Protein Complex Assemblies in Epithelial Cell Polarity and Asymmetric Cell Division

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Abstract

Asymmetric local concentration of protein complexes on distinct membrane regions is a fundamental property in numerous biological processes and is a hallmark of cell polarity. Evolutionarily conserved core polarity proteins form specific and dynamic networks to regulate the establishment and maintenance of cell polarity, as well as distinct polarity-driven cellular events. This review focuses on the molecular and structural basis governing regulated formation of several sets of core cell polarity regulatory complexes, as well as their functions in epithelial cell polarization and asymmetric cell division.

Introduction

The morphological and molecular asymmetries of cells are defined as cell polarity, which is intimately correlated with unique cellular functions. For example, eggs and early embryos generate the anterior–posterior axis with polarized distributions of RNAs and proteins to give rise to daughter cells with different developmental fates [1]; epithelial cells polarize into apical and basolateral domains anchored with distinct lipids and proteins to exert their barrier/transporter function between compartments [2]; asymmetrically dividing stem cells and T cells also set up the apical–basal polarity, and partition different molecules to daughter cells for distinct identities [3,4]; migrating cells build a transient front–rear polarity in the direction of movements, creating distinct protrusions and trailing structures [5]; and the planar cell polarity pathway regulates tissue formation, morphogenesis, and cellular functions in various organs [6]. Despite these diverse cell shapes and functions, a common theme in cell polarity establishment and maintenance is recruitment and prominent local condensation of certain regulatory protein complexes at specific membrane regions (e.g., the Par3/Par6/atypical protein kinase C (aPKC), Crumbs (Crb)/PALS1/PATJ, Scribble (Scrib)/Lethal Giant Larvae (Lgl)/Dlg, Prickle/Strabismus, and Frizzled/Dishevelled/Diego complexes) [2,3,6,7]. Among them, the Par (Par3/Par6/aPKC) complex, originally identified in *Caenorhabditis elegans* and *Drosophila*, is now well known as one of the most conserved components of cell polarity regulatory protein complexes throughout the animal kingdom [8–12]. The discovery of the Par complex, its upstream signals and downstream effectors, its functions in cell polarization in diverse cell types, and its polarity-independent functions have been extensively summarized in recent reviews [1–5,8,13].

The goal of this article is to provide an overview of the molecular logics and structural bases of the Par

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complex-associated protein networks, including how Par proteins interact with each other and how they are linked to other related proteins for polarity establishment and maintenance using the epithelial apical–basal polarity as the paradigm. We begin by introducing the Par, Crb, and Scrib complexes and their roles in establishing epithelial polarity. We summarize the molecular basis underlying the assembly of these core protein polarity complexes and describe how their mutual exclusions at distinct membrane domains are achieved in polarized epithelia. We then highlight recent findings that connect apical–basal polarity with asymmetric cell division (ACD) by focusing on how the Par complex and its associated proteins interface with the mitotic spindle orientation complexes from the structural biology angle.

Cell polarity establishment and maintenance in epithelial cells

The epithelial apical–basal polarity

In multicellular organisms, epithelial cells are the most common cell-type functioning as diffusion barriers to subdivide their body into morphologically and physiologically different compartments, and to selectively secret molecules and absorb nutrients. To achieve this function, epithelial cells must be highly polarized. In vertebrate epithelia, the plasma membrane can be divided into an apical domain facing the external environment, a lateral domain contacting neighboring cells, and a basal domain facing the extracellular matrix. The lateral and basal membranes are often named together as basolateral membranes since they are quite different from the contact-free apical membranes. The separation between the apical and basolateral membranes is mediated through inter-cellular tight junctions (TJs). The apical TJ, which functions as a paracellular diffusion barrier, forms by homophilic interactions between Junctional Adhesion Molecules (JAMs), Occludins, and Claudins [1]. Adherens junctions (AJs), which localize on the lateral side beneath TJs, provide the main mechanical link with neighboring epithelial cells composed of Cadherins and Nectins. The *Drosophila* epithelia are also polarized, with its apical and basolateral membrane domains separated by AJs and septate junctions (SJs, similar to TJs in vertebrates, although their relative position to AJs are reversed in non-endoderm-derived epithelia) [1,5].

Specification of these highly polarized epithelial surfaces is achieved by precise temporal and spatial localization of polarity proteins as well as other molecules including lipids (e.g., phosphatidylinositide phosphates (PIPs) with PIP_2 concentrated in the apical membranes and PIP_3 restricted to the basolateral membranes). Despite increased complexities of tissue structures along the evolution, the establishment and maintenance of the apical–basal polarity in epithelia are controlled by the same set of master polarity regulatory complexes (Fig. 1a): the apical Par and Crb complexes, and the basolateral Scrib tripartite [2,8,13]. The Par complex is ubiquitously expressed and functions in diverse polarity-related cellular events, whereas the Crb and Scrib complexes are epithelia-specific [2]. Additional polarity regulating proteins include, but not limited to, MARK1/2 (Par1), 14–3–3 (Par5), LKB1 (Par4), YMO1 (Yurt in *Drosophila*), Neurexin IV, EPB41 (Coracle in *Drosophila*), syntaxins, podocalyxin, and Na^+/K^+ ATPase [1,14] (see Supplementary Table S1 for a list of polarity proteins from *Drosophila* and their corresponding orthologs in mammals). It is well established that proper assembly and disassembly of these polarity complexes in responding to extrinsic or intrinsic cues are critical for cell polarity development and maintenance. These polarity complexes dissolve and disperse from cell cortices when cells lose polarity [15].

The Par complex

The Par complex includes Par3 (Bazooka in *Drosophila*), Par6, and aPKC (Fig. 1a). Originally identified in *C. elegans* embryos as regulators for the anterior–posterior polarity establishment [16,17], the Par proteins are highly conserved and also regulate initial stages of polarization in *Drosophila* embryos [18], ACD of *Drosophila* neuroblasts (NBs) [19], and establishments of the mammalian epithelial cell apical–basal polarity [20–22] and the axon–dendrite polarity of neurons [23].

The Par proteins are multi-modular scaffold proteins capable of binding to each other as well as a diverse range of other cell polarity regulating proteins (Fig. 1a) [2]. These specific interactions ensure the precise temporal and spatial localization of the Par complex at restricted membrane domains [13]. Par3 (Baz) contains a conserved N-terminal domain (NTD; also called CR1), three central PDZ domains, and the C-terminal region containing multiple protein binding sites including the aPKC-binding motif (CR3, Fig. 1a). Par6 contains an N-terminal PB1 domain, a C-terminal PDZ domain, and a semi-CRIB motif immediately preceding the PDZ domain. aPKC contains an N-terminal PB1 domain and a C-terminal kinase domain. Through electrostatic interactions, the PB1 domain of Par6 forms a hetero-dimer with the PB1 domain of aPKC in a front and back manner (Fig. 1b4) [24–26]. This interaction has been suggested to inhibit aPKC activity [24–27], although a recent study proposes that Par6 activates aPKC by releasing its auto-inhibited conformation [28]. The semi-CRIB motif plus the PDZ domain of Par6 cooperatively binds to the GTP-bound form of Rho GTPase family member Cdc42, in which the semi-CRIB forms a β-strand

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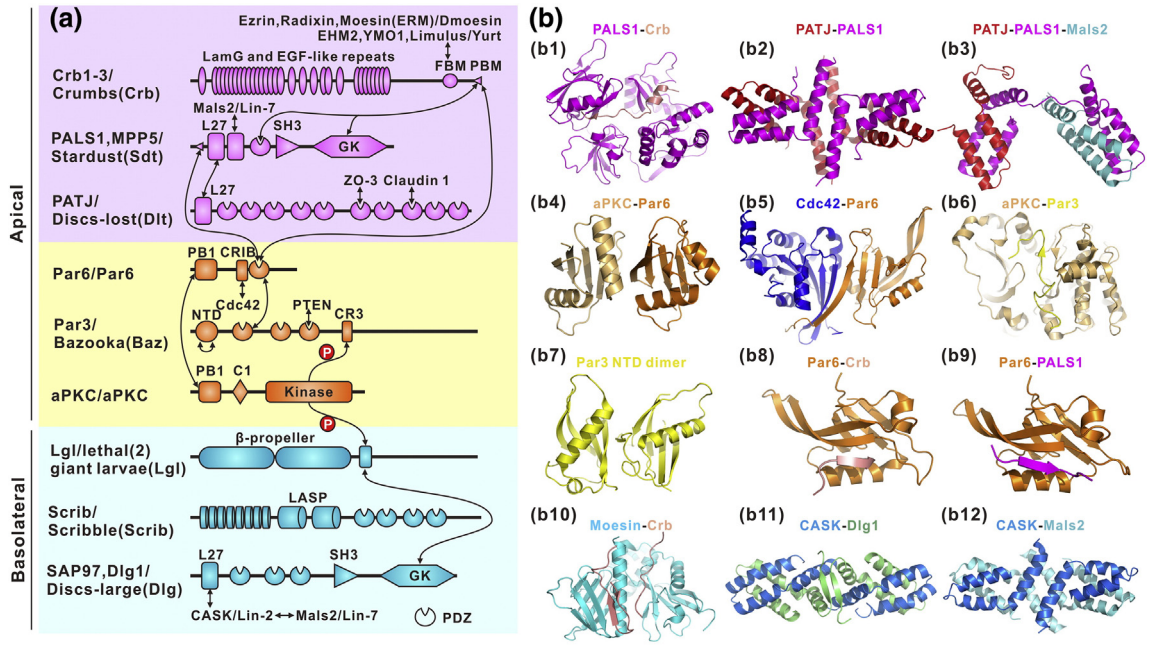

Fig. 1. Apical–basal polarity complexes. (a) Cartoon diagrams of the conserved apical (purple and orange) and basolateral (cyan) polarity proteins, and their interactions with two-way arrowed lines. (b) Summary of the known structures of the apical–basal polarity complexes. (b1) PALS1 PSG–Crb CT complex (PDB ID: 4WSI); (b2) PATJ L27–PALS1 L27N complex (PDB ID: 1Y76); (b3) PATJ L27–PALS1 L27N–L27C–Mals2 L27 complex (PDB ID: 3UIT); (b4) aPKC PB1–Par6 PB1 complex (PDB ID: 1WMH); (b5) Cdc42 PB1–Par6 PB1 complex (PDB ID: 1NF3); (b6) aPKC KD–Par3 CR3 complex (PDB ID: 5LI1); (b7) Par3 NTD dimer (PDB ID: 4I6P); (b8) Par6 PDZ–Crb PBM complex (PDB ID: 5I7Z); (b9) Par6 PDZ–PALS1 complex (PDB ID: 1X8S); (b10) Meosin FERM–Crb CT complex (PDB ID: 4YL8); (b11) CASK L27N–Dlg1 L27 complex (PDB ID: 1RSO); (b12) CASK L27C–Mals2 L27 complex (PDB ID: 1Y74).

inserting between strands of Cdc42 and PDZ to form a continuous β-sheet (Fig. 1b5) [29], and this interaction regulates Par6 PDZ's binding affinity with its major partner Crb [30]. The first PDZ domain of Par3 (Baz) is responsible for recruiting Par6 via a PDZ–PDZ domain interaction [22,31], although the underlying molecular mechanism is still unclear. Par3 (Baz) acts both as a substrate and as an inhibitor of aPKC [22]. Par3 (Baz) CR3 contains the aPKC consensus phosphorylation sequence and can regulate aPKC activity through the inhibitory binding of CR3 to the aPKC kinase domain (Fig. 1b6) and thus blocking substrate access and keeping Par3 (Baz) in complex with Par6 and aPKC [32]. Phosphorylation of a Ser residue in CR3 by activated aPKC with a still unclear regulatory mechanism weakens the Par3 (Baz)/aPKC interaction, dissociates phosphorylated Par3 (Baz) from the Par6/aPKC complex, and shifts it from apical membrane to TJs (AJs) in polarized epithelia [32–35].

The initial recruitment of the Par complex to the junctional region, a process required for TJ formation in vertebrates (AJ formation in flies) as well as for establishing the apical–basal polarity of epithelia, is mainly mediated by interactions of Par3 (Baz) with junctional proteins and lipids [1,36]. Par3 PDZ1 has been reported to interact with cytoplasmic tails (CTs) of cell adhesion molecules such as Nectin [37] and JAM [38,39]; similarly, Baz PDZ domains bind to the C-terminus of *Drosophila* Nectin-like protein Echinoid [40]. The PDZ2 and PDZ3 tandem of Par3 (Baz) is known to interact with PIPs in the lipid bilayer [41,42]. Subsequent studies suggested that PIP binding also involves a polybasic sequence in the C-terminal part of the protein after the PDZ domains [43,44]. Par3 (Baz) PDZ3 recruits the lipid phosphatase PTEN (phosphatase and tensin homolog), which converts PIP_3 to PIP_2 to maintain the apical accumulation of PIP_2 as well as Par3 (Baz) itself [41,45]. The NTD domain of Par3 (Baz) adopts a PB1-like structure and can self-associate to form a homo-oligomer with a front-to-back PB1 dimer as the basic structural unit (Fig. 1b7), thus providing a platform for efficient enrichments of the Par complex and their binding partners to the apical junctions in both *Drosophila* and vertebrate epithelia [46–49]. In addition, NTD plus PDZ1–3 of Par3 (Baz) also exhibits microtubule binding and bundling activity, which can be suppressed by its own C-terminal tail via an intra-molecular auto-inhibition mechanism. Disruption of the microtubule bundling activity of Par3 (Baz) impairs its function in axon specification in neurons and apical–basal polarity establishment in epithelia [50]. Other Par3 (Baz) binding partners have been

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extensively reviewed elsewhere [1,13,36] and will not be repeated here.

Although Par6, aPKC, and Par3 (Baz) form a complex in many polarized cells, they do not always colocalize in *Drosophila* primary epithelia and mammalian epithelia. For example, in *Drosophila* epithelia, Par6 and aPKC localize at the more apical marginal zone together with the Crb complex, whereas Baz is localized slightly more basally at AJs [51–53]. Following Par3 (Baz)-mediated initial recruitment of Par6/aPKC to the apical membrane [54], the binding of Cdc42 to Par6 activates the associated aPKC (possibly by disrupting the inhibitory Par3 (Baz)/aPKC interaction with an unknown mechanism) [53], which subsequently phosphorylates Par3 (Baz) and causes Par3 (Baz) to dissociate from Par6/aPKC [34,35,54]. The released Par6/aPKC complex is then segregated into the apical domain in a Cdc42 effector MRCK and actomyosin-dependent manner [55], where it interacts with Crb tail through Par6 PDZ in a canonical PDZ-target binding mode (Fig. 1b8) [30,56]. Reducing Cdc42 activity leads to delocalization of Crb, Par6, and aPKC from the apical membrane, resulting in defects in actin organization, endocytosis, and AJ remodeling [53,57–59]. Interestingly, Par6 PDZ can also interact with the N-terminal internal sequence of PALS1 (Fig. 1b9) [21,60] as well as Lgl with an unknown mechanism [61], suggesting regulated assembly of Par6 PDZ-orchestrated multi-protein complexes in cell polarity. In addition, aPKC has been reported to phosphorylate the CT of Crb *in vitro*, which appears to be essential for Crb activity *in vivo* [62]. aPKC can also phosphorylate Lgl to regulated its subcellular localization [20,61,63]. Finally, aPKC has recently been shown to phosphorylate and activate Ezrin in the apical domain of intestinal epithelial cells, thereby facilitating the formation of the apical cytoskeleton [64]. Together, the Par6/aPKC complex in association with the Crb complex is a key determinant for the apical domain organization, both through the recruitment and activation of downstream apical complexes and through antagonizing basolateral determinants (see below for more details).

The Crb complex

The Crb complex is a key regulator of epithelial cell architecture by promoting the apical membrane domain formation. The Crb complex comprises the transmembrane protein Crb1–3 (Crb in *Drosophila*), protein associated with Lin-7 I (PALS1)/membrane-associated palmitoylated protein 5 (MPP5, Stardust in *Drosophila*), and the PALS-associated tight junction protein (Patj, Disc-lost in *Drosophila*) (Fig. 1a) [9,13].

As an apical domain marker, the Crb complex is also a crucial determinant for organizing TJs in mammals and AJs in flies, and thus regulates the boundary between the apical and lateral domains. It is enriched at the apical membrane domains of epithelia in both mammals and *Drosophila* [65,66]. Loss-of-function mutation of *Drosophila* Crb abolishes the cell's apical domain in ectodermally derived embryonic epithelia [67], whereas overexpression of Crb3 (Crb) expands the apical domain at the expense of the lateral domain both in fly epithelium and in MDCK cells [56,68,69]. Moreover, expression of Crb3 can induce TJ formation in cells that do not normally form such junctions [70]. Crb is composed of an extracellular region, a transmembrane domain, and a 37-residue highly conserved CT containing a protein 4.1/Ezrin/Radixin/Moesin (FERM)-binding motif (FBM) and an "ERLI" PDZ-binding motif (PBM). PALS1 contains two L27 domains (L27N and L27C), a PDZ domain, a SH3 domain, and a guanylate kinase (GK) domain, whereas PATJ consists of one N-terminal L27 and multiple PDZ domains (Fig. 1a). The efficient assembly of the Crb–PALS1 complex is mediated by Crb CT and the PDZ–SH3–GK (PSG) tandem of PALS1, with all three domains of the tandem contributing to the tight binding to Crb-CT (Fig. 1b1). Formation of the PSG supramodule dramatically increases the Crb binding affinity by ~100-fold than the PDZ domain alone [71]. Tempering the PSG supramodule conformation impairs PALS1-mediated cell-polarity establishment [71]. Assembly of the PATJ–PALS1 complex requires the specific recognition between PATJ L27 domain and the L27N domain of PALS1 through the formation of L27 tetramer, where two units of PATJ L27–PALS1 L27N heterodimer pack together to form a dimer-of-dimers assembly (Fig. 1b2) [72,73]. No direct interaction between Crb and PATJ has been reported so far.

The Crb complex also serves as a scaffold for the recruitment of multiple apical and TJ proteins. PALS1 L27C can recruit mammalian homolog-2 of Lin-2 (Mals2)/Lin-7 through its L27 domain, which functions to stabilize the PALS1–PATJ complex in the apical region (Fig. 1b3) [74]. In this organization, each L27 domain from PALS1 packs with their own cognate L27 domain forming a $L27_{PATJ}$/L27N–$L27C_{PALS1}$/$L27_{Lin\text{-}7}$ hetero-trimer assembly instead of the dimer-of-dimers organization (Fig. 1b2 *versus* b3). Crb FBM can recruit β_H-spectrin via Moesin in S2 cells, thus the Crb/β_H-spectrin complex may stabilize the apical spectrin-based membrane skeleton in fly epithelia [75]. Both the FBM and PBM of Crb contribute to Crb's binding to Moesin, in that the Moesin FERM domain simultaneously recognizes the Crb FBM using its F3 lobe and the PBM via a cleft between the F1 and F3 lobes of the FERM domain (Fig. 1b10) [76]. Therefore, the bindings of Crb to PALS1 and to Moesin are mutually exclusive. Interestingly, aPKC-mediated phosphorylation of Crb CT disrupts the Crb–Moesin association without disturbing the Crb–PALS1 interaction [76]. Thus,

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upon the establishment of the apical–basal polarity in epithelia, apical-localized aPKC can actively prevent the Crb–Moesin complex formation and thereby shift Crb to form complex with PALS1 at apical junctions. Crb FBM can also transiently recruit the basolateral FERM protein YMO1/EHM2/Limulus (Yurt in *Drosophila*) to the apical membranes at late stages during epithelial development, which negatively regulates Crb activity [77]. Through its PDZ domains, PATJ interacts with both ZO-3 and Claudin1 and facilitates their TJ localization [78].

The Scrib/Dlg/Lgl tripartite

The basolateral Scrib tripartite consists of Scrib, SAP97/Dlg1 (Discs Large in *Drosophila*) and Lgl. These three proteins are essential for the establishment and maintenance of epithelial cell polarity by excluding apical complexes from the lateral membranes. Cells with functional losses of any of these proteins lose their polarity and usually overproliferate [79,80]. Distinct from the Par and Crb complexes, though Dlg, Lgl and Scrib are known to interact with each other genetically, there is limited evidence for their direct physical interactions. *In vivo* imaging data have demonstrated that Dlg, Lgl, and Scrib show interdependent colocalization at the lateral membranes of epithelial cells [1,80]. The membrane targeting of Lgl is mediated by its conserved polybasic domain, which binds to membrane PIPs via electrostatic interactions [81]. aPKC-mediated phosphorylation of the polybasic domain neutralizes the positive charges and thus excludes Lgl from apical membranes [53]. It was reported that an adapter protein GUK-holder could bridge Scrib and Dlg together in *Drosophila* [82]. Interestingly, the mammalian homolog of GUK-holder, Nance-Horan syndrome-like 1b (Nhsl1b), was also reported to form a complex with Dlg4 and Scrib, through its role in apical–basal polarity is unclear [83,84]. It was recently shown that the association between mammalian Dlg and Lgl is aPKC-phosphorylation dependent (see below for more structural details) [85]. Phosphorylation of any one of three conserved Ser residues located in the central linker region of Lgl is sufficient for its recognition by Dlg GK domain at the lateral membrane, indicating that the formation of the Scrib tripartite is a dynamically regulated event tightly coupled to the establishment of cell polarity. During mitosis, the first and third Ser in the Lgl central linker can also be phosphorylated by Aurora kinases, which triggers its cortical release [86].

Like the apical polarity proteins, the Scrib tripartite consists of three scaffold proteins that can interact with many other proteins to execute their polarity functions. Through associating with the SH_2 domains of the PI3-kinase p85 regulatory subunit, Dlg1 recruits PI3 kinase to the lateral membranes, where PIP_3 is enriched [87]. Second, through L27-mediated hetero-dimerization, Dlg1 associates with a number of related MAGUK (membrane-associated guanylate kinase) family proteins, including CASK/Lin-2 (Fig. 1b11) [88,89]. The structures of the PATJ–PALS1 (Fig. 1b2), CASK–Dlg1 (Fig. 1b11), and CASK–Mals2 (Fig. 1b12) complexes reveal a general heterodimer assembly mode for cognate pairs of L27 domains [73,89]. Proteins containing multiple L27 domains can further act as a platform for supramodular assemblies in cell polarity. Lgl associates with the actin cytoskeleton through the direct binding of its C-terminal domain to Myosin II, implying that Lgl specifies the lateral cortex in connection with the Myosin II activity [90]. The PDZ3 and PDZ4 of Scrib form a supramodule with enhanced binding to PDZ binding motifs from target proteins [91].

Maintenance of the apical–basolateral polarity through antagonistic polarity complex interactions

In mature epithelia, Par3 (Baz) resides in the TJ (AJ), separating the Crb complex and the Par6/aPKC complex in the apical domain from the Scrib tripartite in the basolateral domain. Once established, the apical–basal polarity is maintained through mutual antagonism between the polarity complexes that define distinct cortical domains [92–94]. The apical exclusion of Par3 (Baz) requires aPKC phosphorylation on the CR3 domain of Par3 (Baz), which disrupts the Par3 (Baz)–aPKC interaction [34,35]. This phosphorylation is not sufficient to exclude Par3 (Baz) apically, as Par3 (Baz) can bind directly with Par6 (Fig. 1). However, as the Crb complex competes with Par3 (Baz) for binding to Par6 PDZ, the second link between Par3 (Baz) and Par6/aPKC is disrupted, and the released Par3 (Baz) is then moved slightly toward the lateral domain to define the apical/lateral boundary. This is proposed to be a major function of Crb [34,35], as in non-epithelia cells, such as oocyte or NBs, phosphorylated Par3 (Baz) still forms a complex with aPKC and Par6 [1].

How Par3 (Baz) is basally excluded by the Scrib tripartite is less clear. A possible mechanism is through Lgl-mediated Par1 distribution at the lateral cortex, where Par1 can phosphorylate and exclude Par3 (Baz) [1]. Par1-mediated phosphorylation of Par3 (Baz) generates 14–3–3 (Par5) binding sites, inhibits Par3 (Baz) from self-oligomerization as well as from binding to aPKC, all of which can prevent the basolateral localization of Par3 (Baz) [92]. Par1 deficient or mutating the Par1 phosphorylation sites in Par3 (Baz) leads to mislocalization of Par3 (Baz) along the lateral cortex in epithelia [92].

The apical and basolateral complexes are also mutually exclusive. For instance, aPKC can phosphorylate Lgl [20,61,63] and Par1 [18] to prevent their association with the apical membrane. The

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aPKC-mediated phosphorylation of Lgl can in return promote Lgl's basolateral binding to Dlg1 [85]. In addition, basal Lgl can exclude Par6 from basolateral domains [53], although the precise mechanism of Lgl–Par6 antagonism remains unclear. Such mutual reinforcement actions are likely important for the stability of the apical–basal polarity in epithelia. Consistently, overexpression of aPKC or knocking down of Lgl expands the apical domain at the expense of lateral domain, whereas loss of aPKC function or overexpression of Lgl enlarges the lateral domain [53,95,96]. In sum, the balance between these mutually antagonistic polarity complexes is crucial for the establishment and maintenance of distinct apical and basal membrane domains and for promoting domain-specific signaling events in epithelia.

It is gradually recognized that, although the common theme for establishing and maintaining the apical–basal polarity through the above polarity proteins is generally conserved throughout the evolution, variations do seem to occur in some tissues. For example, it has been found that Par3 (Baz) is dispensable for polarity in *Drosophila* follicular epithelial cells [97]; Crb is not even expressed in *Drosophila* intestinal epithelia, and the protein is not required to maintain epithelial polarity in some fly tissues [13]. Thus, additional cell-type specific components and other mechanisms must exist for the development and maintenance of the apical–basal polarity.

Cell polarity and ACD

In addition to maintaining the morphological asymmetry for exerting unique cellular processes as found in neurons and epithelia, cell polarity also functions to allow for the differential inheritance of organelles, RNAs, and cortical proteins, which is essential for the generation of diverse cell types from stem cells as well as T lymphocytes [98]. This process, named ACD that produces daughter cells with distinct fates, is crucial for tissue morphogenesis and homeostasis across the animal kingdoms [3,4,99,100]. Cell polarity affects ACD in distinct but likely closely connected ways, for example, through the planar polarity, anterior–posterior polarity, and apical–basal polarity [3,101], and we will focus on the apical–basal polarity.

As the model system for investigating ACD, *Drosophila* NBs possess an apical–basal polarity with several unevenly distributed protein complexes (Fig. 2): the conserved Par3 (Baz)/Par6/aPKC/Cdc42 complex and its related proteins Inscuteable (Insc; mInsc in mammals), Partner of inscuteable (Pins; LGN in mammals), and Gαi on the apical cortex;

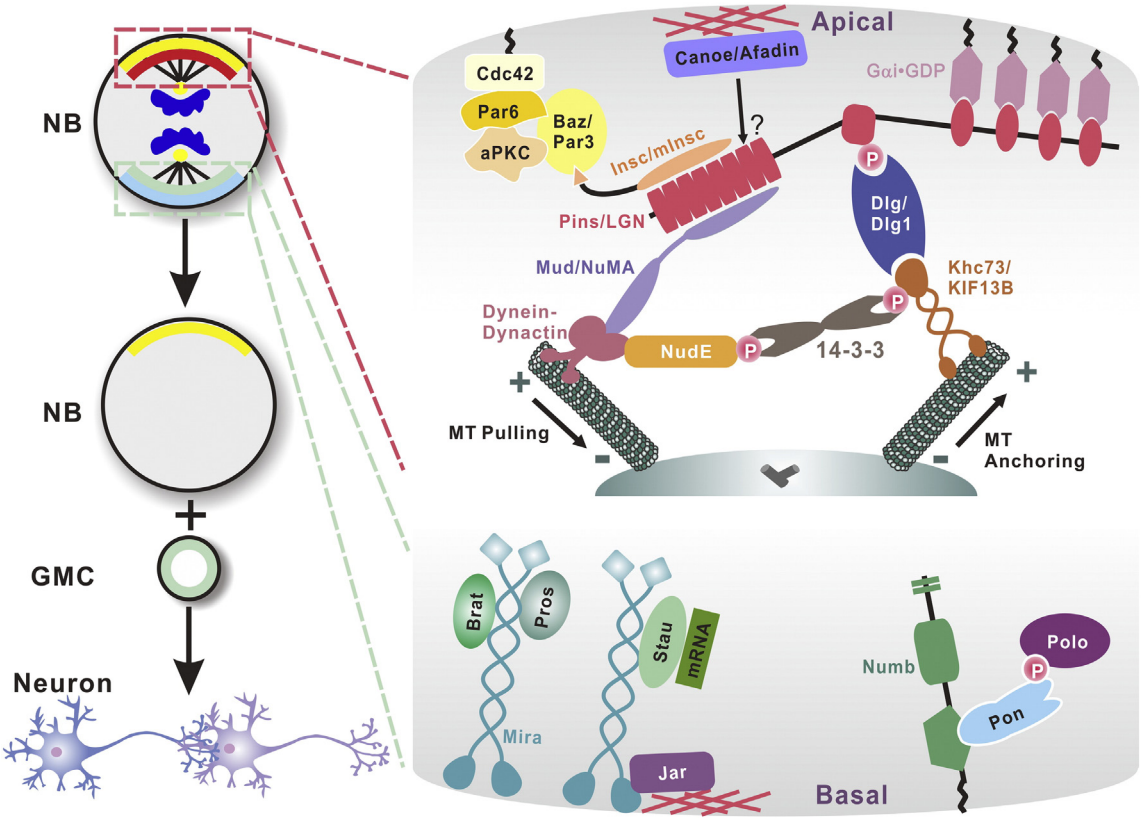

Fig. 2. A schematic diagram showing protein complexes that regulate ACD in *Drosophila* NBs.

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complexes of cell fate determinants including Numb, Prospero (Pros; Prox1/2 in mammals), Brain tumor (Brat; TRIM2/3/32 in mammals), and Staufen (Stau; STAU1/2 in mammals) and their adaptor proteins Partner of Numb (Pon) and Miranda (Mira) on the basal cortex [3,102]. Intriguingly, both the apical and basal protein complexes each forms a crescent at the opposite poles of NB during ACD instead of uniformly distributed on either the apical or basal halves of the cell cortex [19,103–109]. During the ACD of NBs, Pins orchestrates the force generating cortical protein complexes with the mitotic spindle complexes leading to the alignment of the mitotic spindle along the apical–basal axis [110–113]. After mitosis, the two daughter cells inherit their own unique cellular components, with one remains as NB and the other becomes to be a basal ganglion mother cell (GMC) capable of differentiating into neurons/glia (Fig. 2). Malfunction or disruption of the normal localization of the polarity proteins leads to tumor-like over-proliferation of NBs in *Drosophila* [103].

The apical protein complex during ACD of NBs

In developing embryos, NBs delaminate from the neuroectoderm, from which they inherit the apical Par complex [114]. During ACD of NBs, the apical Par proteins act both to restrict fate determinants to the basal pole of the cell, and to orient the spindle poles along the apical–basal axis. After division, the apical daughter retains the Par proteins and stemness, whereas the fate determinants are segregated into the basal daughter to initiate its differentiation (Fig. 2) [3]. The retention of the Par proteins, in particularly aPKC, is important for the self-renewal of NBs [115].

Despite that the Par complex and the fate determinants occupy opposite poles of NBs during the mitosis, the localization of these proteins is highly dynamic. Initially, Par6/aPKC forms a complex with Lgl. At the onset of asymmetric division, the mitotic kinase Aurora A phosphorylates Par6 PB1, which disrupts its inhibitory association with aPKC [27]. Phosphorylation of Lgl by activated aPKC leads to its dissociation from Par6/aPKC and replacement by Par3 (Baz). Par3 (Baz) then anchors the adaptor protein Insc to the apical cortex [19,109], likely through the interaction between Par3 (Baz) PDZ domains and Insc C-terminal PBM (Fig. 3a) [112]. Insc sits at the top of the hierarchy by facilitating the Par complex to provide positional information for the basal localization of the fate determinants and the mitotic spindle orientation [108,116]. Then the core scaffold protein Pins (LGN) is recruited to the apical Par/Insc complex, via interaction between its

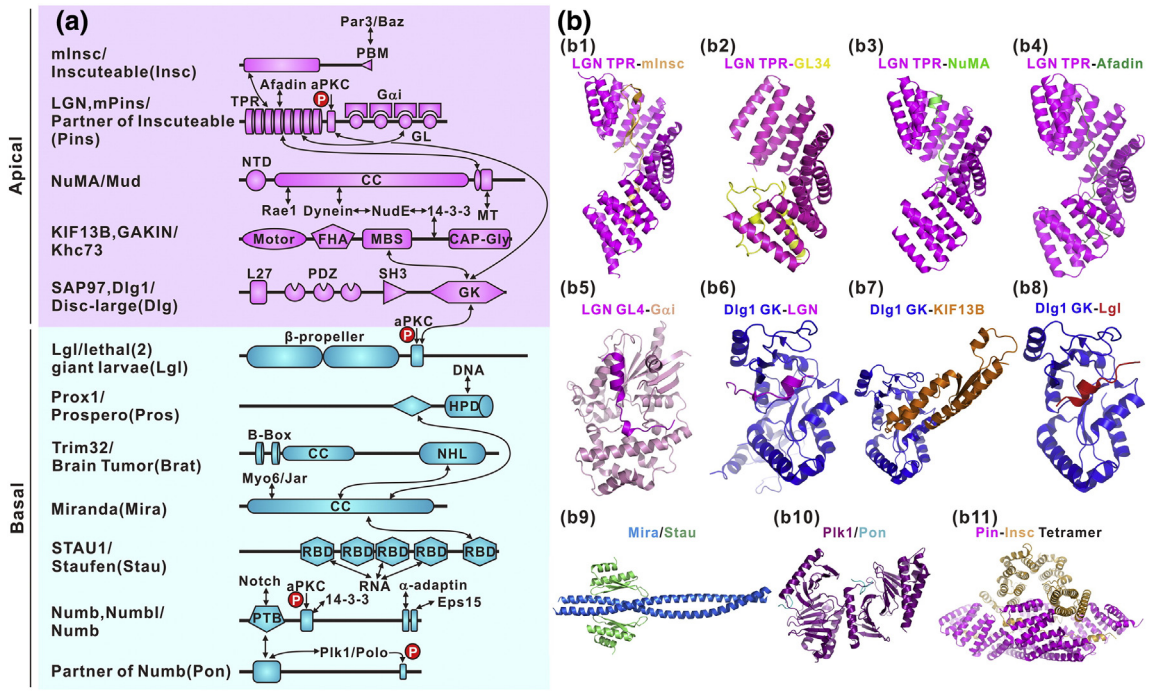

Fig. 3. Polarity complexes in ACD. (a) Cartoon diagrams of the conserved apical (purple) and basal (cyan) polarity proteins in ACD. Their interactions are indicated. (b) Summary of the known structures of the apical–basal polarity complexes in ACD. (b1) LGN TPR–mInsc complex (PDB ID: 3SF4); (b2) LGN TPR–GL34 (PDB ID: 4JHR); (b3) LGN TPR–NuMA complex (PDB ID: 3RO2); (b4) LGN TPR–Afadin complex (PDB ID: 5A6C); (b5) LGN GL4–Gαi complex (PDB ID: 4G5Q); (b6) Dlg1 GK–pLGN complex (PDB ID: 3UAT); (b7) Dlg1 GK–KIF13B complex (PDB ID: 5B64); (b8) Dlg1 GK–pLgl complex (PDB ID: 3WP1); (b9) Mira CBD–Stau dsRBD5 (PDB ID: 5CFF); (b10) Plk1 PBD–pPon complex (PDB ID: 5J19); (b11) Pins TPR–Insc tetramer (PDB ID: 5A7D).

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tetratricopeptide repeats (TPRs) and an N-terminal fragment of Insc (mInsc) (Fig. 3b1) [110–112]. In this complex, the Insc (mInsc) fragment adopts an elongated structure running antiparallel along the concave surface of the Pins (LGN) TPR superhelix. Mapelli and coworkers have recently provided a tetrameric Pins TPR/Insc complex in which a much longer fragment of Insc is involved in the Pins binding (PDB ID: 5A7D; Fig. 3b11), though the physiological function of such organization is unclear. Although the initial apical localization of Insc is Pins independent, the maintenance of apical localization of the Par complex, Insc, and Pins is interdependent [117]. Importantly, the function and molecular mechanism of the above apical polarity proteins in driving mitotic spindle orientation in *Drosophila* NBs are conserved in vertebrate stem cell divisions, albeit with certain tissue-specific modifications [102,118]. Polarity regulators such as aPKC, Par3, Insc, and LGN set the balance for symmetric and asymmetric stem cell divisions during mouse skin [119,120] and eye [121] developments, whereas Cdc42 controls division symmetry to block leukemia cell differentiation [122]. The Par3-like polarity protein Par3L, but not Par3, is essential for mammary stem cell maintenance by binding to LKB1 and inhibiting its kinase activity [123].

The basal enrichment of cell fate determinants

The localization of the cell fate determinants and their adaptors are also highly dynamic and cell cycle dependent. In the interphase, Numb, Pros, *pros* mRNA, Stau, Mira, and *mira* mRNA are found to colocalize with the Par complex at the apical region. Aurora A-induced aPKC activation leads to phosphorylation of Numb and the adaptors Pon and Mira, thus excluding them from the cortical region where aPKC is localized [27,63,124–126]. These apically precluded proteins then reach the basal cortex via passive diffusion and/or myosin-mediated transport [127,128]. How they are anchored at the basal crescent is still largely unclear. It was recently shown that the maintenance of Mira basal localization involves interaction with cognate mRNA [129]. Mira cargo proteins (such as Pros, Brat, and Stau) are likely translocated in the same way as Mira is, as they are always colocalized together during mitosis. How Mira recognizing cargos has been recently characterized. The central cargo binding domain (CBD) of Mira forms a parallel coiled-coil dimer, which can simultaneously recruit two molecules of Stau by binding to the exposed β-sheet of Stau's dsRBD5 (Fig. 3b9) [130]. The dimerization of the Mira CBD is essential for its cargo (e.g., Stau, Pros, and Brat) recognition and translocation to the basal cortex during ACD of NBs [130].

The basal localization of the Notch antagonist Numb is also regulated by its adaptor Pon and cell cycle kinase Polo. Pon is required for the asymmetric segregation of Numb into the GMC daughter, where Numb inhibits Notch activity by regulating its degradation to drive GMC daughter toward differentiation process [105,131]. Polo phosphorylates Pon at its basal localization domain, thus directing its basal recruitment together with Numb [105,132,133]. It is suggested that the efficient Pon phosphorylation by Polo is Cdk1 dependent [134]. Cdk1-mediated phosphorylation of Pon provides a docking site for the PBD domain of Polo-like kinase 1 (Plk1). Phospho-Pon binding-induced PBD dimerization leads to partial activation of Plk1, possibly through the release of the intramolecular auto-inhibitory interaction between PBD and the kinase domain (Fig. 3b10) [134]. However, the molecular basis of Pon-mediated Numb localization is still not clear.

Polarity-driven asymmetric or symmetric cell division

The direction in which a cell divides, symmetrically or asymmetrically, is determined by the mitotic cleavage plane with respect to the cell polarity axis at metaphase. Studies in multiple cell types have identified an evolutionarily conserved core protein complex, Mud/Pins/Gαi (NuMA/LGN/Gαi in mammals) together with dynein exerts a pulling force on astral microtubules for the spindle orientation [135,136] (Fig. 2). Thus, the localization of Mud/Pins/Gαi complex at restricted membrane regions determines how the mitotic spindle is oriented at the metaphase of NB cell cycle.

After the set-up of the apical–basal polarity in *Drosophila* NBs, the apically anchored Pins (via Baz/Insc) builds up the Mud/Pins/Gαi complex at the apical cortex, which functions in a receptor-independent G-protein pathway to orient mitotic spindles along the apical–basal axes of cells (Fig. 2) [137–140]. The vertebrate NuMA/LGN/Gαi complex functions in a similar manner in orchestrating ACD [120,141–144]. Briefly, the membrane attached GDP-bound Gαi (through its myristoyl group inserting into lipid membrane bilayer) anchors Pins (LGN) by associating with its C-terminal four GoLoco (GL) motifs (Fig. 3b5) [138,145,146]. As Pins (LGN) adopts a closed conformation in the steady state with its four GL motifs sequestered by its own the N-terminal TPR domain (Fig. 3b2), Gαi binding to GL motifs relieves the auto-inhibitory TPR-GL interaction and releases the TPR domain for engaging other targets [147–150]. Once in its open conformation, Pins (LGN) TPR efficiently recruits Mud (NuMA) to form the core Mud/Pins/Gαi (NuMA/LGN/Gαi) complex [110,112,137,139–141,147]. As Mud (NuMA) can physically bind to astral microtubules [137,142], this direct interaction fixes one spindle pole on the apical cortex, thus contributing to the lining up of the mitotic spindle along the apical–basal axis via the pulling force generated by

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the minus-end-directed dynein attached to the astral microtubules (Fig. 2) [139,151]. In the Mud/Pins/Gαi (NuMA/LGN/Gαi) complex, a C-terminal fragment of Mud (NuMA) adopts a similar conformation to what Insc does, residing antiparallel along the concave surface of the Pins (LGN) TPR superhelix (Fig. 3b3). Mud (NuMA) and Gαi function cooperatively to keep Pins (LGN) in the open state for the spindle orientation [147]. It is noted that the NuMA/LGN/Gαi complex does not constantly attached to the cortical membranes during mitosis, but instead cycles between the cortex and the spindle poles in a dynein and astral microtubule-dependent manner [152], suggesting the existence of other cortical polarity factors for anchoring the complex. Recent evidence suggests that different types of epithelia use distinct cortical cues to position their mitotic spindles, and some of the identified cues for connecting the NuMA/LGN/Gαi complex with cell cortex include Dlg, Canoe/Afadin, and E-cadherin [136].

In dividing NBs, when Insc is absent, Pins can assemble a compensatory complex containing Dlg and motor protein kinesin heavy chain 73 (Khc73; GAKIN/KIF13B in mammals) to rescue the spindle orientation defects later in the cell cycle, a process known as "telophase rescue" [3,153]. The GK domain of Dlg (Dlg1) binds to the central linker region of Pins (LGN) in an aPKC phosphorylation-dependent manner (Fig. 3b6). The phosphorylation-dependent interaction between LGN and Dlg1 is required for the lateral membrane localization of LGN during mitosis and subsequent spindle orientation in mammalian epithelial cells [113,154]. Structural analysis revealed that the Dlg1 GK domain has evolved into a specific phosphor-Ser/Thr binding module, and this phospho-target recognition mode is conserved in many other MAGUK scaffold proteins [113]. Later in the cell cycle, astral microtubule plus-end localized Khc73 (KIF13B) facilitates the cortical microtubule capture pathway via direct interaction with Dlg (Dlg1) GK [155]. Interestingly, unlike other known GK interactions, the association between Dlg1 GK and KIF13B is very unique and does not require KIF13B to be phosphorylated. Instead, part of the cargo binding tail of KIF13B folds into a stable domain and binds to Dlg1 GK with a very high affinity (Fig. 3b7) [156]. Structural analysis reveals that Dlg GK cannot bind to Khc73 and Pins at the same time (Figs. 3b and 4a). One possible explanation is that Dlg could function as a dimer or even oligomer, which can crosslink Pins and Khc73 simultaneously (Fig. 2) [157]. This hypothesis is supported by the finding that, in *Drosophila* S2 cells with induced cell polarity, Khc73 and Dlg can cooperate with the GK-binding Pins linker region to partially orient mitotic spindles [86]. In addition, the dimeric protein 14–3–3, which can bind to phosphorylated Khc73 and NudE [158], may function to form a bridge between Khc73 on one astral microtubule and the dynein complex on another (Fig. 2). This interaction network may further stabilize the spindle orientation machinery by physically linking the microtubule anchoring (through Dlg/Khc73) and pulling (through Mud/dynein) processes together [86].

Canoe (Afadin in mammals), which plays a key role at AJs [159], has recently been implicated in regulating spindle orientation by interacting directly with Pins (LGN) TPR in a way similar to what Insc (mInsc) and Mud (NuMA) do (Fig. 3b4) [160–162]. In *Drosophila* NBs, Canoe localizes to the apical cortex, where it controls spindle orientation by facilitating the Pins–Mud interaction [161,162], through unknown mechanisms. Canoe can be phosphorylated by Baz/Insc-associated Hippo pathway kinase Warts, and the phosphorylation of Canoe is required for Pins localization and proper spindle orientation [163]. Interestingly, Warts also phosphorylates Mud to promote Pins-mediated mitotic spindle orientation [164]. In mammalian epithelial cells, though Afadin functions in the lateral localization of LGN by connecting it to cortical actins, structural analysis suggests that it competes with NuMA for LGN

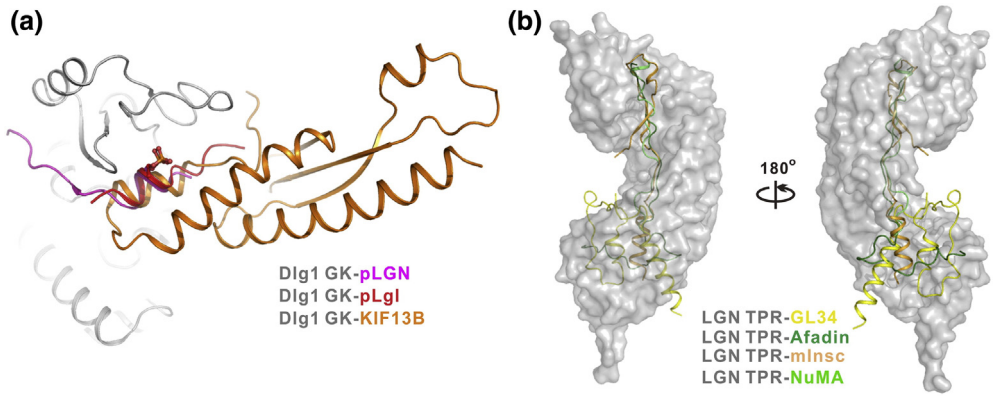

Fig. 4. Competitive interactions during ACD. (a) Superimposition of Dlg1 GK domain (gray) in complex with phospho-LGN (magenta), phospho-Lgl (red), and KIF13B (orange). The phosphate group is shown in the stick-and-ball model. (b) Superimposition of LGN TPR domain (gray) in complex with GL34 (yellow), Afadin (dark green), mInsc (orange), and NuMA (green).

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binding instead of facilitating the NuMA/LGN interaction (Fig. 4a) [160]. Recently, another AJ protein, E-cadherin, has been shown to function as an instructive cue for spindle orientation by recruiting LGN to cell–cell contacts [165]. As is the case for Afadin, LGN cannot be bound to both E-cadherin and NuMA simultaneously. It is proposed that E-cadherin serves as an initial positional cue for the mitotic assembly of the LGN/NuMA complex, which is then anchored by Gαi at cell–cell adhesions during mitosis [165]. In contrast, Suppressor APC domain containing 2 (SAPCD2) controls spindle orientation and ACD by negatively regulating the NuMA/LGN/Gαi complex, likely by competing with NuMA for LGN binding [166].

Recently, another protein complex, small kinetochore associated protein (SKAP)/Astrin, has also been implicated in the regulation of spindle orientation at astral microtubule plus ends [167], possibly through mediating the interaction between astral microtubules and NuMA [136,168]. In addition to generating the pulling force, the spindle-orienting machinery may also regulate microtubule shrinkage by NuMA [169]. In the fly male germline, Baz provides a platform for the centrosome orientation, and Baz-centrosome association informs the cell to recognize the correct cell polarity for productive ACD [170]. Furthermore, it is recognized that in addition to the canonical Mud/Pins/Gαi complex, other system(s) exists in mediating spindle orientation [136]. For example, in the *Drosophila* pupal notum and larval wing, although Mud-mediated pulling is needed to orient spindles, neither Pins nor Dlg is required for the spindle orientation during mitosis [171,172].

Regulation of ACD

Genetic and molecular biological studies have identified numerous regulators controlling the cell polarity and ACD [3]. However, how these factors coordinate with each other to achieve cell polarity establishment and maintenance, as well as proper spindle orientation during ACD remains poorly understood. Recent structural studies have shed some light on the temporal and spatial assembly of the core polarity regulatory complexes.

During the assembly of the LGN-nucleated apical complex and spindle orientation machinery, Insc, NuMA, Afadin, and E-adherin all bind to the inner groove of the LGN TPR superhelix, and thus, their interactions are mutually exclusive (Figs. 3b and 4b). The competitive binding of Insc and Mud toward Pins argues against the commonly accepted model of ACD in *Drosophila* NB: that Insc functions as the linker to connect the Par complex with the Pins/Gai/Mud spindle orientation regulatory complex. However, the colocalization of Insc, Pins, Gαi, and Mud (and their vertebrate counterparts) in the apical cortices of asymmetrically dividing cells is indeed observed. A possible working model is that, after the recruitment of Pins to the apical cortex by Insc-Pins interaction, Pins has to be freed from Insc for the assembly of Mud/Pins/Gαi complex for spindle orientation [112]. How Pins is freed from the Insc sequestration is still unknown, possibly by Insc degradation, accumulation of Mud released from the nucleus in metaphase, or regulated dissociation of the Insc/Pins complex. The higher affinity of mInsc over NuMA in binding to LGN also suggests that both the level and timing of Insc and Mud expression as well as their turnover rates during development could serve as important regulatory factors for asymmetric divisions in stem/progenitor cells. The stable apical localization of Insc-free Pins/Mud complex is likely via the membrane-bound Gαi. In return, Gαi promotes Pins/Mud interaction via the Gαi/GL binding-mediated release of Pins auto-inhibition [147,148]. The binding of Insc to Pins, instead, does not require the Gαi-mediated opening of the auto-inhibited conformation of Pins [112], further suggesting that Insc is capable of targeting Pins to apical cortices at the early stage in ACD cycle. This working model may also be applied to the LGN/NuMA cortical anchoring through Afadin or E-cadherin [136].

Like Pins (LGN), structural studies revealed that the Scrib tripartite component Dlg (Dlg1) also acts as a key regulator in ACD. Dlg (Dlg1) GK can bind to Pins (LGN) linker region upon aPKC phosphorylation and orient the spindle in the “telophase rescue” process [113,173]. Another Scrib protein Lgl may play a regulatory role in this interaction, as Lgl can also bind to the GK domain of Dlg when phosphorylated by aPKC (Fig. 3b8) [85]. It has been suggested that Lgl physically prevents the binding between Pins (LGN) and Dlg (Fig. 4a) until mitosis when Lgl is released from the plasma membrane upon Aurora A-mediated phosphorylation [174,175], while for Khc73 (KIF13B), the situation differs. Binding to Dlg1 GK does not require phosphorylation of KIF13B [156]. Given the competitive binding of phospho-LGN and KIF13B to Dlg1 (Fig. 4a), Dlg1 cannot simultaneously bind to LGN and KIF13B either. A possible explanation is that Dlgs may form high-order oligomers via the intermolecular interactions between the SH3 domain and GK domain [157], thus linking phospho-LGN and KIF13B. Alternatively, Dlg1 may adopt a sequential target binding model like LGN. In this model, the Par/Insc/LGN/Gαi complex first recruits Dlg1 to the apical cortex. Later, KIF13B takes over to interact with Dlg1, which provides an anchor site for astral microtubule at the cell cortex.

Perspectives

Cell polarity proteins regulate a diverse array of cellular processes during development and homeostasis. We have summarized the assembly and regulation mechanisms of the conserved polarity protein complexes that play key roles in the establishment and maintenance of epithelial apical–basal

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polarity, and apical–basal polarity-induced ACD. Although epithelial cell polarization and ACD are fundamental and independent events, they are tightly coupled and share a common regulatory Par protein complex, which coordinates with several sets of other protein complexes to achieve polarized distributions of many cellular components. However, there are still lots of questions remained to be answered. First, the mechanisms for polarity establishment and maintenance vary in different cell types. Although the core proteins are evolutionarily conserved and have been extensively characterized in the model systems regarding their assembly to form protein complexes and their functions in cell polarity, it became obvious that these complexes function differently in distinct cell types. Therefore, additional cell-type specific components may be required to facilitate their specific functions in different tissues or even the same tissue at different developmental stages. Other polarization mechanisms may also exist. Second, as the key polarity proteins are multi-modular scaffolds, which interact with diverse and competitive partners, it remains unclear whether all these proteins are simultaneously present in a single complex, or whether the interactions are dynamic and how their assemblies are regulated. As the subcellular localization of these competitive binding partners is largely overlapping, understanding the temporal partitioning of possible complexes and the role of these complexes in establishing and maintaining cell polarity will require further elaborated *in vitro* and *in vivo* analysis.

For ACD, there are also many open questions. For example, how the Par proteins are maintained on the apical cortex after delamination? How the external signals from the niche are received and transduced into the cell to initiate ACD? How the Par-mediated ACD mechanism coordinates with epigenetic cues and transcription factors to generate daughter cells with distinct fates? Besides these general questions, there are some interesting phenomena. For example, both the apical and basal protein complexes each forms a crescent at the opposite poles of NB during ACD instead of uniformly distributed on either the apical or basal half of the cell cortex [19,103–109]. What prevents them from dispersing over the entire half of the cell cortex? The previous mutual exclusion model from the opposite cell cortices cannot fully explain this. Moreover, polarity proteins within the condensed crescents are found to be in fast equilibrium with the cytoplasmic proteins [127,128,132]. How do the highly concentrated protein crescents autonomously form in restricted membrane regions? How are the large concentration gradients between proteins within the crescent and in cytoplasm maintained? A likely mechanism for restricting polarity protein dispersions is protein oligomerization (e.g., Par3 clustering), which may further recruit other clusters (e.g., cytoskeletal and adherens junction molecules) to form a larger complex network with low diffusion rates. A following question is that, what are the anchoring factors for the apical and basal protein crescents? aPKC-mediated phosphorylation can explain how fate determinants are excluded from the cortical region where aPKC is localized, but they do not explain how they are anchored at the opposite pole. In addition, it is not known what are the mammalian orthologs of *Drosophila* adaptor proteins Mira and Pon, and whether the related localization mechanisms are also employed in mammals. Additional ACD regulators will surely be identified in the future, and cell-type specific mechanisms in driving ACD also need to be investigated. Nevertheless, the *Drosophila* NB has been serving as a very powerful, though simplified model system for investigating mechanisms regulating ACD in the entire animal kingdom.

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Par complex

Abbreviations used:
Par complex, Par3/Par6/aPKC complex; Crb, Crumbs; Scrib, Scribble; TJ, tight junction; AJ, adherens junction; PIP, phosphatidylinositide phosphate; aPKC, atypical protein kinase C; NB, neuroblast; NTD, N-terminal domain; Insc, Inscuteable; Pins, Partner of inscuteable; Pros, Prospero; Brat, brain tumor; Stau, Staufen; Pon,

Partner of Numb; Mira, Miranda; GMC, ganglion mother cell; TPR, tetratricopeptide repeat; CBD, cargo binding domain; GL, GoLoco; Khc73, kinesin heavy chain 73; CT, cytoplasmic tail; FBM, FERM-binding motif; PBM, PDZ-binding motif; GK, guanylate kinase; PSG, PDZ–SH3–GK; Lgl, Lethal Giant Larvae; ACD, asymmetric cell division; JAM, Junctional Adhesion Molecule.

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