A signaling complex in which atypical protein kinase C associates with a regulatory protein, Par6, plays an essential role in establishing cell polarity. Recent studies in organisms ranging from worms to mammals have highlighted some of the conserved mechanisms by which the assembly, localization and activity of this complex are regulated. Recent work is also beginning to unravel how this complex acts in concert with additional molecular complexes to establish and maintain polarity.

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Abbreviations

- A–P: anterior–posterior
- Apc: Adenomatous polyposis coli
- aPKC: atypical protein kinase C
- CRIB: Cdc42/Rac-interactive-binding
- FRET: fluorescence resonance energy transfer
- GBD: GTPase-binding domain
- GFP: green fluorescent protein
- GSK-3β: glycogen synthase kinase-3β
- JAM-1: junctional adhesion molecule 1
- Lgl: lethal giant larvae
- MDCK: Madin-Darby canine kidney
- PDZ: PSD95/Discs large/ZO1
- TJ: tight junctions
- ZA: zonula adherens

Introduction

Polarity is a fundamental property of all eukaryotic cells. During the development of metazoans, regulation of cell polarity is essential for the spatio-temporal regulation of cell and tissue morphogenesis. Cell polarity is also involved in the regulation of cell identity. During asymmetric cell division, fate asymmetry relies on the polarized distribution and unequal segregation of cell-fate determinants at mitosis. The polarized distribution of key regulatory molecules not only underlies the generation of differentiated cell types in developing tissues but also controls the establishment of the anterior–posterior (A–P) body axis in flies and worms. Finally, cell polarity is a key aspect of cell differentiation, underlying cell migration and morphogenesis.

In recent years, a signaling complex of three proteins, Par3, Par6 and atypical protein kinase C (aPKC), has emerged as a central player in the mechanisms that regulate cell polarity in the different cell types of various organisms ranging from worms to mammals (for review, see [1,2]). Recent work indicates that the core of this signaling complex is composed of Par6 and aPKC. Here, we review some recent studies that shed light on how the output of the Par6–aPKC complex is regulated and how this complex directs the establishment of cell polarity. In particular, we discuss findings suggesting that the signaling potential of the complex resides with aPKC and its kinase activity, with Par6 functioning as a key regulator of aPKC kinase activity and with other components, like Par3, functioning as structural scaffolds or as regulators of the kinase activity and/or specificity.

The Par proteins: from Caenorhabditis elegans to mammals

The C. elegans par genes (par1 to par6) were first identified in a genetic screen for maternal-effect mutations affecting the unequal partitioning of polar granules to the posterior cell during the asymmetric division of the one-cell embryo [3]. Two of these genes, par-3 and par-6, encode PSD95/Discs large/ZO1 (PDZ) domain proteins that co-localize at the anterior pole of the embryo [4,5]. A third protein, aPKC, encoded by the pck-3 gene, was later shown to bind Par3 and to co-localize with Par3 and Par6, leading to the proposal that the three proteins form a complex [6] (Figure 1, Table 1). Work from several laboratories has established the pivotal role of the Par3–Par6–aPKC complex in establishing A–P polarity and in regulating asymmetric division in the C. elegans one-cell embryo (for review, see [7,8]).

The initial cue for the anterior localization of the Par3–Par6–aPKC complex involves the microtubule-nucleating activity of the sperm asters, which act to exclude the complex from the posterior cortex of the cell [9–11]. Although little is known about the mechanisms that mediate this activity, a recent study has revealed that the anterior localization of the Par3–Par6–aPKC complex results from a two-step process [12**]. In a first initiation step, the Par3–Par6–aPKC complex is excluded from the posterior cortex in response to the activity of the sperm asters. In a second step, another Par protein, Par2 (a RING
finger protein encoded by the par-2 gene [13]) acts to prevent the posterior spreading of the Par3–Par6–aPKC complex. The Par2 protein has no role in the first step, and its localization to the posterior cortex, where it maintains the exclusion of the Par3–Par6–aPKC complex, only happens when the complex is displaced anteriorly by the sperm asters [12**]. Two other proteins, the class-II non-muscle myosin NMY2 and the 14.3.3 protein family member Par5, were also shown to participate in the initial exclusion of the Par3–Par6–aPKC complex from the posterior cortex [12**,14]; NMY-2 is necessary to prevent accumulation of Par3–Par6–aPKC at the posterior cortex and Par5 seems to have a dual function, first promoting the posterior removal of the Par3–Par6–aPKC complex induced by the sperm asters and later mediating the ability of Par3–Par6–aPKC to remove Par2 from the anterior cortex [12**,14].

Thus, an important question concerns how the Par3–Par6–aPKC complex associates with the cell cortex, a process in which Par3 seems to play a specific role. Indeed, Par3 is initially present at the cortex of both par-6 and pck-3 mutant embryos, whereas the converse is not true: in par-3 mutants, Par6 and aPKC never reach the cortex [5,15,16]. This suggests that one of the roles of Par3 in the complex is to recognize binding partners at the cell membrane. Such an interaction may either provide a platform for the assembly of the complex at the membrane or target the pre-formed complex to the cortex. Future work should elucidate these points and reveal the mechanisms involved in localizing the Par3–Par6–aPKC complex to the anterior pole of the one-cell embryo, where it triggers a molecular cascade that directs the establishment of A–P polarity.

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**Table 1**

<table>
<thead>
<tr>
<th>Fly homologue</th>
<th>Mouse homologue</th>
<th>Domains</th>
<th>Partners</th>
</tr>
</thead>
<tbody>
<tr>
<td>aPKC</td>
<td>PKCδ/PKCε</td>
<td>Kinase, C1, OPR</td>
<td>Par6, Par3, GSK-3β*</td>
</tr>
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<td>Par6A-D</td>
<td>CRIB, PDZ, OPR</td>
<td>aPKC, Par3, Lgl, Std/PALS1</td>
</tr>
<tr>
<td>Bazooka (Par3)</td>
<td>ASIP (Par3)</td>
<td>PDZ</td>
<td>aPKC, Par6, JAM-1</td>
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<tr>
<td>Cdc42</td>
<td>Cdc42</td>
<td>–</td>
<td>Par6</td>
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<tr>
<td>Crumbs (Crb)</td>
<td>Crb</td>
<td>EGF, LamG, TM</td>
<td>Std/PALS1</td>
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<tr>
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<td>PALS1</td>
<td>L27, PDZ, SH3, GUk</td>
<td>Crumbs, Dit/PATJ, Par6</td>
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<tr>
<td>Discs-lost (Dlt)</td>
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<td>MRE, PDZ</td>
<td>–</td>
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<td>GUkHolder</td>
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<td>Lethal giant larvae (Lgl)</td>
<td>mLgl</td>
<td>WD40</td>
<td>MyosinII, syntaxin-4, Par6</td>
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</table>

Summary of our current knowledge on structural domains and interacting partners of the proteins that form the three molecular complexes considered in this review. *The interaction may be indirect. † Many interactors of Dlg have been characterized in flies and mammals; see for instance [65] and references therein.
Which molecular mechanisms are involved downstream of the Par3–Par6–aPKC complex is an important question that has become even more crucial with the realization that similar complexes, involving homologues of the C. elegans proteins, are core regulators of cell polarity in other organisms, from flies to vertebrates (Table 1). Indeed, the proteins encoded by the Drosophila par-6, aPKC and par-3 (bazooka) genes also form a complex [17–19]. This complex is known to regulate several developmental processes (for review, see [1,8]): A–P polarization of the presumptive oocyte in the female germ-line, apical–basal polarization of epithelial cells in the embryo, establishment of asymmetry in mitotic neuroblasts and sensory organ precursor cells, and polarized migration of border cells in the egg chamber. In all these processes, the three proteins appear to co-localize extensively, suggesting that, like their C. elegans homologues, the Drosophila proteins Par-6 and aPKC, together with Par3, act as a signaling complex.

In mammals, homologues of Par6 (Par6α–D [20]), aPKC (αPKCζ and αPKCζ [21]) and of Par3 (also known as ASIP [6]) have also been identified (Table 1). In fact, an interaction between Par6 and aPKC proteins was first shown in Madin–Darby canine kidney (MDCK) cells, where they form a complex with Par3 [20,22]. The three proteins were shown to co-localize at tight junctions (TJs) [6,20,23,24] and studies in cultured epithelial cells indicate that the Par3–Par6–aPKC complex promotes the formation of epithelial TJs and thereby contributes to the establishment and maintenance of apical–basal polarity [23–25]. Finally, Par6 and aPKC have also been described as regulators of cell polarity in migrating primary rat astrocytes [26]. In these cells, Par3 does not appear to co-localize with Par6 and aPKC and is therefore not part of the complex [26].

These studies have highlighted the fundamental importance of the Par6–aPKC signaling complex and introduced two basic questions; the first concerns what is upstream (i.e. how the complex is localized and activated) and the second concerns what is downstream (i.e. how the complex regulates polarity). We address these two questions below in the context of epithelial cells.

**Activation of the Par6–aPKC complex by Cdc42**

An important advance towards an understanding of how aPKC activity can be regulated within the complex with Par6 has come from the identification of Cdc42 as a Par6 partner (Figure 1). In a search for novel effectors of the GTPase Cdc42 using a yeast two-hybrid approach, three groups have identified human Par6 as a direct interactor with an activated form of Cdc42, Cdc42(V12) [20,27,28]. In a parallel study, Par6 was also shown to co-immunoprecipitate with Cdc42(V12) [22]. Cdc42, in the GTP-bound state, interacts with proteins that contain a short conserved sequence called a Cdc42/Rac-interactive-binding (CRIB) motif [29]. However, the Par6 CRIB motif is not complete, lacking two preserved histidines at the C-terminal half, and alone is not sufficient to bind Cdc42 [20]. The Par6 GTPase-binding domain (GBD) is actually formed by the semi-CRIB motif and the adjacent PDZ domain, which has been shown to be absolutely required to form a stable Cdc42∗-GTP–Par6 complex [20]. Recently, the crystal structure of a complex consisting of Par6 bound to activated Cdc42 with the mutation Q61L has been solved [30**], confirming that the PDZ domain indeed contributes to the normal binding of activated Cdc42, by extending and stabilizing the β-sheet structure formed by the partial CRIB motif. In addition, the peptide-binding region of the PDZ domain maps to a region opposite to the intra-molecular β-sheet formed by the PDZ domain and the partial CRIB motif [30**], suggesting that the PDZ domain can promote the simultaneous binding of Cdc42∗-GTP and Par3 (or other PDZ interactors), further modulating the aPKC activity in the complex.

Cdc42 is an important regulator of cell polarity [31,32]. In yeast, Cdc42 is not only required to establish polarity in response to extrinsic or intrinsic spatial cues but is also sufficient to establish polarity in the absence of any such cues via a self-organizing mechanism [33**]. Cdc42 is also important for the polarization of various cell types in metazoans, and accumulating evidence suggests that this involves its participation in the Par6–aPKC complex. For instance, it has been reported that Cdc42, Par6 and aPKC regulate the scratch-induced repolarization of migrating astrocytes [26]. In this experimental model, scratching a confluent monolayer of primary rat astrocytes leads to a reorganization of the microtubule network and a re-positioning of the Golgi apparatus along an axis defined by the wound (Figure 2a). Scratching induces localized activation of integrins at the leading edge of astrocytes, which leads to a fourfold increase in activation of Cdc42. This is accompanied by the recruitment of Par6–aPKC to the leading edge and by an increase in aPKC activity, which is dependent on Cdc42 activation. These findings imply that the correct polarization of astrocytes results from the localized activation of the Par6–aPKC complex at the leading edge by Cdc42∗-GTP. Consistent with this, overexpression of a kinase-dead aPKC or of dominant-negative versions of Cdc42 and Par6 was shown to inhibit the repolarization of the astrocytes [26].

This raises the question of how the binding of Cdc42∗-GTP to Par6 stimulates the kinase activity of the associated aPKC. The N-terminal region of Par6 binds to the regulatory domain of aPKC [20] and overexpression of this region by itself in COS cells significantly increases the kinase activity of the linked aPKC [24]. This N-terminal region of Par6 must therefore have an intrinsic potential to bind and stimulate the kinase activity of aPKC. However, this potential seems to be suppressed by the adjacent GBD, as overexpression of the full Par6 protein is not able
to stimulate aPKC activity [24]. This is not the case if activated Cdc42 is present, which results in a significant increase of the kinase activity. Together, these results led to a model (Figure 1) in which the kinase activity of the Par6–aPKC complex is turned on by the binding of Cdc42-GTP to Par6’s GBD, relieving the GBD-mediated inhibition [24]. In support of this model, FRET analysis [30**] indicates that the binding of Cdc42-GTP to Par6 induces a conformational change in Par6 that might increase the affinity of its N-terminal domain for aPKC and allow a switch in the kinase activity of the complex.

Thus, the emerging picture is that aPKC activation within the Par6 complex is mediated by Cdc42-GTP through double-negative regulation, whereby activated Cdc42 inhibits the Par6-dependent inhibition of aPKC. This suggests a simple mechanism for producing specific and highly localized aPKC activity within a cell: targeting the inactive Par6–aPKC complex to a defined cortical domain and regulating its timely activation by Cdc42. In such a scenario, additional components of the Par6–aPKC complex might regulate its targeting to specific cortical domains where it can be activated.

Functional analysis of the Par6–aPKC complex. (a) Migrating astrocyte: the active Par6–aPKC complex localizes at the leading edge of migrating astrocytes and reorganizes microtubule polarity via the regulation of Apc. (b) Asymmetrically dividing neuroblast: the apical Par3–Par6–aPKC complex inactivates Lgl at the apical cortex, via direct aPKC-mediated phosphorylation. Lgl activity is thereby restricted to the basal cortex, where it directs the localization of cell-fate determinants. (c) Epithelial cell: the crb and Par3–Par6–aPKC complexes localize apical to the ZA whereas the Lgl and the Scrib complexes localize along basolateral membranes. Direct interaction between the Crb and Par3–Par6–aPKC complex is mediated by a Std–Par6 interaction. Genetic evidence suggests that the main function of the Crb complex is to antagonize the activity of the Scrib complex, whereas the main function of the Scrib complex is to antagonize the activity of the Par3–Par6–aPKC complex. Also, the apical localization of the Crb complex depends on the activity of the Par3–Par6–aPKC complex. See text for details.
Regulatory role(s) of Par3
Par3 is a good candidate to be such a regulatory partner of the Par6–aPKC core. Par3 directly binds aPKC [6] and is phosphorylated in vitro by aPKC [22,34], and co-localization of Par6–aPKC with Par3 is seen in the C. elegans one-cell embryo [5,15], in Drosophila epithelial cells [18] and neuroblasts [17,19,35], and in mammalian cells [6,20,23,24]. Par3 may therefore be a conserved scaffold for the whole complex. As discussed above, C. elegans Par3 plays a role in targeting the Par6–aPKC complex to the cortex. However, recent results indicate that Par3 is not always found associated with Par6–aPKC (see below), raising the possibility that it may act as a site-specific anchor for the cytosolic Par6–aPKC complex. In mammalian cells, Par3 directly binds, via its PDZ domains, the C terminus of the junctional adhesion molecule 1 (JAM-1) [36,37], a single-pass membrane protein belonging to the immunoglobulin superfamily. JAM-1 directly associates with the PDZ3 domain of ZO1, which itself directly binds the C terminus of claudin, a major structural constituent of the TJ [36]. Thus, JAM-1 may recruit Par3 to the claudin-based structures that will eventually form TJJs, thereby providing a scaffold to assemble an active Par6–aPKC complex, which is necessary for correct TJ formation. In another recent study [38], Par3 was shown to directly bind, via its PDZ1 domain, to the C terminus of the immunoglobulin-like cell–cell adhesion molecules Nectin-1 and -3 in neuroepithelial cells. Whether Nectin-1 and -3, which localize at adherens junctions, recruit the Par6–aPKC complex via Par3 remains to be investigated.

Whereas genetic data from worms and flies indicate that Par3 plays a primarily positive role in aPKC signaling, a negative regulatory function has also been proposed on the basis of the observation that addition of the aPKC-binding domain of Par3 to purified aPKC leads to a twofold reduction in the kinase activity [22]. Additional experiments are, however, required to validate this hypothesis, which is for the moment solely based on an in vitro result obtained in the absence of Par6. We note, however, that inhibition of aPKC by Par3 might help to prevent ‘ectopic’ aPKC activation until the complex arrives at the right place to be activated by Cdc42-GTP in a timely fashion. We further speculate that aPKC activation might then lead to Par3 phosphorylation and thereby avert Par3-mediated inhibition.

Phosphorylation targets of aPKC
As mentioned above, the identification of direct phosphorylation targets of aPKC is expected to provide key insights into how asymmetrically localized Par6–aPKC regulates polarity. Recently, two targets of aPKC that might act as polarity regulators have been identified. The first, Lethal giant larvae (Lgl), is a membrane-associated protein with five WD40 repeats that was originally identified in Drosophila as the product of a tumor-suppressor gene [39]. New work in Drosophila [40**] and mammals [41*,42*] has now shown that Lgl interacts directly with Par6, via its partial CRIB and PDZ domains, and with aPKC. Furthermore, sequential co-immunoprecipitation experiments reveal that Lgl, Par6 and aPKC are present in the same complex. However, Par3 does not appear to be part of this complex either in flies or in mammals [40**,41**], and further studies suggest that Lgl and Par3 compete to bind to the Par6–aPKC complex [42*].

The presence of Lgl in the Par6–aPKC complex leads to its direct phosphorylation by aPKC at highly conserved serine residues [40**,41*,42*], and this phosphorylation has been shown, in both Drosophila neuroblasts and mammalian cells, to control the intracellular localization of Lgl and its function in regulating cell polarity.

In Drosophila neuroblasts, Lgl activity is known to be required for the basal localization of the cell-fate determinants Numb and Pros during asymmetric division [43,44]. It is not, however, required for the apical localization of the Par3–Par6–aPKC complex, suggesting that Lgl acts downstream of the apical complex to promote the localization of cell-fate determinants to the basal pole. Lgl itself is not asymmetrically distributed, and is found uniformly distributed at the cortex and in the cytoplasm [43,44]. However, as aPKC localizes apically, this might mean that apical Lgl is phosphorylated whereas the remaining pool of Lgl across the cell is unphosphorylated. In the absence of specific antibodies to confirm this, Betschinger et al. [40**] used functional assays to investigate which pool of Lgl is functional. It was found that overexpression of a mutant version of Lgl that lacks the aPKC phosphorylation sites leads to the mislocalization of Numb, Pros and Miranda, an adaptor protein of Pros, around the whole cortex. This effect is not seen with wild-type Lgl, suggesting that the active form of the protein is the unphosphorylated version. In these experiments, the apical localization of the Par6–aPKC complex is unaffected. This led the authors to suggest a model (Figure 2b) in which aPKC phosphorylates and thereby inhibits Lgl at the apical cortex [40**]. As a consequence, active Lgl is only present at the basal cortex, where it promotes the proper localization of Miranda, Numb and Pros (Figure 2b). How Lgl acts in this process is still unclear. Lgl directly interacts with myosin II [45], and the basal localization of cell-fate determinants has recently been shown to involve myosin-based transport [43,44,46,47], suggesting that Lgl might regulate this type of transport. Alternatively, by analogy to the role of yeast Lgl-like proteins in vesicle docking during exocytosis [48,49], active Lgl might direct vesicle trafficking within the cell. In either case, the localized apical activity of the Par6–aPKC complex is translated into a clear asymmetry of Lgl activity within the neuroblast, which eventually leads to the proper localization of fate determinants to the opposite, basal pole.
In mammals, a Lgl homologue (mLgl) is present in the baso-lateral domain of fully polarized MDCK cells, and this localization was shown to be dependent on its phosphorylation [42,50]. These findings are consistent with the notion that apically-located aPKC phosphorylates Lgl and thereby prevents localization of Lgl to the apical membrane of MDCK cells. The non-phosphorylated ‘active’ mLgl will thereby accumulate at the baso-lateral domain, where it might regulate baso-lateral exocytosis [50] via an interaction with syntaxin-4, a component of the baso-lateral exocytic machinery.

However, in partially polarized MDCK cells, mLgl was also found to colocalize with aPKC and Par6 at points of cell–cell contact [42]. Interestingly, phosphorylation of mLgl is increased during polarization of MDCK cells and this is coincident with the progressive segregation of mLgl from Par6–aPKC along the apicobasal axis. This reinforces the suggestion that aPKC-mediated phosphorylation promotes the segregation of mLgl from these points of cell–cell contact to the baso-lateral membrane during the polarization of MDCK cells. On the basis of these results, a model was proposed by Yamanaka et al. [42] in which mLgl is bound to the inactive Par6–aPKC complex during polarization of MDCK cells and antagonizes the Par3-dependent anchoring of this complex to points of cell–cell contacts. This inhibition is counteracted by Cdc42, which would activate aPKC at points of cell–cell contact and thereby lead to mLgl being phosphorylated, released from the Par6–aPKC complex and restricted to the baso-lateral domain [42*].

In another recent study [41*], the role of mLgl phosphorylation was assayed during the polarization of migrating astrocytes. In these cells, mLgl colocalizes with aPKC at the leading edge, and a mutant version of mLgl that lacks the aPKC phosphorylation sites inhibits, albeit weakly, the cells’ polarization. Together, these studies show that the aPKC-dependent phosphorylation of Lgl is an important step in the mechanisms downstream of the Par6–aPKC complex that regulate the establishment of polarity in different cell types.

Another target of the Par6–aPKC complex has been recently identified in rat primary astrocytes: the glycogen synthase kinase-3β (GSK-3β) [51**]. It was shown that GSK-3β physically associates in a complex with aPKC, Par6 and possibly Cdc42. However, only unphosphorylated GSK-3β kinase could be immunoprecipitated with aPKC, indicating that phosphorylation leads to its dissociation from the complex. To address the role of this phosphorylation event (which occurs at serine 9, and which is thought to inhibit the kinase activity of GSK-3β [52]), the authors used a scratch assay in primary astrocytes; as discussed above, this assay has previously shown that the complex Cdc42–Par6–aPKC controls cell polarization following scratching [26]. GSK-3β was shown to become phosphorylated at the leading edge of polarizing astrocytes, where the activity of the Cdc42–Par6–aPKC complex is localized, and further studies indicate that GSK-3β phosphorylation is indeed dependent on the activity of Cdc42, Par6 and aPKC. Thus, polarizing astrocytes have low GSK-3β activity at the leading edge, due to its phosphorylation by the Cdc42–Par6–aPKC complex. This localized inactivation of GSK-3β may promote the accumulation of non-phosphorylated Adenomatous polyposis coli (Apc), which is known to be a direct target of GSK-3β and which regulates the interactions of microtubule ends with the cortex [53]. In fact, Apc was found to accumulate at the leading edge, in a Cdc42- and aPKC-dependent manner, where it associates with the plus ends of microtubules [26]. This seems to be important for the correct polarization of astrocytes, as overexpression of Apc molecules that are unable to bind microtubules leads to disruption of the process. Therefore, active, unphosphorylated Apc is needed at leading edges to regulate microtubule dynamics and the polarization of migrating astrocytes. Together, these findings illustrate another mechanism by which localized activity of the Par6–aPKC complex might lead to polarization of a cell — the modulation of microtubule dynamics via inhibition of the GSK-3β-dependent phosphorylation of Apc (Figure 2a).

Three distinct complexes act in a hierarchical manner to initiate epithelial polarity

The establishment of apico-basal polarity in epithelial cells may be the process in which the function of the Par3–Par6–aPKC complex is best understood. Genetic and biochemical studies in both Drosophila and mammalian cells have uncovered the organizing role of the Par3–Par6–aPKC complex during the biogenesis of epithelia (for review, see [1]). In this process, the Par3–Par6–aPKC complex acts together with two other conserved protein complexes (Figure 2c and 3, Table 1). One complex is apical and contains the transmembrane protein Crumbs [54], the protein Stardust [55,56] (PALS1 in mammalian cells [57]) and the protein Discs-lost [58] (PATJ in mammalian cells [59,60]). This complex will be referred to here as the Crb complex. The other localizes basal to the zonula adherens (ZA), a specialized region of the cell that separates the apical and baso-lateral membrane domains and plays a central role in establishing the correct apico-basal polarity of epithelia [61]. It includes the proteins Scribble [62,63] and Dlg [64], which are physically linked via adaptor molecules [65]. This complex will be referred to here as the Scrib complex. As mentioned above, this complex exhibits a strong functional interaction with Lgl but there is to date no evidence for a direct physical interaction between Lgl and components of the Scrib complex.

Two recent papers [66**,67**] have analyzed in great detail how these three complexes — Par3–Par6–aPKC,
Crb and Scrib — functionally interact to establish apical–basal polarity in early Drosophila embryos. Although losing par3, crb, std, scrib or lgl functions affects the generation of the ZA at gastrulation similarly, both genetic interactions and analysis of late embryonic phenotypes indicate that these three complexes are functionally distinct and that the apically located Par3–Par6–aPKC complex is at the top of a genetic hierarchy regulating epithelial polarity.

First, loss of Crb complex activity delays but does not prevent the acquisition of normal polarity, with crb and std mutant cells exhibiting an enlarged baso-lateral domain at late embryogenesis. Conversely, the absence of the Scrib complex or Lgl does not block polarity establishment, with lgl and scrib mutant cells eventually acquiring a normal polarity and showing an enlarged apical domain. Thus, the Crb and Scrib complexes act antagonistically to confer an apical and basal character, respectively, to the membrane.

Second, double mutant analysis revealed that the primary function of the Crb complex is to antagonize the activity of the Scrib complex and of Lgl, which in turn act together to inhibit the function of the Par3–Par6–aPKC complex (Figure 2c). The results of these genetic studies are entirely consistent with the inhibitory role proposed for mLgl in the regulation of the Par3–Par6–aPKC complex in polarizing MDCK cells [42].

Third, the Par3–Par6–aPKC complex appears to act before and upstream of the Crb and Scrib complexes. Indeed, the Par3–Par6–aPKC complex localizes to the forming ZA before Crb, and the early ZA defects seen in par3 mutant embryos preceded those seen in crb mutants. Moreover, apical localization of the Crb complex is dependent on the Par3–Par6–aPKC complex, but not vice-versa. Thus, the Par3–Par6–aPKC complex functions before the Crb complex and is required for the apical localization of the latter, whereas the Crb complex mainly acts to maintain the apical localization of the Par3–Par6–aPKC complex.

However, little is known about the molecular details underlying these genetic interactions. This gap is being filled by biochemical work on mammalian MDCK cells that has uncovered not only a direct interaction between Par6–aPKC and mLgl [41,42] but also a direct interaction between the Par3–Par6–aPKC and Crb complexes, with Par6 directly binding to PALS1 [68]. This interaction involves the PDZ domain of Par6 and a new domain of PALS1, named U1. Furthermore, this interaction seems to be facilitated by activated Cdc42. As PALS1 binds to the cytoplasmic end of the transmembrane protein Crb, the Par6–PALS1 interaction might explain how the Crb complex maintains the apical localization of the Par3–Par6–aPKC complex (Figure 2c). Alternatively, this interaction might explain how the Par3–Par6–aPKC complex controls the correct apical localization of the Crb complex; this may involve the phosphorylation of Crb by aPKC (S Sotillos and S Campuzano, personal communication). Future studies should reveal the details of the molecular intimacy displayed by these three molecular complexes. Nevertheless, a question remains. How does the forming ZA functionally interact with the Par3–Par6–aPKC complex? Establishing the molecular and functional links between these two complexes is an important goal for future research.
Conclusions

An emerging conclusion from the studies described here is the central role of the Par6–αPKC complex in regulating the establishment of polarity in a variety of distinct cell types, from the one-cell embryo in *C. elegans* to migrating astrocytes and epithelial cells in mammals. In all these cells, the function of the Par6–αPKC complex involves fine-tuned spatio-temporally tight regulation of the signaling activity of αPKC, which, in turn, regulates the activity of proteins organizing cytoskeletal architecture (ApC) and vesicular trafficking (Lgl).

The protein Par6 is an unique and versatile molecule in the way it regulates αPKC activity within the cell. It constitutively inhibits the kinase activity of αPKC via a unique structure that shields the regulatory domain of αPKC, thereby preventing ectopic signaling; via its partial CRIB–PDZ domain, it receives temporal and spatial inputs from various extracellular stimuli converging onto Cdc42, thus releasing inhibition of the αPKC’s kinase activity in a highly accurate manner; via its PDZ domain, it interacts with Par3 and PALS1 and thereby regulates the targeting of αPKC to specific microenvironments within the cell; and finally, via PALS1, it also helps to recruit substrates such as Lgl or Crb to αPKC.

These studies have also illustrated how the activity of the Par6–αPKC complex integrates with other molecular machinery to implement and stabilize the initial asymmetry created by the localized activation of αPKC. In fact, the integration of Par6–αPKC signaling activity with other signaling pathways may be a general feature of cell polarization. This notion is nicely illustrated by a recent and elegant study showing that, in mitotic neuroblasts of *Drosophila*, the apical Par6–αPKC complex acts redundantly with a second signaling complex that includes the Gzi subunit of the heterotrimeric G protein to regulate mitotic spindle asymmetry during unequal division [69**]. A major challenge in the future will be to understand how localized activation of Par6–αPKC acts globally through distinct pathways to orchestrate, in time and space, physiological changes in cell polarity.

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References and recommended reading

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

• of special interest

**•• of outstanding interest


13. GFP fusions of several proteins involved in A–P polarity were created and their localization followed by time-lapse microscopy during polarization in the living *C. elegans* one-cell embryo. This analysis provides evidence for an establishment phase and a maintenance phase during AP polarization. The authors were also able to establish a temporal and functional hierarchy between the par genes known to control A–P polarity. For instance, analysis of GFP–Par6 localization in par2 embryos revealed that initial localization of Par6 is independent of Par2.


30. Garrard SM, Capaldo CT, Gao L, Rosen MK, Macara IG, • Tomchick DR: Structure of Cdc42 in a complex with the GTPase-binding domain of the cell polarity protein Par6. EMBO J 2003, 22:1125-1133. This paper describes the crystal structure of a complex between Cdc42 with the mutation Q61L and the GBD of Par6 (which consists of semiCrib and PDZ), providing a coherent molecular basis to understand the biological function of this complex. These structural studies revealed that the way in which Par6 interacts with Cdc42-GTP is unique and different from the mode in which Cdc42-GTP binds WASP or PAK. This is due to the contribution of the Par6’s PDZ domain to Cdc42-GTP binding, which stabilizes the interaction with the partial CRIB domain. These studies further indicate that the structural motifs in the PDZ domain that allow it to interact with classical PDZ partners are still available for protein-protein interactions in presence of Cdc42-GTP. Thus, Par6 can simultaneously bind Cdc42 and other PDZ partners, such as Par6. Finally, FRET analysis indicated that binding of Cdc42-GTP induces a conformational alteration in Par6 that may in turn regulate apPKC activity.


33. Wedlich-Soldner R, Altschuler S, Wu L, Li R: Spontaneous cell polarization through actomyosin-based division of the Cdc42 GTPase. Science 2003, 299:1231-1235. Remarkable study on the ability of yeast cells to polarize in the absence of any spatial cue, and on the central roles of Cdc42 and the actin cytoskeleton in the process. The mechanism should involve a positive feedback loop involving Cdc42 and actin polymerization. The process can be initiated randomly at the plasma membrane at a point where a stochastic rise in the concentration of activated Cdc42 leads to localized formation of actin cables. This results in a higher delivery rate of vesicles to that point, which, by carrying more activated Cdc42, thus reinforce and stabilize the initial asymmetry. The end-result is full polarization of the yeast cell. The authors suggest that in asymmetric and intrinsic polarity signals might use this feedback loop to establish polarity along a defined axis.


40. Betschinger J, Mechtler K, Knoblich JA: The Par complex directs asymmetric cell division by phosphorylating the cytoskeletal protein Lgl. Nature 2003, 422:326-330. This paper identifies Lgl as a Par−/−apPKC phosphorylation target and proposes a simple model for the accumulation of fate determinants at the basal pole of asymmetrically dividing neuroblasts based on the inhibition of Lgl at the opposite pole. Using immunoprecipitation with anti-Par6 antibodies in Drosophila embryos followed by mass spectrometry sequencing, the authors report the presence of Lgl in the Par6−/−apPKC complex. The authors show that Lgl is phosphorylated by apPKC and that phosphorylated Lgl is inactive and cytoplasmic. They therefore suggest that phosphorylation of Lgl by the apical Par6−/−apPKC complex serves to inactivate it at the apical pole of neuroblasts, thus restricting its activity to the basal pole, where it directs the process of localizing Miranda and associated basal determinants.

41. Plant PJ, Favvett JP, Lin DC, Holdorf AD, Binns K, Kulkami S, • Pawson T: A polarity complex of mPar-6 and atypical PKC binds, phosphorylates and regulates mammalian Lgl. Nat Cell Biol 2003, 5:301-308. This paper reports the interaction in mammalian cells of mLgl and the Par6−/−apPKC complex and characterizes this interaction biochemically. The role of mLgl phosphorylation was assessed in a fibroblast scratch assay and nicely complements the results shown in [40**] on Drosophila neuroblasts.

42. Yamanaka T, Horikoshi Y, Sugiyama Y, Ishiyama C, Suzuki A, Hirose T, Iwamatsu A, Shinohara A, Ohno S: Mammalian Lgl forms a protein complex with PAR-6 and apPKC independently of PAR-3 to regulate epithelial cell polarity. Current Biology 2003, 13:734-743. This study identifies mLgl-1 and mLgl-2 as direct phosphorylation targets of apPKC. Initially identified as Par6 interactions, mLgl-1 and mLgl-2 were found in complexes containing apPKC and Par6, but not Par3. mLgl is shown to transiently colocalize with apPKC and Par6 at points of cell–cell contact in polarizing MDCK cells. As polarity is established, mLgl is segregated from the complex to the baso-lateral domain and this correlates with an increase in its phosphorylation by apPKC.


Pattern formation and developmental mechanisms


This paper describes the localization of mLgl in polarized MDCK cells and correlates this to the phosphorylation status of the protein. Consistent with studies on the yeast Lgl homologue, the authors report that mLgl binds to syntaxin4, raising the possibility that mLgl may be involved in targeting specific proteins to the basolateral membrane during polarization of MDCK cells.


The authors describe the GSK-3β kinase as a downstream target of aPKC that can be specifically inactivated by aPKC phosphorylation. Inactivation of GSK-3β occurs specifically at the leading edge of polarizing astrocytes and might lead to accumulation of Apc at microtubule ends. Although the contribution of Apc to the polarization of these cells is not clear, the findings in this paper link two pathways that together might coordinate several aspects of cell polarity in various cell types.


See annotation [67][*].


These two papers present complementary results that identify a temporal and genetic hierarchy in the way three interacting molecular complexes regulate epithelial apico-basal polarity in Drosophila embryos. A precise temporal observation of mutant phenotypes, alone and in combination, together with a detailed analysis of alterations in protein localization, allowed the authors to show that a major function of the apical Crumbs complex is to restrict the activity of the Scribbel complex to a more basal position. In turn, the Scribbel complex mainly acts to antagonize the activity of the more apical Baz/Pars–Par6–aPKC complex, which appears to play a more central role in positioning the ZA.


Through the analysis of double mutant combinations, the authors elegantly show that the apical Baz/Pars–Par6–aPKC complex acts redundantly with a second signaling complex, the Pins–G3s1 complex, to regulate mitotic spindle positioning in asymmetrically dividing neuroblasts in Drosophila.