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New insights into apical-basal polarization in epithelia

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The establishment of an apical–basal axis of polarity is essential for the organization and functioning of epithelial cells. Polarization of epithelial cells is orchestrated by a network of conserved polarity regulators that establish opposing cortical domains through mutually antagonistic interactions and positive feedback loops. While our understanding is still far from complete, the molecular details behind these interactions continue to be worked out. Here, we highlight recent findings on the mechanisms that control the activity and localization of apical–basal polarity regulators, including oligomerization and higher-order complex formation, auto-inhibitory interactions, and electrostatic interactions with the plasma membrane.

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Introduction

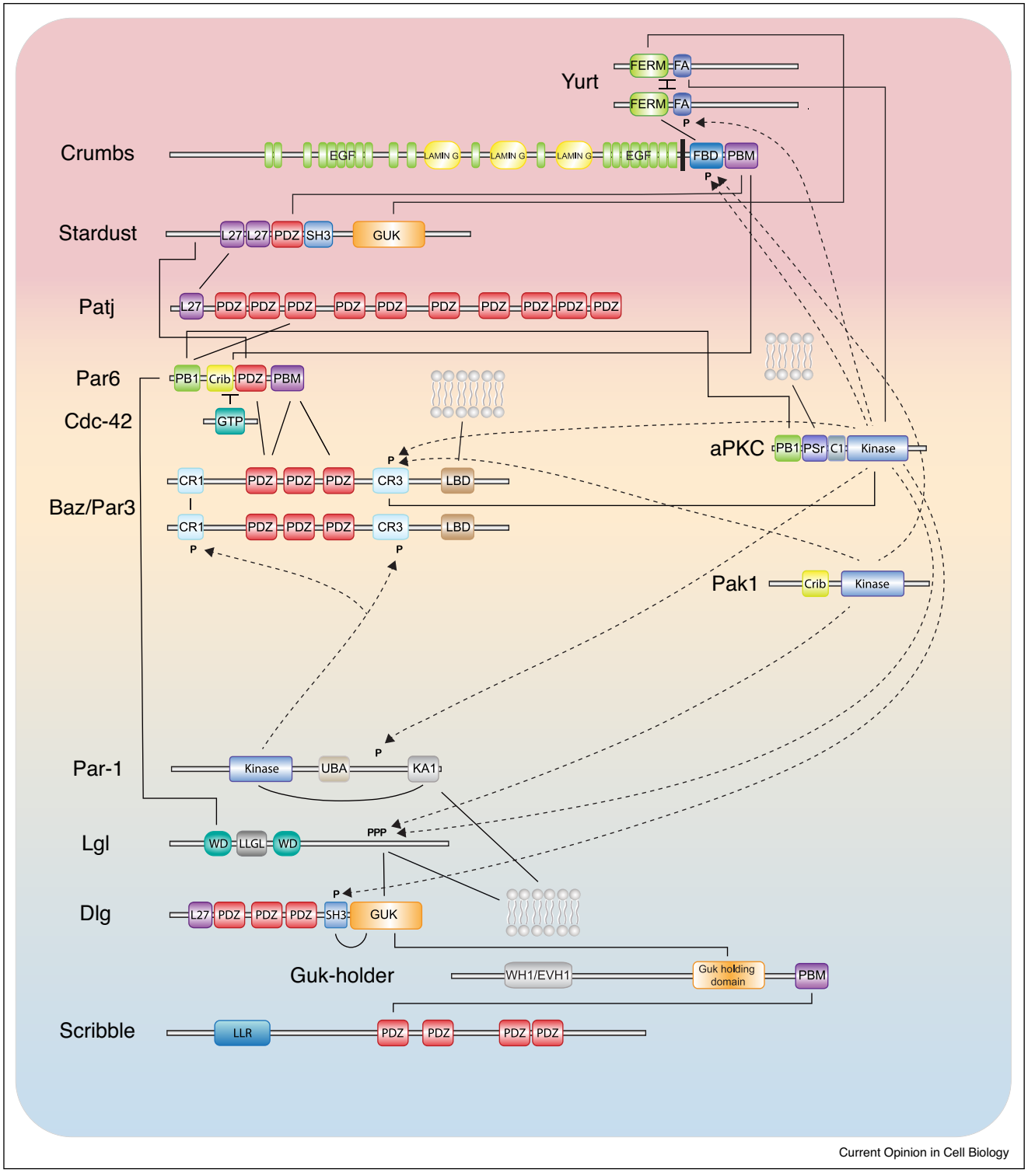
Cell polarity lies at the heart of the establishment of proper cellular architecture and function. To be able to form selectively permeable barriers, epithelial cells polarize along an apical–basal axis and establish molecularly and functionally distinct apical, basal and lateral membrane domains. At the interface between apical and lateral domains, adherens junctions (AJs) provide mechanical strength and cohesion, while occluding junctions — tight junctions (TJs) in vertebrates and septate junctions (SJs) in invertebrates — prevent the passage of molecules between cells and help maintain the segregation of apical and basolateral membrane components. Epithelial polarization and morphogenesis are controlled by an epithelial polarity program, in which polarity proteins, protein trafficking, membrane lipid regulators, and actomyosin and microtubule cytoskeletons all contribute important roles [1,2]. The establishment of opposing cortical domains is the result of a complex web of interactions between a series of evolutionarily conserved polarity proteins, most of which were identified through genetic studies in *Caenorhabditis elegans* and *Drosophila melanogaster* (Figure 1) [3,4]. Members of the *Par*titioning defective (Par) and Crumbs complexes function together in a semi-redundant fashion to establish the apical domain and position cell junctions at the apical/lateral border, while basolateral identity is promoted by the Scribble group proteins, the kinase PAR-1, and the phospho-protein interacting 14-3-3 protein Par5. More recent studies in *Drosophila* added the band 4.1-Ezrin-Radixin-Moesin (FERM) domain proteins Yurt and Coracle, and the membrane proteins Neurexin IV (Nrx-IV) and Na^+K^+-ATPase to the roster of proteins regulating epithelial polarity. In this review, we will highlight recent advances in our understanding of the molecular mechanisms that underpin the interactions that result in the correct spatial segregation of these components.

The apical polarity network

Apical domain identity is established by the coordinated activities of Par proteins and the Crumbs complex [2,3]. The apical PAR proteins include Par3 (Bazooka or Baz in *Drosophila*) and Par6, the small GTPase Cdc42, and the atypical protein kinase C (aPKC) [5]. The Crumbs complex consists of the transmembrane protein Crumbs (Crb in *Drosophila*, CRB1-3 in mammals), the intracellular binding partner Stardust (Sdt, known as protein associated with Lin-7 — PALS1 — in vertebrates), and the Sdt binding partners PATJ (protein associated with tight junctions) and Lin-7 [6]. The proteins in these groups engage in complex and interdependent interactions, and together form an apical polarization network (Figure 2). Par6, aPKC, and the Crumbs proteins co-localize at the apical membrane domain. Par3 engages in transient interactions with Sdt and with Par6–aPKC to mediate their apical localization [7–9]. However, in mature *Drosophila* epithelia and in mammalian epithelial cells, the bulk of Par3/Baz segregates to the apical/lateral border, where it plays an essential role in the positioning and assembly of AJs in *Drosophila* and TJs in mammalian cells [10,11]. Cdc42 plays a central role in the recruitment of Par6–aPKC, but has many other effector proteins important in epithelial morphogenesis, and its activity is controlled by numerous regulatory proteins [12].

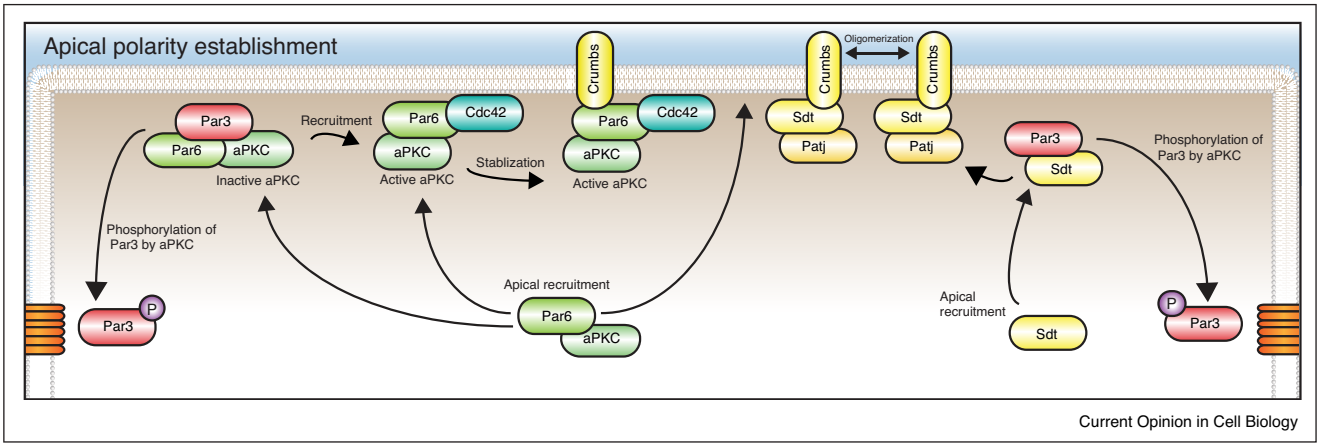
The kinase activity of aPKC is central to the mutual antagonism between cortical polarity regulators, and a major role of the apical polarity proteins is to tightly control aPKC localization and activity. aPKC is complexed with the adapter protein Par6, which mediates

Figure 1



Cell polarity is established and maintained by a complex web of interactions between apical and basolateral polarity regulators. Solid lines indicate physical interactions. Dashed lines indicate phosphorylation events. Canonical domain organizations are shown. Abbreviations: FERM, 4.1-Ezrin-Radixin-Moesin; FA, FERM-adjacent (FA) domain; EGF, epidermal growth factor domain; L27, Lin-2 and Lin-7 domain; SH3, SRC homology 3 domain; GUK, guanylate kinase-like; FBD, FERM binding domain; PBM, PDZ domain-binding motif; PB1, Phox and Bem1 domain; Crib, Cdc42-interactive and Rac-interactive binding motif; CR1, CR3, conserved region 1 and 3; LBD, lipid binding domain; KA1, kinase associated domain; WD, WD40 repeat; LLGL, lethal giant larvae specific domain; WH1/EVH1, Enabled/VASP homology 1; LLR, leucine rich repeat domain; PSr, pseudosubstrate region of aPKC.

Figure 2



The apical domain is established by coordinated interactions between proteins of the Par and Crumbs complexes.

most of the physical interactions that localize aPKC. In addition, Par6 controls the kinase activity of aPKC. It is still unclear, however, whether Par6 acts as an activator or an inhibitor, as studies into the effects of Par6 on aPKC kinase activity have yielded conflicting results [13,14•,15].

The localization of Par6–aPKC to the apical membrane is mediated by physical interactions with Par3, the active GTP-bound version of Cdc42, and Crumbs [11]. The Par3–Par6–aPKC complex is thought to be inactive, which would help to prevent inappropriate phosphorylation of target proteins by aPKC [13,16,17••,18,19]. Inhibition of aPKC was reported to be mediated by formation of high affinity interactions of two motifs within the Par3 conserved region 3 (CR3) with the aPKC kinase domain, blocking substrate access [18]. However, the effects on catalytic activity and its relevance *in vivo* are a point of contention [20,21]. As discussed below, Par3 is an aPKC substrate itself, and its phosphorylation is required for junctional localization. How to reconcile roles of Par3 as both an inhibitor and a substrate of aPKC is an important future challenge.

The Par3–Par6 association was reported to involve a non-canonical interaction between the Par3 PDZ1 domain and Par6 Crib-PDZ domains [19,22,23]. However, recent data indicate that, at least in *Drosophila* and mammals, the Par3–Par6 association is mediated by interaction of the Par3 PDZ1 and PDZ3 domains with a PDZ binding motif (PBM) at the N-terminus of Par6 [24]. Par3 could interact simultaneously with two Par6 proteins, potentially facilitating higher order complex formation, though this has not been shown to occur *in vivo*. The PBM domain is absent from *C. elegans* PAR-6 [24], and hence the mode of the Par3–Par6 interaction is not fully resolved.

At least two mechanisms can contribute to membrane localization of Par3. First, the N-terminal conserved region (CR1) mediates homo-oligomerization of Par3/Baz, which promotes the formation of cortical Par3–Par6–aPKC clusters and has been reported to be essential for Par3 localization [17••,25,26,27••,28]. Second, a C-terminal lipid binding (LB) domain promotes membrane localization through binding to phospholipids [29–31]. Recent studies in *Drosophila* indicate that oligomerization and phospholipid binding can contribute redundantly to the membrane association of Baz [32,33]. How Par3 is transported apically is not completely clear. Dynein-mediated transport is important for Baz positioning in *Drosophila* epithelia and oocytes [34–36], but based on other systems reaction–diffusion processes and advective transport play roles as well [37]. It is also not clear yet to what extent Par6–aPKC is transported apically in a complex with Par3, versus being recruited from the cytoplasm by previously localized Par3.

At the apical domain, the release of Par3/Baz from Par6–aPKC and Sdt critically depends on phosphorylation of Par3 by aPKC on a conserved Serine residue within the conserved region three (CR3), which reduces the affinity of Par3 for aPKC and Sdt [8,9,38–40]. Phosphorylation by aPKC alone is, however, not sufficient for the segregation of Par3 from Par6–aPKC, possibly due to the association of Par3 with Par6, and further depends on interactions of Par6 with GTP-Cdc42 and with Crb [8,9,34,38,41••]. GTP-Cdc42 may outcompete Par3 for binding to Par6, and furthermore induces a conformational switch in the Par6 PDZ domain that greatly increases the affinity for the C-terminal PBM in Crumbs [42–44]. In a recent study, Nunes de Almeida *et al.* used a series of Par6 alleles that specifically disrupt binding to

Cdc42, Crb, or aPKC to investigate the order of events in *Drosophila* photoreceptor cells and follicle epithelium [41••]. Their results support a model, in which recruitment of Par6–aPKC by GTP-Cdc42 promotes the subsequent binding of Par6 to Crb, which results in mutual stabilization of both Par6–aPKC and Crb at the apical membrane.

The shuttling of aPKC between an inactive Par3 complex that promotes localization and a Par3-independent active complex appears to be a common feature of polarized cells. In the one-cell *C. elegans* embryo, PKC-3 shuttles between an inactive PAR-3/PAR-6/PKC-3 complex that promotes anterior segregation, and an active CDC-42/PAR-6/PKC-3 complex [17••,26,45,46]. An important difference however is the role of aPKC. While in *Drosophila* epithelia aPKC phosphorylates Par3/Baz to promote its release from the complex, in *C. elegans* aPKC activity was required to couple the behavior of PAR-3 and PAR-6/PKC-3 [17••]. A similar observation was recently made in *Drosophila* neuroblasts, where acute inhibition of aPKC resulted in uncoupling of the localizations of Baz and aPKC–Par6 [47]. These observations may reflect differences between epithelial cells and asymmetrically dividing cells. For example, in non-epithelial cells which lack apical junctions, phosphorylation of Par3 by aPKC is not essential. However, it may also reflect an incomplete understanding of a common underlying mechanism.

The relative importance of the interactions with Par6–aPKC described above are cell context dependent. For example, in the follicular epithelium and adult mid gut epithelium, Baz is dispensable for the localization of Par6 [48,49••], while in the follicular cells of stage 10 *Drosophila* embryos, Crb is not required for apical enrichment of aPKC [50]. In these cells, direct recruitment of Par6–aPKC by Cdc42 may be the dominant mechanism. However, in the *C. elegans* embryonic epidermis, Cdc42 was found to be dispensable for polarization and for apical localization of PAR-6, indicating that Cdc42 is not essential under all conditions [51•]. Interestingly, a recent preprint proposes an alternative localization mechanism for *Drosophila* and mammalian aPKC, based on direct binding of a polybasic domain within the aPKC pseudosubstrate region to the membrane lipids PI4P and PIP2 [14•]. This interaction did not require Cdc42, but does depend on binding to Par6, which the authors postulate induces an allosteric change that exposes the polybasic domain [14•]. The same study also finds that the binding of Crb activates the kinase activity of aPKC, although this cannot represent a general mechanism as aPKC is also active in cell types that do not express Crumbs.

A recent study in MDCK cells points to yet another mechanism that may contribute to the apical segregation of Par6–aPKC [52]. In these cells, apical activation of Cdc42 stimulates actomyosin contractility, while aPKC inhibits actomysosin contractility at the level of cell junctions, potentially setting up a contractility gradient. Interfering with apical actomyosin contractility blocked the separation of Par3 from Par6–aPKC, suggesting that cortical contractility mediates the segregation of Par6–aPKC. How cortical contractility promotes Par3 segregation in this cell type, however, is unclear [52]. Taken together, it is evident that the localization and activation of aPKC can be controlled by several mechanisms that may act together or play specific roles in particular cell types.

Basolateral polarity regulators

The main basolateral determinants are the PAR-1/MARK serine/threonine kinases, which mediate exclusion of proteins from the basolateral domain through phosphorylation, and the Scribble group proteins Scribble (Scrib), Discs large (Dlg) and Lethal giant larvae (Lgl), which exclude apical proteins from the basolateral domain by virtue of the ability of Lgl to inhibit aPKC [53–56]. In addition, the Scribble module may regulate the polarized trafficking of proteins. In *Drosophila*, the Scribble module was shown to regulate trafficking of Crumbs and other cargo from early endosomes to the Golgi system through the retromer pathway [57]. In the *C. elegans* intestine, the Scrib homolog LET-413 acts as a Rab5 effector, to promote the activation of RAB-10 and endocytic recycling [58]. Scrib, Dlg, and Lgl each contain multiple protein–protein interaction domains, and scaffold a bewildering array of proteins linked to many processes besides cell polarity, including tissue growth, differentiation, and cell migration [53].

In *Drosophila*, the Scribble proteins are dependent on each other for their localization and act in a common pathway to restrict apical determinants to the apical domain, control AJ formation and regulate cell growth [56]. With respect to its role in junction formation, a recent preprint unexpectedly identified a very early role in organizing AJs in the developing *Drosophila* embryo, upstream of other polarity regulators including Baz/par3 [59]. Recently, the hierarchy in the relationships between Scrib, Dlg, and Lgl was elucidated in the midgut of adult *Drosophila*, a tissue, in which the Scribble proteins are present at septate junctions but not essential for polarization [49••]. In these cells, Scrib recruits Dlg to the septate junctions, which in turn recruits Lgl. In *C. elegans* and mammalian systems, the Scribble proteins also play key roles in cell polarity, but the importance of their contribution varies, and the proteins often only partially overlap in localization [54]. Unlike the apical polarity determinants, few physical interactions have been identified within the Scribble module. In mammalian cells, Lgl2 directly interacts with the GUK domain of Dlg4 when phosphorylated by aPKC on one of three conserved serine residues [60]. In the synapses of *Drosophila* neurons, Dlg was also shown to indirectly associate with Scrib

through the linker protein GUK-holder (Gukh) [61]. Recently, the Scrib PDZ1 domain was shown to interact with a PDZ binding motif in C-terminus of Gukh [62]. The same study also provides evidence for a broader role for Gukh in controlling epithelial polarity, and it will be important to investigate the role of Gukh homologs in other organisms in epithelial morphogenesis.

Mechanisms of mutual exclusion

The major mutual antagonistic interactions in epithelial cells are mediated by the kinases aPKC and PAR-1, and by Lgl. aPKC phosphorylates PAR-1, Par3, and Lgl to mediate their exclusion from the apical domain [15]. Conversely, Lgl inhibits aPKC by binding to Par6–aPKC, which competes with Par3 binding, inhibits aPKC kinase activity, and prevents membrane association of Par6–aPKC [53,54]. Crumbs has also been shown to be a target for phosphorylation by aPKC [63], but the importance of this is unclear as mutating the aPKC target sites does not appear to affect Crb localization, epithelial polarization, or viability in *Drosophila* [64]. In the basolateral domain, the main target of PAR-1 is Par3. PAR-1 phosphorylates Baz/Par3 on two conserved serine residues, which prevents oligomerization, creates a binding site for the 14-3-3 protein Par5, and prevents binding of aPKC, all of which contribute to the exclusion of Baz/Par3 from the basolateral domain [65,66]. The combined activities of aPKC and PAR-1 thus position Par3 at the apical/lateral border, which is critical for the correct positioning and assembly of junctional complexes.

The mechanism of exclusion of Lgl is now well established. Several aPKC target proteins, including Lgl, the cell fate regulators Mira and Numb, and the *C. elegans* polarity protein PAR-2, localize to the plasma membrane via electrostatic interactions between basic and hydrophobic (BH) motifs and phospholipids [67,68]. Phosphorylation of aPKC consensus sites within BH motifs is thought to prevent membrane association by neutralizing positive charges [67,68]. Recent studies on *C. elegans* LGL-1 and human Lgl2 confirm this model and further show that aPKC phosphorylation counteracts formation of a membrane-binding α-helix conformation of the BH motif [69•,70]. In mitotic divisions of epithelial cells, the cell-cycle kinases Aurora A and B also phosphorylate Lgl on target sites within the BH motif to induce a complete loss from the cortex [71,72]. At the end of mitosis, when cells need to repolarize, the phosphatase PP1 dephosphorylates these sites [73•].

The mechanisms that mediate plasma membrane localization and exclusion of PAR-1 are also becoming increasingly clear. PAR-1 proteins contain a kinase domain, a linker region that harbors a conserved aPKC phosphorylation site, and a C-terminal kinase associated (KA1) domain that can mediate membrane association by binding to acidic phospholipids [55]. Phosphorylation by aPKC results in exclusion from the membrane by creating a binding site for the 14-3-3 protein Par5, and has also been reported to reduce PAR-1 kinase activity [74–77]. A second level of control over PAR-1 activity is exerted by the KA1 domain, which had been suggested to form an auto-inhibitory interaction with the kinase domain [55]. Recent investigations of the human PAR-1 kinase MARK1 revealed that the KA1 domain can indeed bind to the kinase domain, blocking substrate access, thereby rendering the kinase inactive [78•,79]. Binding of MARK1 to the plasma membrane via the KA1 domain releases this interaction, resulting in kinase activation [78•,79]. Recent data in *C. elegans* confirm an inhibitory role for the KA1 domain and furthermore indicate that inhibition of PAR-1 activity by aPKC/PKC-3 and the KA1 domain act in parallel to restrict PAR-1 activity to the posterior domain [80].

In addition to aPKC, PAR-1, and Lgl, the FERM domain protein Yurt plays a role in determining apical and basolateral domain sizes in *Drosophila*, zebrafish and cultured mammalian cells [81–86]. Yurt physically interacts with and negatively regulates Crumbs and aPKC, and is itself a target for inactivation by aPKC [81–83,85]. In developing *Drosophila* embryos, Yurt displays a dynamic localization and activity pattern, initially preventing Crumbs localization to the basolateral domain, and later acting at the apical domain to limit Crumbs activity and prevent apical overexpansion [82,83,85]. How Yurt inhibits aPKC is not known, but the binding to Crumbs was recently shown to depend on Yurt oligomerization [87•]. Phosphorylation of Yurt by aPKC prevents this oligomerization, explaining at least in part how aPKC counteracts Yurt [87•].

Finally, aPKC may not be the only kinase involved in excluding proteins from the apical domain. A recent study reported that, in the *Drosophila* follicular epithelium, the kinase Pak1 may act redundantly with aPKC downstream of Cdc42, and phosphorylates an overlapping set of substrates [88]. Inhibition of Pak1 also resulted in polarity defects in mammalian cell cultures, indicating that the role of Pak1 may be conserved [88].

Conclusion

The past couple of years have seen great progress in our understanding of the regulatory interactions between polarity proteins, and several novel mechanisms of control have emerged. It is clear that polarity proteins are regulated at multiple levels, as shown by the dual control over PAR-1 activity by aPKC and membrane association, the redundant control over Baz/Par3 membrane association by oligomerization and phospholipid binding, and the multiple mechanisms involved in targeting aPKC to the apical domain. While we have highlighted commonalities, the organization of cell polarity is not the same in all tissues and organisms. In *Drosophila*, *C. elegans*, and mouse embryos the requirements for Par3, Par6, Cdc42, aPKC

and Crumbs all vary between epithelial tissues [48,51•,89–93], and recent investigations in the *Drosophila* midgut point to polarization mechanisms that do not rely on Par or Crumbs proteins at all [49••]. For the future, it will be important to clarify how conserved and generally used each mechanism of regulation is in different organisms and polarized cell types.

Conflict of interest statement

Nothing declared.

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References and recommended reading

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

- • of special interest
- •• of outstanding interest

1. Roignot J, Peng X, Mostov K: **Polarity in mammalian epithelial morphogenesis**. *Cold Spring Harb Perspect Biol* 2013, **5**.

2. Rodriguez-Boulan E, Macara IG: **Organization and execution of the epithelial polarity programme**. *Nat Rev Mol Cell Biol* 2014, **15**:225-242.

3. Tepass U: **The apical polarity protein network in Drosophila epithelial cells: regulation of polarity, junctions, morphogenesis, cell growth, and survival**. *Annu Rev Cell Dev Biol* 2012, **28**:655-685.

4. St Johnston D, Ahringer J: **Cell polarity in eggs and epithelia: parallels and diversity**. *Cell* 2010, **141**:757-774.

5. Goldstein B, Macara IG: **The PAR proteins: fundamental players in animal cell polarization**. *Dev Cell* 2007, **13**:609-622.

6. Bulgakova NA, Knust E: **The Crumbs complex: from epithelial-cell polarity to retinal degeneration**. *J Cell Sci* 2009, **122**:2587-2596.

7. Franz A, Riechmann V: **Stepwise polarisation of the Drosophila follicular epithelium**. *Dev Biol* 2010, **338**:136-147.

8. Krahn MP, Bückers J, Kastrup L, Wodarz A: **Formation of a Bazooka-Stardust complex is essential for plasma membrane polarity in epithelia**. *J Cell Biol* 2010, **190**:751-760.

9. Morais-de-Sá E, Mirouse V, St Johnston D: **aPKC phosphorylation of Bazooka defines the apical/lateral border in Drosophila epithelial cells**. *Cell* 2010, **141**:509-523.

10. Ebnet K, Iden S, Gerke V, Suzuki A: **Regulation of epithelial and endothelial junctions by PAR proteins**. *Front Biosci J Virtual Libr* 2008, **13**:6520-6536.

11. Laprise P, Tepass U: **Novel insights into epithelial polarity proteins in Drosophila**. *Trends Cell Biol* 2011, **21**:401-408.

12. Pichaud F, Walther RF, Nunes de Almeida F: **Regulation of Cdc42 and its effectors in epithelial morphogenesis**. *J Cell Sci* 2019, **132**.

13. Graybill C, Wee B, Atwood SX, Prehoda KE: **Partitioning-defective protein 6 (Par-6) activates atypical protein kinase C (aPKC) by pseudosubstrate displacement**. *J Biol Chem* 2012, **287**:21003-21011.

14. • Dong W, Lu J, Zhang X, Wu Y, Lettieri K, Hammond GR, Hong Y: **A polybasic domain in aPKC mediates Par-6-dependent control of plasma membrane targeting and kinase activity**. *bioRxiv* 2019 http://dx.doi.org/10.1101/588624
This preprint describes a novel mechanism for targeting aPKC to the plasma membrane that is independent of Cdc42. This could explain the observation that certain cells, like *C. elegans* epidermal cells, do not seem to require Cdc42 for apical localization of Par6 and aPKC.

15. Hong Y: **aPKC: the kinase that phosphorylates cell polarity**. *F1000Research* 2018, **7**.

16. David DJV, Wang Q, Feng JJ, Harris TJC: **Bazooka inhibits aPKC to limit antagonism of actomyosin networks during amnioserosa apical constriction**. *Dev Camb Engl* 2013, **140**:4719-4729.

17. •• Rodriguez J, Peglion F, Martin J, Hubatsch L, Reich J, Hirani N, Gubieda AG, Roffey J, Fernandes AR, St Johnston D *et al.*: **aPKC cycles between functionally distinct PAR protein assemblies to drive cell polarity**. *Dev Cell* 2017, **42**:400-415.e9
Finds that aPKC shuttles between PAR-3 and CDC-42 containing complexes in the *C. elegans* one cell embryo, linking cue-sensing roles and effector roles within the apical network.

18. Soriano EV, Ivanova ME, Fletcher G, Riou P, Knowles PP, Barnouin K, Purkiss A, Kostelecky B, Saiu P, Linch M *et al.*: **aPKC Inhibition by Par3 CR3 flanking regions controls substrate access and underpins apical-junctional polarization**. *Dev Cell* 2016, **38**:384-398.

19. Lin D, Edwards AS, Fawcett JP, Mbamalu G, Scott JD, Pawson T: **A mammalian PAR-3-PAR-6 complex implicated in Cdc42/Rac1 and aPKC signalling and cell polarity**. *Nat Cell Biol* 2000, **2**:540-547.

20. Holly RW, Prehoda KE: **Phosphorylation of Par-3 by atypical protein kinase C and competition between its substrates**. *Dev Cell* 2019, **49**:678-679.

21. Thompson BJ, McDonald NQ: **Competitive inhibition of aPKC by Par-3/bazooka and other substrates**. *Dev Cell* 2019, **49**:680.

22. Joberty G, Petersen C, Gao L, Macara IG: **The cell-polarity protein Par6 links Par3 and atypical protein kinase C to Cdc42**. *Nat Cell Biol* 2000, **2**:531-539.

23. Li J, Kim H, Aceto DG, Hung J, Aono S, Kemphues KJ: **Binding to PKC-3, but not to PAR-3 or to a conventional PDZ domain ligand, is required for PAR-6 function in *C. elegans***. *Dev Biol* 2010, **340**:88-98.

24. Renschler FA, Bruekner SR, Salomon PL, Mukherjee A, Kullmann L, Schütz-Stoffregen MC, Henzler C, Pawson T, Krahn MP, Wiesner S: **Structural basis for the interaction between the cell polarity proteins Par3 and Par6**. *Sci Signal* 2018, **11**.

25. Harris TJC: **Protein clustering for cell polarity: Par-3 as a paradigm**. *F1000Research* 2017, **6**:1620.

26. Wang S-C, Low TYF, Nishimura Y, Gole L, Yu W, Motegi F: **Cortical forces and CDC-42 control clustering of PAR proteins for *Caenorhabditis elegans* embryonic polarization**. *Nat Cell Biol* 2017, **19**:988-995.

27. •• Dickinson DJ, Schwager F, Pintard L, Gotta M, Goldstein B: **A single-cell biochemistry approach reveals PAR complex dynamics during cell polarization**. *Dev Cell* 2017, **42**:416-434.e11
Uses a highly innovative single cell interaction assay to elucidate the regulation of PAR complex composition and stoichiometry in the one-cell *C. elegans* embryo.

28. Kono K, Yoshiura S, Fujita I, Okada Y, Shitamukai A, Shibata T, Matsuzaki F: **Reconstruction of Par polarity in apolar cells reveals a dynamic process of cortical polarization**. *bioRxiv* 2019 http://dx.doi.org/10.1101/523589.

29. Krahn MP, Klopfenstein DR, Fischer N, Wodarz A: **Membrane targeting of Bazooka/PAR-3 is mediated by direct binding to phosphoinositide lipids**. *Curr Biol* 2010, **20**:636-642.

30. Horikoshi Y, Hamada S, Ohno S, Suetsugu S: **Phosphoinositide binding by par-3 involved in par-3 localization**. *Cell Struct Funct* 2011, **36**:97-102.

31. Claret S, Jouette J, Benoit B, Legent K, Guichet A: **PI(4,5)P2 produced by the PI4P5K SKTL controls apical size by tethering PAR-3 in Drosophila epithelial cells**. *Curr Biol* 2014, **24**:1071-1079.

32. Kullmann L, Krahn MP: **Redundant regulation of localization and protein stability of DmPar3**. *Cell Mol Life Sci* 2018, **75**:3269-3282.

33. McKinley RFA, Yu CG, Harris TJC: **Assembly of Bazooka polarity landmarks through a multifaceted membrane-association mechanism**. *J Cell Sci* 2012, **125**:1177-1190.

34. Harris TJC, Peifer M: **The positioning and segregation of apical cues during epithelial polarity establishment in Drosophila**. *J Cell Biol* 2005, **170**:813-823.

35. Jouette J, Guichet A, Claret SB: **Dynein-mediated transport and membrane trafficking control PAR3 polarised distribution**. *eLife* 2019, **8**.

36. McKinley RFA, Harris TJC: **Displacement of basolateral Bazooka/PAR-3 by regulated transport and dispersion during epithelial polarization in Drosophila**. *Mol Biol Cell* 2012, **23**:4465-4471.

37. Goehring NW, Grill SW: **Cell polarity: mechanochemical patterning**. *Trends Cell Biol* 2013, **23**:72-80.

38. Walther RF, Pichaud F: **Crumbs/DaPKC-dependent apical exclusion of Bazooka promotes photoreceptor polarity remodeling**. *Curr Biol* 2010, **20**:1065-1074.

39. Hirose T, Izumi Y, Nagashima Y, Tamai-Nagai Y, Kurihara H, Sakai T, Suzuki Y, Yamanaka T, Suzuki A, Mizuno K *et al.*: **Involvement of ASIP/PAR-3 in the promotion of epithelial tight junction formation**. *J Cell Sci* 2002, **115**:2485-2495.

40. Nagai-Tamai Y, Mizuno K, Hirose T, Suzuki A, Ohno S: **Regulated protein-protein interaction between aPKC and PAR-3 plays an essential role in the polarization of epithelial cells**. *Genes Cells Devoted Mol Cell Mech* 2002, **7**:1161-1171.

41. •• de Almeida FN, Walther RF, Pressé M, Vlassaks E, Pichaud F: **Cdc42 defines apical identity and regulates epithelial morphogenesis by promoting apical recruitment of Par6-aPKC and Crumbs**. *Development* 2019, **146** dev175497

Using a series of Par6 alleles that disrupt specific interactions, this study investigates the recruitment of Par6-aPKC, and finds that Cdc42 promotes apical identity by coordinating the apical recruitment of Par6-aPKC and Crb.

42. Peterson FC, Penkert RR, Volkman BF, Prehoda KE: **Cdc42 regulates the Par-6 PDZ domain through an allosteric CRIB-PDZ transition**. *Mol Cell* 2004, **13**:665-676.

43. Whitney DS, Peterson FC, Volkman BF: **A conformational switch in the CRIB-PDZ module of Par-6**. *Struct Lond Engl 1993* 2011, **19**:1711-1722.

44. Whitney DS, Peterson FC, Kittell AW, Egner JM, Prehoda KE, Volkman BF: **Binding of Crumbs to the Par-6 CRIB-PDZ module is regulated by Cdc42**. *Biochemistry* 2016, **55**:1455-1461.

45. Beers M, Kemphues K: **Depletion of the co-chaperone CDC-37 reveals two modes of PAR-6 cortical association in *C. elegans* embryos**. *Dev Camb Engl* 2006, **133**:3745-3754.

46. Aceto D, Beers M, Kemphues KJ: **Interaction of PAR-6 with CDC-42 is required for maintenance but not establishment of PAR asymmetry in *C. elegans***. *Dev Biol* 2006, **299**:386-397.

47. Hannaford M, Loyer N, Tonelli F, Zoltner M, Januschke J: **A chemical-genetics approach to study the role of atypical Protein Kinase C in Drosophila**. *Dev Camb Engl* 2019, **146**.

48. Shahab J, Tiwari MD, Honemann-Capito M, Krahn MP, Wodarz A: **Bazooka/PAR3 is dispensable for polarity in Drosophila follicular epithelial cells**. *Biol Open* 2015, **4**:528-541.

49. •• Chen J, Sayadian A-C, Lowe N, Lovegrove HE, St Johnston D: **An alternative mode of epithelial polarity in the Drosophila midgut**. *PLoS Biol* 2018, **16**:e3000041

Provocative study that indicates that polarity in the *Drosophila* midgut can be achieved without critical roles for the canonical cortical polarity regulators.

50. Sherrard KM, Fehon RG: **The transmembrane protein Crumbs displays complex dynamics during follicular morphogenesis and is regulated competitively by Moesin and aPKC**. *Dev Camb Engl* 2015, **142**:2226.

51. • Zilberman Y, Abrams J, Anderson DC, Nance J: **Cdc42 regulates junctional actin but not cell polarization in the *Caenorhabditis elegans* epidermis**. *J Cell Biol* 2017 http://dx.doi.org/10.1083/jcb.201611061

Cdc42 is not required for apical localization of PAR-6 and PKC-3 in *C. elegans* embryonic epithelia, in contrast to its role regulating the activity and localization of PAR-6/PKC-3 in the one-cell embryo, suggesting that the regulation of PAR-6/PKC-3 by Cdc42 is context dependent.

52. Zihni C, Vlassaks E, Terry S, Carlton J, Leung TKC, Olson M, Pichaud F, Balda MS, Matter K: **An apical MRCK-driven morphogenetic pathway controls epithelial polarity**. *Nat Cell Biol* 2017, **19**:1049-1060.

53. Stephens R, Lim K, Portela M, Kvansakul M, Humbert PO, Richardson HE: **The scribble cell polarity module in the regulation of cell signaling in tissue development and tumorigenesis**. *J Mol Biol* 2018, **430**:3585-3612.

54. Bonello TT, Peifer M: **Scribble: a master scaffold in polarity, adhesion, synaptogenesis, and proliferation**. *J Cell Biol* 2019, **218**:742-756.

55. Wu Y, Griffin EE: **Regulation of cell polarity by PAR-1/MARK kinase**. *Curr Top Dev Biol* 2017, **123**:365-397.

56. Yamanaka T, Ohno S: **Role of Lgl/Dlg/Scribble in the regulation of epithelial junction, polarity and growth**. *Front Biosci J Virtual Libr* 2008, **13**:6693-6707.

57. de Vreede G, Schoenfeld JD, Windler SL, Morrison H, Lu H, Bilder D: **The Scribble module regulates retromer-dependent endocytic trafficking during epithelial polarization**. *Development* 2014, **141**:2796-2802.

58. Liu H, Wang S, Hang W, Gao J, Zhang W, Cheng Z, Yang C, He J, Zhou J, Chen J *et al.*: **LET-413/Erbin acts as a RAB-5 effector to promote RAB-10 activation during endocytic recycling**. *J Cell Biol* 2018, **217**:299-314.

59. Bonello TT, Peifer M: **Scribble and Discs-large direct adherens junction positioning and supermolecular assembly to establish apical-basal polarity**. *J Cell Biol* 2017, **216**:3729-3744.

60. Zhu J, Shang Y, Wan Q, Xia Y, Chen J, Du Q, Zhang M: **Phosphorylation-dependent interaction between tumor suppressors Dlg and Lgl**. *Cell Res* 2014, **24**:451-463.

61. Mathew D, Gramates LS, Packard M, Thomas U, Bilder D, Perrimon N, Gorczyca M, Budnik V: **Recruitment of scribble to the synaptic scaffolding complex requires GUK-holder, a novel DLG binding protein**. *Curr Biol* 2002, **12**:531-539.

62. Caria S, Magtoto CM, Samiei T, Portela M, Lim KYB, How JY, Stewart BZ, Humbert PO, Richardson HE, Kvansakul M: ***Drosophila melanogaster* Guk-holder interacts with the Scribbled PDZ1 domain and regulates epithelial development with Scribbled and Discs Large**. *J Biol Chem* 2018, **293**:4519-4531.

63. Sotillos S, Díaz-Meco MT, Caminero E, Moscat J, Campuzano S: **DaPKC-dependent phosphorylation of Crumbs is required for epithelial cell polarity in Drosophila**. *J Cell Biol* 2004, **166**:549-557.

64. Cao H, Xu R, Shi Q, Zhang D, Huang J, Hong Y: **FERM domain phosphorylation and endogenous 3′UTR are not essential for regulating the function and subcellular localization of polarity protein Crumbs**. *J Genet Genomics* 2017, **44**:409-412.

65. Benton R, St Johnston D: ***Drosophila* PAR-1 and 14-3-3 inhibit Bazooka/PAR-3 to establish complementary cortical domains in polarized cells**. *Cell* 2003, **115**:691-704.

66. Krahn MP, Egger-Adam D, Wodarz A: **PP2A antagonizes phosphorylation of Bazooka by PAR-1 to control apical-basal polarity in dividing embryonic neuroblasts**. *Dev Cell* 2009, **16**:901-908.

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

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

69. • Almagor L, Ufimtsev IS, Ayer A, Li J, Weis WI: **Structural insights into the aPKC regulatory switch mechanism of the human cell polarity protein lethal giant larvae 2**. *Proc Natl Acad Sci U S A* 2019, **116**:10804-10812

aPKC mediated phosporylation of Lgl does not cause conformational changes in the crystal structure of the protein. However, it induces a charge neutralization that favors the association of Lgl with negatively charged lipids such PIP2 of the plasma membrane.

70. Visco I, Hoege C, Hyman AA, Schwille P: **In vitro reconstitution of a membrane switch mechanism for the polarity protein LGL**. *J Mol Biol* 2016, **428**:4828-4842.

71. Bell GP, Fletcher GC, Brain R, Thompson BJ: **Aurora kinases phosphorylate Lgl to induce mitotic spindle orientation in Drosophila epithelia**. *Curr Biol* 2015, **25**:61-68.

72. Carvalho CA, Moreira S, Ventura G, Sunkel CE, Morais-de-Sá E: **Aurora A triggers Lgl cortical release during symmetric division to control planar spindle orientation**. *Curr Biol* 2015, **25**:53-60.

73. • Moreira S, Osswald M, Ventura G, Gonçalves M, Sunkel CE, Morais-de-Sá E: **PP1-mediated dephosphorylation of Lgl controls apical-basal polarity**. *Cell Rep* 2019, **26**:293-301.e7

Upon mitotic exit, the protein phosphatase PP1 dephosphorylates Lgl on serines phosphorylated by aPKC and Aurora A, enabling Lgl to reload onto the cortex to promote re-polarization after cell division.

74. Hurov JB, Watkins JL, Piwnica-Worms H: **Atypical PKC phosphorylates PAR-1 kinases to regulate localization and activity**. *Curr Biol* 2004, **14**:736-741.

75. Suzuki A, Hirata M, Kamimura K, Maniwa R, Yamanaka T, Mizuno K, Kishikawa M, Hirose H, Amano Y, Izumi N *et al.*: **aPKC acts upstream of PAR-1b in both the establishment and maintenance of mammalian epithelial polarity**. *Curr Biol* 2004, **14**:1425-1435.

76. Kusakabe M, Nishida E: **The polarity-inducing kinase Par-1 controls *Xenopus* gastrulation in cooperation with 14-3-3 and aPKC**. *EMBO J* 2004, **23**:4190-4201.

77. Motegi F, Zonies S, Hao Y, Cuenca AA, Griffin E, Seydoux G: **Microtubules induce self-organization of polarized PAR domains in *Caenorhabditis elegans* zygotes**. *Nat Cell Biol* 2011, **13**:1361-1367.

78. • Emptage RP, Lemmon MA, Ferguson KM: **Molecular determinants of KA1 domain-mediated autoinhibition and phospholipid activation of MARK1 kinase**. *Biochem J* 2017, **474**:385-398

Elucidates the mechanism behind autoinhibition and phospholipid activation of the human MARK1 kinase.

79. Emptage RP, Lemmon MA, Ferguson KM, Marmorstein R: **Structural basis for MARK1 kinase autoinhibition by Its KA1 domain**. *Struct Lond Engl 1993* 2018, **26**:1137-1143.e3.

80. Folkmann AW, Seydoux G: **Spatial regulation of the polarity kinase PAR-1 by parallel inhibitory mechanisms**. *Dev Camb Engl* 2019, **146**.

81. Hsu Y-C, Willoughby JJ, Christensen AK, Jensen AM: **Mosaic eyes is a novel component of the Crumbs complex and negatively regulates photoreceptor apical size**. *Dev Camb Engl* 2006, **133**:4849-4859.

82. Laprise P, Lau KM, Harris KP, Silva-Gagliardi NF, Paul SM, Beronja S, Beitel GJ, McGlade CJ, Tepass U: **Yurt, Coracle, Neurexin IV and the Na(+),K(+)-ATPase form a novel group of epithelial polarity proteins**. *Nature* 2009, **459**:1141-1145.

83. Laprise P, Beronja S, Silva-Gagliardi NF, Pellikka M, Jensen AM, McGlade CJ, Tepass U: **The FERM protein Yurt is a negative regulatory component of the Crumbs complex that controls epithelial polarity and apical membrane size**. *Dev Cell* 2006, **11**:363-374.

84. Lee JD, Silva-Gagliardi NF, Tepass U, McGlade CJ, Anderson KV: **The FERM protein Epb4.1l5 is required for organization of the neural plate and for the epithelial-mesenchymal transition at the primitive streak of the mouse embryo**. *Dev Camb Engl* 2007, **134**:2007-2016.

85. Gamblin CL, Hardy ÉJ-L, Chartier FJ-M, Bisson N, Laprise P: **A bidirectional antagonism between aPKC and Yurt regulates epithelial cell polarity**. *J Cell Biol* 2014, **204**:487-495.

86. Schell C, Rogg M, Suhm M, Helmstädter M, Sellung D, Yasuda-Yamahara M, Kretz O, Küttner V, Suleiman H, Kollipara L *et al.*: **The FERM protein EPB41L5 regulates actomyosin contractility and focal adhesion formation to maintain the kidney filtration barrier**. *Proc Natl Acad Sci U S A* 2017, **114**:E4621-E4630.

87. • Gamblin CL, Parent-Prévost F, Jacquet K, Biehler C, Jetté A, Laprise P: **Oligomerization of the FERM-FA protein Yurt controls epithelial cell polarity**. *J Cell Biol* 2018, **217**:3853-3862

Oligomerization of the polarity protein Yurt is essential for binding to Crumbs. aPKC-mediated phosphorylation dismantles the Yurt oligomer, inhibiting Yurt function and excluding the protein from the apical domain.

88. Aguilar-Aragon M, Elbediwy A, Foglizzo V, Fletcher GC, Li VSW, Thompson BJ: **Pak1 kinase maintains apical membrane identity in epithelia**. *Cell Rep* 2018, **22**:1639-1646.

89. Achilleos A, Wehman AM, Nance J: **PAR-3 mediates the initial clustering and apical localization of junction and polarity proteins during *C. elegans* intestinal epithelial cell polarization**. *Development* 2010, **137**:1833-1842.

90. Totong R, Achilleos A, Nance J: **PAR-6 is required for junction formation but not apicobasal polarization in *C. elegans* embryonic epithelial cells**. *Dev Camb Engl* 2007, **134**:1259-1268.

91. Hirose T, Karasawa M, Sugitani Y, Fujisawa M, Akimoto K, Ohno S, Noda T: **PAR3 is essential for cyst-mediated epicardial development by establishing apical cortical domains**. *Dev Camb Engl* 2006, **133**:1389-1398.

92. Tepass U, Knust E: **Phenotypic and developmental analysis of mutations at the crumbs locus, a gene required for the development of epithelia in *Drosophila melanogaster***. *Rouxs Arch Dev Biol* 1990, **199**:189-206.

93. Waaijers S, Ramalho JJ, Koorman T, Kruse E, Boxem M: **The *C. elegans* Crumbs family contains a CRB3 homolog and is not essential for viability**. *Biol Open* 2015, **4**:276-284.