The PAR-aPKC system: lessons in polarity

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Summary

Ten years ago, *par-1* and *par-3* were cloned as two of the six *par* genes essential for the asymmetric division of the *Caenorhabditis elegans* zygote. PAR-1 is a protein kinase, whereas PAR-3 is a PDZ-domain-containing scaffold protein. Work over the past decade has shown that they are part of an evolutionarily conserved PAR-aPKC system involved in cell polarity in various biological contexts. Recent progress has illustrated the common principle that the PAR-aPKC system is the molecular machinery that converts initial polarity cues in the establishment of complementary membrane domains along the polarity axis. In most cases, this is achieved by mutually antagonistic interactions between the aPKC–PAR-3–PAR-6 complex and PAR-1 or PAR2 located opposite. However, accumulating evidence has also revealed that mechanisms by which the asymmetrically localized components of the PAR-aPKC system are linked with other cellular machinery for developing polarity are divergent depending on the cell type.

Key words: Polarity, PAR, aPKC, Asymmetric cell division, Lgl

Introduction

One of the essential features of life is its ability to create order against the universal tendency towards disorder. A typical example is cell polarity: the asymmetric distribution of constituents within a single cell that produces the asymmetry of cellular functions. One cell type showing extreme polarity is the epithelial cell, which plays essential roles in multicellular organisms by forming physiological and mechanical barriers and controlling tissue architecture (Rodriguez-Boulan and Nelson, 1989). Epithelial cells exhibit an apicobasal polarity with respect to their cytoskeletal organization, the distribution of membrane proteins and the specific junctional complex at the tip of the lateral membrane (Yeaman et al., 1999). Developmental biologists have observed another type of cell polarity that is tightly coupled with the asymmetric cell division that is crucial for differentiation to form distinct cell types. Particularly at the start of the development of multicellular organisms, cell fate determinants are segregated asymmetrically within a single cell before division (Horvitz and Herskowitz, 1992). Genetic studies have led to the identification of various gene products that are indispensable for this (Jan and Jan, 1998; Way et al., 1994).

In 1995, Kemphues and co-workers cloned two of the six partition-defective (*par*) genes that are essential for the asymmetric division of the *Caenorhabditis elegans* zygote (Etemad-Moghadam et al., 1995; Guo and Kemphues, 1995). This heralded a dynamic decade during which studies of the different aspects of cell polarity alluded to above converged into a single stream. Currently, there is convincing evidence that many cell polarity events that superficially appear very different are commonly regulated by a set of evolutionarily conserved proteins called the PAR-aPKC (for 'atypical protein kinase C') system (Ohno, 2001). The generality and essential importance of the PAR-aPKC system have been demonstrated in diverse examples of polarity control from various species (Fig. 1). Its crucial roles in development have also been established at the level of the organism in both invertebrates and vertebrates, including zebrafish (Horne-Badovinac et al., 2001), *Xenopus* (Kusakabe and Nishida, 2004) and mice (K. Akomoto, T. Noda and S.O., unpublished). However, accumulating evidence has also revealed diversity and complexity in the means by which different cells utilize the PAR-aPKC system to establish polarity. Here, we review recent progress in understanding the PAR-aPKC system in light of this generality and diversity.

The PAR-aPKC system creates complementary membrane domains in the *C. elegans* zygote

The PAR-aPKC system (Table 1) comprises three serine/threonine protein kinases (aPKC, PAR-1 and PAR-4), two PDZ-domain-containing scaffold proteins (PAR-3 and PAR-6), one RING-finger protein (PAR-2) and one member of the 14-3-3 family of proteins (PAR-5). aPKC, PAR-3 and PAR-6 interact with each other and often work as a functional unit. Here, we call this the aPKC complex, although the interaction between the aPKC–PAR-6 complex and PAR-3 has been suggested to be dynamic (see below).

In *C. elegans*, the polarization of the egg begins after fertilization. An unidentified polarity signal associated with the sperm centrosome specifies the posterior pole and causes marked cytoplasmic reorganization (Nance, 2005). Upon fertilization, the aPKC complex accumulates at the cortex of the anterior half of the cell, whereas PAR-1 and PAR-2 accumulate in the posterior (Table 1, Fig. 1A) (Boyd et al., 1996; Etemad-Moghadam et al., 1995; Guo and Kemphues, 1995; Hung and Kemphues, 1999; Tabuse et al., 1998). Genetic analyses have indicated that the establishment of these asymmetric distributions of PAR-aPKC proteins is crucial for the polarity of the zygote. Time-lapse analyses of green fluorescent protein (GFP)-tagged PAR proteins have revealed that, in an initial 'establishment phase', the aPKC complex

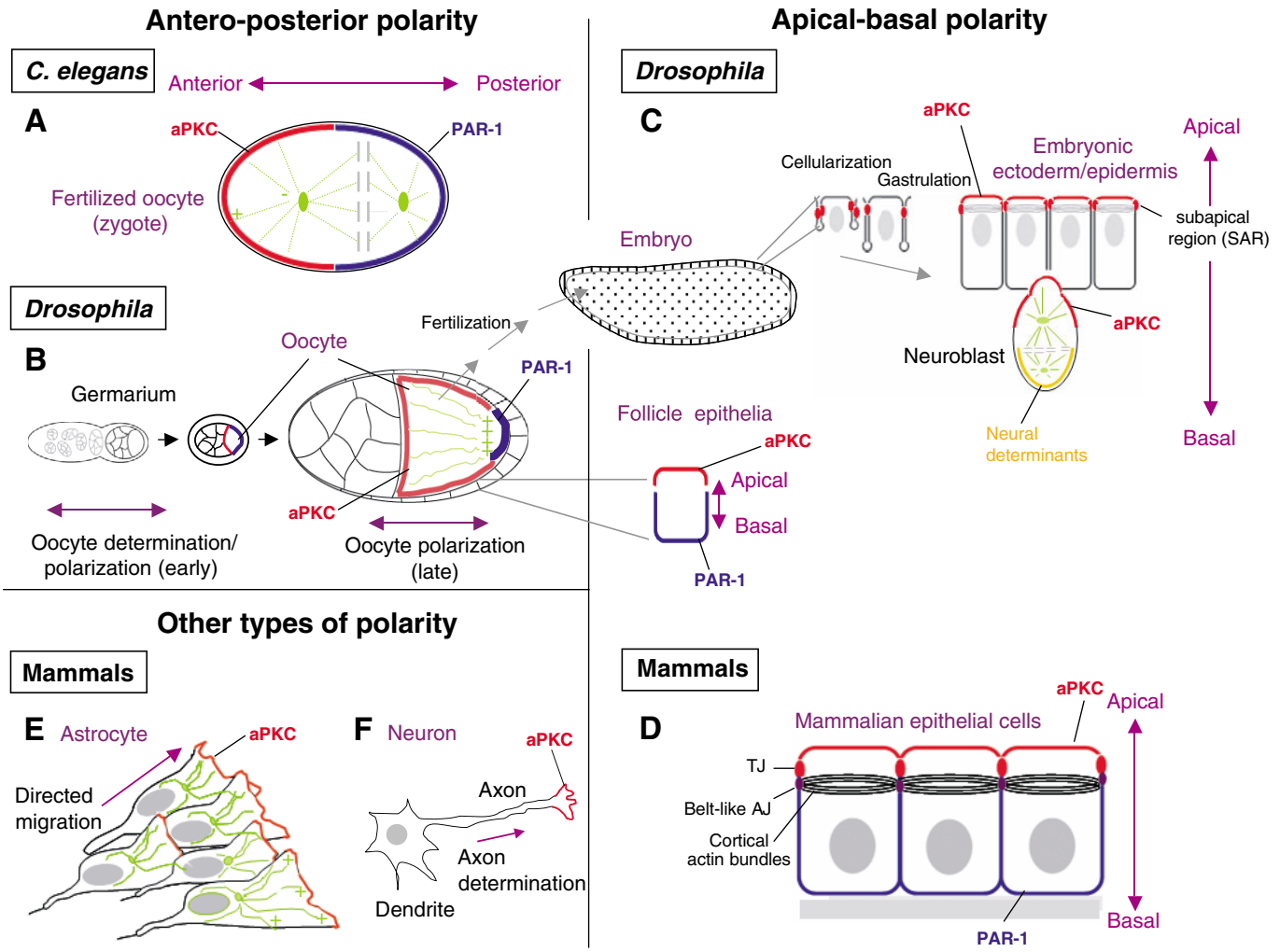

Fig. 1. Various types of cell polarity in which the PAR-aPKC system is involved. (A) Anterior-posterior polarization of the *C. elegans* zygote just after fertilization. (B) Anterior-posterior polarizations (early and late) of *Drosophila* oocytes during oogenesis. (C) Development of apical-basal polarity of *Drosophila* blastoderm epithelium formed just beneath the egg membrane. (D) Apical-basal polarity observed in mammalian cultured epithelial cells. (E) Polarization of mammalian primary-cultured astrocytes to the front during directed migration. (F) Polarization of a mammalian primary-cultured neuron that specifies one of the immature neurites as an axon. Red lines and dots represent the localization of the aPKC complex, whereas blue lines indicate the PAR-1 distribution. The distribution of PAR-1 in the *Drosophila* embryonic ectoderm and its derivative neuroblasts, as well as in mammalian astrocytes and neurons has not been clearly described. Gray ovals represent nuclei, green lines represent microtubules.

starts to accumulate in the anterior cortex in response to sperm entry, excluding PAR-2, which is initially distributed uniformly throughout the membrane (Fig. 2A) (Cuenca et al., 2003; Pellettieri and Seydoux, 2002). In a later 'maintenance' phase, PAR-2 plays a crucial role in excluding the aPKC complex from the posterior and stabilizing its anterior localization (Fig. 2A). Although the posterior localization of PAR-1 is downstream of the aPKC complex and PAR-2, PAR-1 is also suggested to exert feedback regulation stabilizing the anterior localization of the aPKC complex (Cuenca et al., 2003).

A recent elegant study using GFP-tagged myosin II provided strong evidence that these dynamic movements of PAR-aPKC proteins are driven by asymmetric actomyosin contraction induced by sperm entry (Munro et al., 2004). Upon fertilization, limited clearance of a symmetrically tensioned meshwork of cortical actomyosin is induced near sperm pronuclei where the sperm-aster contacts the posterior cortex. Then, the resultant asymmetric contraction generates a cytoplasmic flow that carries the aPKC complex to the anterior. Importantly, in embryos lacking PAR-3, PAR-6 or PKC-3 (*C. elegans* aPKC), a contractile meshwork of myosin II exists and is cleared near sperm pronuclei. However, asymmetric contraction of the meshwork towards the anterior does not occur (Cheeks et al., 2004; Kirby et al., 1990; Munro et al., 2004). Therefore, the activity of the aPKC complex itself is required to generate the forces that drive its asymmetric distribution.

PAR-aPKC-dependent generation of complementary membrane domains is a general mechanism for cell polarity

Homologs of PAR-2, a putative E3 ubiquitin ligase, have not been identified In *Drosophila* and mammals. In these species,

Table 1. Localization of proteins of the PAR-aPKC complex in different species

				Localization				
				C. elegans	*Drosophila*			Mammal
S. pombe	*C. elegans*	*Drosophila*	Mammal	zygote	late oocyte	epithelia	neuroblast	epithelia
–	PAR-3	Bazooka	PAR-3/ASIP*	Anterior cortex	Anterior cortex	SAR/apical membrane	Apical cortex	TJ†/apical membrane
–	PAR-6	PAR-6	PAR-6α,β,γ					
–	PKC-3	aPKC	aPKCλ,ζ					
Kin1	PAR-1	PAR-1	PAR-1a/MARK3/C-TAK PAR-1b/MARK2/EMK1 PAR-1c/MARK1‡	Posterior cortex	Posterior cortex	Basolateral membrane	?	Basolateral membrane
–	PAR-2	–	–	Posterior cortex	–	–	–	–
–	PAR-4	LKB1	LKB1/STK11	Uniformly cytoplasmic (faintly cortical)	(Uniformly cortical)	(Uniformly cortical)	?	(Uniformly membraneous)§
BMH1/2	PAR-5	14-3-3ϵ/Leo 14-3-3ζ	14-3-3β,γ,ϵ,η,ζ¶	Uniformly cytoplasmic	Cytoplasm	Cytoplasm	?	(Uniformly cytoplasmic/ membraneous)

SAR, subapical region; TJ, tight junction. Parentheses indicate the localization of overexpressed protein. See text for details. For the localization of PAR-4 and PAR-5/14-3-3, see the following references: PAR-4 (Martin and St Johnston, 2003; Watts et al., 2000); PAR-5/14-3-3 (Benton et al., 2002; Hurd et al., 2003a; Morton et al., 2002).

*In mammals, the PAR-3-related protein PAR-3L, showing an overall homology with PAR-3, has been identified (Gao et al., 2002). Although its characterization is limited, it has been shown to localize to TJs when overexpressed; it cannot bind to aPKC.

†As discussed in the text, the localization of PAR-3 often slightly differs from that of aPKC and/or PAR-6. In particular, in mammalian epithelial cells, PAR-3 tends to show a clearer concentration in TJs than do aPKC and PAR-6.

‡MARK4/MARKL1 is not included because this variant of mammalian PAR-1 does not show asymmetric localization in mammalian epithelial cells when overexpressed (A.S. and S.O., unpublished).

§A.S. and S.O., unpublished.

¶14-3-3 isoforms for which the interaction with PAR-1b was confirmed are only listed (M. Hirata, A.S. and S.O., unpublished).

PAR-1 appears to replace PAR-2. The aPKC complex and PAR-1 localize at the anterior and posterior cortices of *Drosophila* eggs (Benton and St Johnston, 2003b; Vaccari and Ephrussi, 2002), and the apical and basolateral membranes of *Drosophila* epithelial cells (Cox et al., 2001a; Hutterer et al., 2004; Kuchinke et al., 1998; Shulman et al., 2000; Wodarz et al., 2000) and mammalian cultured epithelial cells (Bohm et al., 1997; Izumi et al., 1998; Suzuki et al., 2001), respectively (Table 1, Fig. 1B-D). As in the *C. elegans* zygote, the asymmetric distributions of the aPKC complex and PAR-1 are essential for the polarity of these cells. Recent studies have revealed not only the presence of mutually antagonistic interactions between the aPKC complex and PAR-1 but also the molecular basis of these interactions (Fig. 2B). In *Drosophila* oocytes and follicle cells, PAR-1 phosphorylates conserved serine residues on PAR-3 and induces the destabilization of the aPKC complex, thereby preventing the invasion of the aPKC complex into the posterior and basolateral membranes (Benton and St Johnston, 2003b). By contrast, in mammalian epithelial cells, aPKC phosphorylates a conserved threonine residue on PAR-1b (one of the PAR-1 variants) at tight junctions and thus induces its dissociation from the basolateral membrane (Suzuki et al., 2004). Notably, in both cases, the 14-3-3 protein PAR-5 specifically interacts with the phosphorylated forms of *Drosophila* PAR-3 or

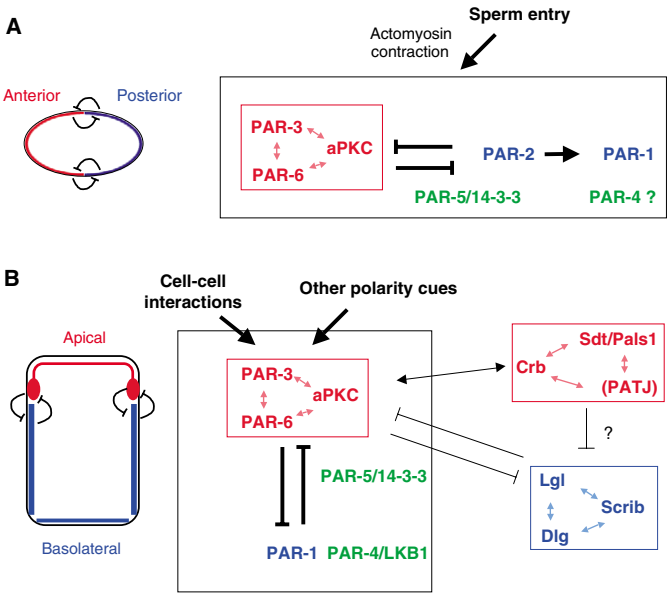

Fig. 2. Antagonistic interactions between oppositely localized PAR-aPKC system components that lead to the establishment of complementary membrane domains. (A) *C. elegans* zygote. (B) *Drosophila*/mammalian epithelial cells. In epithelial cells, the involvement of additional evolutionarily conserved polarity proteins (Crb/Pals1/PATJ and Lgl/Scrib/Dlg) has also been demonstrated. The components of the PAR-aPKC system are shown in black boxes. Red indicates anterior or apical polarity proteins, whereas blue indicates posterior or basolateral polarity proteins. Green indicates proteins that do not show clear asymmetric localization. Red- and blue-colored boxes enclose proteins categorized into functional groups whose localizations are mutually dependent (the requirement of PATJ for the localization of Crb and Sdt/Pals1 has been shown only in MDCK cells not in *Drosophila*) (Pielage et al., 2003; Shin et al., 2005). The role of PAR-4 in these interactions has not been well clarified, although its close functional relationship with PAR-1 has been demonstrated in both types of cell (see text).

mammalian PAR-1b and assists the mutual antagonism between the aPKC complex and PAR-1 (Fig. 2B). Phosphorylation by aPKC might also be involved in the exclusion of PAR-1 and PAR-2 from the anterior cortex in the *C. elegans* embryo (G. Seydoux, personal communication) (Gotta, 2005).

The third kinase, PAR-4/LKB1, is also essential for epithelial cell and oocyte polarities (Baas et al., 2004; Martin and St Johnston, 2003). Mammalian PAR-4/LKB1 activates PAR-1 by phosphorylating the activation loop of the PAR-1 kinase domain (Lizcano et al., 2004). Thus, PAR-4/LKB1 is thought to work upstream of PAR-1, although *Drosophila* genetics indicate it might reside downstream of PAR-1 (Martin and St Johnston, 2003) (Fig. 2). The PAR-aPKC system thus appears to be evolutionarily conserved molecular machinery that amplifies initial polarity cues to establish complementary membrane domains along the polarity axis (Fig. 2). However, the mechanisms by which the asymmetrically distributed PAR proteins direct the subsequent global polarization of cells vary depending on the cell type. In addition, not all polarized cells use antagonistic interactions between the oppositely localized aPKC complex and PAR-1 and PAR-2. Some cells only explore one of their activities in combination with other protein machinery (see Fig. 1E,F). Below, we focus on some recent studies of the aPKC complex that further describe the molecular basis of polarity regulation by the PAR-aPKC system. Note that, because of space limitations, we cannot cover the significant progress in our understanding of PAR-1 functions (Benton and St Johnston, 2003b; Cohen et al., 2004; Navarro et al., 2001; Ossipova et al., 2005).

Mutual molecular interactions within the aPKC complex

The components of the aPKC complex – aPKC, PAR-3 and PAR-6 – regulate each other to establish their asymmetric localization (Fig. 2) (Macara, 2004; Nagai-Tamai et al., 2002; Ohno, 2001). aPKC and the scaffold protein PAR-6 interact through their N-terminal PB1 domains (Fig. 3) (Suzuki et al., 2003; Suzuki et al., 2001). The scaffold protein PAR-6 works as an adaptor molecule that links Rac1/Cdc42 GTPase activity to aPKC by specifically interacting with the active form of Rac1/Cdc42 through its semi-CRIB motif and the adjacent PDZ domain (Fig. 3) (Garrard et al., 2003; Gotta et al., 2001;

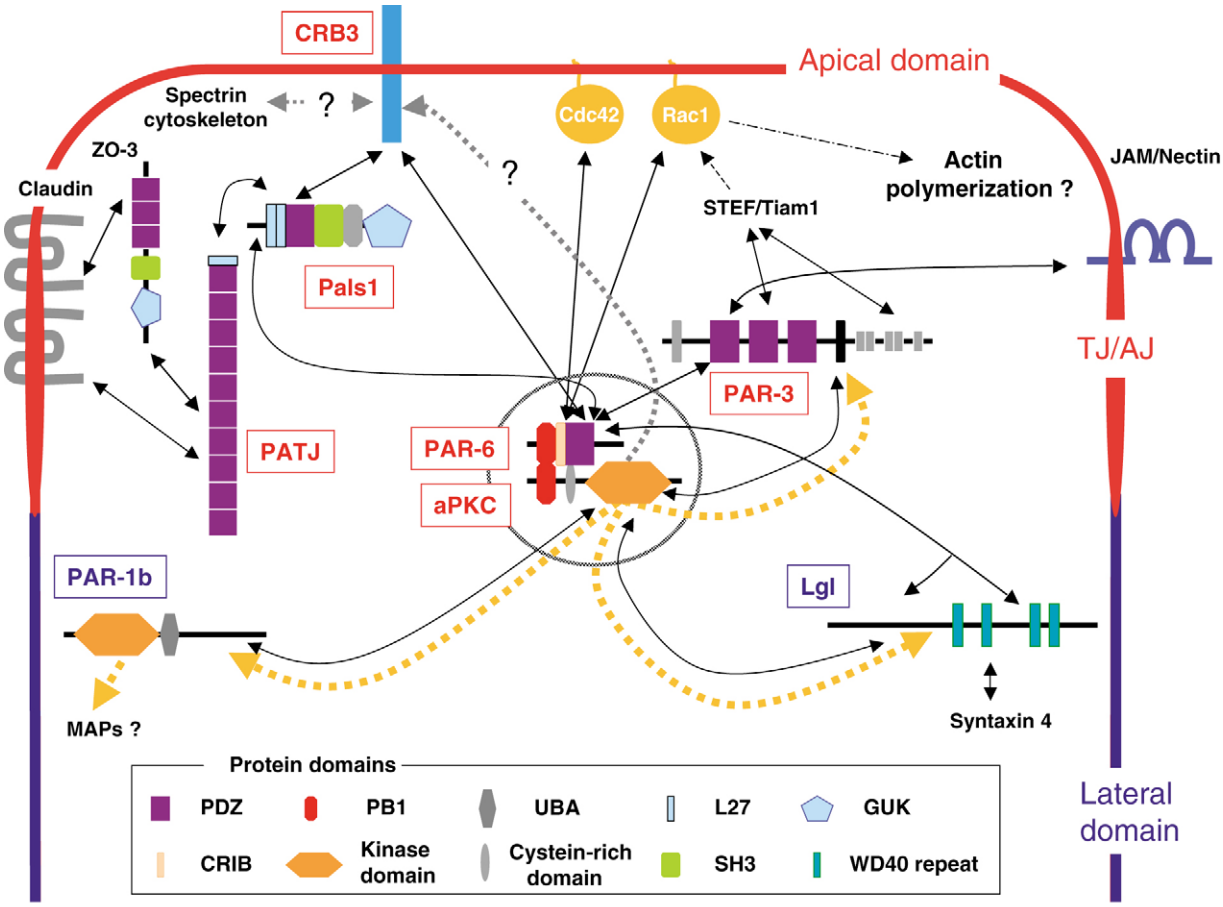

Fig. 3. The protein-protein interaction network involving polarity proteins in mammalian epithelial cells. The key shows the protein domains involved. Black arrows indicate direct physical interactions, whereas orange dotted arrows indicate phosphorylation. Putative interactions suggested from the data obtained using *Drosophila* epithelia are indicated by gray dotted arrows with question marks. The involvement of PAR-5/14-3-3 is not shown in this figure to avoid complication, although PAR-5/14-3-3 has been demonstrated to interact with PAR-1b and PAR-3. PAR-1b is suggested to phosphorylate microtubule-associated proteins (MAPs) and thereby affect microtubule stability (Drewes et al., 1997; Cohen et al., 2004). AJ, adherens junction; TJ, tight junction.

Hutterer et al., 2004; Joberty et al., 2000; Lin et al., 2000; Qiu et al., 2000; Yamanaka et al., 2001). This is highly consistent with the idea that Cdc42 plays a central role in establishing cell polarity from yeast to mammals irrespective of the biological context (Etienne-Manneville, 2004). Indeed, accumulating evidence suggests that Cdc42 is generally involved in transmitting polarity cues to the aPKC complex (Etienne-Manneville and Hall, 2001; Gotta et al., 2001; Hutterer et al., 2004; Yamanaka et al., 2001).

PAR-3, another scaffold protein, interacts with the kinase domain of aPKC through its central conserved region, the phosphorylation of which by aPKC decreases its affinity for aPKC (Fig. 3) (Izumi et al., 1998; Nagai-Tamai et al., 2002). Mutation of the aPKC phosphorylation site in PAR-3 impairs the normal function of the aPKC complex. This suggests that the interaction between aPKC and PAR-3 is dynamically regulated. Indeed, in contrast to biochemical co-immunoprecipitation data, PAR-3 often shows a slightly distinct localization from aPKC and/or PAR-6 in polarized cells when closely inspected (Harris and Peifer, 2005; Nam and Choi, 2003; Tabuse et al., 1998). Therefore, despite the additional interaction between the first PDZ domain of PAR-3 and the PAR-6 PDZ domain (Joberty et al., 2000; Lin et al., 2000), phosphorylated PAR-3 might be apt to dissociate from the aPKC–PAR-6 complex. PAR-3 is thought to target the aPKC–PAR-6 complex to particular membrane regions, since it is often observed to arrive earlier than aPKC and/or PAR-6 and can stay there – albeit transiently – even if aPKC or PAR-6 is depleted (Harris and Peifer, 2005; Suzuki et al., 2002; Tabuse et al., 1998). However, aPKC kinase activity and the binding of PAR-6 to Cdc42 have been reported to be indispensable for the maintenance of PAR-3 localization at the cortex (Hutterer et al., 2004; Suzuki et al., 2002). Therefore, phosphorylation by aPKC might also be important for the stable association of PAR-3 with the initial targeting sites.

Coupling the aPKC complex to junctional structures essential for epithelial polarity

The molecular basis for the PAR-3-mediated targeting of the aPKC complex to the membrane has been described in detail in the repolarization of mammalian epithelial cells induced by cell-cell contact (Ebnet et al., 2004). The process can be experimentally induced by subjecting an epithelial monolayer to Ca^{2+} depletion/repletion (Ca^{2+} switch) or wound healing; the resulting cell-cell adhesion mediated by E-cadherin molecules provides a cue for the development of apicobasal polarity. At first, nascent junctional structures – spot-like adherens junctions (AJs) – are formed at cell-cell contact sites by gradually recruiting various junctional proteins, including future tight junction (TJ) components. In the next phase, these AJs fuse and differentiate into epithelium-specific AJs and TJ that encircle the apex of the cells and are both essential for epithelial polarity (Fig. 1D). Upon cell-cell contact, the aPKC complex is recruited early to the initial spot-like AJs (Suzuki et al., 2002). This is thought to be mediated by a direct interaction between the first PDZ domain of PAR-3 and the C-terminal PDZ-domain-binding sequences of immunoglobulin-like cell adhesion molecules: JAM-1 and nectin-1/3 (Fig. 3) (Ebnet et al., 2001; Itoh et al., 2001; Takekuni et al., 2003). Then, as repolarization progresses, the aPKC complex accumulates in TJs and there is slight leakage into the apical membrane (Table 1, Fig. 1D). Importantly, overexpression of a dominant-negative mutant of aPKC or PAR-3 blocks the maturation of spot-like AJs into belt-like AJs and TJs (Mizuno et al., 2003; Suzuki et al., 2002). Therefore, the aPKC complex is assumed to play an indispensable role in epithelial polarity by promoting the development of epithelium-specific junctional structures. This is consistent with the results obtained in *C. elegans* zygotes showing that the aPKC complex regulates the development of asymmetric structures to which it finally localizes.

Drosphila PAR-3, Bazooka (Baz), was independently identified as a gene product essential for the polarization of the embryonic ectoderm (Kuchinke et al., 1998), which is the first epithelium formed immediately before gastrulation by a unique process called ‘cellularization’ (Fig. 1C). A fertilized *Drosophila* egg first undergoes 13 nuclear divisions in a syncytium. Then, the egg membrane invaginates around each nucleus into the cytoplasm to produce thousands of independent epithelial cells around the circumference of the embryo. Again, spot-like nascent AJs are formed early during cellularization, but the establishment of belt-like AJs that corresponds to the completion of epithelialization is accomplished only later in development (Fig. 1C) (Tepass et al., 2001). Maternal/zygotic *baz* mutants exhibit defects in the apical accumulation and coalescence of spot-like AJs into beltlike AJs (Muller and Wieschaus, 1996), which is consistent with the above conclusion in mammalian epithelial cells that the aPKC complex is indispensable for junctional development. Defects in *Drosophila* aPKC as well as PAR-6 have now been shown to result in polarity defects similar to those in *baz* mutants (Harris and Peifer, 2005; Petronczki and Knoblich, 2001; Wodarz et al., 2000). However, a recent study demonstrated that the apical accumulation of Baz/PAR-3 observed at the mid-stage of cellularization occurs even in embryos lacking spot-like AJs (Harris and Peifer, 2004), which suggests that Baz/PAR-3 does not target to the nascent junctional structures for initial localization. Clear orthologs of JAM and nectin are absent in *Drosophila*. Moreover, the subapical region (SAR) to which the aPKC complex finally localizes in *Drosophila* epithelia lacks TJ-like electron-dense ultrastructures (the barrier function of *Drosophila* epithelia is exerted by a septate junction that is basal to belt-like AJs) (Table 1, Fig. 1C) (Knust and Bossinger, 2002; Tepass et al., 2001). The apical anchoring mechanism of the aPKC complex in *Drosophila* epithelia is still unknown (Harris and Peifer, 2005).

The molecular mechanisms by which the aPKC complex promotes junctional development remain to be clarified. Recent work has suggested that TJ development is promoted by an interaction between the C-terminal region of PAR-3 and a Rac-specific guanine nucleotide exchange factor (GEF) STEF/Tiam1 (Fig. 3) (Chen and Macara, 2005). However, the significance of this interaction is still controversial (Mertens et al., 2005; Nishimura et al., 2005). Phosphorylation by aPKC might be involved in PAR-3-mediated junctional development (Hirose et al., 2002). Oligomerization of PAR-3 mediated by its conserved N-terminal domain might also be involved in junctional maturation (Benton and St Johnston, 2003a; Mizuno et al., 2003).

The aPKC complex cooperates with other conserved proteins

As discussed earlier, PAR-1 has an antagonistic role stabilizing the asymmetric localization of the aPKC complex.

Interestingly, another conserved protein, Lethal (2) giant larvae (Lgl), plays a similar role to PAR-1 and cooperates with the aPKC complex to regulate apicobasal polarity of epithelial cells and neuroblasts (Fig. 2B). Lgl, Scribble (Scrib) and Discs large (Dlg) are basolaterally localized tumor suppressor proteins that were identified in *Drosophila* genetic studies (Bilder et al., 2000; Bilder and Perrimon, 2000). They depend on each other for correct subcellular localization and thus form a functional group (the Lgl group). Lgl group proteins are recruited to the basolateral membrane of the embryonic ectoderm slightly later than the aPKC complex and thereafter play an indispensable role maintaining the apical localization of the aPKC complex (Bilder et al., 2003; Hutterer et al., 2004; Tanentzapf and Tepass, 2003) (Fig. 2B). The aPKC complex in turn is essential for restricting Lgl localization to the basolateral membrane (Fig. 2B) (Hutterer et al., 2004). The molecular basis of this antagonism is well understood: Lgl competes with PAR-3 for binding to the aPKC–PAR-6 complex and thus sequesters the aPKC–PAR-6 complex away from PAR-3 (Fig. 3) (Yamanaka et al., 2003). In addition, aPKC phosphorylates Lgl to induce a conformational change to an auto-inhibited state in which it interacts with neither the membrane nor the actin cytoskeleton (Fig. 3) (Betschinger et al., 2005). In mammalian epithelial cells, the antagonizing effect of Lgl group proteins on the apical localization of the aPKC complex has not been confirmed. However, Lgl is proposed to antagonize the polarization of a mammalian Madin-Darby canine kidney (MDCK) epithelial cell line by sequestering the aPKC–PAR-6 complex, which is released by cell-cell-contact-induced activation of aPKC and can then interact with PAR-3 (Yamanaka et al., 2003).

Interestingly, the Lgl group cooperates with the aPKC complex in *Drosophila* neuroblasts in a different manner (Albertson and Doe, 2003; Betschinger et al., 2003; Ohshiro et al., 2000). *Drosophila* neuroblasts that delaminate from the embryonic ectoderm inherit the apically localized aPKC complex from the overlying ectoderm to maintain their apicobasal polarity. This is essential for the cells to accumulate neural determinants basally during cell division and undergo asymmetric cell divisions to produce ganglion mother cells. In contrast to epithelial cells, in neuroblasts, Lgl group proteins are distributed throughout the cortex, are not excluded from the apical cortex and do not interfere with the apical localization of the aPKC complex. Nevertheless, they play an essential role in the basal accumulation of neural determinants. Recent studies suggested that this is achieved through the inactivation of Lgl by aPKC-mediated phosphorylation without its clear dissociation from the cortex (Betschinger et al., 2005; Betschinger et al., 2003). The basal accumulation of neural determinants is proposed to be driven by the contraction of cortical myosin II filaments. These are activated at the apical cortex, where aPKC inhibits Lgl and thus releases myosin II from Lgl-mediated suppression (Barros et al., 2003; Betschinger and Knoblich, 2004). It is unknown whether a similar activity of Lgl operates at the basolateral membrane of epithelial cells. Instead, Lgl is proposed to interact with syntaxin 4, a component of the exocytic machinery at the basolateral membrane, and thereby regulate polarized protein trafficking (Fig. 3) (Musch et al., 2002). This is consistent with the facts that yeast homolog of Lgl, Sro7/Sro77p, binds to Sec9p, a t-SNARE, and functions in the docking and fusion of post-Golgi vesicles (Lehman et al., 1999), and that Lgl is highly enriched in the Golgi apparatus together with PAR-6 in some mammalian non-epithelial cells (Plant et al., 2003).

Another set of conserved proteins cooperates with the aPKC complex in epithelial cell polarity (Fig. 2B): the Crumb (Crb) complex, which is composed of Crb, Stardust (Sdt) and PATJ, and shows an apical localization similar to that of the aPKC complex (Roh and Margolis, 2003). Crb and Sdt are essential for *Drosophila* embryonic ectoderm polarity (Tepass and Knust, 1993). Genetic analyses showed that the aPKC complex is required for the stable apical localization of the Crb complex. In turn, Crb stabilizes the subapical localization of the aPKC complex during late gastrulation (Bilder et al., 2003; Tanentzapf and Tepass, 2003). Mammalian CRB3, Pals1 (the Sdt homolog) and PATJ also affect the localization of the aPKC complex, TJ biogenesis and the polarity of MDCK epithelial cells (Roh et al., 2003; Shin et al., 2005; Straight et al., 2004). Epistatic analysis in *Drosophila* suggested that the Crb complex suppresses the antagonistic effects of the Lgl group on the aPKC complex (Johnson and Wodarz, 2003). However, accumulating evidence of multiple interactions between PAR-6 and Crb complex components (Hurd et al., 2003b; Lemmers et al., 2004; Nam and Choi, 2003) and the lack of biochemical evidence for the interaction between the Crb complex and the Lgl group seem to indicate that the Crb complex directly stabilizes the apical localization of the aPKC complex in a PAR-3-independent manner (Fig. 2B and Fig. 3) (Gibson and Perrimon, 2003). In fact, in *Drosophila* follicle epithelia, the apical localization of the aPKC complex is stabilized redundantly by PAR-1-mediated lateral exclusion and Crb-complex-mediated apical recruitment (Benton and St Johnston, 2003b). The Crb complex might also be responsible for the development of apical membrane identity after the completion of junctional maturation, because *Drosophila* Crb mediates the recruitment of the actin-binding protein Dmoesin and the components of the apical spectrin-based membrane skeleton (Medina et al., 2002). Note that *Drosophila* aPKC phosphorylates the juxtamembrane domain of Crb and thus affects the development of the apical membrane domain (Fig. 3) (Sotillos et al., 2004).

Microtubules: major targets of the aPKC complex

A polarized distribution of cell fate determinants is not sufficient for asymmetric division. To segregate determinants asymmetrically into two daughter cells, the mother cell must adjust the orientation of the mitotic spindle along the polarity axis (Fig. 1A,C) (Ahringer, 2003). Spindle shape and position can also be controlled asymmetrically to produce daughter cells of unequal size. How the aPKC complex regulates these processes is a major problem in this field and is covered in several excellent reviews (Ahringer, 2003; Betschinger and Knoblich, 2004; Wodarz et al., 2005). However, it should be pointed out that the studies of the *C. elegans* zygote and *Drosophila* neuroblast reached the same conclusion: that the aPKC complex cooperates with heterotrimeric G proteins activated by specific guanine nucleotide dissociation inhibitors (GDIs) in a receptor-independent manner, although the mode of the cooperation varies depending on the species. One way by which these proteins adjust spindle position is by regulating the interaction between the cell cortex and astral microtubules (MTs) emanating from spindle poles. For example, the aPKC

complex and heteromeric G proteins have been demonstrated to regulate MT pulling force at the cortex of the *C. elegans* zygote (Grill et al., 2001; Grill et al., 2003). This might be achieved by regulating the minus-end-directed MT motor dynein, which is involved in orienting/positioning mitotic spindles in a wide variety of systems. It is also possible that the aPKC complex and heterotrimeric G proteins enhance MT stabilization and polymerization at the cortex to push the spindle pole.

The aPKC complex also regulates polarized localization of the MT-organizing center (MTOC) and thereby plays an indispensable role in the maintenance of oocyte fate during *Drosophila* oogenesis (Fig. 1B) (Cox et al., 2001b; Huynh et al., 2001). This role of the aPKC complex has been investigated in more detail in several different contexts: polarized migration of wound-edge astrocytes (Fig. 1E) (Etienne-Manneville and Hall, 2003), keratinocyte monolayers (Kodama et al., 2003), and shear-stressed endothelial cells (Tzima et al., 2003). In these cases, the aPKC–PAR-6 complex but not PAR-3 plays an indispensable role in polarized migration by locating the MTOC between the leading edge and the nucleus (Etienne-Manneville and Hall, 2001). A polarity cue is thought to trigger spatially restricted activation of Cdc42, which causes the accumulation of the aPKC–PAR-6 complex at the tip of the leading edge and aPKC activation. Then, aPKC phosphorylates and inactivates glycogen synthase kinase 3 (GSK-3β), which allows the adenomatous polyposis coli (APC) protein to interact with MT plus-ends and stabilize MTs at the leading edge. The aPKC–PAR-6 complex might also regulate dynein localization or activity and thus tether MTs at the cell cortex (Etienne-Manneville and Hall, 2001; Gomes et al., 2005; Palazzo et al., 2001). An independent study has demonstrated another mechanism by which the aPKC–PAR-6 complex regulates directional cell migration: it recruits Smurf1, a ubiquitin ligase, to the leading edge and induces Rho degradation to ensure the rear-end-specific localization of Rho, which promotes tail retraction in this region (Wang et al., 2003).

Note that the above case is unique in that the aPKC complex accumulates at a limited region of the cell membrane to develop cell polarity without generating complementary membrane domains. Another example of this is provided by mammalian primary-cultured hippocampal neurons (Fig. 1F), in which the aPKC complex accumulates at the tip of one of the immature neurites and thus specifies the axon to generate neuronal polarity (Nishimura et al., 2004; Shi et al., 2004; Shi et al., 2003). It is interesting that GSK3β, APC and MTs are not downstream targets of the aPKC complex in this process. Instead, they are suggested to be required for KIF3-mediated transport of PAR-3 to the plus-ends of rapidly growing MTs at the nascent axon tip (KIF3 is a plus-end-directed kinesin-family MT motor). The downstream target could be Rac, whose activation is induced by the interaction between PAR-3 and STEF/Tiam1 and might be required for the growth of a specified axon (Nishimura et al., 2005). Contradictory studies indicate that the aPKC complex is not involved in axon or dendrite specification in *Drosophila* in vivo (Rolls and Doe, 2004). Further study is therefore necessary to clarify the exact role of the aPKC complex in this respect. But note that the aPKC complex co-immunoprecipitates with KIF3A in MDCK cells. In these cells, this interaction is suggested to be involved in cilium formation (Fan et al., 2004).

Conclusions and perspectives

Except for PAR-1 and PAR-5/14-3-3, counterparts of the PAR-aPKC system components are absent in yeast (Table 1). Yeast PKC lacks the PB1 domain that enables aPKC to cooperate with PAR-6 in multicellular organisms (Ohno and Nishizuka, 2002). It is therefore tempting to speculate that the establishment of the PAR-aPKC system was a crucial event for the evolution of multicellular organisms, for which the generation of multiple types of cell polarity is required. As we mentioned, Cdc42 lies at the center of cell polarity from yeast to mammals (Etienne-Manneville, 2004). Therefore, the PAR-aPKC system might have developed as molecular machinery that amplifies a Cdc42-mediated polarity cue and links it to various types of machinery for establishing cell polarity. The diversity of the membrane-targeting mechanisms and downstream effectors of the PAR-aPKC system could have developed in parallel with the evolution of complexity in multicellular organisms. Accumulating evidence indicates that the PAR-aPKC system regulates actomyosin and the tubulin-dynein or tubulin-kinesin system. Its linkage with the vesicle transport machinery has also been suggested. In the past decade, we have uncovered the striking conservation of the basic cell polarity machinery. In the next decade, how this evolutionarily conserved polarity machinery realizes the complexity and dynamics of cell polarity in multicellular organisms will be revealed in more detail.

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References

Ahringer, J. (2003). Control of cell polarity and mitotic spindle positioning in animal cells. *Curr. Opin. Cell Biol.* **15**, 73-81.

Albertson, R. and Doe, C. Q. (2003). Dlg, Scrib and Lgl regulate neuroblast cell size and mitotic spindle asymmetry. *Nat. Cell Biol.* **5**, 166-170.

Baas, A. F., Kuipers, J., van der Wel, N. N., Batlle, E., Koerten, H. K., Peters, P. J. and Clevers, H. C. (2004). Complete polarization of single intestinal epithelial cells upon activation of LKB1 by STRAD. *Cell* **116**, 457-466.

Barros, C. S., Phelps, C. B. and Brand, A. H. (2003). *Drosophila* nonmuscle myosin II promotes the asymmetric segregation of cell fate determinants by cortical exclusion rather than active transport. *Dev. Cell* **5**, 829-840.

Benton, R. and St Johnston, D. (2003a). A conserved oligomerization domain in *Drosophila* Bazooka/PAR-3 is important for apical localization and epithelial polarity. *Curr. Biol.* **13**, 1330-1334.

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

Benton, R., Palacios, I. M. and St Johnston, D. (2002). *Drosophila* 14-3-3/PAR-5 is an essential mediator of PAR-1 function in axis formation. *Dev. Cell* **3**, 659-671.

Betschinger, J. and Knoblich, J. A. (2004). Dare to be different: asymmetric cell division in *Drosophila*, *C. elegans* and vertebrates. *Curr. Biol.* **14**, R674-R685.

Betschinger, J., Mechtler, K. and Knoblich, J. A. (2003). The Par complex directs asymmetric cell division by phosphorylating the cytoskeletal protein Lgl. *Nature* **422**, 326-330.

Betschinger, J., Eisenhaber, F. and Knoblich, J. A. (2005). Phosphorylation-induced autoinhibition regulates the cytoskeletal protein Lethal (2) giant larvae. *Curr. Biol.* **15**, 276-282.

Bilder, D. and Perrimon, N. (2000). Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. *Nature* **403**, 676-680.

Bilder, D., Li, M. and Perrimon, N. (2000). Cooperative regulation of cell polarity and growth by *Drosophila* tumor suppressors. *Science* **289**, 113-116.

Bilder, D., Schober, M. and Perrimon, N. (2003). Integrated activity of PDZ protein complexes regulates epithelial polarity. *Nat. Cell Biol.* **5**, 53-58.

Bohm, H., Brinkmann, V., Drab, M., Henske, A. and Kurzchalia, T. V. (1997). Mammalian homologues of *C. elegans* PAR-1 are asymmetrically localized in epithelial cells and may influence their polarity. *Curr. Biol.* **7**, 603-606.

Boyd, L., Guo, S., Levitan, D., Stinchcomb, D. T. and Kemphues, K. J. (1996). PAR-2 is asymmetrically distributed and promotes association of P granules and PAR-1 with the cortex in *C. elegans* embryos. *Development* **122**, 3075-3084.

Cheeks, R. J., Canman, J. C., Gabriel, W. N., Meyer, N., Strome, S. and Goldstein,

B. (2004). *C. elegans* PAR proteins function by mobilizing and stabilizing asymmetrically localized protein complexes. *Curr. Biol.* **14**, 851-862.
Chen, X. and Macara, I. G. (2005). Par-3 controls tight junction assembly through the Rac exchange factor Tiam1. *Nat. Cell Biol.* **7**, 262-269.
Cohen, D., Brennwald, P. J., Rodriguez-Boulan, E. and Musch, A. (2004). Mammalian PAR-1 determines epithelial lumen polarity by organizing the microtubule cytoskeleton. *J. Cell Biol.* **164**, 717-727.
Cox, D. N., Lu, B., Sun, T. Q., Williams, L. T. and Jan, Y. N. (2001a). *Drosophila* par-1 is required for oocyte differentiation and microtubule organization. *Curr. Biol.* **11**, 75-87.
Cox, D. N., Seyfried, S. A., Jan, L. Y. and Jan, Y. N. (2001b). Bazooka and atypical protein kinase C are required to regulate oocyte differentiation in the *Drosophila* ovary. *Proc. Natl. Acad. Sci. USA* **98**, 14475-14480.
Cuenca, A. A., Schetter, A., Aceto, D., Kemphues, K. and Seydoux, G. (2003). Polarization of the *C. elegans* zygote proceeds via distinct establishment and maintenance phases. *Development* **130**, 1255-1265.
Drewes, G., Ebneth, A., Preuss, U., Mandelkow, E. M. and Mandelkow, E. (1997). MARK, a novel family of protein kinases that phosphorylate microtubule- associated proteins and trigger microtubule disruption. *Cell* **89**, 297-308.
Ebnet, K., Suzuki, A., Horikoshi, Y., Hirose, T., Meyer Zu Brickwedde, M. K., Ohno, S. and Vestweber, D. (2001). The cell polarity protein ASIP/PAR-3 directly associates with junctional adhesion molecule (JAM). *EMBO J.* **20**, 3738-3748.
Ebnet, K., Suzuki, A., Ohno, S. and Vestweber, D. (2004). Junctional adhesion molecules (JAMs): more molecules with dual functions? *J. Cell Sci.* **117**, 19-29.
Etemad-Moghadam, B., Guo, S. and Kemphues, K. J. (1995). Asymmetrically distributed PAR-3 protein contributes to cell polarity and spindle alignment in early *C. elegans* embryos. *Cell* **83**, 743-752.
Etienne-Manneville, S. (2004). Cdc42–the centre of polarity. *J. Cell Sci.* **117**, 1291-1300.
Etienne-Manneville, S. and Hall, A. (2001). Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKCzeta. *Cell* **106**, 489-498.
Etienne-Manneville, S. and Hall, A. (2003). Cdc42 regulates GSK-3beta and adenomatous polyposis coli to control cell polarity. *Nature* **421**, 753-756.
Fan, S., Hurd, T. W., Liu, C. J., Straight, S. W., Weimbs, T., Hurd, E. A., Domino, S. E. and Margolis, B. (2004). Polarity proteins control ciliogenesis via kinesin motor interactions. *Curr. Biol.* **14**, 1451-1461.
Gao, L., Macara, I. G. and Joberty, G. (2002). Multiple splice variants of Par3 and of a novel related gene, Par3L, produce proteins with different binding properties. *Gene* **294**, 99-107.
Garrard, S. M., Capaldo, C. T., Gao, L., Rosen, M. K., Macara, I. G. and Tomchick, D. R. (2003). Structure of Cdc42 in a complex with the GTPase-binding domain of the cell polarity protein, Par6. *EMBO J.* **22**, 1125-1133.
Gibson, M. C. and Perrimon, N. (2003). Apicobasal polarization: epithelial form and function. *Curr. Opin. Cell Biol.* **15**, 747-752.
Gomes, E. R., Jani, S. and Gundersen, G. G. (2005). Nuclear movement regulated by Cdc42, MRCK, myosin, and actin flow establishes MTOC polarization in migrating cells. *Cell* **121**, 451-463.
Gotta, M. (2005). At the heart of cell polarity and the cytoskeleton. *Dev. Cell* **8**, 629-633.
Gotta, M., Abraham, M. C. and Ahringer, J. (2001). CDC-42 controls early cell polarity and spindle orientation in *C. elegans*. *Curr. Biol.* **11**, 482-488.
Grill, S. W., Gonczy, P., Stelzer, E. H. and Hyman, A. A. (2001). Polarity controls forces governing asymmetric spindle positioning in the *Caenorhabditis elegans* embryo. *Nature* **409**, 630-633.
Grill, S. W., Howard, J., Schaffer, E., Stelzer, E. H. and Hyman, A. A. (2003). The distribution of active force generators controls mitotic spindle position. *Science* **301**, 518-521.
Guo, S. and Kemphues, K. J. (1995). par-1, a gene required for establishing polarity in *C. elegans* embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. *Cell* **81**, 611-620.
Harris, T. J. and Peifer, M. (2004). Adherens junction-dependent and –independent steps in the establishment of epithelial cell polarity in *Drosophila*. *J. Cell Biol.* **167**, 135-147.
Harris, T. J. and Peifer, M. (2005). The positioning and segregation of apical cues during epithelial polarity establishment in *Drosophila*. *J. Cell Biol.* **170**, 813-823.
Hirose, T., Izumi, Y., Nagashima, Y., Tamai-Nagai, Y., Kurihara, H., Sakai, T., Suzuki, Y., Yamanaka, T., Suzuki, A., Mizuno, K. et al. (2002). Involvement of ASIP/PAR-3 in the promotion of epithelial tight junction formation. *J. Cell Sci.* **115**, 2485-2495.
Horne-Badovinac, S., Lin, D., Waldron, S., Schwarz, M., Mbamalu, G., Pawson, T., Jan, Y., Stainier, D. Y. and Abdelilah-Seyfried, S. (2001). Positional cloning of heart and soul reveals multiple roles for PKC lambda in zebrafish organogenesis. *Curr. Biol.* **11**, 1492-1502.
Horvitz, H. R. and Herskowitz, I. (1992). Mechanisms of asymmetric cell division: two Bs or not two Bs, that is the question. *Cell* **68**, 237-255.
Hung, T. J. and Kemphues, K. J. (1999). PAR-6 is a conserved PDZ domain-containing protein that colocalizes with PAR-3 in *Caenorhabditis elegans* embryos. *Development* **126**, 127-135.
Hurd, T. W., Fan, S., Liu, C. J., Kweon, H. K., Hakansson, K. and Margolis, B. (2003a). Phosphorylation-dependent binding of 14-3-3 to the polarity protein Par3 regulates cell polarity in mammalian epithelia. *Curr. Biol.* **13**, 2082-2090.
Hurd, T. W., Gao, L., Roh, M. H., Macara, I. G. and Margolis, B. (2003b). Direct interaction of two polarity complexes implicated in epithelial tight junction assembly. *Nat. Cell. Biol.* **5**, 137-142.
Hutterer, A., Betschinger, J., Petronczki, M. and Knoblich, J. A. (2004). Sequential roles of Cdc42, Par-6, aPKC, and Lgl in the establishment of epithelial polarity during *Drosophila* embryogenesis. *Dev. Cell* **6**, 845-854.
Huynh, J. R., Petronczki, M., Knoblich, J. A. and St Johnston, D. (2001). Bazooka and PAR-6 are required with PAR-1 for the maintenance of oocyte fate in *Drosophila*. *Curr. Biol.* **11**, 901-906.
Itoh, M., Sasaki, H., Furuse, M., Ozaki, H., Kita, T. and Tsukita, S. (2001). Junctional adhesion molecule (JAM) binds to PAR-3: a possible mechanism for the recruitment of PAR-3 to tight junctions. *J. Cell Biol.* **154**, 491-497.
Izumi, Y., Hirose, T., Tamai, Y., Hirai, S., Nagashima, Y., Fujimoto, T., Tabuse, Y., Kemphues, K. J. and Ohno, S. (1998). An atypical PKC directly associates and colocalizes at the epithelial tight junction with ASIP, a mammalian homologue of *Caenorhabditis elegans* polarity protein PAR-3. *J. Cell Biol.* **143**, 95-106.
Jan, Y. N. and Jan, L. Y. (1998). Asymmetric cell division. *Nature* **392**, 775-778.
Joberty, G., Petersen, C., Gao, L. and Macara, I. G. (2000). The cell-polarity protein Par6 links Par3 and atypical protein kinase C to Cdc42. *Nat. Cell Biol.* **2**, 531-539.
Johnson, K. and Wodarz, A. (2003). A genetic hierarchy controlling cell polarity. *Nat. Cell Biol.* **5**, 12-14.
Kirby, C., Kusch, M. and Kemphues, K. (1990). Mutations in the par genes of *Caenorhabditis elegans* affect cytoplasmic reorganization during the first cell cycle. *Dev. Biol.* **142**, 203-215.
Knust, E. and Bossinger, O. (2002). Composition and formation of intercellular junctions in epithelial cells. *Science* **298**, 1955-1959.
Kodama, A., Karakesisoglou, I., Wong, E., Vaezi, A. and Fuchs, E. (2003). ACF7: an essential integrator of microtubule dynamics. *Cell* **115**, 343-354.
Kuchinke, U., Grawe, F. and Knust, E. (1998). Control of spindle orientation in *Drosophila* by the Par-3-related PDZ- domain protein Bazooka. *Curr. Biol.* **8**, 1357-1365.
Kusakabe, M. and Nishida, E. (2004). The polarity-inducing kinase Par-1 controls *Xenopus* gastrulation in cooperation with 14-3-3 and aPKC. *EMBO J.* **23**, 4190-4201.
Lehman, K., Rossi, G., Adamo, J. E. and Brennwald, P. (1999). Yeast homologues of tomosyn and lethal giant larvae function in exocytosis and are associated with the plasma membrane SNARE, Sec9. *J. Cell Biol.* **146**, 125-140.
Lemmers, C., Michel, D., Lane-Guermonprez, L., Delgrossi, M. H., Medina, E., Arsanto, J. P. and Le Bivic, A. (2004). CRB3 binds directly to Par6 and regulates the morphogenesis of the tight junctions in mammalian epithelial cells. *Mol. Biol. Cell* **15**, 1324-1333.
Lin, D., Edwards, A. S., Fawcett, J. P., Mbamalu, G., Scott, J. D. and Pawson, T. (2000). A mammalian PAR-3-PAR-6 complex implicated in Cdc42/Rac1 and aPKC signalling and cell polarity. *Nat. Cell Biol.* **2**, 540-547.
Lizcano, J. M., Goransson, O., Toth, R., Deak, M., Morrice, N. A., Boudeau, J., Hawley, S. A., Udd, L., Makela, T. P., Hardie, D. G. et al. (2004). LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. *EMBO J.* **23**, 833-843.
Macara, I. G. (2004). Parsing the polarity code. *Nat. Rev. Mol. Cell. Biol.* **5**, 220-231.
Martin, S. G. and St Johnston, D. (2003). A role for *Drosophila* LKB1 in anterior-posterior axis formation and epithelial polarity. *Nature* **421**, 379-384.
Medina, E., Williams, J., Klipfell, E., Zarnescu, D., Thomas, G. and Le Bivic, A. (2002). Crumbs interacts with moesin and beta(Heavy)-spectrin in the apical membrane skeleton of *Drosophila*. *J. Cell Biol.* **158**, 941-951.
Mertens, A. E., Rygiel, T. P., Olivo, C., van der Kammen, R. and Collard, J. G. (2005). The Rac activator Tiam1 controls tight junction biogenesis in keratinocytes through binding to and activation of the Par polarity complex. *J. Cell Biol.* **170**, 1029-1037.
Mizuno, K., Suzuki, A., Hirose, T., Kitamura, K., Kutsuzawa, K., Futaki, M., Amano, Y. and Ohno, S. (2003). Self-association of PAR-3-mediated by the conserved N-terminal domain contributes to the development of epithelial tight junctions. *J. Biol. Chem.* **278**, 31240-31250.
Morton, D. G., Shakes, D. C., Nugent, S., Dichoso, D., Wang, W., Golden, A. and Kemphues, K. J. (2002). *The Caenorhabditis elegans* par-5 gene encodes a 14-3-3 protein required for cellular asymmetry in the early embryo. *Dev. Biol.* **241**, 47-58.
Muller, H. A. and Wieschaus, E. (1996). armadillo, bazooka, and stardust are critical for early stages in formation of the zonula adherens and maintenance of the polarized blastoderm epithelium in *Drosophila*. *J. Cell Biol.* **134**, 149-163.
Munro, E., Nance, J. and Priess, J. R. (2004). Cortical flows powered by asymmetrical contraction transport PAR proteins to establish and maintain anterior-posterior polarity in the early *C. elegans* embryo. *Dev. Cell* **7**, 413-424.
Musch, A., Cohen, D., Yeaman, C., Nelson, W. J., Rodriguez-Boulan, E. and Brennwald, P. J. (2002). Mammalian homolog of *Drosophila* tumor suppressor lethal (2) giant larvae interacts with basolateral exocytic machinery in Madin-Darby canine kidney cells. *Mol. Biol. Cell* **13**, 158-168.
Nagai-Tamai, Y., Mizuno, K., Hirose, T., Suzuki, A. and Ohno, S. (2002). Regulated protein-protein interaction between aPKC and PAR-3 plays an essential role in the polarization of epithelial cells. *Genes Cells* **7**, 1161-1171.
Nam, S. C. and Choi, K. W. (2003). Interaction of Par-6 and Crumbs complexes is essential for photoreceptor morphogenesis in *Drosophila*. *Development* **130**, 4363-4372.
Nance, J. (2005). PAR proteins and the establishment of cell polarity during *C. elegans* development. *BioEssays* **27**, 126-135.
Navarro, C., Lehmann, R. and Morris, J. (2001). Oogenesis: Setting one sister above the rest. *Curr. Biol.* **11**, R162-R165.
Nishimura, T., Kato, K., Yamaguchi, T., Fukata, Y., Ohno, S. and Kaibuchi, K. (2004). Role of the PAR-3-KIF3 complex in the establishment of neuronal polarity. *Nat. Cell Biol.* **6**, 328-334.

Nishimura, T., Yamaguchi, T., Kato, K., Yoshizawa, M., Nabeshima, Y., Ohno, S., Hoshino, M. and Kaibuchi, K. (2005). PAR-6-PAR-3 mediates Cdc42-induced Rac activation through the Rac GEFs STEF/Tiam1. *Nat. Cell Biol.* **7**, 270-277.

Ohno, S. (2001). Intercellular junctions and cellular polarity: the PAR-aPKC complex, a conserved core cassette playing fundamental roles in cell polarity. *Curr. Opin. Cell Biol.* **13**, 641-648.

Ohno, S. and Nishizuka, Y. (2002). Protein kinase C isotypes and their specific functions: prologue. *J. Biochem. (Tokyo)* **132**, 509-511.

Ohshiro, T., Yagami, T., Zhang, C. and Matsuzaki, F. (2000). Role of cortical tumour-suppressor proteins in asymmetric division of *Drosophila* neuroblast. *Nature* **408**, 593-596.

Ossipova, O., Dhawan, S., Sokol, S. and Green, J. B. (2005). Distinct PAR-1 proteins function in different branches of Wnt signaling during vertebrate development. *Dev. Cell* **8**, 829-841.

Palazzo, A. F., Joseph, H. L., Chen, Y. J., Dujardin, D. L., Alberts, A. S., Pfister, K. K., Vallee, R. B. and Gundersen, G. G. (2001). Cdc42, dynein, and dynactin regulate MTOC reorientation independent of Rho-regulated microtubule stabilization. *Curr. Biol.* **11**, 1536-1541.

Pellettieri, J. and Seydoux, G. (2002). Anterior-posterior polarity in *C. elegans* and *Drosophila* – PARallels and differences. *Science* **298**, 1946-1950.

Petronczki, M. and Knoblich, J. A. (2001). DmPAR-6 directs epithelial polarity and asymmetric cell division of neuroblasts in *Drosophila*. *Nat. Cell. Biol.* **3**, 43-49.

Pielage, J., Stork, T., Bunse, I. and Klambt, C. (2003). The *Drosophila* cell survival gene discs lost encodes a cytoplasmic Codanin-1-like protein, not a homolog of tight junction PDZ protein Patj. *Dev. Cell* **5**, 841-851.

Plant, P. J., Fawcett, J. P., Lin, D. C., Holdorf, A. D., Binns, K., Kulkarni, S. and Pawson, T. (2003). A polarity complex of mPar-6 and atypical PKC binds, phosphorylates and regulates mammalian Lgl. *Nat. Cell Biol.* **5**, 301-308.

Qiu, R. G., Abo, A. and Steven Martin, G. (2000). A human homolog of the *C. elegans* polarity determinant Par-6 links Rac and Cdc42 to PKCzeta signaling and cell transformation. *Curr. Biol.* **10**, 697-707.

Rodriguez-Boulan, E. and Nelson, W. J. (1989). Morphogenesis of the polarized epithelial cell phenotype. *Science* **245**, 718-725.

Roh, M. H. and Margolis, B. (2003). Composition and function of PDZ protein complexes during cell polarization. *Am. J. Physiol. Renal Physiol.* **285**, F377-F387.

Roh, M. H., Fan, S., Liu, C. J. and Margolis, B. (2003). The Crumbs3-Pals1 complex participates in the establishment of polarity in mammalian epithelial cells. *J. Cell Sci.* **116**, 2895-2906.

Rolls, M. M. and Doe, C. Q. (2004). Baz, Par-6 and aPKC are not required for axon or dendrite specification in *Drosophila*. *Nat. Neurosci.* **7**, 1293-1295.

Shi, S. H., Jan, L. Y. and Jan, Y. N. (2003). Hippocampal neuronal polarity specified by spatially localized mPar3/mPar6 and PI 3-kinase activity. *Cell* **112**, 63-75.

Shi, S. H., Cheng, T., Jan, L. Y. and Jan, Y. N. (2004). APC and GSK-3beta are involved in mPar3 targeting to the nascent axon and establishment of neuronal polarity. *Curr. Biol.* **14**, 2025-2032.

Shin, K., Straight, S. and Margolis, B. (2005). PATJ regulates tight junction formation and polarity in mammalian epithelial cells. *J. Cell Biol.* **168**, 705-711.

Shulman, J. M., Benton, R. and St Johnston, D. (2000). The *Drosophila* homolog of *C. elegans* PAR-1 organizes the oocyte cytoskeleton and directs oskar mRNA localization to the posterior pole. *Cell* **101**, 377-388.

Sotillos, S., Diaz-Meco, M. T., Caminero, E., Moscat, J. and Campuzano, S. (2004). DaPKC-dependent phosphorylation of Crumbs is required for epithelial cell polarity in *Drosophila*. *J. Cell Biol.* **166**, 549-557.

Straight, S. W., Shin, K., Fogg, V. C., Fan, S., Liu, C. J., Roh, M. and Margolis, B. (2004). Loss of PALS1 expression leads to tight junction and polarity defects. *Mol. Biol. Cell* **15**, 1981-1990.

Suzuki, A., Akimoto, K. and Ohno, S. (2003). Protein Kinase C lambda/iota (PKClambda/iota): A PKC Isotype Essential for the Development of Multicellular Organisms. *J. Biochem. (Tokyo)* **133**, 9-16.

Suzuki, A., Yamanaka, T., Hirose, T., Manabe, N., Mizuno, K., Shimizu, M., Akimoto, K., Izumi, Y., Ohnishi, T. and Ohno, S. (2001). Atypical protein kinase C is involved in the evolutionarily conserved par protein complex and plays a critical role in establishing epithelia-specific junctional structures. *J. Cell Biol.* **152**, 1183-1196.

Suzuki, A., Ishiyama, C., Hashiba, K., Shimizu, M., Ebnet, K. and Ohno, S. (2002). aPKC kinase activity is required for the asymmetric differentiation of the premature junctional complex during epithelial cell polarization. *J. Cell Sci.* **115**, 3565-3573.

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

Tabuse, Y., Izumi, Y., Piano, F., Kemphues, K. J., Miwa, J. and Ohno, S. (1998). Atypical protein kinase C cooperates with PAR-3 to establish embryonic polarity in *Caenorhabditis elegans*. *Development* **125**, 3607-3614.

Takekuni, K., Ikeda, W., Fujito, T., Morimoto, K., Takeuchi, M., Monden, M. and Takai, Y. (2003). Direct binding of cell polarity protein PAR-3 to cell-cell adhesion molecule nectin at neuroepithelial cells of developing mouse. *J. Biol. Chem.* **278**, 5497-5500.

Tanentzapf, G. and Tepass, U. (2003). Interactions between the crumbs, lethal giant larvae and bazooka pathways in epithelial polarization. *Nat. Cell Biol.* **5**, 46-52.

Tepass, U. and Knust, E. (1993). Crumbs and stardust act in a genetic pathway that controls the organization of epithelia in *Drosophila* melanogaster. *Dev. Biol.* **159**, 311-326.

Tepass, U., Tanentzapf, G., Ward, R. and Fehon, R. (2001). Epithelial cell polarity and cell junctions in *Drosophila*. *Annu. Rev. Genet.* **35**, 747-784.

Tzima, E., Kiosses, W. B., del Pozo, M. A. and Schwartz, M. A. (2003). Localized cdc42 activation, detected using a novel assay, mediates microtubule organizing center positioning in endothelial cells in response to fluid shear stress. *J. Biol. Chem.* **278**, 31020-31023.

Vaccari, T. and Ephrussi, A. (2002). The fusome and microtubules enrich Par-1 in the oocyte, where it effects polarization in conjunction with Par-3, BicD, Egl, and dynein. *Curr. Biol.* **12**, 1524-1528.

Wang, H. R., Zhang, Y., Ozdamar, B., Ogunjimi, A. A., Alexandrova, E., Thomsen, G. H. and Wrana, J. L. (2003). Regulation of cell polarity and protrusion formation by targeting RhoA for degradation. *Science* **302**, 1775-1779.

Watts, J. L., Morton, D. G., Bestman, J. and Kemphues, K. J. (2000). The *C. elegans* par-4 gene encodes a putative serine-threonine kinase required for establishing embryonic asymmetry. *Development* **127**, 1467-1475.

Way, J. C., Wang, L., Run, J. Q. and Hung, M. S. (1994). Cell polarity and the mechanism of asymmetric cell division. *BioEssays* **16**, 925-931.

Wodarz, A., Ramrath, A., Grimm, A. and Knust, E. (2000). *Drosophila* atypical protein kinase c associates with bazooka and controls polarity of epithelia and neuroblasts. *J. Cell Biol.* **150**, 1361-1374.

Wodarz, A., Yoshida, S., Muller, H. A., Ephrussi, A., Huttner, W. B., Grawe, F. and Knust, E. (2005). Molecular control of cell polarity and asymmetric cell division in *Drosophila* neuroblasts. *Curr. Opin. Cell Biol.* **17**, 475-481.

Yamanaka, T., Horikoshi, Y., Suzuki, A., Sugiyama, Y., Kitamura, K., Maniwa, R., Nagai, Y., Yamashita, A., Hirose, T., Ishikawa, H. et al. (2001). PAR-6 regulates aPKC activity in a novel way and mediates cell-cell contact-induced formation of the epithelial junctional complex. *Genes Cells* **6**, 721-731.

Yamanaka, T., Horikoshi, Y., Sugiyama, Y., Ishiyama, C., Suzuki, A., Hirose, T., Iwamatsu, A., Shinohara, A. and Ohno, S. (2003). Mammalian Lgl forms a protein complex with PAR-6 and aPKC independently of PAR-3 to regulate epithelial cell polarity. *Curr. Biol.* **13**, 734-743.

Yeaman, C., Grindstaff, K. K. and Nelson, W. J. (1999). New perspectives on mechanisms involved in generating epithelial cell polarity. *Physiol. Rev.* **79**, 73-98.

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