Untangling interactions in the PAR cell polarity system

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Animal cells establish polarity *via* the partitioning–defective protein system. Although the core of this system comprises only four proteins, a huge number of reported interactions between these members has made it difficult to understand how the system is organized and functions at the molecular level. In a recent JBC article, the Prehoda group has succeeded in reconstituting some of these interactions *in vitro*, resulting in a much clearer and simpler picture of partitioning–defective complex assembly.

Cell polarity, defined as the structural and functional asymmetry of a cell's membrane, cytoplasm, cytoskeleton, and organelles, is a fundamental feature of nearly all eukaryotic cells. In animals, a group of partitioning–defective (PAR) proteins has been shown to be important for establishing polarity in diverse cell types including epithelia, crawling cells, neurons, and asymmetrically dividing stem cells (Fig. 1*A*) (1). Mutations that disrupt cell polarity cause severe developmental defects by interfering with tissue formation, and mis-regulation of cell polarity can contribute to cancer progression (2).

The PAR system is a signaling network composed of several protein kinases and their associated scaffolding and regulatory proteins, which localize asymmetrically on the plasma membrane of polarized cells (1). Atypical protein kinase C (aPKC) is one of the central and most highly conserved players in this network; aPKC is recruited to the apical membrane in epithelial cells, to the leading edge in migrating cells, and to a cortical crescent in asymmetrically dividing cells such as *Drosophila* neuroblasts and the *Caenorhabditis elegans* zygote (Fig. 1*A*). Polarized, membrane-associated aPKC is thought to drive polarity by locally phosphorylating substrates that then carry out polarized cell behaviors (1). Therefore, a key question in the field is how aPKC becomes localized and activated in a polarized fashion. A series of recent papers from the Prehoda lab (3–5), culminating in a new paper from Vargas & Prehoda in the JBC (5), addresses the assembly of protein complexes that polarize aPKC on the plasma membrane.

It has been shown that aPKC interacts with three other conserved proteins—Par6, Par3, and Cdc42—that together form the evolutionarily conserved "core" of the PAR system. The scaffolding protein Par6 forms a very tight (subnanomolar affinity) complex with aPKC (6), such that aPKC/Par6 can be thought of as a single functional unit (Fig. 1*B*). The aPKC/Par6 heterodimer can bind to the scaffold protein Par3 and to the Rho-family small GTPase Cdc42 (7, 8), and either of these partners can recruit aPKC/Par6 to the plasma membrane (Fig. 1, *C* and *D*). The relationship between Par3, Cdc42, and aPKC/Par6 has been a source of confusion in the field almost since these interactions were discovered. An early paper reported that all four proteins could be immunoprecipitated together, suggesting they might form a single complex (7), but other data muddied this picture. Par3 and Cdc42 were found to have distinct localizations in cells (9) and to bind to overlapping regions on Par6 (7, 8, 10), suggesting that these interactions might be mutually exclusive (although this was never directly tested). Moreover, aPKC is known to phosphorylate Par3, and binding assays performed in the absence of ATP (trapping the kinase domain in complex with its substrate) were misinterpreted as indicating a stable interaction between the aPKC kinase domain and the region of Par3 that is phosphorylated (7, 11). Thus, the nature of aPKC–Par6–Par3–Cdc42 complex(es) was unclear, and further progress was hindered by difficulty purifying the necessary proteins (especially Par3 and Par6) for *in vitro* biochemical analyses.

Progress finally came in 2020, when the Prehoda lab partially reconstituted an aPKC–Par6–Par3 complex, using Par6 that was coexpressed with aPKC and a fragment of Par3 that excluded the N-terminal oligomerization domain but contained the domains that were reported to be involved in aPKC–Par6 interactions (3). Strikingly, aPKC was found to bind directly to Par3 not *via* its kinase domain but *via* a previously unknown PDZ ligand (also known as PDZ-binding motif) at aPKC's C-terminus (Fig. 1*C*) (3). A subsequent paper showed that direct interactions between Par6 and Par3, if they occur at all, do not contribute measurable binding energy to the complex (4). Thus, it became clear that the C-terminus of aPKC is the primary site of interaction with Par3 (3, 4), while the CRIB domain of Par6 constitutes its binding site for Cdc42 (10). Since Par3 and Cdc42 have different, nonoverlapping-binding sites on aPKC/Par6, they could conceivably bind simultaneously. But do they?

The new work from Vargas and Prehoda (5) addresses this question directly, using *in vitro*–binding assays with purified proteins. The authors first assembled a Cdc42–aPKC–Par6 complex and then added purified Par3 to see whether it would join the complex. Strikingly, it did not; instead, Par3 bound only to aPKC/Par6, displacing Cdc42 in the process.

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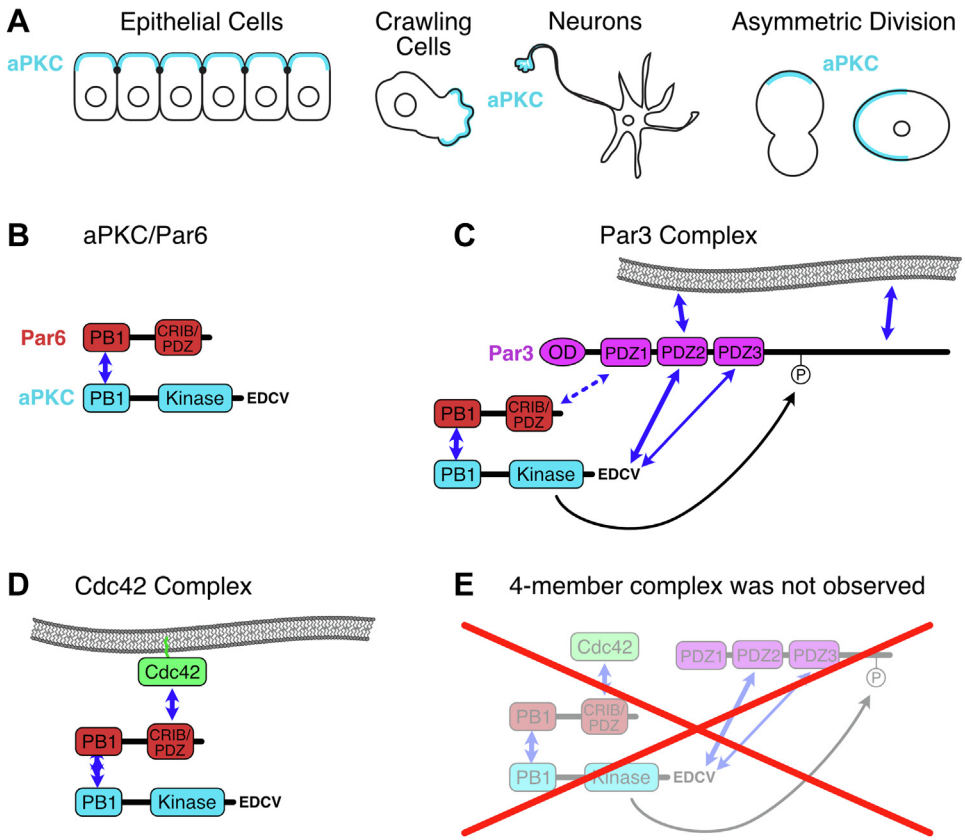

Figure 1. PAR polarity complexes. *A*, illustration of the localization of aPKC in different polarized cell types. *B–E*, schematics of PAR protein complexes. *Blue double-headed arrows* indicate protein–protein or protein–lipid interactions. *B*, the aPKC/Par6 heterodimer. The letters "EDCV" represent the C-terminal residues of aPKC that comprise the PDZ ligand. *C*, the Par3 complex. aPKC interacts with second and third PDZ domains of Par3 *via* its PDZ ligand and phosphorylates a conserved site on Par3 (*black arrow*). Interactions between Par6 and Par3 were reported (*dashed blue arrow*) but later found to be less significant. *D*, the Cdc42 complex. Cdc42 is a Rho-family small GTPase whose C-terminus carries a lipid anchor. *E*, in principle, aPKC/Par6 could bind simultaneously to Cdc42 and to Par3, but these two interactions were found to be mutually exclusive.

The converse experiment gave the same result: addition of Cdc42 to a preformed Par3–aPKC–Par6 complex resulted in the displacement of Par3 and formation of a Cdc42–aPKC–Par6 complex. Thus, aPKC–Par6 interactions with Cdc42 and with Par3 are mutually exclusive (Fig. 1*E*). Does this mean that Cdc42 and Par3 compete for a single binding side on aPKC/Par6 after all? No; the interaction between aPKC's C-terminus and Par3's PDZ domains was necessary and sufficient to displace Cdc42, even though this interaction does not involve the Par6 CRIB domain. Together, these results suggest that allosteric modulation of aPKC/Par6 must occur, preventing Par3 and Cdc42 from binding simultaneously to aPKC/Par6.

Overall, we are left with a simple and clear picture of aPKC's interactions with its partners. aPKC/Par6 form two distinct complexes, one with Par3 and one with Cdc42 (5), in agreement with *in vivo* data that suggest two distinct aPKC pools (9, 12). The Par3 and Cdc42 interactions are mutually exclusive, most likely due to aPKC/Par6 adopting different conformations when in complex with each partner. However, several important questions remain. Most importantly, how do distinct interactions with Par3 *versus* Cdc42 affect aPKC's kinase activity toward its substrates? Cdc42 is believed to activate aPKC (12, 13), while the activity of aPKC when bound to Par3 has been controversial (14, 15). *In vivo*, different binding partners could influence aPKC's access to different substrates in addition to regulating its intrinsic catalytic activity. Additionally, what cellular mechanisms regulate association of aPKC/Par6 with Par3 *versus* Cdc42? Given the tight spatiotemporal control of cell polarity signaling, there are likely to be more sophisticated mechanisms at work than just simple competition. The stage is now set for pursuing these important questions.

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Abbreviations—The abbreviations used are: aPKC, atypical PKC; PAR, partitioning–defective.

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