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Review Article

The Par3/Par6/aPKC complex and epithelial cell polarity

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ABSTRACT

Apical–basal polarity is the basic organizing principle of epithelial cells, and endows epithelial cells to function as defensive barriers and as mediators of vectorial transport of nutrients in and out of organisms. Apical–basal polarity is controlled by a number of conserved polarity factors that regulate cytoskeletal organizations, asymmetric distributions of cellular components, and directional transports across cells. Polarity factors often occupy specific membrane regions in response to the adhesion forces generated by cell–cell and cell–extracellular matrix interactions. Both internal polarity factors and the external extracellular matrices play fundamental roles in epithelial cell polarity establishment and maintenance. This review focuses on recent developments of the Par3/Par6/aPKC complex and its interacting proteins in epithelial cell polarity.

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Abbreviations: ECM, Extracellular matrix; TJ, Tight junction; AJ, Adherens junction; SJ, Septate junction; ZA, Zonula adherens; MZ, Marginal zone; Par, Partition defective; EMT, Epithelial to mesenchymal transition; MET, Mesenchymal to epithelial transition; Dlg, Disc-large; Lgl, Lethal (2) giant larvae; MARK, Map/microtubule affinity regulating kinase; LKB1/STK11, Liver kinase B1/serine-threonine protein kinase 11; PI(4,5)P2/PIP2, Phosphatidylinositol 4,5-bisphosphate; PI(3,4,5)P3/PIP3, Phosphatidylinositol (3,4,5)-triphosphate; PALS, Protein Associated with *Caenorhabditis elegans* Lin-7 protein; PATJ, PALS1-associated TJ protein; S/T kinase, Serine/threonine kinase; PTEN, Phosphatase and tensin homolog; PI3K, Phosphoinositide 3-kinases; NuMa, Nuclear mitotic apparatus protein; LGN, Leu-Gly-Asn repeat-enriched protein; MARCKS, Myristoylated alanine-rich protein kinase C substrate; GSK3β, Glycogen synthase kinase 3 beta; Baz, Bazooka; LamG, Laminin G domain; EGF-like, Epidermal growth factor-like domain; TM, Transmembrane region; FERM, 4.1, Ezrin, Radixin and Moesin; PDZ, Postsynaptic-density-95/Disc-large/Zona-occludens 1; FBM, FERM binding motif; PBM, PDZ binding motif; FA, FERM-adjacent region; L27, Lin-2/Lin-7 domain; SH3, Src homology 3 domain; Guk, Guanylate Kinase like domain; NTD, N-terminal domain; PB1, Phox and Bem1 domain; CRIB, Cdc42/Rac interactive binding domain; CCA, Cell–cell adhesion; CMA, Cell–matrix anchorage; MDCK, Madin-Darby Canine Kidney cell line.

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Epithelial cell polarity

Cell polarity refers to a difference in structure, composition or function between two poles. The general concept of cell polarity could be divided into anterior–posterior polarity in one-cell embryos in *Caenorhabditis elegans* and *Drosophila melanogaster* oocytes, apical–basal polarity in epithelial cell, transient polarity exemplified in directed migrating cells as well as planar cell polarity during tissue formation. Among those, epithelial tissue is a prevalent system in animal kingdom and an easily accessible system for *in-vitro* cellular studies, thus the cell polarity following apical–basal axis in epithelial cells is most commonly studied. This review tries to summarize the current understanding and some of the recent progresses in the area of apical–basal polarity of epithelial cells regulated by the Par3/Par6/aPKC complex (the Par complex) and the proteins associated with the Par complex.

In multicellular organisms, epithelial cells are the most common cell-type and form as an organized layer that subdivide the body into morphologically and physiologically different compartments. Functional-wise, epithelia act as diffusion barriers between the inside and outside of the organism, and allow vectorial transport from one side to the other. To achieve this function, epithelial cells must be highly polarized. Therefore at the outermost layer of a cell–plasma membrane, we could observe different shape and polarized distribution of molecules. The plasma membrane of epithelial cells can be subdivided into an apical region facing the external environment, a lateral domain that contacts neighboring cells, and a basal domain that contacts the extracellular matrix (ECM) and the interstitial space of the body. The lateral surface is characterized by the formation of inter-cellular junctions, such as the Tight Junctions (TJ) (In *Drosophila*, the similar structure is named the Septate Junction (SJ), and its relative position to AJ is different (Fig. 1C)) and the Adherens Junctions (AJ). TJs act as barriers to paracellular diffusion, and AJs provide the main mechanical link with neighboring epithelial cells. The basal side of polarized epithelia is marked by desmosomes, which contact with the ECM (Fig. 1) [1,2]. The lateral and basal membranes are often named together as basolateral membrane since they are different from the contact-free apical membrane.

Cell polarity determinants and their complexes

Specification of the highly polarized surfaces is fulfilled by precisely temporal and spatial localization of polarity proteins or other molecules including various lipids. At the molecular level, epithelial cell polarity is mainly controlled by a conserved set of polarity proteins in both vertebrates and invertebrates (Fig. 2). Such polarity regulating proteins include, but not limited to, Par3 (Bazooka in *Drosophila*), Par6/aPKC, Crumbs/PALS1 (Stardust in *Drosophila*)/PATJ, Dlg/Scrib/Lgl, MARK1/2 (Par1), 14-3-3 (Par5), and LKB1 (Par4) [2]. Studies on epithelial cell development in *Drosophila* have revealed that the apical-basal polarity is established differently in the primary blastoderm epithelium that gives rise to the ectoderm and the secondary epithelia which are formed via mesenchymal to epithelial transitions (MET) [3]. In the primary epithelium, such as the *Drosophila* oocyte follicle cells, the cytoskeleton and associated small GTPases provide the initial polarity cue by directing the localization of the Par6/aPKC complex to the apical side of the cell, Bazooka to the apical/lateral junction, and Par1, the Dlg/Scrib/Lgl complex to the basolateral membranes. This polarity is later strengthened by the recruitment of Crumbs and Stardust to the apical cortex. In the secondary epithelia, such as midgut epithelia, dorsal vessels and glia sheets forming blood–nerve barrier, their polarization requires basal cues, such as laminin and its effector integrins [4–6], although this process has not been well studied in detail *in vivo*.

Mammalian embryogenesis is different from that of insects in a way that the process of epithelialization happens before gastrulation in mammal's embryos. As such, the requirement of basal matrix arrangement is more stringent in mammalian embryogenesis [7]. In human/mouse early embryo, before the first epithelial layer–trophectoderm is formed, cells with adhesive forces generated by E-cadherin have already emerged [8]. However, in adult mammals, the epithelial cell type can originate from either one of three germ-layers through epithelial to mesenchymal transition (EMT)/MET processes similar to the insect epithelial developing processes, and the generation and maintenance of epithelial cell polarity are controlled by the similar set of polarity factors [2].

The Par complex

The genes encoding for Par3, Par6 and aPKC were originally identified in searching for genes that are required to establish anterior/posterior polarity of *C. elegans* zygotes (hence named *par* for partitioning-defectiveness of zygotes after mutation of the genes) [9]. Subsequently, growing evidences showed that Par3 and Par6 function together with aPKC to regulate initial stages of polarization in *C. elegans* and *Drosophila* embryos [10], *Drosophila* neuroblast asymmetric cell division [11], and establishments of the mammalian epithelial cell apical–basal polarity and the axon–dendrite polarity of neurons [12–15]. In the last decade, mechanistic and functional characterizations of the Par complex and their associated proteins have greatly enhanced our understanding of the signaling events in such symmetry breaking and polarity maintaining processes.

A central feature of Par3 and Par6 is that both proteins are multi-modular scaffold proteins capable of binding to each other as well as a diverse range of other cell polarity regulating proteins [16] (Fig. 2). These specific interactions ensure that the Par complex is correctly localized at the specific membrane domains in a spatial and temporal manner [17]. The initial targeting of the Par complex to the junctional region is mainly through the Par3-nectin interaction [18]. In the Par complex, Par3 was found to associate with the Par6/aPKC hetero-dimer via the PDZ-PDZ domain interaction at the onset of epithelial polarization [14,19,20]. Par6 contains an N-terminal PB1 domain, a C-terminal PDZ domain and a semi-CRIB motif immediately

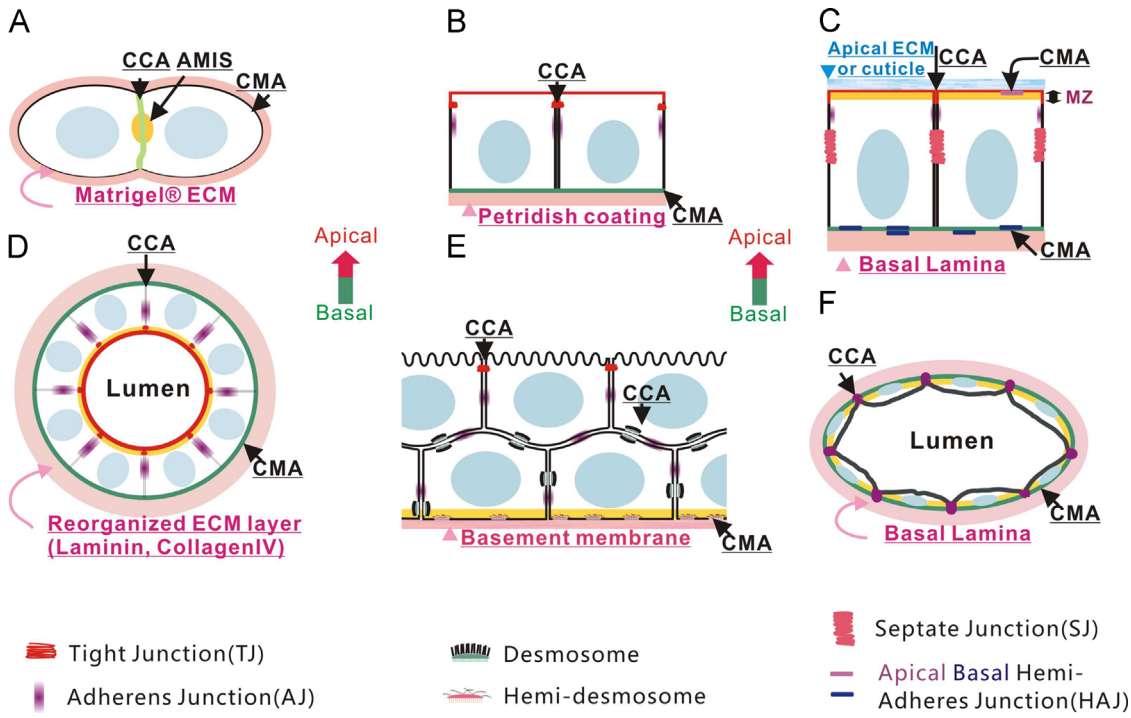

Fig. 1 – Different forms of the organizations of epithelial cells. (A) *In-vitro* epithelial cell culture in Matrigel® medium at the 2-cell stage. Cell–cell adhesion (CCA) is formed by E-cadherin interaction between neighboring cells. Several junctional proteins including future basolateral polarity proteins (such as Na^+–K^+–ATPase) are recruited in the cell–cell adhesion site. Par3 and aPKC can be found at the apical membrane initiation site (AMIS) [69]. Cell–matrix anchorage (CMA) is not obvious at this stage [69]. (B) *In-vitro* 2D epithelial cells cultured in flat petridish surface coated with one of the following coating reagents: lectin, gelatin, poly-D-Lysine, fibronectin, collagen, or Matrigel®. Epithelial cells (such as MDCKII cells) grown on such flat surface would develop into a cuboidal (Z dimension) and honeycomb (X–Y dimension) shape with recognizable TJ and AJ structures. The CMA structure is also formed due to the interaction of cells with the coating surface. The separation of two poles (apical and basolateral) of epithelial cells ensures that both lipid and membrane protein contents will not freely diffuse between the two poles. Par3 is found mainly localized at the TJ region, while aPKC and Par6 is not restricted to the TJ region. (C) In *Drosophila* epithelial cells, lateral junctions are composed of AJ (Zonula Adherens, ZA in *Drosophila*, where the cadherin–catenin complex resides) and the septate junction (SJ) with AJ at the more apical position. SJ in *Drosophila* is functionally analogous to but morphologically different from TJ in vertebrate epithelia. Several basolateral polarity proteins such as Dlg, Scrib, coracle, Nrx IV and Na^+–K^+–ATPase are restricted at the SJ instead of the entire basolateral membranes as in mammalian epithelial cells. Besides, there is a marginal zone (MZ) at the sub-apical region where most apical polarity complexes including Baz are located, while Par6 and aPKC are localized at apical membrane (labeled as orange) [17] Although desmosome proteins are absent in invertebrate proteome, similar CMAs known as hemi-AJs can be found at both basal side (contacting with basal lamina) and apical side (with secreted apical ECM or cuticle layer) of epithelia [1]. (D) Epithelial 3D cyst formed by culturing cells in ECM-like medium such as Matrigel®. CMA can be found at the outer-surface in accordance with the remodeled ECM layer including laminin and collagen IV [64]. Par3 is mainly located at TJ region while aPKC is at apical membrane and TJ (labeled in orange) [69]. (E) In zebrafish larvae bi-layered epidermis, outer layer is called periderm and is linked with the underlying layer-basal epidermis through AJ and desmosomes (CCA). The basal epidermal cells are connected to the extracellular basal lamina with hemidesmosome (CMA). In this system, aPKC is localized at the basal domain of basal epidermal cells (labeled in orange), and its localization requires hemidesmosome formation mediated by Integrin α6 and regulated via cross-talk between Lgl and E-cadherin [44]. (F) In mouse blood vessels, the vascular lumen is surrounded by a single layer of squamous endothelial cells which are in contact with basal lamina (CMA). Interestingly Par3 is localized at the basal side of endothelial cells of arteries and acts downstream of β1 integrin signaling (labeled in orange) [45].

preceding the PDZ domain. The PB1 domain of Par6 forms a hetero-dimer with the PB1 domain of aPKC and the semi-CRIB domain can specifically bind to the GTP-bound form of Cdc42. The binding of Cdc42 to Par6 activates the associated aPKC, which subsequently phosphorylate Par3 and cause Par3 to dissociate Par6/aPKC [21]. In *Drosophila* primary epithelium, it was found that, following Baz-mediated apical membrane localization of Par6/aPKC, the binding of Crumbs tail to the PDZ domain of Par6 dissociates Baz from the Par6/aPKC complex. Thus, Baz and Par6/aPKC reside at distinct membrane regions: Baz at AJ and Par6/aPKC at the more apical marginal zone (MZ) (Fig. 1C) [22,23]. Similarly, in mammalian epithelia Par3 is found at TJ and Par6/aPKC are at more apical apex [24], although this difference of localization is not prominent in 2D cultures [25].

In addition to its role in forming the Par complex, Par3 also has Par6/aPKC-independent functions. Par3 contains a conserved N-terminal domain (NTD, also called CR1), three central PDZ domains, and the C-terminal region containing multiple protein

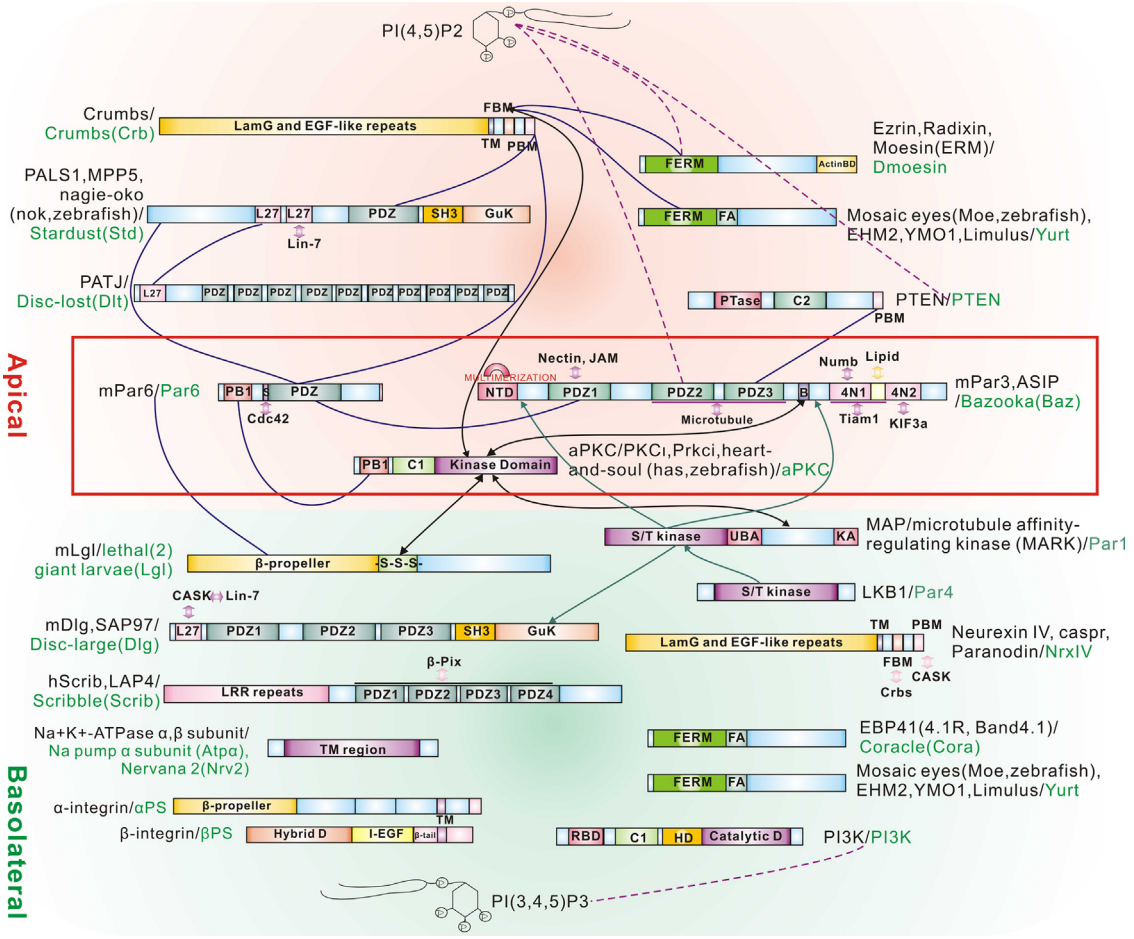

Fig. 2 – Epithelial cell polarity complexes. Apical-basal axis of epithelial cells is generated and maintained through mutual competitive interactions between polarity protein factors which are distinctly localized at the apical (red) and basolateral (green) domains. The domain organizations and interaction networks of the selected mammalian polarity factors are shown here. Solid lines represent direct binding, solid lines with single arrow head represent kinase phosphorylation events, solid lines with double arrow heads represent both the direct association and phosphorylation events between two proteins, dashed lines represent direct binding of polarity proteins to PIP-lipids. The corresponding orthologs name for the polarity factors from *Drosophila* are marked in green. Par complex is marked with red square frame. Among these polarity proteins, apical Crbs, basolateral integrins and Na^+K^+–ATPase are membrane proteins responsible for receiving extracellular signals. Additionally, the E-cadherin/catenin (Shotgun (Shg)/Armadillo (Arm) in *Drosophila*, and Hammerhead (HMR)/Humpback (HMP) in *C. elegans*) complex is another crucial adhesion signaling molecules for AJ formation in epithelial cells. Several scaffold proteins containing protein–protein interacting modules including PDZ, L27, FERM and PB1 domains mediate polarity complex formation. Among these polarity proteins, Dlg, Lgl, Scrib, Crbs, aPKC, PTEN and LKB1, are all demonstrated as tumor suppressor proteins.

binding sites including the aPKC-binding-motif. The conserved NTD domain can self-associate [26,27] and this NTD-mediated oligomerization of Par3 provides a molecular basis for enrichments of the Par complex [28] and their binding partners in specific membrane regions of polarized cells. Several cell adhesion molecules (CAMs) including p75, nectin, and JAM, are reported to interact with the first PDZ domain of Par3 [29–32] (Fig. 2). These interactions are likely to serve as membrane targeting signals of Par3. The first PDZ domain of Par3 is also responsible for recruiting Par6 via a PDZ–PDZ domain interaction [14,19,20], although with unknown underlying molecular mechanism. The PDZ2 and PDZ3 tandem of Baz and mPar3 are known to bind to lipid membranes, and such PDZ/membrane interaction is critical for Par3's localization at TJ and its function in establishing the apical–basal polarity of epithelia [33,34]. Together with the lipid phosphatase PTEN which directly binds to the third PDZ domain with its C-terminal PDZ binding motif (PBM), Par3 may serve as an ideal scaffold for integrating PIP signaling events during cellular polarization [34,35]. Interestingly, the N-terminal half of mPar3 also exhibits strong microtubule binding and bundling activity, which can be suppressed by its own C-terminal half via an intra-molecular auto-inhibition mechanism. Disruption of this microtubule bundling activity of Par3 impairs its function in axon specification in neurons and apical–basal polarity establishment in epithelia [36].

The C-terminal half of Par3 contains an aPKC binding site and three conserved regions named 4N1/2/3 each with no recognizable domain composition (Fig. 2). As a major component of the Par complex, aPKC is known to phosphorylate numerous polarity proteins including GSK3β, MARCKS, LGN, Par3, Lgl, Crumbs, and

Lin5/NuMA, thereby exerts its regulatory roles in cellular polarization. The recently solved crystal structure of PKCι in complex with its binding peptide from Par3 elucidated how aPKC recognizes and phosphorylates its target proteins [37]. The coiled-coil region 4N1 is reported to interact with cell fate determining molecule Numb/Numbl [38]. In the middle of 4N1, a stretch of evolutionary conserved positively charged residues are also found to bind to membrane lipids and thus contribute to the cortical localization of Par3 [39]. 4N1 is also capable of interacting with Rac1-specific guanine nucleotide exchange factor (GEF) Tiam1/2, which in return regulates TJ formation in epithelia and axon/dendrite development in neuronal system [40–42]. The 4N2 region binds to microtubule motor KIF3A and this interaction is required for the proper neuronal polarity [43]. Despite of the multiple functional binding sites identified in the Par3 C-terminal half, the protein structures of any individual 4 N regions or their complexes with cognate interacting proteins have not been solved till now to explain their detailed interaction mechanisms.

Although Par3 and Par6/aPKC have been found mostly functional at the apical side as relatively upstream polarity factors [2], recent evidence suggest the Par complex may also locate at basal side in zebrafish larvae bi-layered epidermis (aPKC) [44] (Fig. 1E) and in mouse blood vessels (Par3) [45](Fig. 1F), although the functions of the Par complex there are not clear yet.

Maintaining distinct cortical domains in epithelial cells depends on mutually antagonistic interactions between polarity complexes. For example, aPKC can phosphorylate Par1 to prevent it from associating with the apical membrane. Conversely, Par1 can phosphorylate Par3 to generate 14-3-3 (Par5) binding sites and to interfere with its self-oligomerization and its interaction with aPKC, thus preventing Par3 from residing at the basolateral membrane [46](Fig. 2). Although Lgl can physically interact with Par6/aPKC, aPKC-mediated phosphorylation of Lgl prevents Lgl from associating with the apical membrane cortex [12,47–49] (Fig. 2). Over-expression of aPKC or knocking-down of Lgl enlarges the apical domain at the expense of lateral domain, whereas loss of aPKC activity or over-expression of Lgl reduces the apical domain [48,50,51]. However, *Drosophila* embryos lacking both the Crumbs and Scrib/Lgl complexes still form clusters of polarized epithelial cells by the end of embryogenesis, indicating that epithelial polarity is controlled by redundant polarity factors [52,53]. Recently it's found that Yurt and Neurexin IV/coracle/Na^+–K^+–ATPase function as a second group of lateral proteins that antagonize the activity of the apical Crumbs complex during mid-embryogenesis in *Drosophila* [54,55], and Yurt depletion can also cause loss of basolateral membranes in MDCK cells [54]. Finally, additional polarity factors remain to be identified since correctly polarized epithelial cells can be formed at the end of *Drosophila* embryogenesis in the absence of both scribble and Yurt [54].

Adhesive forces and cell polarity

In one-cell embryos such as *C. elegans* embryos where the anterior-posterior polarity is believed to be generated stochastically by actomyosin flow at the sperm entry site [2]. In multicellular system, “touching” by neighbors is a resemblance of fertilization process and is crucial for the later differentiation processes. The initiation and maintenance of apical–basal polarity inside epithelial sheets in multicellular system is closely related to cell–cell adhesion (CCA) generated by junctional protein complexes (*e.g.*, E-cadherin) between neighboring cells, and cell–matrix anchorage (CMA) points (*e.g.*, integrin on the epithelial membrane, laminin and collagen etc as the basal lamina layer) [56] (Figs. 1, 2). Taking kidney cells as an example, applying antibodies against E-cadherin in pre-implanted mouse embryos can inhibit compaction and formation of the trophectoderm, and consequently cause the cells to become morphologically mesenchymal [8]. Similarly, in the developing mouse kidney, adding antibodies against laminin A has been reported to inhibit polarization of the pro-metanephrogenic mesenchymal cells [6].

In-vitro cultured epithelial cells such as Madin-Darby Canine Kidney (MDCK) II cells, human mammary gland non-tumorigenic epithelial cell line-MCF10A, and human epithelial colorectal adenocarcinoma cells-Caco2 [57], have been very useful models to study functions of intrinsic factors or external cues in polarity regulation (*e.g.* by knocking-down/over-expression polarity proteins [28], changing the basal coating pattern [58], lowering calcium concentration in culture media to disengage CCA and CMA [59], or introducing external forces such as scratch wounding [60], etc). These studies have revealed that: (1) CCAs including AJ and TJ are the primary residing points for several polarity factors including the Par complex, Lgl/Dlg/Scrib, PALS1/PATJ, Na^+–K^+–ATPase, LKB1, and the cadherin/catenin complexes. Mislocalization or changing of their expression level would impair the apical–basal polarity [61](Fig. 2); (2) CMA not only provides anchoring scaffold but also negatively regulates CCA formation and antagonizes the position of cell–cell junctions to be stabilized away from ECM [56]; (3) Conversely, CCA also plays similar negative feedback effect on CMA, both via intra- and intercellular cytoskeleton force generating network [58]. In a way, the interplay between CCA and CMA is also resembled by the counter-action between apical and basolateral polarity factors in polarized epithelia (Fig. 2).

Culturing of epithelial cells on two-dimensional petridish surface coated with compounds mimicking ECM would force cells into honeycomb shaped single layer, thus generating a well-organized membrane domains with an apical–basal polarity, similar to the simple epithelia sheets *in vivo* (Fig. 1B). However, the dish coating or simple plastic cannot recapitulate the *in vivo* basal lamina of its complexity and rigidity. Additionally, formation of epithelial tissues often require hollow lumens, which are formed by ordered line-up of a layer of epithelial cells (or a single cell such as in *Drosophila* trachea [1] as an extreme case) and function as nutrient uptake in digestive tract, gas exchange in trachea, and blood circulation in vessels [62]. Therefore, to mimic the epithelial tubule structure *in vivo*, 3D epithelial cell cultures are frequently used, as cells cultured in ECM containing media form cysts each with an internal apical lumen [63](Fig. 1D). At the 2-cell stage during cyst formation, CCA is sufficient to trigger the enrichment of several polarity proteins such as E-cadherin, Na^+–K^+–ATPase, and gp135 at the contact zone (Fig. 1A). From 2-cell stage onwards, the generation of a single lumen with appropriate orientation of apical–basal polarity and specification of apical domain of 3D cysts require ECM to engage with CMA [63]. Except for the aforementioned internal polarity factors, the laminin–integrin signaling for CMA formation is also shown to regulate the apical–basal polarity orientation through PI(3)K and Rac1 signaling [64], while apical crumbs may act as an antagonist for the PI

(3)K–Rac1 module to generate and maintain appropriate apical–basal membrane ratio [65]. Besides the 3D cyst model, a recent work using confined micropattened chambers coated with ECM of different surface areas to support cyst formation, demonstrated that laminin signaling from the coated surface contributes to lumen initiation by inhibiting cell spreading and limiting peripheral actin contractility through LKB1/Par4 acting upon Rho/Rho Kinase (ROCK)/myosin II pathway [66].

Concluding remarks

Epithelial cells are the most common cell-type in the human body, and are also the first differentiated structure to emerge during embryogenesis to form tissues including gut, lung, kidney, liver, skin and all of the secretory organs of the body. More than 80% of tumors are of epithelial origin and several polarity genes such as Dlg, Lgl, Scrib, Crbs, aPKC, PTEN and LKB1 also serve as tumor-suppressors (Fig. 2). Asymmetric cell divisions, which are responsible for generating differentiated cells as well as maintaining stem-cell pools, are known to occur in various epithelial tissues including epidermis, gut, mammary glands and lung [67]. During asymmetric cell division, epithelial apical–basolateral polarity is transformed into bipolar polarity and its adhesion sites are used as niches instructing mitotic spindle orientations. The symmetry of stem cell divisions are also regulated by epithelial cell polarity proteins [68]. Research in the last decade via combined efforts from genetic, cell biology and biophysical studies has generated a great deal of knowledge about the mechanisms controlling cell polarity establishment and maintenance. These studies also paved ways for further discovery of additional polarity factors and their downstream effectors and elucidation of their roles in organogenesis, stem cell differentiation, as well as cancer development. However, the current working model needs further revision for the evidence that loss of major polarity factors often does not disrupt the epithelial polarity when cells/tissues are given sufficient time to develop [54]. Additionally, there is much to be learned about the cross-talks between the apical and basolateral polarity complexes. Finally, our understanding on the connections between the extrinsic polarity cues and intrinsic cellular polarity determinants are still rather primitive. It is assured that many exciting findings in these areas will come to light in the coming few years.

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