclusion from the PPM (Figure 3C, 3D, 3E). PAR3 distribution along the anterior posterior axis tends to be isotropic (Figure 3C, 3D). We can conclude that SKTL kinase activity, hence the production of PI(4,5)P\textsubscript{2}, is essential to regulate PAR3 distribution.

**SKTL can bypass PAR1 dependant posterior exclusion**

Upon phosphorylation, PAR1 excludes PAR3 from the posterior domain of the oocyte (Benton and St Johnston, 2003). Accordingly, with our quantification method, we find that PAR3 failed to be excluded from the PPM upon PAR1 knockdown by RNAi (Figure 4B, 4D and Figure 4-Figure supplement 1). Likewise, a PAR3-AA mutant form, non phosphorylatable by PAR1 (Benton and St Johnston, 2003), is not excluded from the PPM (Figure 4C-D). Thus, PAR1 through its role in PAR3 phosphorylation is required to exclude PAR3 from the PPM. As SKTL also controls the posterior exclusion of PAR3, we combined the knockdown of PAR1, which presents normally no PAR3 exclusion, with the overexpression of SKTL, which increases the exclusion. In this case we observe that SKTL is still able to exclude PAR3 even in absence of PAR1 (Figure 4E). We then confirm this observation by using the PAR3-AA mutant form (Figure 4E). In this case too, SKTL overexpression is still able to exclude PAR3 from the posterior membrane. These results suggest that SKTL leads to PAR3 exclusion independently of PAR3 phosphorylation by PAR1.

**Endocytosis RAB5 dependent is important to PAR3 PPM exclusion**

In order to understand how SKTL could induce the PPM exclusion of PAR3, we investigate the implication of endocytosis in this process. PI(4,5)P\textsubscript{2} has numerous functions in the cell of which a role in the first steps of endocytosis (for review (Posor et al., 2015)). In *Drosophila* oocyte, PIP(4,5)P\textsubscript{2} is required for endocytic-vesicle formation and the small GTPase RAB5 is required for the maturation of these early endocytic vesicles (Compagnon et al., 2009).
As we previously described, in addition to a plasma membrane localisation, PAR3 is also detected in dotted structures. By immunostaining and by colocalisation using Mander’s overlap coefficient, we identified that a half of PAR3 vesicles (50.2% ± 0.05 SEM, n=10) are RAB5-positive, an early endosomes marker (Figure 5A). Moreover, when RAB5 activity is impaired by the expression of a dominant negative form, RAB5S43N (Entchev and Gonzalez-Gaitan, 2002), as revealed by lipophilic dye uptake, the endocytosis is strongly reduced at the posterior of the oocyte (Figure 5-Figure supplement 1A-B) and PAR3 distribution is affected (Figure 5D, 5E). The same result is observed by using a RAB5 knockdown (Figure 5C and 5E, RAB5 RNAi). PAR3 accumulation at the APM is lost and its density significantly increases at the PPM, leading to a lost of its PPM exclusion (Figure 5E, 5F). Thus, the proper PAR3 distribution along the antero-posterior axis seems to rely on endocytosis to be removed from the PPM and to be enriched at the APM.

Recycling pathway RAB11 dependant is required to PAR3 APM enrichment

To precise the nature of all of the PAR3 dotted structures that can be detected in wild type context, we immunostained the different compartments of the cell and measured their colocalisation with PAR3 (Figure 6A).

PAR3 associated vesicles are enriched in PI(4,5)P2, and colocalise with RAB5-positive compartment (early endosome) but also with RAB11-positive compartment (26.8% ± 0.06 SEM, n=13) of cytoplasmic PAR3 colocalise with RAB11-recycling endosomes, Figure 6A, 6B). There is no colocalisation with endoplasmic reticulum (KDEL), Golgi compartment (SYX16) or late endosome (HRS) markers (Figure 6A and Figure 6-Figure supplement 1). Taken together, these results indicate that PAR3 can be associated with RAB5 and RAB11 endosomes. However, endosomes could be a mosaic of several RAB domains present in continuity on the same membrane vesicle (Sonnichsen et al., 2000; Wandinger-Ness and Zerial, 2014). Here we do not know if PAR3 is associated with a unique endosome supporting RAB5 and RAB11 or with a specific compartment containing only one RAB.

In order to know if RAB11 is involved in the asymmetrical distribution of PAR3, we knock down RAB11 in the oocyte through germline clones with rab11p2148 allele, which does preclude oocyte development (Jankovics et al., 2001) and monitor PAR3 distribution (Figure 6F). There is no significant difference on PAR3 PPM exclusion between control (Figure 6D-E) and rab11p2148 mutant clones (Figure 6D and 6F). However we noticed a RAB11 effect on
PAR3 APM enrichment (Figure 6C). Thus RAB11 seems to be more important for APM accumulation than for the PPM exclusion.

Finally we monitored whether PAR3, which comes from the nurse cells through the ring canals, is also connected to membrane traffic. We noticed that PAR3 accumulates in the vicinity of the ring canals and that it is associated with PI(4,5)P2 membrane and with RAB11 endosomes (Figure 6- Figure supplement 2). This suggests that neo-synthetized PAR3 is also transported in the oocyte in association with endosomes.

**DLIC, PAR3 and SKTL are interacting partners**

In order to uncover new interacting partners for SKTL upon polarity establishment of PAR3, we performed a proteomic screen by immunoprecipitation and then mass spectrometry analysis. We identify, after immunoprecipitation of SKTL-GFP, a peptide of the dynein light intermediate chain (DLIC), which is absent in the control. DLIC is an essential subunit of the MT-based dynein motor (Reck-Peterson et al., 2018). This interaction between SKTL and DLIC was confirmed by western blot with anti-DLIC antibody (Figure 7E).

Interestingly, LIC2, DLIC mammalian homologue, interacts with PAR3 through the N-terminal dimerization and PDZ1 domains of PAR3 (Schmoranzer et al., 2009). Thus we investigate whether this interaction is conserved in *Drosophila*. We immunoprecipitated DLIC-GFP in ovarian extract and identify a weak association with endogenous PAR3 (Figure 7D). Interestingly, it seems that we coimmunoprecipitate predominantly a high molecular weight PAR3 form (Figure 7 - Figure supplement 2). This weight could correspond to an oligomeric form of PAR3 (Kullmann and Krahn, 2018).

Since DLIC interacts with PAR3 and SKTL, we wonder whether an interaction between PAR3 and SKTL is also occurring. Through immunoprecipitation, we found a physical interaction between PAR3 and SKTL (Figure 7E). Hence, as described for other PI(4,5)P2 effectors (Choi et al., 2015), PAR3 seems to form a tripartite complex with PI(4,5)P2 and SKTL, the enzyme that produces it (Figure 3H).

Dynein regulates the transport of PAR3 to the anterior plasma membrane but has only a modest impact on posterior exclusion
We have shown that MTs are important to asymmetric localisation of PAR3 and that PAR3 interacts with DLIC. In oocytes (stage 8-10), the MT network forms a gradient following the antero/posterior axis (Januschke et al., 2006). As MTs are more abundant and more nucleated (minus ends) at the anterior pole of oocyte (Khuc Trong et al., 2015; Parton et al., 2011), dynein, as a minus end directed motor, could be necessary for PAR3 accumulation at the APM. To investigate this possibility, we therefore knocked down dynein by expression of ShRNAs directed against the dynein heavy chain isoform (DHC64c) in the oocyte (Sanghavi et al., 2013). In this condition, PAR3 accumulation is lost at the APM and its level significantly increases at the LPM (Figure 7A-B). Nevertheless PAR3 is still excluded from PPM (Figure 7C) consistently with the weak MT requirement for this process (Figure 2B). Taken together these data indicate that PAR3 could be transported in complex with the dynein motor on MTs toward the APM.

Moreover, we noticed that upon Dhc64C inactivation, PAR3 is not evenly distributed along the plasma membrane but is retained in dotted structures below (Figure 7A and Figure 7-Figure supplement 1A). Moreover PAR3 is still associated with PI(4,5)P2 vesicles when MTs are impaired in presence of colchicin (Figure 7- Figure supplement 1B). Thus, the PAR3 transport to the APM depends on MTs, but the association of PAR3 with vesicles does not.

**Connection between posterior exclusion and anterior accumulation of PAR3**

Our results indicate that PAR3 oocyte distribution relies on two separated processes an exclusion from the posterior plasma membrane and an accumulation to the anterior plasma membrane; we then investigate whether the two processes can be connected. To this end, we monitor PAR3 dynamics in the oocyte through fluorescence recovery after photobleaching (FRAP) of PAR3-GFP. Since a substantial part of PAR3 that accumulates at the anterior part of the oocyte could come from synthesis and subsequent transport from the nurse cells through the ring canals (Figure 6 – Supplement 2 and (Doerflinger et al., 2010)), we wanted to dissociate this PAR3 arrival from the potential relocalisation of PAR3 in the oocyte from the posterior. To do so, we chose to photobleach PAR3-GFP localised in the nurse cells and at the APM (Figure 8A), at stage 9A (video 1). We then followed both the evolution of the PAR3-GFP fluorescence at the APM and at the PPM (Figure 8A). By this way, we monitor only the PAR3 relocalisation process within the oocyte. We observe that after photobleaching, both by quantity and through normalized fluorescent measurement, PAR3 accumulates progressively to the anterior while it is excluded from the posterior (Figure 8B...
and 8C). This indicates that PAR3, already present in oocyte, can be transported to the APM. The difference in quantity between the PAR3 which accumulates at the anterior and the one which is excluded from the posterior, could be explain by the presence of PAR3 in transit into the cytoplasm.

We next address whether this transport in the oocyte relies on MT cytoskeleton as predicted from our results. We repeated PAR3 FRAP experiments in presence of colchicine (Figure 8D). We observe that after photobleaching, the anterior accumulation and the posterior exclusion of PAR3 are significantly reduced (Figure 8C). Thus, this result shows that MT network is important for the anterior relocalisation of PAR3 fraction from the posterior in the oocyte.

**IKKe/IK2 controls asymmetrical localisation of PAR3**

We have shown that PAR3 localisation depends on the MT network and its associated dynein motor. Moreover, in the cytoplasm PAR3 localised on RAB11-positive vesicles and RAB11 is important for PAR3 APM accumulation. Our results suggest that PAR3 is transported in association with MTs and recycling endosome vesicles to the APM. Interestingly in the proteomic screen for SKTL partners, we also identified the IKKe/IK2 kinase. IKKe has been shown to regulate RAB11 associated cargos transport with dynein in developing bristles (Otani et al., 2011). Furthermore, IKKe phosphorylates a RAB11 cofactor, NUF, in order to release the cargos from the dynein motor at the MT minus end. Interestingly, in oocyte, IKKe is specifically enriched at the APM (Dubin-Bar et al., 2008). We then monitor whether IKKe is required for PAR3 polarized distribution. Upon IKKe RNAi mediated knocked down in the oocyte, PAR3 becomes isotropic all along the plasma membrane, without clear accumulation at the APM (Figure 9B). Moreover PAR3 piles up in the cytoplasm (Figure 9C) in large circular structures surrounded by actin mesh that we called actin clumps (ACs) thereafter (Figure 9A and Figure 9 - Figure supplement 1A). We further notice that aPKC, a partner of PAR3, is also present on ACs (Figure 9 - Figure supplement 1C). Interestingly, ACs accumulates near MT minus ends close to the oocyte nucleus as revealed by Nod-LacZ transgene (Clark et al., 1997) (Figure 9 - Figure supplement 2B-C).

We then investigate the nature of those structures and in particular their association with endocytic vesicles. ACs colocalise with RAB11 (Figure 9D) and NUF positive-vesicles (Figure 9 - Figure supplement 1B). RAB5 vesicles are not enriched in the ACs but are all
around them (Figure 9E). Furthermore, there is no colocalisation with Syntaxin16 highlighting Golgi and Lysosome compartment (Akbar et al., 2009) (Figure 9 - Figure supplement 1D).

These structures also contain SKTL (Figure 9G) and PI(4,5)P2 (Figure 9F), suggesting that PAR3, is still associated with PI(4,5)P2/SKTL membrane during its transport. Moreover ACs are strongly reduced when SKTL kinase dead form is expressed (SKTL$^{DNRQ}$, Figure 9H), or disappear in sktl mutant (sktl$^{2.3}$/sktl$^{Δ5}$, Figure 9I). Hence in absence of IKKε, SKTL activity and PI(4,5)P2 are necessary for ACs accumulations.

Furthermore in absence of IKKε, the dynein subunit DLIC, which interacts with PAR3 and SKTL, is held back on structures similar to ACs (Figure 9 - Figure supplement 2A) at the MTs minus ends (Figure 9 - Figure supplement 2B-C). Importantly, in absence of MTs, upon colchicin treatment, ACs disappear indicating that their accumulation depends on the MT network (Figure 9K compared to Figure 9J). Thus, in oocyte, IKKε seems to regulate the connection between PAR3 associated to RAB11-positive vesicles and MT minus ends.
DISCUSSION

Taken together our results shed light on a dual step process that sustains PAR3 asymmetry in the *Drosophila* oocyte (Figure 10). The first one occurs at the middle of stage 9 and is in charge of PAR3 exclusion from the PPM. It involves PAR1, the actin cytoskeleton, PI(4,5)P2 and RAB5 dependant endocytosis. The second step relates to PAR3 accumulation at the oocyte anterior side. It brings into play MT associated transport with the minus end directed motor, dynein in association with vesicular trafficking. Furthermore, both of these steps rely on PAR3 interactions with PI(4,5)P2 endosomal vesicles.

To exclude PAR3 from the PPM, the precise role of the actin cytoskeleton remains to be clarified. An attractive hypothesis is that the posterior actin network could act on PAR3 removal from the plasma membrane, possibly by regulating directly endocytosis process (Figure 10C1). In support of this idea, a connection between actin and a specific Oskar dependant endocytosis pathway has already been highlighted at the oocyte posterior pole (Tanaka and Nakamura, 2011; Vanzo and Ephrussi, 2002; Vazquez-Pianzola et al., 2014). However, the actin requirement could also be connected with the role of actin in PAR1 posterior localisation (Doerflinger et al., 2006). Indeed, in presence of latrunculin, PAR1 is not focused to the PPM but its localisation become isotropic. Consequently posterior PAR3 would be less phosphorylated by PAR1 and so less excluded.

How is achieved the posterior to the anterior redistribution of PAR3 in the oocyte? Recent analyses on MT-associated transport of mRNA particles in the oocyte have shown that the MT network is polarized with a slight bias for MT plus ends towards the posterior (Trovisco et al., 2016; Zimyanin et al., 2008). Since PAR3 is associated with the MT minus end directed dynein motor, this MT polarity bias could be used to relocate PAR3 associated with vesicles towards the APM (Figure 10A). However this anterior redistribution is unlikely to be direct due to the weak MT polarization bias (Khuc Trong et al., 2015). Hence, it may involve intermediate re-localisation steps along the LPM before being redirected toward the APM, which is in accordance with the observed PAR3 distribution.

In the last few decades, the mutual inhibitions of PAR1 and PAR3 have been well studied at a functional level, and the molecular mechanism relies on some direct or indirect phosphorylations (Figure 10C2). However, the fate of PAR3 after phosphorylation by PAR1 at the posterior is unknown. PAR3 does not seem to be degraded in a SLMB/E3 ubiquitin ligase-dependent manner at the PPM, unlike aPKC or PAR6 (Morais-de-Sa et al., 2014). By FRAP approach, we have shown that posterior fraction of PAR3 proteins contributes after re-
localisation through a MT-dependant process to the anterior pool of PAR3. It is important to mention, that this anterior fraction of PAR3 coming from the posterior of the oocyte is likely to be minor compare to the anterior pool of PAR3 which is directly coming from the nurse cells.

Our results indicate here that PAR3 and PAR1 coexist at the PPM until stage 9A indicating that the relations between them both are more complex and that an additional process may be required to exclude PAR3 from the PPM. Starting from stage 9B, PAR1 excludes PAR3 from the PPM but has little effect on PAR3 anterior accumulation. However, the strict exclusion of PAR3 on this small posterior domain is sufficient to obtain an important anterior/posterior asymmetry.

PAR3 is a cytoplasmic protein, but here we show that it is often found in association with membranes, plasma membrane or endocytic membrane (early/recycling endosome). Interestingly in mammalian epithelial cells PAR3 associate with exocyst components (Ahmed and Macara, 2017). This association is present in the oocyte, but is also observed in nurse cells, particularly at the front of the ring canals where PAR3 accumulates in PI(4,5)P2 and RAB-positive compartments. How, PAR3 as cytosolic protein could be transported with endocytic membranes? PAR3 could be associated to a cargo bound to vesicles, which are then transported to the anterior. In parallel, PAR3 could also control its own association with the vesicles. Indeed, PAR3 interacts with SKTL that in return produces PI(4,5)P2 that stabilises PAR3 to the membrane (Claret et al., 2014; Krahn et al., 2010b). This further points the importance of SKTL in PAR3 APM accumulation and PPM exclusion. PI(4,5)P2 being a well-known regulator for the first steps of endocytosis (Posor et al., 2015), we hypothesize that SKTL acts on PAR3 PPM exclusion by regulating the formation of vesicles required for future PAR3 anterior targeting. Our results provide also evidences for a role of PI(4,5)P2/SKTL in recycling endosome sorting. PI(4,5)P2 has been described to be present both at the cell surface and on the distal portions of the tubular endosome (Brown et al., 2001). PI(4,5)P2 is required for the recruitment to the membrane of many proteins involved in vesicle formation, fusion and actin polymerization (Posor et al., 2015). This suggests that PI(4,5)P2 and SKTL would be important on membranes, including that of recycling endosomes, for the budding and the sorting of specific elements of which PAR3.

Here, we show that IKKe participate to the establishment of PAR3 asymmetry in the oocyte. Upon IKKe knockdown, PAR3 accumulates with aPKC on RAB11 positive-vesicle aggregates suggesting that PAR3 transport at the MT minus ends is arrested. In polarized
bristles, IKKε was previously shown to take the RAB11 endocytic vesicles down to the MTs minus ends (Otani et al., 2011). In oocyte, the mechanism could to be similar (Figure 10B). In IKKε−/− knockout, vesicle aggregates accumulates at the MT minus ends with dynein subunit DLIC. This accumulation depends on MTs but also on PIP(4,5)P2/SKTL. These vesicles enriched for PI(4,5)P2, SKTL and able to nucleate actin present common characteristics with the anterior plasma membrane. They could constitute a pre-built platform in the cytoplasm before being targeted to the appropriate position at the plasma membrane. This idea of a preformed platform has already been suggested in the tracheal system where PAR3 associates with recycling endosomes containing E-cadherin during adherens junction rearrangements (Le Droguen et al., 2015). Moreover, during de novo apical lumen formation in MDCK cells and in human pluripotent stem cells, there is an accumulation of apical components in RAB11 positive-vesicles that are subsequently delivered to the apical plasma membrane (Overeem et al., 2015; Taniguchi et al., 2017). The first sign of apical domain formation in these cells is the relocation of the polarity protein PAR3.
**Materials and Methods**

**Fiji macro and quantification methods**

To quantify membrane and cytoplasm repartition of proteins in oocytes, we developed a macro on Fiji (cf. Oocyte Analysis macro). In each oocyte, we selected 3 points (two on both sides of the anterior membrane and one in the middle of the posterior membrane). 4 sections of its plasma membrane were then automatically generated (anterior; lateral 1; lateral 2; posterior) independently of the oocyte stage (See Figure 1-Figure supplement 3 for details). After delimitation of the plasma membrane with the plot profile tool, we obtained its intensity profile. After delimitation of each plasma membrane domains with the polygon tool, we obtained the mean fluorescent intensity and the length of these domains. The anterior signal corresponds to the signal from the APM of the oocyte and the signal from the plasma membrane of the neighbouring nurse cells. Therefore, for each oocyte individually, we quantified the mean fluorescent intensity of a simple and a double plasma membrane of adjacent nurse cells. We then removed, from the anterior signal, the mean fluorescent intensity of the nurse cells plasma membrane to precisely quantify the signal coming only from the APM of this oocyte (Figure 1-Figure supplement 3C). Finally, after delimitation of the cytoplasm we obtained its mean signal intensity. For each oocyte individually, the measured intensity signals were normalised in two steps. First, all the raw quantities (grey level intensity) are divided by the total intensity in the oocyte (APM + PPM + LPM + Cytoplasm). Second, the plasma membrane values are expressed in density i.e. that the length of the corresponding membranes divides them (see intermediate quantification in Figure 1-Figure supplement 2). This process was performed on oocyte individually, and then the results were pooled by genotypes and/or conditions and/or developmental stages. In our representation of the density, only the plasma membrane domains are indicated and not the cytoplasm. So the density of the different cortical zones does not add up to one. The quantity corresponds to the grey level intensity measured on the image. The density represents the same values divided by the length of corresponding membranes. Between a stage 9A and a stage 10, the oocyte plasma membrane overgrowths. The density shows how PAR3 are concentrated at the membrane by avoiding the oocyte and plasma membrane size variation. The posterior exclusion ratio is the ratio of LPM density on PPM density. The asymmetry ratio is the ratio of APM density on PPM density.
To quantify the co-localisation of PAR3 with other proteins, the Coloc 2 plugin (Fiji) was used. Oocytes (between 7 and 13) of several females per genotype were analysed; the cytoplasmic contour was delimitated and the quantification was performed only for one plan in this zone. The Pearson correlation coefficient (PC) or the Manders overlap coefficient (MOC) was determined in single confocal images. The PC value ranges from -1 (no correlation) to +1 (complete correlation) with values in between indicating different degrees of partial correlation. The negative control was provided by quantifying Pearson’s coefficient for the same images, but after rotation of one by 90 degrees, a condition in which only random colocalisation is observed. The MOC measures the fraction of PAR3 signal overlapping with red signal and vice versa.

**Fly stocks**

Mutant sktl^{2,3} has been described in (Gervais et al., 2008), sktl^{A5} in (Hassan et al., 1998) and par-1^{w3} and par-1^{6323} in (Shulman et al., 2000). The following fly stocks were also used: canton-S as wild type; UASp-PAR3-GFP and UASp-PAR3^{4A}-GFP (Benton and St Johnston, 2003); UASp-GFP-PAR1(NIS) (Doerflinger et al., 2006); Ubi-PH_{PLC}-RFP (Claret et al., 2014); pUbi-DLIC-GFP (Pandey et al., 2007); Pubi-PDI-GFP (Bobinnec et al., 2003); UASp-RAB5DN^{(S43N)} (Pelissier et al., 2003); FRT82B rab11^{P2148;2D1} (Jankovics et al., 2001); Bac PAR3-GFP = P[w+, FRT9-2]18E; f, baz[815.8], P[CaryP, PB[BAC Baz-sjGFP2]attP18] (Besson et al., 2015); the knockdown stocks from the Transgenic RNAi Project (TRiP, Bloomington Drosophila stock center) UAS-RNAi par1^{GL00253}; UAS-RNAi dhc64^{GL00543} (Sanghavi et al., 2013); UAS-RNAi ikk^{GL00160}; UAS-RNAi Rab5^{HMS00147} (BL34832); UAS-RNAi mCherry (BL35785); PAR3-Trap = P[PTT-GC]baz^{CO1941} (BL51572); HspFLP; FRT82B RFP; the stocks UASp-Myc-SKTL and UASp-Myc-SKTL^{DNRQ}, that we established. Tub67c-GAL4 (Januschke et al., 2002) and Osk-GAL4 VP16 (Telley et al., 2012) were used to express transgenes in germinal cells. Tub67c-GAL4 was used to express all UAS transgenes except the knockdown stock UASp-RNAi dhc64^{GL00543} where Osk-GAL4 VP16 was used. The crosses were kept at 25°C. However, to express two UAS transgenes, the fly were kept at 29°C, 24 hour before dissection. rab11^{P2148} germline clones were generated as described in (Compagnon et al., 2009) and selected against RFP.

**Drug treatment**
Flies were fed with yeast paste, on vinegar agar plates, containing 1mM of latrunculin B (Sigma), in DMSO, (sigma) or DMSO alone as control for 48h or 16µM of colchicin (Sigma) for 24h or 48h. Ovaries were then dissected, fixed and stained using standard procedures.

**Immunohistochemistry**

Immunostainings were performed using standard protocols. The following primary antibodies were used: rabbit anti-SKTL at 1:20000 (Claret et al., 2014); mouse anti-Myc 9E10 (Santa Cruz) at 1:250; rabbit anti-RAB11 at 1:8000 (Nakamura); rabbit anti-RAB5 at 1:50 (Marcos Gonzalez-Gaitan); guinea pig anti-HRS at 1:500 (H. Bellen); mouse anti-KDEL at 1:300 (abcam 10C3, RRID: AB_ab12223); rabbit anti-NUF at 1:500; mouse anti-spNF at 1:50 (Abdu Uri); rabbit anti-Syntaxin16 at 1:1000 (R.Leborgne); rabbit anti-PKC (Santa Cruz) at 1:1000; mouse anti-HTS (Hybridoma, RRID: AB_528289) at 1:10; mouse anti-β-Gal (Promega) at 1:250; rabbit anti-Staufen at 1:200. F-actin was visualized after staining with rhodamine-phalloidin (RRID: AB_2572408) or Alexa488-phalloidin (RRID: AB_2315147; life technology) at 1:200. Alexa594-WGA (Molecular probes) was used at 1:100 to stain nuclear membrane. Images were obtained with a ZEISS LSM700 confocal microscope and a Leica TCS-SP5 AOBS inverted scanning microscope.

**Molecular Biology**

SKTL kinase dead mutant, SKTL\textsuperscript{DNRQ}, was created using the PCR overlap mutagenesis method to insert, as it has been done on mammalian PIP5K\textalpha (Coppolino et al., 2002), two punctual mutations in SKTL sequence (Asp\textsubscript{398} mutated in Asn and Arg\textsubscript{564} mutated in Gln).

Using the gateway recombination cloning, SKTL and SKTL\textsuperscript{DNRQ} sequences were tagged with 6 MYC in N terminal (in the vector pPMW, Murphy Lab). The sequence AttB used by the phiC31 integrase was inserted in the vector pPMW. The constructions were then, integrated by transgenesis into the AttP2 site on chromosome III (BestGene).

**Co-immunoprecipitation and Western Blot**

Ovary extracts were obtained from wild type and DLIC-GFP transgenic flies by dissecting ovaries (20 flies per genotype) into PBS. Ovaries were placed on ice with lysis buffer (10mM Tris/Cl pH7.5; 150mM NaCl; 0.5 mM EDTA; 0.5% NP-40 with Complete Protease Inhibitor cocktail, Roche) and mechanically homogenized using micro pestles in matching tubes. S2-(DGRC (RRID: CVCL_TZ72)) cell lysates were obtained after freeze-thaw lysis of cells transfected with GFP-SKTL with PAR3-HA. Ovaries and cells lysates were then spun at
16,000 g for 10 minutes at 4°C. Using Chromotek standard procedures, the supernatant was collected and GFP-SKTL, PAR3-GFP or DLIC-GFP were precipitated with 15µL GFP-Trap®_MA (Chromotek) magnetic beads for 2 hour at 4 °C with rotation. Input and bound fractions were analysed by SDS-PAGE and western blotting, using NuPage, 4-12% Bis-Tris gels (Life technology). DLIC was detected using 1:5000 guinea pig anti-DLIC C-ter (Satoh et al., 2008), PAR3-HA using rabbit 1:5000 Anti-HA (GenWay), PAR3 using 1:500 rabbit anti-PAR3 N-ter (Wodarz et al., 1999), PAR1 using 1:100 rabbit anti-PAR1 (Rb96, J. McDonald), GFP using 1:1000 mouse anti-GFP (Roche) or 1:5000 rabbit anti-GFP, Tubulin using 1:1000 mouse anti-tub (DM1A sigma). For the immunoprecipitation controls, we used PDI-GFP in fly extracts and GFP-SNAP (kindly gift by M. Sanial) in S2 cells extracts.

**Proteomic SKTL partner screen**

S2 cells (S2-DGRC (RRID: CVCL_TZ72)) were transfected with SKTL-GFP vector or not (control) and harvested 3 days after. Protein extraction and immunoprecipitation were performed using Chromotek standard procedures. After trypsin digestion, the samples were analysed by a LTQ Velos Orbitrap (Thermo Fisher Scientific). Among the potential partner identified, we focused on 2 proteins: DLIC (one peptide identified not present in the control: SGSPGTGGPGAGNPAGPGR score 80) and IKKε (2 peptides identified not present in the control: VMQQQQQEVMAVMR and LLAIEEDQEGR, score 24).

**FRAP experiment**

Egg chambers were dissected in Schneider’s Insect Medium supplemented with 13% FBS, 0.34 U/ml insulin. Egg chambers were cultured in a 150 µm deep micro chamber filled with the same medium and sealed on one side with a 0.17 µm coverslip matching the characteristics of the facing objective lens, and a membrane permeable to oxygen on the other side. Imaging was carried out with spinning disk confocal (Axio Observer Z1 (Zeiss), Spinning Head CSU-X1, Camera sCMOS PRIME 95 (photometrics)) using a Plan-APOCHROMAT 40×/1.25 oil objective lens. For each egg chambers, six prebleach images were taken. Then, the region of interest (ROI) corresponding to nurse cells and oocyte APM was scanned twenty five times with the appropriate laser line at full laser power (473nm) to photobleach GFP fluorescence. Fluorescence recovery into the ROI was monitored immediately after the bleach by time-lapse imaging at low-intensity illumination. Time-lapse recordings were processed with Fiji. The FRAP efficiency has been controlled with the entire thickness of the sample.
The same experiment was realized on egg chambers treated with colcemid. The egg chambers were incubated 30 minutes in Schneider medium, 2% FBS, 0.2 U/ml insulin with 0.82 μM of colcemid, before the onset of the FRAP experiment, and the egg chambers stayed in this medium during the fluorescence recovery.

**Endocytosis assay**

To confirm the effect of RAB5DN expression on endocytosis, we performed an endocytosis assay by using the lipophilic dye FM4-64 (Molecular probes). This dye partitions into membranes where it becomes fluorescent and can be internalized in the cell by endocytosis. Egg chambers were dissected in Schneider’s Insect Medium supplemented with 20% FBS, 10μg/ml of juvenile hormone, 0.2U/ml insulin. Next they were incubated in the dark with the same medium + 20μM FM4-64 during 45 min at 25°C and then rinsed two times and washed 15 min at 4°C in medium without FM4-64. The oocytes were immediately observed by confocal microscopy.

**Statistical analysis**

Data were analysed using Mann-Whitney test and were represented by box plot (with Tukey variation) thanks to Graphpad Prism 6. All plots are expressed as the mean ± standard error of the mean (SEM).
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COMPETING INTERESTS

The authors declare that no competing interests exist.


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Figure 1: Dynamic PAR3 distribution along the oocyte anterior posterior axis

(A) Description of the quantification Fiji Macro. After selecting 3 points in the oocyte (yellow stars) and delimitation of oocyte perimeter, the macro allows us to obtain different data about proteins repartition in oocytes: the intensity profile of the plasma membrane (magenta); the mean fluorescent intensity and the length of each one of the plasma membrane domains that are automatically generated (anterior/APM in red; lateral/LPM in green; posterior/PPM in blue) and the mean signal intensity inside the cytoplasm (cyan).

(B-E) Distribution of PAR3 between stage 8 and 10 in representative examples.

(B) Localisation of PAR3-GFP, expressed in the germline under the control of the maternal driver Tub67c-GAL4, from stage 8 to stage 10. The brackets indicate oocytes.

(C) Representative intensity profiles of plasma membranes distribution in a single oocyte (APM in red, PPM in blue). Green arrows highlight PAR3 posterior accumulation and red arrows PAR3 posterior exclusion.

(D) Raw quantity of PAR3 in each plasma membrane domain from stage 8 to stage 10 (APM in red; LPM in green; PPM in blue).

(E) To avoid size/expression fluctuations of oocytes between the different mutant genotypes, PAR3 distribution has been normalized by the length of the membrane and the total oocyte signal (density/total). In this case, we cannot compare the level between the different stages but only the asymmetrical distribution of PAR3 between the different domains.

(F) Evolution of PAR3 asymmetry from stage 8 to 10. The asymmetry ratio (APM/PPM of PAR3 density) highlights the increase of PAR3 polarity in oocytes from stage 8 to stage 10.

Stage 8, n=8 ; stage 9A, n=9 ; stage 9B, n=15 ; stage 10, n=14. Mann-Whitney test, NS: not significant; **: p-value < 0.01 ; ***: p-value < 0.001 ; ****: p-value < 0.0001. Error bars indicate SEM. The scale bars represent 30 µm in (B) and in all following figures.
Figure 1 - Figure supplement 1:

(A) Egg chamber expressing PAR3-GFP under its own promoter (green / w, P[w+, FRT9-2]18E, f, baz[815.8], P{CaryP, PBAC Baz-sfGFP2}attP18) and nuclear RFP (red) both in the somatic and germinal cells. White arrowheads indicate the signal at the APM membrane, white arrow, the double nurse cells membrane and red arrows, the juxtaposition of the oocyte membrane and the apical membrane of follicular cells.

(B) Schematic representation of a stage 10 egg chamber. The yellow box highlights the proximity between the oocyte APM and the plasma membrane of the adjacent nurse cells. The green boxes show the juxtaposition between the oocyte LPM or PPM and the apical plasma membrane of the neighbouring follicular cells.

(C) Determination of oogenesis stages. The migration of follicular cells (white arrow heads) toward the oocyte was used to determinate the egg chamber stage. The stage 9A comprises the oocytes of which follicular cells have migrated until the middle of nurse cells region. The stage 9B includes the oocytes of which the follicular cells have migrated from the middle of nurse cell region to near the oocyte anterior. Then at stage 10, the follicular cells are positioned around the oocyte.

The scale bars represent 30 µm.
Figure 1 - Figure supplement 2:

(A) PAR3 density from stage 8 to stage 10. The quantity of PAR3 has been normalized by the membrane length of the domain but not by the total oocyte intensity. Stage 8, n=8; stage 9A, n=9; stage 9B, n=15; stage 10, n=14. Mann-Whitney test, NS: not significant; *: p-value < 0.05; **: p-value < 0.01; ****: p-value < 0.0001. Error bars indicate SEM.
Figure 1 - Figure supplement 3:

(A) Localisation of GFP-PAR3 (left) and PAR1-GFP (right) under the control of maternal Tub67c-GAL4 in stage 9B egg chambers. Note the weak density of PAR3 and the strong density of PAR1 in the PPM crescent. For all egg chambers, we defined the three following points: A and B on both sides of the APM and P on the middle of the PPM. Subsequently Fiji Macro automatically segmented the egg chambers tracing three lines (yellow lines). First it traced the perpendicular line going from the middle of AB to P. It then traced the lines going from A or from B and crossing at 40% of P on the preceding perpendicular line. The crossing between this two last lines and the plasma membrane, separates the LPMs from the PPM crescent. The APM is comprised between the points A and B.

(B) Validation of the PPM crescent identification by Fiji Macro. The PPM corresponds to the PAR1 (right, white) and Staufen (left, red) plasma membrane domain. Actin cytoskeleton is stained in green. Scale bars indicate 30µm.

(C) Quantification by our Fiji Macro of the signal intensity at the anterior domain coming both from the oocyte APM and the plasma membrane of the two neighbouring nurse cells in the control lines (Osk-GAL4 and Tub67c-GAL4). The proportion of signal arising from oocytes compared to adjacent nurse cells is relatively stable from stage 8 to stage 10. Error bar indicates SEM.

(D-D') Example of distribution of PAR3-GFP (green) express with Tub67c-GAL4 driver in a stage late 10 oocyte showing the proximity of the oocyte APM with the adjacent nurse cells. The nurse cell membranes are detached partially (D) or totally (D’) of the oocyte APM. White arrows indicate the oocyte APM and the nurse cell adjacent membrane. DNA is stained by DAPI (blue).
Figure 2: Cytoskeleton involvement in PAR3 polarity

(A-D) Role of cytoskeleton on PAR3 distribution. Flies are nourished with latrunculin (Cyan) for 48h, colchicin (yellow) for 24h or only yeast paste (control). (A) Representative distribution of PAR3-GFP in stage 9B oocytes. Note the PPM exclusion of PAR3 (arrow) or the strong APM accumulation (arrowhead). (B) The PAR3 posterior exclusion ratio (ratio LPM/PPM density) is represented between stage 8 and 10 oocyte. Under the value of 1, there is a posterior accumulation of PAR3, and above 1 a posterior exclusion. (C) The antero-posterior asymmetry (ratio APM/PPM density at stage 9B) is strongly affected by colchicin but not by latrunculin. (D) The two drugs lead to an increase of cytoplasmic fraction of PAR3 in stage 9B oocytes. Mann-Whitney test, NS: not significant; *: p-value < 0.05 ; *** : p-value < 0.001 ; ****: p-value < 0.0001. Error bars indicate SEM.

Control (Stage 8, n=8 ; stage 9A, n=9 ; stage 9B, n=15 ; stage 10, n=14); + latrunculin (Stage 8, n=5 ; stage 9A, n=12 ; stage 9B, n=11 ; stage 10, n=9); + colchicin (Stage 8, n=8 ; stage 9A, n=9 ; stage 9B, n=11 ; stage 10, n=6).
Figure 2 - Figure supplement 1: Cytoskeleton involvement in PAR3 distribution

(A) Quantification of PAR3-GFP density between stage 8 and 10 upon latrunculin (cyan) and colchicin (yellow) treatment.

These data were used to obtain the graph in Figure 2B-D. Error bars indicate SEM.

Control (Stage 8, n=8; stage 9A, n=9; stage 9B, n=15; stage 10, n=14); + latrunculin (Stage 8, n=5; stage 9A, n=12; stage 9B, n=11; stage 10, n=9); + colchicin (Stage 8, n=8; stage 9A, n=9; stage 9B, n=11; stage 10, n=6).
Figure 2 - Figure supplement 2: Effect of colchicine on distribution of PAR3 present at an endogenious level

(A) Localisation of PAR3-GFP (Trap line) at a pre-vitellogenic stage egg chamber. We can notice that PAR3 localized at the anterior cortex and also in some small dots in the oocyte cytoplasm (arrowhead). We cannot observe the very weak signal of PAR3 in the cytoplasm at later stage because of vitellus accumulation.

(B-D) Microtubule requirement for endogenous PAR3 distribution. Flies are fed with colchicine (+colchicin) for 48h or only yeast paste (-colchicin). To enhance the GFP signal, an anti-GFP staining has been performed.

(B-C) Representative distribution of PAR3-GFP in absence (B) or presence of colchicine (C). Note the accumulation of dotted structure (arrowhead) with colchicin. The nuclear staining is not specific of PAR3 and is only linked to the use of anti-GFP antibody.

(D) Colchicin leads to an increase of cytoplasmic fraction of PAR3. Mann-Whitney test, *** : p-value < 0.0002. Control (n=10) and + colchicin (n=6).

N indicates the oocyte nucleus position. The scale bars represent 30 µm.
Figure 3: SKTL by producing PI(4,5)P2 controls PAR3 APM accumulation and PPM exclusion

(A-F) Distribution of PAR3 in response to SKTL activity. PAR3-GFP is expressed in germlinal cells at stage 9B in control condition, or with overexpression (OE) of Myc-SKTL, Myc-SKTL^{DNRQ} or in context $sktl^{2.3}/sktl^{A5}$.

(A-C) Representative distribution of PAR3-GFP in oocyte in these different genetic contexts.

(D) Quantification of PAR3 density at each plasma membrane domains in $sktl$ mutant or SKTL overexpressed (OE) contexts in stage 9B oocytes. Error bars indicate SEM.

(E) Antero-posterior asymmetry of PAR3 (ratio APM/PPM density) at stage 9B in control or $sktl$ mutant.

(F) Quantification of PAR3 posterior exclusion ratio in $sktl$ mutant or SKTL overexpressed contexts at stage 9B. For (D-F): Control (stage 9B, n=10); $sktl^{2.3}/sktl^{A5}$ (stage 9B, n=8); Myc-SKTL (stage 9B, n=8); Myc-SKTL^{DNRQ} (stage 9B, n=10). Mann-Whitney test, NS: not significant; *: p-value < 0.05 ; **: p-value < 0.01 ; *** : p-value < 0.001 ; ****: p-value < 0.0001.

(G) PAR3-GFP (green, G') expressed in germline is present occasionally on vesicles containing PI(4,5)P2 visualized with PH_{PLC} RFP (magenta). Arrowheads show the vesicles that are associated with PAR3 and PI(4,5)P2. G', G'' and G''' are magnifications of G (white frame).

(H) Colocalisation of PAR3 (green, H'), PI(4,5)P2 visualized with PH_{PLC} RFP (red, H'') and SKTL visualized with myc tag (white, H'''). Note the coexpression of PAR3 and SKTL increase the cytoplasmic dotted localisation of PAR3.

Scale bars indicate 30µm.
Figure 4: SKTL dependant PAR3 posterior exclusion bypass the regulation by PAR1

(A-C) Representative distribution of PAR3-GFP in stage 9B oocyte in control situation (A), in RNAi PAR1 context (B) or when PAR3 phosphorylation sites by PAR1 are mutated (C).
In the control genotype, PAR3 is excluded of PPM (arrow), unlike the other two genotypes. N indicates the oocyte nucleus position. The scale bars represent 30 µm.

(D) PAR3 posterior exclusion in response to PAR1 at stage 9B. In germinal cells, PAR3AA-GFP, a mutant form, non phosphorylatable by PAR1 or PAR3-GFP are expressed with nothing, with par1 or with mCherry knock-down contexts. The posterior exclusion ratios in stage 9B oocytes are represented. PAR3 (stage9B, n=15); PAR3-AA (stage9B, n=6); PAR3 RNAi mCherry (stage9B, n=10); PAR3 RNAi PAR1 (stage9B, n=10).

(E) SKTL effect on PAR3 posterior exclusion is observed in combination with a PAR1 activity decrease (in green) or with the PAR3-AA non phosphorylatable form (in red). PAR3, RNAi PAR1, RNAi mCherry (stage9B, n=10); PAR3, RNAi PAR1, mycSKTL (stage9B, n=11) PAR3 RNAi PAR1 (stage9B, n=10); PAR3, RNAi mCherry, mycSKTL (stage9B, n=10); PAR3-AA, RNAi mCherry (stage9B, n=10); PAR3-AA, mycSKTL (stage9B, n=10).
Mann-Whitney test, NS: not significant; *: p-value < 0.05 ; **: p-value < 0.01 ; ***: p-value < 0.001 ; ****: p-value < 0.0001.
Figure 4 - Figure supplement 1: Validation of RNAi PAR1 efficiency.

Flies Tub67c-GAL4 or Tub67c-GAL4; UASp RNAi par1 were put on Fly cages to lay eggs on agar plates. The plates were collected every hour to produce the embryonic extracts revealed after western blot. PAR1 was revealed with a rabbit anti-PAR1 antibody.
Figure 5: PAR3 asymmetry depends on RAB5.

(A) PAR3-GFP (A’, A’’’ green) expressed in germline is present occasionally in RAB5 positive early endosomes (A’’, A’’’ magenta). A’, A’’ and A’’’ are magnifications of A (white frame). Arrowheads show the vesicles that are associated with PAR3 and RAB5.

(B-F) PAR3 distribution in response to RAB5 activity impairment. PAR3-GFP is expressed in germinal cells at stage 9B in RAB5 RNAi, RAB5DN (S43N) or in mCherry knock-down (control) contexts.

(B-D) Representative images of PAR3 distribution in control (B), in RAB5 RNAi (C) or in RAB5DN (S43N) (D). Scale bars indicate 30µm.

(E) Quantification of PAR3 density at each plasma membrane domains. Error bars indicate SEM.

(F) Quantification of PAR3 posterior exclusion in different RAB5 mutant contexts. Control (stage9B, n=10); RAB5 RNAi (stage9B, n=12); RAB5DN (S43N) (stage9B, n=6). Mann-Whitney test, ns: not significant; *: p-value < 0.05 ; **: p-value < 0.01.
Figure 5 - Figure supplement 1: Validation of RAB5DN effect on endocytosis

Endocytosis uptake of FM4-64 dye was performed on control (A, Tub67c-GAL4) or on RAB5DN(S43N) expressing egg chambers (B, Tub67c-GAL4; UASp RAB5DN). In panel (A), the dye is internalised in dotted structures all around the plasma membrane with higher rate at the posterior. In panel (B), there is a strong decrease of FM4-64 internalisation, indicating that endocytosis is altered by RAB5DN(S43N) expression. Note that FM4-64 accumulates at the APM without any clear explanations for that.
Figure 6: Role of RAB11 on PAR3 asymmetrical localisation

(A) Colocalisation of PAR3 (PAR3-GFP maternally expressed) with vesicular trafficking markers. To quantify the colocalisation, we measured the Pearson’s coefficient on stage 9B oocytes and presented the results in this box plot. As control, we used the value of colocalisation obtained with the same images but after rotation of one by 90 degrees. The stars represent the p-value with the control. For HRS, KDEL and SYX16: n=7; for PHPLC: n=8; for RAB5: n=10; for RAB11: n=13.

(B) PAR3-GFP (B’, green) expressed in germline is present occasionally in RAB11 positive recycling endosomes (B”, B”” magenta). B’, B” and B”” are magnifications of B (white frame). Arrowheads show the vesicles that are associated with PAR3 and RAB11. The arrow points the vesicle associated only with PAR3.

(C-D) RAB11 effect on asymmetrical distribution of PAR3. The antero-posterior asymmetry (C) and the posterior exclusion (D) of PAR3 at stage 9B have been evaluated in rab11P2148 germline clones. Control (stage9B, n=10); rab11P2148 (stage9B, n=10). Mann-Whitney test, NS: not significant; *: p-value < 0.05; ***: p-value < 0.001; ****: p-value < 0.0001. Error bars indicate SEM.

(E-F) Representative images of PAR3 distribution in rab11P2148 clones. The rab11P2148 mutant cells are indicated by the absence of nuclear RFP staining (nls-RFP). (E) Heterozygote eggchamber (rab11P2148/+ ) is used as control. (F) rab11P2148 mutant oocyte. Note that PAR3 is always excluded of the PPM (arrow). Scale bars indicate 30µm.
Figure 6 - Figure supplement 1: PAR3 and vesicular compartments

(A-C’’) Characterisation of the components of PAR3-GFP vesicular structures. PAR3-GFP (green, A, B and C) is expressed in the germline under the control of the maternal driver 
*Tub67c-GAL4*. By immunostaining, we can observe that PAR3-GFP cytoplasmic vesicular structures do not colocalise with HRS (magenta, A’), a late endosome marker, KDEL (magenta, B’), an endoplasmic reticulum marker, or SYNTAXIN 16 (magenta C’), a TRANS Golgi marker.

The scale bars represent 30 µm.
Figure 6 - Figure supplement 2: PAR3 colocalises with PI(4,5)P2 and RAB11 in front of the ring canal

Localisation of PAR3-GFP near a ring canal between a Nurse Cell (NC) plasma membrane and the oocyte (Oo) APM. PAR3-GFP is expressed in the germline under the control of the maternal driver *Tub67c-GAL4*. In the Nurse Cell (NC), besides being at the plasma membrane, PAR3 can be accumulated in front of the ring canal. In this case, PAR3 is present in vesicular structures RAB11-positive (A) and PI(4,5)P2-positive (B). The scale bars represent 10 µm.
Figure 7: Dynein regulates PAR3 asymmetry

(A-C) Distribution of PAR3 in response to dynein activity decrease. PAR3-GFP is expressed in germinal cells at stage 9B in control (osk-GAL4; UASp PAR3-GFP) or dhc64 knock-down contexts (osk-GAL4; UASp RNAi dhc64; UASp PAR3-GFP). (A) Representative distribution of PAR3 in oocyte (note the dotted accumulation of PAR3 under the plasma membrane in the insert). The scale bars represent 30 µm. (B) Quantification of PAR3 density at each plasma membrane domains. (C) Quantification of PAR3 posterior exclusion (ratio LPM/PPM) in dynein mutant context. For (A-C): Control (stage 9B, n=10); RNAi dhc64c (stage 9B, n=10).

For (B-C), Mann-Whitney tests; NS: not significant; *: p-value < 0.05; **: p-value < 0.01; ***: p-value < 0.001; ****: p-value < 0.0001. Error bars indicate SEM.

(D) Co-Immunoprecipitation (IP) of PAR3 by DLIC-GFP in ovarian extracts. Ovaries were dissected from Pubi-DLIC-GFP flies or control Pubi-PDI-GFP flies. The ovarian extract was incubated on magnetic beads coupled with antibody anti-GFP. PAR3 was revealed by anti-PAR3 antibody.

(E) SKTL interacts with both PAR3 and DLIC. Co-Immunoprecipitation (IP) of PAR3-HA or DLIC with GFP-SKTL in S2 cell extracts. Cells were transfected with GFP-SKTL or GFP-SNAP (as control) and with PAR3-HA. The cell extracts were incubated on magnetic beads with anti-GFP antibody. PAR3 was revealed by anti-HA antibody and DLIC by anti-DLIC antibody.
Figure 7 - Figure supplement 1:

(A) Role of dynein on PAR3 cytoplasmic accumulation (quantification of PAR3 proportion in the cytoplasm related to the whole oocyte intensity). Control (stage 9B, n=10); RNAi dhc64c (stage 9B, n=10). Mann-Whitney test, ***: p-value < 0.001. Error bars indicate SEM.

(B) PAR3 association with PI(4,5)P2 vesicles is not affected by colchicin. Flies are nourished with colchicin for 24h. PAR3-GFP (B’, green) still colocalises with PI(4,5)P2 visualized with PH\textsubscript{PLC} RFP (B”, B’’’ magenta). B’, B’’ and B’’’ are magnifications of B (white frame). The scale bars represent 30 µm.
Figure 7 - Figure supplement 2: Validation of PAR3 detection by antibody

By western blot with anti-PAR3 antibody, we can reveal numerous bands corresponding to several isoforms of PAR3 and to post-transcriptionally modified forms. We hypothesize that the higher band (>250kD) corresponds to an oligomer form of PAR3.

(A) To confirm that this higher band is PAR3, we tried to decrease its intensity by PAR3 RNAi. We immunoprecipitated PAR3-GFP (Tub67c-GAL4; UASp PAR3-GFP) in embryonic extracts expressing or not PAR3 RNAi. We revealed PAR3 by using anti-PAR3 antibody or GFP antibody. We used tubulin as loading control.

(B) We realized the same experiment with a strain expressing PAR3-GFP at an endogenous level (Bac PAR3-GFP). PAR3-GFP was immunoprecipitated by GFP antibody in ovarian or embryonic extracts. In this case, we can also revealed the PAR3 band of high molecular weight, which disappear with PAR3 RNAi.

These results confirm that the high molecular weigh band revealed by anti-PAR3 antibody is a form of PAR3, perhaps a dimer form, in accordance to its weight.

(C) Visualization by Coomassie blue staining of the total proteins in Figure 7E-input samples.
Figure 8: PAR3 recovery after APM photobleaching

(A) PAR3-GFP expressed in ovarian follicle (Tub67c-GAL4; UASp PAR3-GFP) is photobleached in all the nurse cells and at the APM (yellow area, A1). Next the fluorescence recovery was followed during around 1400s (A2).

(B) PAR3 quantity in each domain (anterior and posterior domains) has been quantified by using the same method that before for three ovarian follicles and raised to zero after bleaching. We can observe that, after photobleaching, PAR3 accumulates progressively to the anterior while it is excluded from the posterior.

(C) PAR3 quantity of each zone before FRAP was normalised to 1 and we observed the recovery of the fluorescence.

(D) The same experiment that in (A) was realised on ovarian follicle that was incubated with colcemid. The quantification was shown on graphs in (C) and (D).

In (B) and (C), the error bar represents SEM.

The scale bars represent 20 µm.
Figure 9: IKKe regulates PAR3 microtubule unloading and APM accumulation

(A-C) Distribution of PAR3 in response to IKKe knockdown. (A) IKKe knockdown affects the localisation of PAR3 and in particular leads to an accumulation of circular actin clumps (ACs) enriched in PAR3. (B) Quantification of PAR3 density at each plasma membrane domains from stage 9A to stage 10 oocytes in WT or IKKe knockdown contexts (Tub67e-GAL4; UASp RNAi ikke; UASp PAR3-GFP). (C) Quantification of PAR3-GFP distribution in the cytoplasm related to the whole oocyte intensity at stage 9B in WT or in IKKe knockdown contexts. For (B-C), Control (stage 9A, n=9 ; stage 9B, n=15 ; stage 10, n=14); RNAi ikke (stage 9A, n=15 ; stage 9B, n=10 ; stage 10, n=9). Mann-Whitney test is realised on (C). Error bars indicate SEM. **** indicates that p-value < 0.0001.

(D-E) Actin clumps (D’, E’, green) contain RAB11 (magenta, D’’) but not RAB5 (E’’, red) is around this actin clumps. Actin is visualized after staining with phalloidin. (D’-D’’) and (E’-E’’), are magnifications of D and E (white frame).

(F-G) In an IKKe knockdown oocyte, ACs contain PI(4,5)P2, visualized with PHPLC GFP probe, (F) and Myc-SKTL, visualized with Myc tag (G).

(H-I) PI(4,5)P2 or SKTL are involved in the formation of the ACs. ACs are reduced in SKTL

(DNRQ) context (H) and disappeared in sktl^{2.3}/sktl^{A5} context (I).

(J-K) MTs are necessary for actin rings formation. Flies were nourished with colchicin for 24h (K) or only yeast paste (J). Actin (green) is visualized after staining with phalloidin and the nuclear membranes after staining with WGA (red).

The oocyte nucleus position is indicated by an “N” in (G), (J) and (K).
Figure 9 - Figure supplement 1: Effect of IKKε knockdown

(A) In an IKKε knock-down oocyte, ACs (A and A’ green) are accumulated around the nucleus (red). The oocyte nucleus position is indicated by an “N” in A. (A’) is a magnification of the oocyte presented in (A) and represents 5 Z-plans (13.5µm of depth).

(B-D) ACs (B’, C’, D’ green) contain NUF (B”’, red), aPKC (C”’, red) but not SYX16 (D”’, red). Actin is visualized after staining with phalloidin. (B’-B’’’) and (C’-C’’’), (D’-D’’’) are magnifications of B, C and D (white frame).
Figure 9 - Figure supplement 2: Alteration of microtubules network in IKKε knockdown

(A) Dynein is trapped in ACs. Dynein is visualised thanks to DLIC-GFP transgene (green) and actin (red) is visualised after staining with phalloidin.

(B-C) Effect of IKKε on MT minus ends. MTs minus ends are visualized with the transgene UASp Nod-LacZ after staining with anti-β-galactosidase. In a wild type oocyte, MT minus ends are localised around the nucleus and surround the nucleus at the plasma membrane. (B1) and (B2) are two independent examples of this staining. In an IKKε knock-down oocyte, MT minus ends are isotropically localised at the plasma membrane, around the nucleus (C1) and in the clumps around the nucleus (C2). (C1) and (C2) are two independent examples of this staining. “N” indicates the oocyte nucleus position.
Figure 10: Speculative model of PAR3 localisation regulation

(A) In *Drosophila* oocyte, PAR3 asymmetrical localisation proceeds in at least two steps. The first step (C) occurs at the posterior plasma membrane and leads to the PAR3 exclusion starting from stage 9B. This step implicates PAR3 phosphorylation by PAR1 (C2) and a RAB5 dependant endocytosis (C1). The PIP5K, SKTL, and its product PI(4,5)P2 (orange) are crucial for this process as well as actin cytoskeleton (blue gradient). As PI(4,5)P2 is critical for the endocytosis onset but also for actin cytoskeleton regulation, the link between all these protagonists remains hypothetical. Furthermore, as the polarised localisation of PAR1 depends on actin, we cannot exclude a role of PAR1 on endocytosis directly or indirectly by the PAR3 phosphorylation. The second step (B) takes place at the anterior cortex where PAR3 has to be strongly enriched. The dynein dependent transport brings PAR3 at the MT minus ends likely with recycling endosomal cargo (RE). Then PAR3 cargo would be released from the dynein through an IKKe dependant process. However, how PAR3 reaches the cortex is not known. PI(4,5)P2 is also important for the PAR3 endosomal sorting and we can speculate that an association with vesicles is required to its anterior cortex targeting.

Finally, while we have shown that the posterior fraction of PAR3 provide, through MTs, the anterior pool of PAR3, the neo-synthetized fraction of PAR3 from the nurse cell could also contributes to the anterior pool. Moreover, in the oocyte, as the MT network presents only a slight bias of minus end directed transport in the oocyte, it is thus possible that PAR3 is transported step by step along the lateral cortex before reaching the anterior membrane.
Video 1: Recovery of fluorescence after photobleaching of PAR3 at the APM domain.

Representative FRAP experiment on PAR3-GFP (Tub67c-GAL4; UASp PAR3-GFP) in *drosophila* egg chamber.
Source data files and legends

Figure 1-source data 1: Quantification of TubGal4; UASp PAR3-GFP eggchambers during oogenesis.

Figure 1-figure supplement 3-source data 1: Proportion of anterior PAR3 (APM) in oocyte versus in adjacent nurse cells.

Figure 2-source data 1: PAR3 posterior exclusion ratio in oocyte of flies feed with latrunculin, colchicin or without drugs. (Figure 2B)

Figure 2-source data 2: PAR3 asymmetry ratio in oocyte of flies feed with latrunculin, colchicin or without drugs. (Panel C)

Figure 2-source data 3: PAR3 Quantity (cytoplasm intensity/Total oocyte intensity) in oocyte of flies feed with latrunculin, colchicin or without drugs. (Figure 2D)

Figure 2-figure supplement 1-source data 1: Quantification of PAR3-GFP density (normalised) between stage 8 and 10 upon latrunculin and colchicin treatment.

Figure 2-figure supplement 2-source data 1: Density of cytoplasmic fraction of PAR3-GFP (trap line) at stage 9B upon latrunculin and colchicin treatment. (Panel D)

Figure 3-source data 1: Quantification of PAR3 density (normalised) at each plasma membrane domains in sktl mutant or SKTL overexpressed (OE) contexts in stage 9B oocytes.

Figure 3-source data 2: Quantification of PAR3 asymmetry ratio in sktl mutant or control at stage 9B. (Panel E)

Figure 3-source data 3: Quantification of PAR3 posterior exclusion ratio in sktl mutant or SKTL overexpressed contexts at stage 9B. (Panel F)

Figure 4-source data 1: Quantification of PAR3 posterior exclusion in response to PAR1 at stage 9B. (Panel D)

Figure 4-source data 2: Quantification of PAR3 posterior exclusion in response to PAR1 at stage 9B in combination with SKTL. (Panel E)

Figure 5-source data 1: Quantification of PAR3 density at each plasma membrane domains of stage 9B oocytes in response to RAB5 activity impairment. (Panel E)

Figure 5-source data 2: Quantification of PAR3 posterior exclusion in different RAB5 mutant contexts at stage 9B. (Panel F)

Figure 6-source data 1: Quantification of PAR3 colocalisation with vesicular compartment. (Panel A)

Figure 6-source data 2: Quantification of PAR3 asymmetry ratio in rab11 mutant clones. (Panel C)
**Figure 6-source data 3:** Quantification of PAR3 posterior exclusion ratio ratio in rab11 mutant clones. (Panel D)

**Figure 7-source data 1:** Quantification of PAR3 density at each plasma membrane domains in Dhc64 knockdown at stage 9B oocytes (Panel B).

**Figure 7-source data 2:** Quantification of PAR3 posterior exclusion ratio in Dhc64 knockdown at stage 9B oocytes (Panel C)

**Figure 8-source data 1:** PAR3 quantity variation (grey levels) at the anterior and the posterior of oocyte after anterior FRAP experiment. This experiment has been performed on three oocytes for each conditions. (panel B)

**Figure 8-source data 2:** PAR3 quantity of each zone before FRAP was normalised to 1 and we observed the recovery of the fluorescence

**Figure 9-source data 1:** Quantification of PAR3 density at each plasma membrane domains in IKKe knockdown at stage 9B oocytes (Panel B).

**Figure 9-source data 2:** quantification of PAR3 proportion in the cytoplasm related to the whole oocyte intensity in IKKe knockdown context. (panel C)

**Figure 9-figure supplement 2-source data 1:** quantification of PAR3 proportion in the cytoplasm related to the whole oocyte intensity in IKKe knockdown context. (panel C)
Figure 1
Figure 1 - Figure Supplement 2
Figure 1 - Figure Supplement 3
Figure 2
Figure 2- Figure supplement 1
Figure 2- Figure supplement 2
Figure 3
Figure 4
Embryonic extract (0-1h)

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Figure 5
Figure 5 - Figure supplement 1
Figure 6
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Figure 6 - Figure supplement 2
Figure 7
Figure 7 - Figure supplement 1
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Figure 8
Figure 9
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