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# Organization and execution of the epithelial polarity programme

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## Abstract

Epithelial cells require apical–basal plasma membrane polarity to perform crucial vectorial transport functions and cytoplasmic polarity to generate different cell progenies for tissue morphogenesis. The establishment and maintenance of a polarized epithelial cell with apical, basolateral and ciliary surface domains is guided by an epithelial polarity programme (EPP) that is controlled by a network of protein and lipid regulators. The EPP is organized in response to extracellular cues and is executed through the establishment of an apical-basal axis, intercellular junctions, epithelial–specific cytoskeletal rearrangements and a polarized trafficking machinery. Recent studies have provided insight on the interactions of the EPP with the polarized trafficking machinery and how they regulate epithelial polarization and depolarization.

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The epithelium is perhaps the first tissue that emerged during phylogenesis. Modern cadherin-based epithelia appeared with the dawn of the Metazoa, 600 million years ago; however, epithelial structures are much older, as demonstrated by the recent finding of an ancient non-cadherin-based epithelium in the pre-Metazoan organism *Dictyostelium discoideum* [1]. The evolutionary appearance of modern epithelia reflects the requirement of Metazoa for a tissue structure that is capable of segregating their internal medium from the outside environment [2]. Epithelial cells are particularly well suited for this fundamental function, owing to several attributes of the epithelial phenotype (Figure 1a): they form sheets of uniformly polarized cells that are kept together through specialized adherens junctions on the basis of calcium-dependent cell adhesion molecules (cadherins); they contain sealing junctions between their apical and basolateral domains (tight junctions in vertebrates, septate junctions in invertebrates) that segregate the internal medium from the outside environment; they have segregated apical and basolateral plasma membrane domains with asymmetric compositions of nutrient and fluid transporters required to carry out their vectorial secretory and absorptive functions; they have a polarized trafficking machinery that is composed of secretory organelles (ER, Golgi complex) and endosomal compartments and which is required for the generation and maintenance of the asymmetric distribution of plasma membrane proteins. As the complexity of metazoan organisms evolved, epithelial cells became essential building blocks to generate the various organs required for survival, and to implement their body plan [3]. Mammals have well over 150 different types of epithelial cells that perform key roles in the generation and/or function of

their digestive, respiratory, reproductive, neural, sensory, vascular and hormonal systems[4]. These cells are constantly replaced, and arise from tissue-specific stem cells.

The epithelium is the first tissue that emerges during ontogenesis, and epithelial cells have fundamental roles in embryo morphogenesis and organ development[5]. There are additional features of the epithelial phenotype that make epithelial cells especially suited for these roles. First, the polarized actin and microtubule cytoskeleton enables epithelial cells to constrict their apical surfaces (Figure 1b), and thus participate in key developmental processes such as gastrulation and tubulogenesis [6], and to expand or shrink their lateral membranes, which enables the formation of columnar or squamous epithelia (Figure 1c). Second, epithelial cells can orient the mitotic spindle. This feature enables epithelial cells to divide parallel to the sheet to increase their number (Figure 1d) or perpendicular to the sheet to generate different daughter cells in the case of stem cells (Figure 1e) or to generate stratified epithelia from simple epithelia (Figure 1f) [7]. Third, the primary cilium[8] (Fig 1a) found on the cell surface of vertebrate epithelial cells harbors signaling pathways that have key roles in the organization of planar polarity, which is essential for many developmental processes and in epithelial cell differentiation in response to signals from mesenchymal or endothelial cells. Fourth, epithelial cells can quickly lose or acquire the epithelial phenotype, known as epithelial-mesenchymal transition (EMT) or mesenchymal–epithelial transition (MET), respectively [9]. A process similar to EMT has been proposed to account for the dissemination of cancers, the large majority of which, in humans, arises from epithelia [10, 11].

Therefore, it is of fundamental importance to understand the molecular programmes that drive epithelial polarization and depolarization. Over the past three decades, genetic and biochemical studies in *Caenorhabditis elegans, Drosophila melanogaster* and monolayer or three-dimensional (3D) cultures of mammalian epithelial cells have generated an extensive body of knowledge on a network of polarity proteins and lipids that provide identity to the apical and basolateral domains of epithelial cells [12-15] (Figure 2). Moreover, cell biological and biochemical studies in mammalian epithelial cells, such as the prototype epithelial cell line MDCK, provided detailed information on the polarized trafficking machinery used by epithelial cells to distribute their plasma membrane proteins into apical, basolateral and ciliary domains [16-18] (Figure 1a). Here, we refer to the overall process through which the network of epithelial polarity proteins and lipids mediate the organization of a polarized epithelial cell as the epithelial polarity programme (EPP). An important caveat is that although there is a striking preservation of the key EPP players and mechanisms, there is also considerable variation in their use by different epithelial cells in different organisms, tissues and developmental contexts. In this Review, we discuss the interactions of the EPP and the polarized trafficking machinery and how, in turn, vesicular trafficking contributes to the organization of the EPP.

## Key players mediating the EPP

Multiple distinct but interacting groups of proteins make up the EPP. The Par proteins are ubiquitously expressed and function in many different contexts to regulate polarity, cell proliferation, and differentiation, while the Crumbs and Scribble groups are more restricted in their distribution. The Scribble group is restricted to epithelial cells and localize to lateral

membranes. The Crumbs group is localized apically or at apical junctions. These components of the EPP are discussed in detail below.

## Par proteins

An elegant screen for maternal embryonic lethal mutants in *C. elegans*, that are required for the correct partitioning of cytoplasmic components between the anterior and posterior cells of the early embryo [19]. All but one of these genes has since been found in other animals, but not in plants or fungi. For example, screens in *Drosophila* [CEd:D. melanogaster throughout] embryos for defective epidermal development revealed fly homologues of the *par* genes[13]. The Par proteins involved in epithelial morphogenesis are the protein kinases Par1 and Par4, a phospho-protein interactor Par5 (an isoform of 14-3-3) and two scaffold and adapter proteins that contain PDZ domains, Par3 and Par6. In addition, atypical protein kinase C (aPKC) and the CDC42 GTPase are also considered to be part of the group (Figure 2, *Par* proteins are shown in orange). Although only single copies of the Par genes exist in nematodes and flies, this gene family has expanded in vertebrates, although the reasons for this remain unknown (for example, there are three genes encoding Par6, two gene encoding Par3 and two genes encoding aPKC). Presumably the isoforms have different roles, but so far only one study has provided evidence for this hypothesis. It showed that replacement of the zebrafish Par6 gene with a different isoform did not rescue the wild type phenotype [20].

## Crumbs, Scribble and Coracle groups

Although Par proteins function in many different contexts, forward genetic screens in *Drosophila* also uncovered additional polarity components that are more specific for epithelial cells. These proteins are also conserved throughout Metazoa and interact both genetically and physically with the Par proteins. One group of epithelial polarity proteins , known as the Crumbs complex, consists of Crumbs (Crb), Stardust, (Sdt; called Palsl in vertebrates) and Dpatj (Patj) [15, 21]. Another group, known as the Scribble group, contains Discs-large (Dlg), Lethal giant larvae (Lgl), and Scribble (Scrb) [22]. In addition, at least in *Drosophila*, other proteins that participate in the EPP have been revealed, including FERM domain proteins (Moesin and Yurt), Coracle, Neurexin IV and Na,K-ATPase[23]; together these proteins make up the Coracle group of EPP proteins. The Scribble and Coracle groups have similar roles in establishing basolateral identity but operate at different times during *Drosophila* development (gastrulation and organogenesis, respectively) [23].

## Rho GTPases

The Rho (Ras homologous) family of GTPases comprises over 20 members, including Cdc42, Racl and RhoA [24]. They constitute molecular switches that cycle between active (GTP-bound) and inactive (GDP-bound) conformations. This switch, as well as the localization of GTPases within the cell, is controlled by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). The best studied function of GTPases is their role as organizers of the actin cytoskeleton: RhoA controls the generation of contractile forces through the regulation of actin-myosin filament assembly, whereas Racl and Cdc42 organize actin modules at the cell periphery to generate lamellipodia and filopodia, respectively[25]. Rho GTPases have key functions in the organization of the EPP, and some of them are independent of their actin-organizing roles [26]. Furthermore, they cross-talk to

regulate each other's activity [27]. Cdc42 is an evolutionarily conserved master regulator of cell polarity. It was originally shown to be a key organizer of polarized budding and secretion in *S. cerevisiae*, and has many roles in the organization and execution of the EPP28. RhoA generates contractile forces that antagonize the adhesive forces generated by Racl26.

### Polarity lipids

Phosphoinositides constitute just 1% of the total cell lipids, but they have many fundamental biological roles as precursors of lipid messengers and as membrane docking sites for effectors of signaling cascades [29]. There are seven phosphoinositide isoforms that differ in the location and number of phosphate groups in the inositol ring. They are spatio-temporally regulated by organelle-specific phosphoinositide kinases and phosphatases. Thus, individual phosphoinositides can serve as unique lipid signatures for cellular organelle identity; through their ability to recruit proteins that possess one or more of the 11 known phosphoinositide-binding modules they fine-tune the composition of the membrane–cytosol interphase. Phosphoinositides and other lipids are implicated in the generation of epithelial polarity [3031] {Shewan, 2011 #377}. An important advance was the uncovering of key roles for PI(3,4,5)$P_3$ ($PIP_3$) in basolateral plasma membrane identity[32] and PI(4,5)$P_2$ ($PIP_2$) in apical plasma membrane identity [33]. PTEN, a phosphatase that generates $PIP_2$ from $PIP_3$, is localized apically in fly embryonic epithelia[34]; however, it is not clear yet whether PIP2 and PIP3 have roles in establishing and maintaining polarity in *Drosophila* epithelia similar to those described in mammalian epithelia A recent genome-wide screen in *C. elegans* revealed that the biosynthetic pathways of glycosphingolipids are important for the generation of apical membranes in intestinal cells [35]. This is in agreement with the long-postulated role of glycosphingolipids and cholesterol in the formation of lipid rafts for apical transport [36]. Finally, phosphatidylserine (PS) has been shown to play a key part in regulating Cdc42-mediated polarization in yeast [37], but polarity roles for PS in the EPP have not yet been reported.

## Organizing the EPP

### Feed-back loops

A striking feature of the EPP proteins is their differential localization at the epithelial cell cortex. Among the core polarity proteins, Crumbs, Na,K-ATPase and Neurexin are transmembrane proteins, whereas all other proteins are peripheral cytoplasmic proteins that localize at the cell cortex through interactions with membrane proteins or other polarity proteins. An exception is Par5 (which encodes a 14–3–3 protein), which is distributed throughout the cytoplasm.

How are these domains formed and maintained? One important and recurring mechanism is the mutual exclusion of proteins from one domain by those in the other. Two protein kinases have key roles in this process: aPKC and Par1. Atypical PKC is recruited to the apical cortex, where it excludes basolateral proteins, while Par1 is recruited to the basolateral cortex and excludes apical proteins. Strikingly, intercellular adhesion proteins such as E-cadherin, proteins involved in endocytosis (such as Numb) and proteins that control spindle

orientation (such as Pins and its mammalian homologue LGN) are mislocalized to the apical surface when aPKC positioning is lost. This disrupts epithelial morphogenesis [38, 39]. However, these mechanisms are context dependent, as, for example, the localization of Pins is independent of aPKC in neuroepithelial cells [40] and in the *Drosophila* follicular epithelium[41].

Exclusion of 'trespassing' proteins from the apical domain is driven by aPKC-dependent phosphorylation. Phosphorylated residues in basolateral proteins attract Par5, which releases the proteins from the cell cortex [13, 42]. Conversely, Par1 located on the lateral membrane phosphorylates and excludes apical proteins from this domain. One important Par1 target is Par3. Par5 binds to phosphorylated Par3 present on the lateral membrane, which triggers the dissociation of the Par3-Par5 complex into the cytoplasm, where the Par3 is dephosphorylated [42]. In this manner, EPP proteins maintain distinct apical and basolateral domains at the cell cortex [13, 42].

What determines aPKC and Par1 localization? The mechanism of Par1 attachment to the cell cortex remains unclear, but it is excluded from the apical domain through direct phosphorylation by aPKC [43-45]. Atypical PKC is attached to the apical cortex through a Crb-Par6-Pals1 complex (Figure 2a). Par6 helps to recruit substrates to aPKC and to regulate the kinase activity of this protein [4647]. The association of Cdc42-GTP with Par6 helps recruit the complex to the apical domain and promotes aPKC activity. The CDdc42-specific GEFs Tuba [48] or Dbl3 [49] generate Cdc42-GTP at the apical cortex. Studies investigating the formation of apical lumens in MDCK cysts have shown that an early event in this process is the apical concentration of PTEN, which leads to exclusion of $PIP_3$, the apical enrichment of $PIP_2$ and the consequent recruitment of annexin and Cdc42[33].

Par3 resides at tight junctions in vertebrate epithelial cells and is not part of the apical complex; however, it is required for apical delivery of aPKC. Par3 binds aPKC in two distinct modes: first, through the kinase domain of aPKC; and second through an indirect association via Par6 (Figure 2b). A mechanism has been proposed whereby phosphorylation of Par3 by aPKC releases the kinase, which can then associate with the Crb–Pals1 complex –through Par6 [50]. A different model suggests that phosphorylation of Par3 triggers the dissociation of Pals1/Stardust, which can then bind to Crb and recruit Par6 [51]. However, these proposed models do not explain why Par3 is needed for the apical localization of aPKC. Why could aPKC and Pals1 not simply diffuse to the apical surface and interact with the Crb complex, and why would aPKC not continually phosphorylate Par3 to be released before it arrives at the apical cortex? It is likely that Par3 functions as a chaperone to prevent the inappropriate phosphorylation of substrates in the cytoplasm. The PP1 phosphatase, which is known to associate with Par3 [52], might rapidly reverse any premature phosphorylation by aPKC to prevent cytoplasmic release. Loss of Par3, or expression of a mutant that cannot bind aPKC, results in the mislocalization of aPKC and in the inappropriate activation of signaling pathways that promote tumor invasion and metastasis [53].

### Feed-forward loops

Crb is a key component of the EPP. Overexpression of Crb increases the size of the apical domain, and the apical domain is frequently lost or reduced in epithelial cells lacking Crb [54]. Although the exact mechanism of how Crb functions to promote apical identity remains unclear, it has been shown that the recruitment of other EPP proteins such as Pals1, Par6 and aPKC plays a part[55](Figure 2a). However, another potential pathway, in which Crb inhibits a Rac1-PI3K feedforward loop, has been proposed56. In *Drosophila* embryos, PI3K signaling is required for Rac activation, which in turn stimulates PI3K signaling. Crb at the apical surface might contribute to the attenuation of this feedback loop by inhibiting Rac activation, thereby reducing $PIP_3$ production. However, the underlying molecular mechanisms remain unknown. Another mechanism for the establishment of apical identity involves the control of phosphoinositide production. In many epithelial cells, $PIP_3$ is constitutively produced at basolateral membranes but is absent from the apical surface [33]. One reason for this asymmetric distribution is that the phosphoinositide kinase PI-3K associates with the E-cadherin–β-catenin complex at adherens junctions [57]. E-cadherin is in turn recruited by $PIP_3$, resulting in a feed-forward loop that amplifies and stabilizes $PIP_3$ formation by PI3K.

## Execution of the EPP

The epithelial phenotype is established very early during embryogenesis. Mammalian embryogenesis starts by zygotic cell divisions that generate a blastula epithelium through cavitation, compaction and fluid transport after the 8–16 cell stage [58] (Figure 3a). During embryogenesis, cells at the primitive streak in the ectoderm lose their epithelial phenotype and differentiate into mesenchymal cells, (epithelial–mesechymal transition ((EMT)). These cells are internalized and form the mesoderm. They regain epithelial character (mesenchymal–epithelial transition (MET)) at their destination, for example during kidney formation[959] (Figure 3B). Insect zygotes (for example, *D. melanogaster*) undergo multiple nuclear divisions that generate ~6000 nuclei, which are then compartmentalized into epithelial cells by expansion and invaginations of the plasma membrane [60] (Figure 3C).

### Orientation of the apicobasal axis

The establishment and orientation of the polarity axis is a key event during the acquisition of the epithelial phenotype, for example during developmental MET (Figure 3Bb (left)). Important regulators of MET are external cues that are provided by neighboring cells, which generate cell–cell contacts and intercellular junctions. In spite of its developmental importance, our understanding of the steps involved in the generation of the epithelial axis is based almost exclusively on studies carried out in cultured epithelial cells, for example in MDCK cells in 3D culture (figure 3B). In suspension culture, MDCK cells form cysts with the apical surface facing out; exposure of thee cysts to collagen gels results in re-orientation of polarity with the lumen facing in[6162]. Polarity reorientation depends on a pathway in which collagen activation of β1 integrins activates Racl, which promotes the assembly of a basement membrane via laminin secretion [6364]. A β1 integrin antibody or dominant negative Racl blocks polarity reorientation, which requires the activation of RhoA and its downstream effectors Rockl and Myosin2 [65]. External cues act together with EPP polarity

proteins, lipids and Rho GTPases (Cdc42, Racl and RhoA) to generate junctions and to organize the molecular trafficking necessary to form apical and basal domains (Fig 3Bb(left)). Single MDCK cells suspended in matrigel orient their lumen centrally as they divide; studies using this model have shown that lumen formation involves the transcytosis of apical proteins at the end of mitosis from the plasma membrane to the midbody, where the lumen will form [6617](figure 4a). A similar type of transcytotic process occurs in vivo in the mouse mammary gland [67]. Microtubules that are tethered to β1 integrin at the basal cortex deliver apical proteins from the basal membrane to the apical membrane (Figure 3Bb (left)). Deletion of β1 integrin results in a disruption of microtubule polarization, which in turn disrupts cell polarity and lumen formation. Integrin-linked kinase (ILK) mediates the integrin-dependent attachment of microtubule plus ends to the basal cortex. Whether a similar mechanism occurs in other epithelial cell types remains to be established.

Conversely, the conversion of the epithelial polarity axis into a polarized migration axis is a key event during EMT (Figure 3Bb (right)). EMT is promoted by transcriptional events that often involve TGFβ receptors and culminate with the loss of E-cadherin [968]. As a result, epithelial cells transition into mesenchymal cells, which move along cytokine gradients; their polarized motility is regulated by many polarity proteins and lipids. Key events in EMT are the asymmetric activation of the Rho GTPases Cdc42 and Rac1 at the front edge and RhoA at the back end[266970]. Cdc42acts via a downstream pathway involving Par3-Par6-aPKC, Gsk3β and APC, to control the rearward movement of the nucleus and the central position of the centrosome and via Rac1 to promote lamellipodium formation in the direction of cell migration, whereas RhoA promotes contraction and detachment of the rear end of the cell. [71, 72].

In most epithelial cells, the apical domain is formed at the opposite end of the basal domain. However, in hepatocytes the apical domain is constituted by bile canaliculi, interrupts the lateral cortex of neighboring cells. This remarkable morphogenetic variation of epithelial polarity is controlled by the EPP through the Par1 protein kinase. High Par1 activity induces a hepatic-like localization of apical surfaces in MDCK cells[73], for instance, whereas depletion of Par1 in hepatocytic HepG2 cells induces a columnar type of epithelial polarity[74]. Whether similar mechanisms control apical domain location in vivo remains, however, to be established.

## Assembly of the apical junctional complex

Two important questions in epithelial biology are, first, how the EPP instructs the assembly of intercellular junctions and second, whether such junctions are required for apical–basal polarization. Vertebrates assemble tight junctions, adherens junctions and desmosomal junctions, each of which contains multiple adhesive proteins. E-cadherin is a key molecule in junction formation. The expression of E-cadherin in vertebrate zygotes causes compaction and marks the moment of establishment of the epithelial phenotype [58, 75] (Figure 3a). Albeit different in some details, there are surprising parallels between junction formation in vertebrate and invertebrates. Studies in MDCK cells have shown that adherens junction assembly starts with the formation of E-cadherin puncta at protrusive cell-cell contacts that are later reorganized into belt junctions by the actin cytoskeleton [767778]. During

cellularization of the *Drosophila* embryo, the plasma membrane invaginates via furrows to compartmentalize the nuclei into epithelial cells [60] (Figure 3c); adherens junctions are not required for this process. The initial events in junction formation involve the assembly of cadherin–catenin clusters on the apical surface that are then invaginated deep in the furrows. As the surface further invaginates to form individual cells, Par3 accumulates at apicolateral junctions and recruits the cadherin clusters, which grow into spot adherens junctions[79]. These spots are then reorganized into adherens junction belts around the cells by the actin cytoskeleton, with septate junctions located more basally.

Cdc42 has a key role in the assembly of adherens junctions via Par3–Par6–aPKC (Figure 3Bb (left)). Disrupting Cdc42 expression has context-dependent effects, which reflects the multiple functions of the GTPase in actin dynamics and vesicle transport. In epithelial cells of the *Drosophila* embryo, the loss of Cdc42 primarily blocks apical recycling, but a downstream consequence is a loss of adherens junction integrity, which is consistent with a role for apical recycling endosomes in E-cadherin basolateral sorting and delivery [80]. In the developing fly, however, loss of Cdc42 can cause a defect in E-cadherin endocytosis through disruption of actin dynamics [81, 82]. Interestingly, conditional knockout mice that lack Cdc42 in the skin, exhibit defects in aPKC-dependent phosphorylation of GSK3β, which results in enhanced degradation of β-catenin and the consequent loss of adherens junctions [83]. Whether these diverse effects can be reconciled into a general model for the regulation of E-cadherin trafficking remains to be seen. The situation is particularly complicated because multiple components of the EPP affect E-cadherin stability and localization. The polarity protein Scribble colocalizes with E-cadherin and stabilizes its association with p120-catenin, which is important in maintaining its cortical localization [84]. In addition, Scribble ensures that internalized E-cadherin is targeted to the lysosomes for degradation. In the absence of Scribble, E-cadherin is instead diverted to the retrograde pathway and accumulates in the Golgi [56].

The vertebrate EPP also plays an important part in tight junction assembly and maintenance (Figure 3B (left)). The EPP first directs the formation of immature junctions, which are later segregated into separate tight junctions, located more apically, and adherens junctions, located more basally[85] (Figure 3Bb (left)). In *C. elegans*, adherens and tight junctions remain together in a ‘combined junction’with both adhesion and sealing properties whereas in *Drosophila* septate junctions form basally to the adherens junctions (Figure 1a). In addition to their sealing functions, tight junctions form barriers that separate the apical and basolateral domains of the plasma membrane, and thus contribute to epithelial polarization (the ability of septate junctions to segregate apical and basolateral domains has not been demonstrated yet, though). However, Par proteins and Scribble polarity complex have minor effects on tight junction. For example, siRNA-mediated knockdown of either Par3 or Scrib delays tight junction assembly but, ultimately, normal junctions form that are indistinguishable from wild-type junctions [84, 86]. The key polarity proteins in this process are Crb and Pals1 (Figure 3Bb (left)). Loss of Crb3 or Pals1 causes persistent defects in tight junction assembly and maintenance [87, 88]; junctions begin to form but remain incomplete and lack barrier function. Interestingly, genetic evidence from studies in *Drosophila* supports a hierarchical mechanism in which Par3 is required for the recruitment or stabilization of Crb and Pals1 at the apical surface. However, this is clearly not the case in mammals, in

which Pals1 (and probably Crb3) seems to localize correctly to the apical cortex and remain functional in the absence of Par3. The underlying mechanism through which these polarity proteins stabilize tight junctions remains obscure. Tight junction transmembrane proteins such as occludin and possibly also claudins are delivered by the basolateral sorting machinery [89], whereas Crb is likely transported by the apical machinery [17, 90], and therefore a direct control of tight junction assembly by Crb is unlikely. However, it is feasible that Crb and Pals1 stabilize newly delivered tight junction proteins, although the underlying mechanism remains to be determined.

Are junctions necessary for apical-basal polarization? During early mammalian embryonic development, the inhibition of E-cadherin activity with antibodies prevents the formation of epithelial tissue[75], and several cell culture studies suggest that cadherin-based intercellular adhesion provides an initial cue required for cell polarization[9192]. Moreover, E-cadherin contains a juxtamembrane domain that confers retention at the lateral membrane and transcytosis of apically mis-sorted protein to the lateral membrane, and disruption of this domain causes a complete loss of polarity [93]. However, single cells such as spermatozoids polarize in the absence of such cues, and as mentioned above, adherens junctions are dispensable for the initial polarization of *Drosophila* embryos during cellularization. Cells lacking tight junctions can also polarize, and it has been demonstrated that the activation of the Par4 (LKB1 in mammals) kinase can induce the spontaneous polarization of individual intestinal epithelial cells in the absence of contact with any other cells [94]. Thus, the weight of evidence suggests that junctions are dispensable for apical-basal polarity, although they likely stabilize and enhance the polarized state.

## EPP-induced cytoskeletal and organelle rearrangements

The EPP controls the dramatic reorganization of the cytoskeleton and organelles that characterizes the acquisition of the epithelial phenotype, for example during MET (Figure 3Bb (left)). Changes in the localization and function of the centriole is a result and also a key determinant of the changes in microtubule organization [95]. In polarized epithelial cells, the centriole is recruited to the apical surface, where the mother centriole nucleates the nine microtubule pairs that form the primary cilium. In epithelial cells microtubules are mostly non-centrosomal and more stable than in non polarized cells [96]. Some microtubules nucleated by the basal body or by intermediate filaments under the apical surface [97] may be released and captured by the developing apical junctional complex, which in turn they contribute to develop [98]. It has been shown that E-cadherin expression has microtubule-stabilizing properties, consistent with the higher stability of epithelial microtubules [99]. The kinesin KIF17, EB1 and APC stabilize MT at the basal plus ends which contributes to establishing the height of epithelial cells [100101] . Taken together, all of this accounts for the characteristic apical–basal orientation of cortical microtubules, with basally oriented plus ends [102, 103]. A second population of centrally located microtubules radiates apically from non-centrosomal supranuclear nucleation sites [104105], mainly the Golgi apparatus [106, 107]. The epithelial microtubule organization mediates the typical supranuclear localization of the Golgi apparatus, common recycling endosomes (CREs) and apical recycling endosomes (ARE) and the peripheral localization of apical and basolateral sorting endosomes, albeit the details of these localization mechanisms are poorly understood[18, 108]. The Ser/Thr kinase

Par1b is a major regulator of microtubule organization in fly epithelia and mammalian epithelial cells [18, 73, 109], as well as in *D. melanogaster* eggs [110]. Inhibition of Par1b function in MDCK cells by the expression of a dominant negative Par1b mutant caused the microtubule cytoskeleton to remain centrosomal, similar to their organizationin nonpolarized or migrating cells even after the cells contacted neighboring cells [73]. However, the details of how Par1B controls centriolar function and microtubule organization remain unknown.

Acquisition of apical–basal polarity also entails a dramatic reorganization of the actin cytoskeleton, which is controlled by an epithelial polarity sub-programme. Actin organization differs at apical, lateral and basal membranes. CDC42, recruited to the apical plasma membrane by $PIP_2$ and the GEF Tuba1, promotes actin polymerization, but the formation of microvilli requires the participation of several FERM domain proteins such as ezrin in mammalian epithelia and moesin in flies [111]. The polarity protein and kinase Par4 / LKB1 induces the formation of microvilli through Mst4-dependent phosphorylation of Ezrin [112]. This polarization depends on actin but not on microtubules [113]. However, this sub-programme is independent of other Par proteins, and in many epithelial cells, Mst4 is not the predominant Ezrin kinase [114]. Other kinases, including LOK and SLK, can also phosphorylate and regulate Ezrin and maintain its localization at the apical cortex [114]; therefore the primary mechanism that controls microvillus formation remains uncertain.

The organization of actin at the lateral membrane is determined to a large extent by E-cadherin and associated catenins [77, 115]. Conversely, the formation and maintenance of adherens junctions and tight junctions is tightly intertwined with actin dynamics [115]. Actin organization is also characteristic in the perinuclear area, where it is in part regulated by CDC42 and plays important parts in regulating vesicular trafficking from the *trans*-Golgi network (TGN) and perinuclear endosomes [116117118, 119].

## Organization of the polarized trafficking machinery

As mentioned earlier, a major goal of the EPP is to organize the epithelial vesicle trafficking machinery to achieve a polarized apical–basal distribution of plasma membrane proteins that perform vectorial transport functions. Mammalian epithelial cells express over 2000 solute transporters, pumps, and nutrient and signalling receptors (encoded by ~5% of the genome). These proteins are expressed in a tissue-specific manner with characteristic apical–basal polarity, which may vary in different organs dependent in specific local needs [120]. The polarity of a given plasma membrane protein arises from its intracellular sorting at the TGN and endosomal compartments during transport along biosynthetic and recycling routes[18] (Box 1).

## Sorting signals and decoding mechanisms

Plasma membrane sorting through biosynthetic and recycling routes is mediated by apical and basolateral sorting signals and mechanisms. Apical sorting signals include N-glycans and O-glycans [121, 122] in the ectodomain, specialized transmembrane domains such as that of influenza hemagglutinin (HA) [123], the glycosyl phosphatidyl-inositol (GPI) anchors [124, 125] and determinants in the cytoplasmic domain, such as those found in

rhodopsin [126-128] and Megalin [129130]. It has been proposed that glycans mediate apical sorting through interaction with specific lectins, for example galectins 3, 4 and 9 [108131, 132]. Galectins are secreted into the apical medium via non-conventional secretion mechanisms (i.e. directly through the plasma membrane) and then endocytosed into sorting and recycling endosomes, reaching even the TGN, where they carry out their sorting function. Transmembrane segments and membrane anchors are thought to mediate sorting in the TGN or endosomal compartments through their affinity for specialized membrane lipid domains or rafts [36, 108, 133]. Lipid rafts, formed by cholesterol and glycosphingolipids, must undergo a clustering process to become functional sorting platforms [134-136]; interaction with cytoskeletal components on the cytosolic side necessary for trafficking might be mediated by transmembrane proteins such as MAL1/VIP17 [133, 137].

Basolateral sorting signals, by contrast, are simple peptide motifs in the cytoplasmic domain of a protein. Some resemble typical endocytic signals (for example, tyrosine (YXXO, NPXY) and dileucine (LL/LI) motifs) [138, 139], which reflects the fact that basolateral sorting is largely a clathrin-mediated process [140], similarly to clathrin-mediated endocytosis (CME). Basolateral sorting is mediated by the clathrin adaptor AP-1, which is present in two forms, AP-1A and AP-1B. They share three of the four subunits (β1,γ,σ1) and differ only in the medium subunit (μ1A or μ1B, respectively). Tyrosine basolateral sorting signals interact with pockets in the μ1A or/and μ1B of AP-1A and AP-1B [141] similar to pockets defined by crystallography studies in the μ2 subunit of AP-2. Whereas AP-1A is ubiquitous, AP-1B is expressed only by epithelial cells [142, 143]. Exactly how AP-1A and AP-1B carry out their basolateral sorting function remains controversial [144145]. It was recently suggested that both adaptors localize to the TGN and CRE, where they perform similar sorting functions, differing only in their affinity for basolateral cargo proteins [146]. However, functional assays suggest that whereas AP-1B sorts basolateral PM proteins in both biosynthetic and recycling routes[138147-149], AP-1A functions preferentially in the biosynthetic route [141, 150]. Some epithelial cells lack AP-1B (for example, the liver, retinal pigment epithelium and proximal tubule[151-153]) and therefore rely only on AP-1A (and other still unknown mechanisms) for basolateral protein sorting, as has been shown for neurons [154]. Cathrin is also involved in basolateral sorting of proteins that do not contain canonical endocytic sorting signal, for example TfR (with GDNS as a basolateral sorting motif) [155] and CD147 (with a sorting motif based on a single leucine)[156]. This suggests that among the ~20 twenty known clathrin adaptors [145], adaptors other than AP-1 may also function in polarized trafficking. Indeed, there is some suggestive evidence that AP-3, AP-4 and ARH may participate in basolateral sorting [138, 157-159]. In addition, non clathrin mechanisms may be involved in basolateral sorting, an example is Naked 2, which binds to motifs in the cytoplasmic domain of transforming growth factor alpha (TGFa) to mediate its basolateral trafficking [160]. To date, the mechanism mediating trafficking of Na,K-ATPase, a key epithelial transporter sorted to the basolateral membrane by clathrin independent machinery [140], remains unknown. Also unknown are the mechanisms that determine its apical localization in neuroepithelia such as RPE and choroid plexus.

## Vesicular trafficking to apical and basolateral domains

Apical and basolateral protein sorting in the biosynthetic, recycling and transcytotic routes is coordinated with the production and fission of different types of post-Golgi apical and basolateral carrier vesicles. Vesicular fission from the sorting compartments seems to be mediated by different mechanisms: dynamin 2 is involved in apical routes [119], and protein kinase D [161] and BARS have a role in basolateral routes [162]. Recent work shows that dynamin 2 mediates fission of apical transport vesicles at the level of apical recycling endosomes [163]. Vesicle transport through the epithelial cytoplasm is facilitated by microtubule and actin motors 18. Although it was initially suggested that microtubule motors participate in both apical and basolateral transport [164], most of the evidence since then has implicated them in apical trafficking. For example dynein and several kinesins (including , KIFC3 [165], KIF5B [104], KIF1A [166] and KIF16B [105]) participate in several different apical transport routes. Interestingly the same protein, p75 neurotrophin receptor, is transported by KIF1A or KIF1Bβ in non polarized MDCK cells [166] and by a different kinesin, KIF5B, in polarized MDCK cells [104]. Myosin 2 and myosin 6 mediate basolateral transport from the TGN [167, 168], whereas myosin 5 mediates apical transport from ARE to the plasma membrane [169]. Rab11a positive ARE are emerging as an important additional sorting compartment (in addition to TGN and CRE) in polarized epithelial cells. Although the ARE has been reported to be a ‘station’ in apical traffic, a surprising finding is that some basolateral proteins, for example E-cadherin, may traffic through the ARE on their way to the plasma membrane [80]. Mutations in myosin 5, a resident of ARE, are a cause of microvillus inclusion disease, a lethal congenital condition that is characterized by the failure to form a mature apical membrane and the appearance of large intracellular vacuoles containing microvilli, mainly in enterocytes[170].

Vesicle tethering to both apical and basolateral plasma membrane domains has been shown to depend on different subunits of the exocyst complex [171, 172]. Exocyst accumulates at the level of the junctional complex [171], which is also the preferential site of fusion of basolateral transport vesicles [173]. Vesicle fusion to the apical membrane is mediated by different t-SNARES, syntaxins 1, 2 and 3, which is compatible with the existence of various apical transport routes and by the V-SNARE TiVAMP [17417]. Fusion with the basolateral membrane is mediated by the t-SNARE syntaxin 4 [174, 175] and the v-SNARE Cellubrevin or Vamp3 [17]. Disruption of the expression or polarity of these SNAREs [173-175] results in depolarization of plasma membrane proteins, congruent with the idea that SNARES are major constituents of the core polarity machinery of the cell.

About a quarter of the ~60 members of the rab family [176177] have been implicated in polarized post-Golgi trafficking operating at the TGN or various endosomal compartments (Table 1). A recent study demonstrated that Rab5 is a master regulator of endosome biogenesis in the liver [178]. Knockdown of Rab5 in adult mouse liver markedly reduced early endosomes, late endosomes and lysosomes and also reduced LDL endocytosis and transcytotic delivery of apical proteins to the bile canaliculi but did not affect rab11 positive recycling endosomes or delivery of apical proteins that utilize a direct route from the Golgi. These findings suggest that recycling endosomes are maintained by trafficking from compartments other than early endosomes, for example the Golgi apparatus.

## EPP proteins and the polarized trafficking machinery

Although previously evidence for any obvious links between EPP proteins and the vesicle trafficking machinery was lacking, there is now substantial support for close integration between them. A screen in *C. elegans* for genes involved in membrane trafficking revealed a general requirement for EPP proteins in endocytosis [179]. Mutants in EPP proteins caused reduced clathrin-mediated cargo uptake and reduced recycling of clathrin-independent cargo. A recent screen in *C. elegans* for genes that control the subapical localization of ARE around the basal body identified Par5, but the downstream mechanisms involved remain unknown[180]. Studying this mechanism in vertebrate cells is difficult, as they express five different Par5 isoforms.

In *Drosophila* embryos, the disruption of Cdc42 causes accumulation of apical proteins in sorting endosomes, probably due to effects on aPKC localization and/or activity [181]. In other tissues, however, Cdc42 controls endocytosis of adherens junctions at lateral membranes through a Par-independent pathway that probably involves actin organization. The situation in mammalian cells is less clear, but Cdc42 is enriched at the Golgi, where it binds to the COP1 vesicle coat protein, coatomer, and has been implicated in basolateral protein sorting[116-118]. Interestingly, the Par3 polarity protein can bind to the exocyst and might also have a role in polarized secretion [182]. However, the molecular mechanisms underlying these regulatory functions are still unclear.

Epithelial syntaxins have been reported to bind to the polarity protein Lgl, and are essential for polarization in *Drosophila* and in MDCK cells in vitro [174]. Additionally, the synaptotagmin-like proteins (Slp) 2-a and 4-a, which control exocytosis, are needed for correct epithelial organization in 3D MDCK cyst cultures 183. Finally, rab11a/exocyst dependent apical transport pathways were found to reinforce the localization of the apical Par complex[66].

Crumbs sorting, localization and recycling. Crumbs is a key organizer of apical polarity and tight junctions and also a transmembrane protein, unlike most polarity proteins. An important question, therefore, is how this transmembrane protein is localized either to the apical surface and tight junctions in mammals, or the subapical domain in *Drosophila*. The trafficking and localization of Crumbs and other polarity proteins has been studied during development of MDCK monolayers in 2D and 3D cultures [66, 184] (Figure 4a-c). When single MDCK cells are suspended in 3D basement membrane cultures that contain laminin, apical and basolateral membrane proteins are distributed over the entire plasma membrane. However, upon entry into mitosis, Crumbs, podocalyxin and other apical proteins are specifically internalized and concentrated in Rab11-positive recycling endosomes, which accumulate at the site of cytokinesis in the region of the midzone microtubules [66]. The delivery of Crumbs and other apical proteins to the plasma membrane at this site triggers the formation of a lumen between the two daughter cells [55]. Podocalyxin is an anti-adhesive, mammalian transmembrane protein that is delivered very early, perhaps simultaneously with Crb, to the presumptive apical surface, and is required for the generation of lumina. Apical transmembrane proteins are delivered by vesicles that traffic along microtubules, and syntaxin 3 is required for apical fusion. Other vesicle transport proteins, including Rab8 and the exocyst, also seem to be required for apical protein delivery [185]. However, it is still

unclear what signals target Crb to the apical surface, because the intracellular domains that are known to bind other EPP proteins are dispensable for its localization.

Once at the apical plasma membrane, Crb must be stabilized to reduce the rate of endocytosis. One proposed mechanism in *Drosophila* involves a positive feedback loop through extracellular domain Crb-Crb interactions and the recruitment of the ERM (Ezrin, Radixin, Moesin) domain protein Expanded (in *Drosophila*) and its binding partner, Kibra [186] (Figure 4d). The basolateral protein Lgl inhibits this stabilization, enabling mis-targeted Crb to be internalized and recycled to the apical membrane. Recycling seems to require the retromer complex, which suggests that Crb needs to be transported back through the TGN[187]. However, the reason for this is not yet understood. Moreover, Expanded and Kibra are components of the Hippo signaling pathway that controls organ size and responds to cell density, and Crb may function, at least in *Drosophila* imaginal disks, to suppress Hippo signaling and prevent tissue overgrowth [188].

## Trafficking to the primary cilium

The primary cilium, which forms at the apical surface of vertebrate epithelial cells, represents a third distinct region of the plasma membrane that is constructed by a process called intraflagellar transport (IFT) [189] (Figure 5a). As cilia lack the protein synthesis machinery, all components for its assembly and function must be transported into and within the cilium. The cilium originates from the basal body, which is generated in post-mitotic cells from a centrosome, and consists of a microtubule-based axoneme covered by a specialized membrane. Cilia components are transported by microtubule motor proteins (Figure 5a). Just as the apical and basolateral membranes are separated by tight junctions, the cilia membrane and apical membrane are separated by diffusion barriers that maintain the cilium as a distinct signaling organelle[190]. A spiral array of fibers that connects the basal body to the ciliary membrane blocks vesicle access to the cilium, and nuclear pore components are also found in this region, perhaps functioning as a diffusion barrier to the entry and exit of soluble proteins (Figure 5a) [191].

The gating of entry and exit is important, because key signaling systems – particularly the Hedgehog (HH) pathway – are segregated in and are dependent on the primary cilium [192]. The HH pathway plays a key part in the differentiation of epithelial cells [192]. Recent work has demonstrated that endothelial cells have key instructive roles in the differentiation of the epithelia in various organs, for example liver, lung, skin and retinal pigment epithelium, through the HH, Wnt and Notch pathways[193, 194195, 196]. In order to exert its function HH, secreted by endothelial cells, must reach its transmembrane receptor, Patched (Ptc), which is localized at the primary cilium membrane. Thus, HH must be transcytosed across the epithelial cell to reach its site of function (Figure 5b); however the mechanism involved remains elusive.

Multiple components of the EPP participate in both the formation and function of the primary cilium. For example, aPKC is required for ciliogenesis [197, 198] but has also been implicated in regulating the function of Gli (a mediator of the HH response) through phosphorylation [199]. Conversely, Gli induces aPKC expression. However, a note of caution is necessary because many of these studies use an aPKC inhibitor– a myristoylated

pseudosubstrate peptide – that has recently been demonstrated to be nonspecific and to induce effects in the brain that are not mimicked by knockout of aPKCζ. Nonetheless, other components of the EPP, including Par6, Par3, and Crb3, are all concentrated in primary cilia and are required for proper cilia formation [104, 198]. Par6, together with the polarity proteins Pals1 and Patj, associate with nephrocystin-1 and -4, which are both important for cilia function [200]. However, the functional consequences of this interaction are not yet understood. The Par proteins also associate with kinesins that are involved in IFT [198]. Finally, the exocyst protein Sec10 is required for ciliogenesis, perhaps through a role in the localization of the cation channel protein polycystin-2 [182, 201]. As Par3 can bind to the exocyst, it seems likely that multiple components of the EPP machinery are involved, in controlling membrane protein sorting and delivery to the primary cilia, although the exact mechanisms are not yet fully understood. One important question for the future is whether the EPP machinery regulates HH signaling by affecting other proteins involved in the pathway, such as Ptc or Smo delivery, in addition to its effects on Gli phosphorylation.

## Outlook

The EPP integrates numerous processes and touches on almost every aspect of cell biology. Many of the mechanistic details of this integration remain to be identified. One complication is that the execution of the EPP may vary markedly in different locations or physiological contexts, often using the same components but in cell-type specific ways. For example, in *Drosophila*, Crb is only essential for apical specification during morphogenesis when adherens junctions are rapidly expanding or turning over 15. Moreover, basolateral polarity proteins such as Lgl are not essential for the maintenance of polarity in late-stage embryogenesis, but are required during gastrulation. As an example from mammalian cells, the initial landmark for the apical domain in single cells grown in 3D culture is the site of abscission during cytokinesis, but this is unlikely to be true during development, when single cells are probably not isolated from each other, and neighboring cells will provide spatial information through cadherin-based adhesion. An important future goal, therefore, will be to understand how the EPP operates in specific, biologically relevant contexts.

It will also be central to gain better temporal and spatial resolution of the initial stages of epithelial polarization. We do not know which proteins first arrive at the presumptive membrane domains, or at the tight junctions that form between the apical and lateral domains. We also need to learn more about the interconnected signaling between sensors, such as the primary cilium, integrins and cadherins, and the EPP. Our knowledge of the links between the effectors of the EPP, particularly the vesicle trafficking machinery and the polarity proteins, is also still very superficial. A comprehensive understanding of these links will surely inform our knowledge of human disease, which so often involves epithelial cells.

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## Box 1. Biosynthetic and recycling routes of epithelial cells

The asymmetric plasma membrane protein distribution of epithelial cells results from various sorting events in the post-endocytic and biosynthetic trafficking routes.

***Recycling and transcytotic routes.*** Fast recycling receptors, such as apical Megalin (which recovers filtrated proteins from the lumen of the kidney proximal tubule) and basolateral low density lipoprotein receptor (LDLR) and transferrin receptor (TfR) (which provide the cell with blood nutrients) are internalized, respectively, into apical sorting endosomes (ASE) and basal sorting endosomes (BSE) and recycled back to the plasma membrane along fast recycling routes (see the figure, 1, 3) or sorted and recycled along slow recycling routes (2, 4) to their respective cell surface [202-204]. The slow apical recycling route (2) has an additional endosomal compartment, the apical recycling endosome (ARE) [203, 204]. The polymeric IgA receptor follows a transcytotic route through the BSE, CRE and ARE (5) The transcytotic route of TfR in MDCK cells lacking the clathrin adaptor AP-1B transits through the same compartments (5), but may also traffic through the ASE (6). Proteins destined for degradation after internalization from the apical or basolateral surface reach lysosomes from the ASE or BSE, respectively (routes 7 and 8)[205]. Fusion of multivesicular bodies (MVB) with the apical or basolateral surface apparently releases different apical and basal exosomes (routes 9 and 10), although the exosome sorting mechanisms remain unknown [206]. Solid lines represent apical and baslateral recycling routes, dashed lines represent transcytotic routes and dotted lines represent route to and from MVB.

***b. Biosynthetic routes.*** Apical and basolateral PM proteins are synthesized at the endoplasmic reticulum (ER), transferred to the Golgi apparatus and sorted at the Trans Golgi Network (TGN) into direct vesicular routes to the plasma membrane (PM). (apical 11; basolateral 15). In addition, newly synthesized cargo proteins may traffic through endosomal compartments before reaching their respective surface domains [108138, 149, 207, 208209]. Apical PM proteins may utilize ARE, CRE or ASE as intermediate stations for apical transport (routes 12, 13 or 14, respectively). Basolateral PM proteins may use CRE (route 16) or basal sorting endosomes (route 17) as intermediate stations.

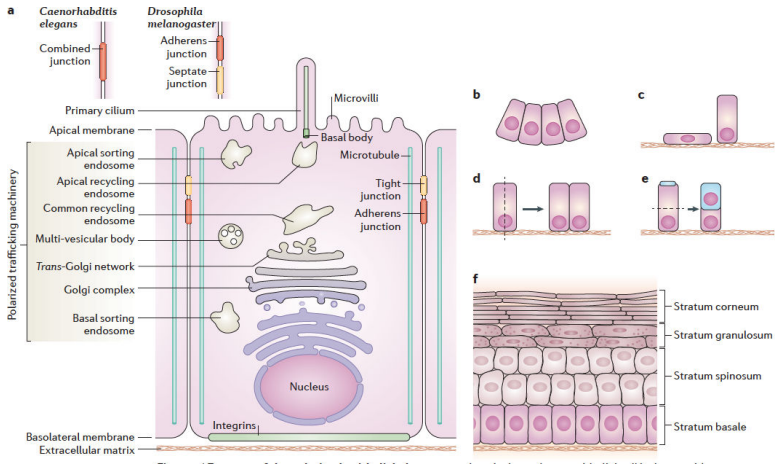


**Figure 1. Features of the polarized epithelial phenotype**

(a) A typical vertebrate epithelial cell is shown with components of the polarized vesicle sorting machinery and the apical junctional complex depicted. Note that invertebrate (for example in *Drosophila*) epithelial cells lack primary cilia and the junctional complex is organized differently with adherens junctions located more apically than the sealing junction (named *septate junction* instead of *tight junction*) (top right insert). In *C. elegans*, adherens junctions and sealing junctions are combined into a single structure (top left insert). (b - f) Epithelial cells organize into different structures through their cytoskeleton and through oriented cell division. (b) actin-mediated constriction of the apical domain causes furrowing; (c) reducing the size of the lateral membrane produces squamous epithelia; (d) cell division (indicated by the dashed line) in the plane of the epithelium expands the sheet; whereas divisions perpendicular to the plane may generate different cell lineages in the case of stem cells (e), or may give rise to stratified epithelia (f)

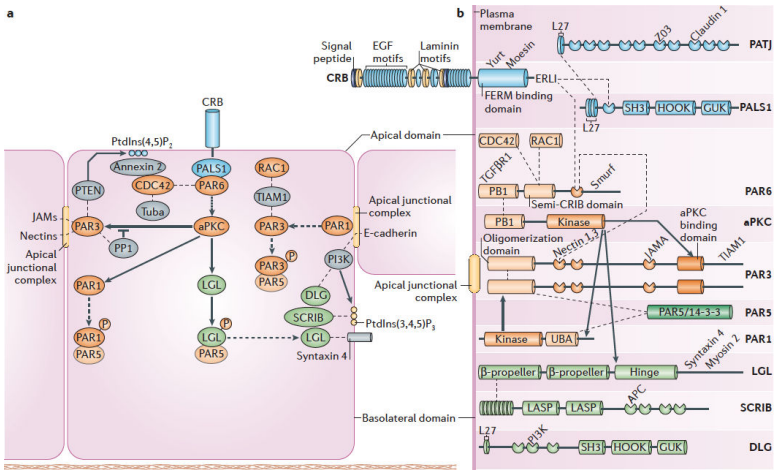


**Figure 2. The EPP players. (a) Feed-back loops between polarity proteins**

The differential localization of polarity proteins at the cell cortex is regulated through binding interactions (dashed lines) and phosphorylations (solid arrows); dashed arrows indicate change to or from a phosphorylated state. The apical polarity protein Crumbs (Crb) recruits Pals1 through its C-terminal PDZ-binding domain (ERLI) which recruits Par6 to phosphorylate Par3, the kinase Par1 and LGL and exclude them (together with other members of the Scribble complex (Dlg Scribble) from the apical domain. Members of the Scribble complex interact genetically with each other but there is no evidence of physical interaction. Conversely, Par1-mediated phosphorylation events prevent basal invasion by the apical polarity determinants, such as Par3. Phosphorylated proteins, including Par1, LGL and Par3, bind Par5 during relocation to their resident domain. Polarity lipids also help generate membrane asymmetries. Specifically, PTEN, recruited to the junctional area through interaction with Par3, generates ptdIns(4,5)P2 ($PIP_2$), which helps recruit Cdc42 via annexin 2. Cdc42 participates in the activation of aPKC via Par6. Basolateral PI3K, recruited to the junctional area through E-cadherin, recruits Dlg and generates $PIP_3$, which additionally contributes to basal membrane identity through the recruitment of Scribble. Lgl contributes to basal identity through interaction with Syntaxin 4, which promotes basolateral secretion. **(b) Modular organization of the EPP players.** EPP proteins are made up of several modular domains, which enable key interactions to occur between among EPP players. They also allow interactions to occur between EPP players and other proteins that are necessary for polarity. Solid arrows indicate phosphorylation events, dashed lines represent binding interactions.

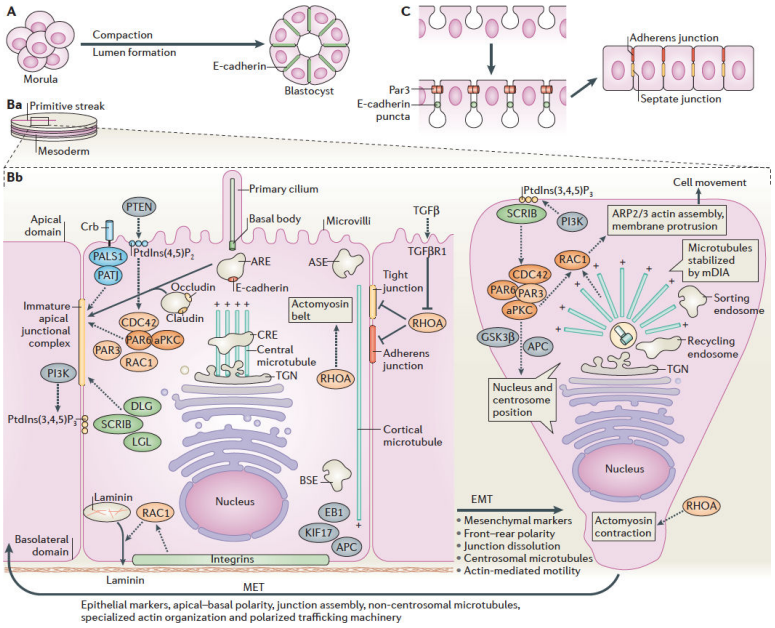


**Figure 3. Execution of the EPP**

The epithelium is the first tissue to appear during development. **(A)** In mammalian embryos, an epithelium arises when morula cells compact and form a lumen upon expression of E-cadherin, to form a blastocyst. **(Ba)** In the bilaminar embryo, epiblast epithelial cells at the primitive streak differentiate into mesenchymal cells (epithelial-mesenchymal transition (EMT)) that migrate to form the intermediate mesoderm. In turn, mesoderm cells convert into epithelial cells (mesenchymal-epithelial transition (MET)), for example during formation of the kidney. **(Bb)** During MET, epithelial cells express epithelial signature markers such as E-cadherin (left), laminin receptors (integrins), Crumb complex proteins and undergo a dramatic cytoskeletal re-organization and organelle repositioning [39, 48, 183, 210-212].The Crumbs complex, the Cdc42-Par3-Par6-aPKC complex and the Scribble complex cooperate to form an immature apical junctional complex (left plasma membrane), which matures into segregated tight and adherens junctions (right plasma membrane). Junction formation involves the delivery of E-cadherin from apical recycling endosomes to form spot adherens junctions and the exocytosis of the tight junction components occludin and claudins by the basolateral sorting machinery to form tight junctions. Rac1 interacts with Par 3 through Tiam1 and thus contributes to the organization of the peri-junctional actin cytoskeleton required for the coalescence of spot into belt adherens junctions. RhoA and myosin2 contribute as well to the formation of an actomyosin belt that enhances cell adhesion [213, 214]. At the basal pole, Rac1 stimulates the secretion of laminin that interacts with basal integrin receptors; this contributes to the orientation of the cell along an apical–basal axis.. The relocalization of the centrosome to the apical pole, the developing junctions, the polarity protein Par1, together with APC, the kinesin KIF17 and EB1 contribute to the reorganization of microtubules, In turn this contributes to the polarized organization of endosomal compartments and the Golgi complex. EMT (right) is promoted by transcriptional events that often involve TGFβ receptors and culminate with the loss of E-cadherin resulting in the disassembly of adherens junctions [968]. The polarized movement of mesenchymal cells along cytokine gradients is regulated by many polarity

proteins and lipids that are also part of the EPP. At the front of the cell, activated CDC42 recruits Par6-aPKC, and ultimately GSK3b and APC to control the position of the nucleus and centrosome. In turn, the centrosome nucleates a centrifugal array of microtubules with peripheral plus ends that mediates: the juxta-nuclear localization of the Golgi apparatus, TGN and recycling endosomes [198, 215] and the peripheral localization of sorting endosomes via plus-end kinesins[216,217]; and the formation of an actin-based leading edge. Microtubules facing the direction of forward motion are stabilized by formins [218] and activate Rac1 (activated also by aPKC), which, in turn promotes actin polymerization and the formation of a actin-driven frontal lamellipodium through Arp 2/3 [219, 220] RhoA is activated at the back end to control the generation of contractile forces through regulation of actomyosin filament assembly and contraction [26] (C) Blastoderm cellularization in *Drosophila melanogaster.* This process illustrates an alternative strategy to generate an epithelial cell. Embryonic development in flies begins with a rapid series of nuclear divisions without cytokinesis that originate a syncytial embryo with the nuclei present at the periphery. Nuclei are segregated from other nuclei by a compartmentalization process that involves the formation of membrane cleavage furrows between cells. E-cadherin, found initially in puncta at apical surface is displaced to the tip of the growing cleavage furrows and is progressively recruited more apically to form belt-like adherens junctions in a process promoted by Par3 and the actin cytoskeleton. Septate junctions form more basally stimulated by the Scribble complex.

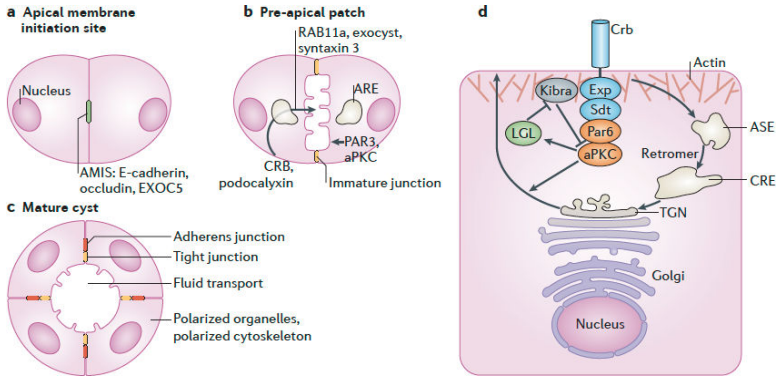


**Figure 4. Trafficking of EPP players during polarization of epithelial cells**

**a. Polarization of MDCK cells in 3D cultures.** The steps that lead to the establishment of apical–basal polarity have been best characterized in MDCK cell cysts, generated by cell division from individual cells in collagen-rich matrigel gels. (a) Polarization starts at the two-cell stage with the accumulation of E-cadherin, occludin and exocyst components (such as Sec10)at the site of cell–cell contact called apical membrane initiation site (AMIS). (b) The AMIS progresses into a pre-apical patch through a series of trafficking events that include transcytosis of Crumbs and podocalyxin from the periphery to apical recycling endosomes and their vesicular delivery to AMIS mediated by Rab11a, exocyst (Sec10) and the t-SNARE syntaxin 3. Exocytosis also functions in the initial recruitment of Par3 and aPKC (to the pre-apical patch, where they contribute to form an immature junctional complex. (c) The mature cyst exhibits segregated tight junctions and adherens junctions, a fully developed lumen expanded through polarized fluid transport and polarized cytoskeleton and organelles. **(d) Control of Crumbs recycling.** The localization of Crumbs is maintained through endocytosis and recycling. Crumbs interacts with a FERM domain protein, Expanded (Exp), and the Pals1–Par6–aPKC complex. The FERM domain protein links Crb to the cortical actin cytoskeleton. A Hippo pathway protein, Kibra, can bind to Exp and inhibits aPKC activity. Kibra is in turn inhibited by a lateral polarity protein, Lgl. Lgl is phosphorylated by aPKC, which causes to dissociate from the membrane. Endocytosis of Crb is followed by retromer-mediated retrograde transport to the trans-Golgi network (TGN), from where it can be recycled back to the apical cortex.

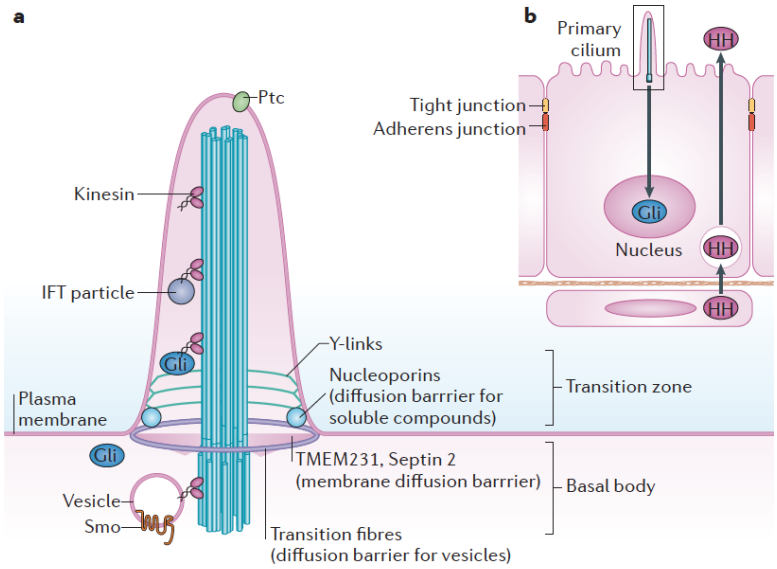


**Figure 5. The primary cilium and Hedgehog signaling**

a. Primary cilium structure and signaling components. Multiple types of diffusion barrier separate the ciliary membrane and apical membrane to control the access to the primary cilium, creating a distinct polarized membrane domain Transition fibers that connect the basal body to the ciliary membrane blocks vesicle access to the cilium, and nuclear pore components are also found in this region and have been implicated to function as a diffusion barrier, perhaps functioning as a diffusion barrier to the entry and exit of soluble proteins. Patched (Ptc) is inactive in the primary cilium, but moves out of this organelle upon binding of Hedgehog (HH) (not shown). Other components of the HH signalling cascade, Smoothened (Smo), which is located on intracellular vesicles, and Gli, also move in and out of the cilium in a dynamic manner, and is recruited to the tip of the cilium by a kinesin. Activation of Ptc triggers the translocation of Smo to the ciliary membrane, where it activates Gli. After a series of post-translational modifications, GLi moves to the nucleus to activate transcription of target genes. ***b. Transcytosis of HH.*** HH is produced by endothelial or mesenchymal cells underlying epithelial cells. Upon binding of HH to its receptor Patched on the surface of the primary cilium, the transcription factor Gli is activated and transported to the nucleus to activate epithelial differentiation genes. The transcytotic pathway of HH has not yet been characterized.

## Table 1

The role of Rabs in polarized epithelial trafficking

| Rab | Trafficking role | Refs |
|---|---|---|
| Rab 4 | Recycling from apical and basal sorting endosomes to plasma membranes | 221 |
| Rab 5 | Endocytosis from plasma membranes into apical and basal sorting endosomes | 178, 222 |
| Rab 6 | Myosin 2-mediated fission of VSV G protein vesicles from the TGN in polarized and non-polarized cells | 167, 223 |
| Rab 7 | Trafficking from early to late apical and basolateral endosomes | 205 |
| Rab 8 | Transport to the basolateral plasma membranes | 224 |
| | Biosynthetic delivery of BL cargo via trans-CRE route | 225 |
| | Transport to the primary cilium in co-operation with AP-1 | 226, 227 |
| | Apical lumen formation in MDCK 3D cysts | 66, 169 |
| Rab 10 | Transport to basolateral PM | 228 |
| Rab 11 | Apical recycling and transcytosis at ARE | 105, 229, 230 |
| | Biosynthetic trafficking of rhodopsin at ARE in MDCK cells | 163 |
| | Apical lumen formation in MDCK 3D cysts | 231 |
| | Apical transcytosis of transferrin receptor in AP-1B KD cells | 105 |
| Rab 13 | Transport between the TGN and CRE | 232 |
| Rab 17 | Apical recycling and transcytosis | 233, 234 |
| Rab 25 | Apical recycling and transcytosis at ARE | 229 |
| | Apical to basal transcytosis of Fc Receptor | 235 |
| Rab 27 | Lysosomal fusion with basolateral plasma membranes, polarized exosome secretion | 236 |
| | Apical lumen formation | 183 |