Protein complexes that control renal epithelial polarity

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Pieczynski J, Margolis B. Protein complexes that control renal epithelial polarity. Am J Physiol Renal Physiol 300: F589–F601, 2011. First published January 12, 2011; doi:10.1152/ajprenal.00615.2010.—Establishment of epithelial apicobasal polarity is crucial for proper kidney development and function. In recent years, there have been important advances in our understanding of the factors that mediate the initiation of apicobasal polarization. Key among these are the polarity complexes that are evolutionarily conserved from simple organisms to humans. Three of these complexes are discussed in this review: the Crumbs complex, the Par complex, and the Scribble complex. The apical Crumbs complex consists of three proteins, Crumbs, PALS1, and PATJ, whereas the apical Par complex consists of Par-3, Par-6, and atypical protein kinase C. The lateral Scribble complex consists of Scribble, discs large, and lethal giant larvae. These complexes modulate kinase and small G protein activity such that the apical and basolateral complexes signal antagonistically, leading to the segregation of the apical and basolateral membranes. The polarity complexes also serve as scaffolds to direct and retain proteins at the apical membrane, the basolateral membrane, or the intervening tight junction. There is plasticity in apicobasal polarity, and this is best seen in the processes of epithelial-to-mesenchymal transition and the converse mesenchymal-to-epithelial transition. These transitions are important in kidney disease as well as kidney development, and modulation of the polarity complexes are critical for these transitions.

Crumbs; apical; basolateral; Scribble; Par proteins

EPITHELIAL POLARIZATION refers to the asymmetric distribution of biomolecules that allow epithelia to form organized membrane subdomains for specialized functions, including secretion, filtration, absorption, and sensory function. Apicobasal polarity is established by an intricate framework combining protein-protein interactions, control of transcription, and ion flux (119, 139). In the kidney, like many organs, polarized epithelia form tubular structures, with the apical membrane facing the lumen and the basolateral membrane formed by cell-cell (lateral) and cell-matrix interactions (basal) (Fig. 1) (123). Cell-cell interactions are mediated by transmembrane proteins such as cadherins and nectins, while cell-matrix interactions are mediated by proteins such as integrins and dystroglycans (119, 123, 152). In between the apical and basolateral membranes sits the tight junction protein (PATJ), all of which are highly conserved from invertebrates to vertebrates (Fig. 2) (139). Crumbs was first identified in Drosophila as a regulator of epithelial polarity and its mutation led to a speckled cuticle abnormality (156). There are three human homologs of the Crb gene, termed Crb1, Crb2, and Crb3. Crb1 is primarily localized to the retinal pigmented epithelium of the eye, and mutations in the gene have been linked to retinitis pigmentosa (24). Crb2 gene expression has been detected in brain, testis, uterus, eye, and embryonic tissue, but its full function remains to be elucidated. Recent work in zebrafish has pointed to an important role for a Crb2 isoform in podocytes (29). The Crb3 gene is highly expressed in the majority of mammalian epithelia and in the case of the kidney is the predominant isoform (98). Since Crb3 is highly expressed in kidney epithelia, it can be readily studied initiating this process. The complexes are the apical Crumbs and Par complexes along with the basolateral Scribble complex (Fig. 2). In general, it is the mutual antagonism of the apical vs. the basolateral complexes as mediated by protein-protein interactions and protein phosphorylation that defines the apical-basolateral boundary that forms at the tight junction. This review will focus its discussion on the composition and function of these polarity complexes.

The Crumbs Complex

Crumbs. In renal epithelia, the Crumbs complex consists of three major proteins and multiple associated proteins. The core complex consists of the proteins Crumbs (Crb), protein associated with Lin-7 1 (PALS1), and PALS1-associated tight junction protein (PATJ), all of which are highly conserved from invertebrates to vertebrates (Fig. 2) (139). Crumbs was first identified in Drosophila as a regulator of epithelial polarity and its mutation led to a speckled cuticle abnormality (156). There are three human homologs of the Crb gene, termed Crb1, Crb2, and Crb3. Crb1 is primarily localized to the retinal pigmented epithelium of the eye, and mutations in the gene have been linked to retinitis pigmentosa (24). Crb2 gene expression has been detected in brain, testis, uterus, eye, and embryonic tissue, but its full function remains to be elucidated. Recent work in zebrafish has pointed to an important role for a Crb2 isoform in podocytes (29). The Crb3 gene is highly expressed in the majority of mammalian epithelia and in the case of the kidney is the predominant isoform (98). Since Crb3 is highly expressed in kidney epithelia, it can be readily studied...
in cultured renal cells, such as Madin-Darby canine kidney (MDCK) cells.

Each member of the Crb family encodes a single span transmembrane protein with an extracellular amino terminus and conserved cytoplasmic carboxy terminus, the latter of which contains a FERM (F for 4.1 protein, E for ezrin, R for radixin, and M for moesin) binding domain and a PDZ [P for postsynaptic density-95, D for discs large, and Z for zonula occludens (ZO) 1] binding domain (Fig. 3) (98). Crb1 and Crb2 share sequence homology in their EGF repeat-rich extracellular domains, while the extracellular domain of Crb3 is markedly shorter and lacks any identifiable domain structure (98). Additionally, Crb3 exists in two variants due to alternate splicing of the final exon of the Crb3 gene. Alternate splicing results in a 22-amino acid difference in the carboxy terminus of each isoform (32), altering the sequence of the PDZ binding domain. Crb3a is termed ERLI for the carboxy-terminal glutamic acid-arginine-leucine-isoleucine (E-R-L-I in single letter code) amino acids which comprise its PDZ binding motif. Crb3b is known as the CLPI isoform, named for the four terminal cysteine-leucine-proline-isoleucine (C-L-P-I in single letter code) sequence comprising its carboxy-terminal residues.

The difference in PDZ binding domains of Crb3 has been shown to alter the function of the Crb3 isoforms. Both isoforms localize to the apical membrane and the primary cilium of polarized MDCK cells (32). However, Crb3b (CLPI) also localizes to the spindle pole of mitotic MDCK cells, and specific knockdown of Crb3b (CLPI) disrupts cell division and cytokinesis, with cells demonstrating a multinuclear phenotype (32). The Crb3a (ERLI) isoform knockdown does not impair cell division, but rather affects the polarization and the formation of tight junctions in MDCK cells (32). The knockdown phenotypes indicate that both isoforms are essential for epithelial formation and function; however, the mechanisms regulating the abundance of each splice variant are not yet determined.

The intracellular domains of Crb3a have been shown to be essential for Crb3 function in epithelial cells (34). Crb3a binds to the conserved polarity proteins, PALS1 and Par6, via its PDZ binding carboxy-terminal sequence, ERLI (Figs. 2 and 3) (57, 87). This interaction functionally links the Crumbs and Par polarity complexes and is essential for Crb3a effector function (87, 131). Insights into the binding partners for the FERM binding domain of Crb3a are just emerging. The protein Yurt was shown to bind the FERM binding domain of Drosophila Crb and function as a negative regulator of Crb (81). Studies have also indicated the mammalian orthologs of Yurt, YMO1, EHM2, and EPB41L5 have a similar function in mammalian cells and form a novel complex with Crb3 and PALS1 (43, 81, 82). Similarly, the ortholog for Yurt in zebrafish, known as mosaic eyes (moe), also codes for a FERM protein, and knockouts of this gene give rise to a phenotype similar to that of a PALS1 knockdown (known as Nagie Oko in zebrafish nomenclature) (68). Recent work in Drosophila has indicated that the Crb FERM binding domain may interact with the Expanded protein (46, 91, 130). Expanded is a FERM domain protein that has been shown to regulate the function of the growth-controlling Hippo pathway (178). Overexpression of Crb in Drosophila leads to loss of Expanded and activation of the Hippo-Warts-Yorkie pathway, leading to cell overgrowth (46, 91, 130). While the role of Crb in mammalian epithelial growth is still unclear, it has been suggested that loss of Crb3 contributes to tumor progression in a mouse model (74).

Although the function of Crb3 in growth control is uncertain, there is no doubt that modulation of Crb3 protein levels has major effects on cell polarity and tight junction formation. Overexpression of Crb3 leads to an expansion of the apical surface, an extended tight junction, and a reduced basolateral membrane (131). Knockdown of Crb3 in mammalian epithelia leads to an extensive loss of the tight junction best seen in three-dimensional culture (136, 159). Additionally, forced expression of high levels of the Crb3 gene in normally unpolarized epithelia can lead to the establishment of tight junctions, including the sequestration of tight junction structural proteins including those of the ZO, claudin, and occludin families (34).

Many groups including ours have been interested in the very early events that lead to lumen formation (123, 135). This would be highly relevant to epithelial lumen that forms when mesenchyme is converted to epithelia in early stages of kidney development. Our group and others have found that single epithelial cells grown in three-dimensional culture can develop a lumen during the first division (67, 136). These studies theorize that lumen formation is closely connected to cytokinetic mechanisms that occur in the first cell division and provide a possible mechanism for how lumens are initially formed in developing renal epithelia (135).

PALS1. PALS1, also referred to as MPP5, is a multidomain adapter protein associated with the conserved crumbs complex in mammalian epithelia (131, 133). In fully polarized epithelia, the PALS1 protein is localized to the tight junction (Fig. 2) (72). PALS1 contains at least six distinct protein interacting domains, including a pair of L27 domains (named L27N and L27C, respectively), a PDZ domain, a Src homology 3 (SH3) domain, a Hook domain, and a guanylate kinase (GUK) domain (Fig. 3) (72). The presence of a GUK domain makes PALS1 a member of the membrane-associated guanylate kinase (MAGUK) family of modular adaptor proteins (17). The GUK domain of PALS1, like other MAGUK proteins, lacks any discernable kinase activity and likely is involved in protein-protein interactions (16, 103, 154). PALS1 has important homologs in lower organisms, including Stardust in Drosophila and nagie Oko in zebrafish. Stardust functions similarly to...
mammalian PALS1 binding Crumbs (8, 51), and mutations in Stardust lead to speckled cuticle defects similar to Crumbs (155). Nagie oko and Stardust have crucial roles in retinal development through interactions with Crumbs proteins (16, 169). In addition, a role for nagie oko in zebrafish heart development is well documented (13, 134).

PALS1 not only binds Crumbs proteins, it also interacts with PATJ (Fig. 2) (131, 133). PALS1 interacts with Crumbs proteins via its PDZ domain and binds PATJ with its L27N domain (88). Domain deletion analysis has illustrated that the interactions with both Crb3 and PATJ are essential for cell polarity in mammals (88, 145). PALS1 also links the Crumbs complex to the Par complex via the binding of the PDZ domain of Par6 to the N terminus of PALS1 (127, 167). Knockdown studies of PALS1 indicate that it is essential for cell polarity, affecting levels of PATJ and the ability of atypical PKC (aPKC) to target to the tight junction (145).

The regulation of PALS1 protein in mammalian epithelia is maintained by its association and binding to other proteins, illustrating the complex nature of the tight junction. Mammalian PALS1 was identified in a screen for proteins associated with Lin-7, a small PDZ domain containing protein necessary for proper development in C. elegans (72). Lin-7 stabilizes PALS1 protein in MDCK cells by binding to the L27C domain found in PALS1 (15, 124, 144). Loss of Lin-7 destabilizes PALS1, leading to tight junction defects (144). The Lin-7c knockout mouse displays a variety of renal defects, including cystic kidneys (124). In this regard, it has been reported that PALS1 can interact with nephrocystins, genes involved in the pediatric cystic kidney disease nephronophthisis (23). Additionally, PALS1 is destabilized by the lack of Crb3 in MDCK cells. Snail-induced epithelial-to-mesenchymal transition (EMT) results in decreased PALS1 protein levels with only a modest decrease in transcription of the gene (171).
It has been demonstrated that PALS1 regulates the architecture of not only the tight junction but also the lateral membrane. Wang et al. (166) illustrated that PALS1 knockdown affected the trafficking of E-cadherin to the lateral surface of MDCK cells through the exocyst complex. This is also found in Drosophila where loss of Stardust or Crumbs affects adherens junctions (44). This reflects the close interplay between polarity proteins, formation of the lateral membrane, and cell adhesion. A knockout of PALS1 was found to be early embryonically lethal, and selective depletion of PALS1 from the cortical progenitors of the medial cortex and hippocampus led to mice with essentially no cerebral cortex (76). This was ascribed in part to defects in mammalian target of rapamycin (mTOR) signaling, as mTOR components have previously been shown to interact with the Crumbs complex (101).

**PATJ and multi-PDZ protein-1.** PATJ and multi-PDZ protein-1 (MUPP1) are two highly related multiple PDZ-domain-containing proteins found in mammalian epithelia. The presence of multiple PDZ domains on both PATJ and MUPP1 gives rise to a multitude of functions for both proteins in mammalian epithelial cells. Both PATJ and MUPP1 contain N-terminal L27 domains (Fig. 3), which have been shown to bind to PALS1 (133), and MUPP1 has also been shown to directly interact with MPP4 (also known as Dlg6) via this L27 domain (Fig. 2) (164). PATJ and MUPP1 both contain multiple PDZ domains, making them molecular scaffolds at the tight junction. PATJ contains 10 PDZ domains, while MUPP1 contains 13. PDZ domains 6 and 8 of PATJ directly bind to ZO-3 and claudin-1, respectively (132). The binding of the ZO and claudin families to PATJ directly links conserved polarity complexes to tight junction structural proteins. Similarly, MUPP1 is also linked to tight junction structural proteins, including those of the claudin and junctional adhesion molecule family (47).

However, while PATJ has been demonstrated to be important for the maintenance and establishment of epithelial integrity, MUPP1 may not be essential for tight junction formation (1). PATJ has been shown to interact with angiomotin, and this interaction may provide clues into the role PATJ plays as a signaling molecule in polarized epithelia (147, 170). Angiomotin binds PDZ 2 and 3 of PATJ and has a large number of interactions that may shed light on how the epithelial barrier is...
maintained. In addition to binding PATJ, angiomotin also associates with Rich1, a Cdc42 GAP, thus directly linking PATJ to the Rhofamily of GTPases and the Par polarity complex through Cdc42 (170). Since both proteins are associated with the tight junction and found within close proximity of the cell membrane, PATJ and MUPP1 can act as anchors of transmembrane receptors localized at or near the tight junction and adherens junction (22, 89). These transmembrane receptors may represent key upstream signaling molecules responsible for maintenance and function of the epithelial monolayer.

The Par Complex

The mammalian Par complex comprises the proteins partitioning defective-3 (Par3), partitioning defective-6 (Par6), and aPKC (42). The Par complex is a cornerstone of apicobasal cell polarity, first identified in the C. elegans zygote (97). Genetic studies in C. elegans provided important insights into the mechanisms of asymmetric distribution of proteins, as removal of the Par proteins led to a failure of the zygote to correctly segregate proteins needed for proper development (31).

Par3. Deletion of the Par3 gene leads to the defective partitioning of proteins during the first cell division in the C. elegans zygote (75). In mammalian epithelia, Par3 has been shown to be necessary for tight junction formation and the localization of other members of the Par complex (38, 78, 151). Par3 is a multidomain scaffold protein, with three PDZ domains that can interact with Par6 and aPKC. (Figs. 2 and 3) (55, 66, 69) The first PDZ domain of Par3 interacts with Par6, and this interaction along with the Par3-aPKC interaction are indispensable for the development of cell polarity (52, 69, 90). It has been demonstrated that Par3 can homodimerize via its N terminus, and this interaction is important for the apical localization of Par3 (107). In the same study, the authors showed that the extreme N terminus of Par3 is necessary for proper localization of the other Par complex and tight junction proteins (107).

In mammals, there are two Par3 proteins, Par3A and Par3B (38), and although expression of these proteins overlap in renal epithelia, Par3B does not interact with aPKC (38). This exclusion is not fully understood, as Par3B can still bind Par6 and overexpression of the N terminus of Par3B can block tight junction formation similarly to overexpression of Par3A (38). There are also three different Par3A protein isoforms due to alternate splicing in mammalian epithelia, and only two of these can bind aPKC (38). Each Par3A isoform is distinguishable due to significant differences in the molecular masses of the translated sequence (180, 150, and 100 kDa, respectively), but they still possess a similar domain structure. The 180- and 150-kDa isoforms of Par3A bind aPKC on their C terminus, which is absent in the 100-kDa isoform (90).

The identity of the Par polarity complex is regulated, at least in part, by the ability of aPKC to interact with and phosphorylate Par3A (49, 66, 109). Phosphorylation of Par3 at S827 by aPKC is required for proper tight junction localization of Par3A (49, 111, 150). Overexpression of either a nonphosphorylatable mutant of Par3A or a dominant negative aPKC results in a similar phenotype, expanded lateral and reduced apical surfaces (111). In Drosophila, the effects of Par3 phosphorylation have been recently studied. Phosphorylation of Par3 by aPKC dissociates Par3 from aPKC and removes Par3 from the apical complex and sends it to the lateral zonula adherens. At the same time, the phosphorylation of Par3 frees Par6 to bind to Crumbs in the apical membrane (109, 165). It has also been suggested that Par3 binds directly to Stardust/PALS1 and that phosphorylation of Par3 releases aPKC and Stardust to move to the apical membrane (80). These results, taken together, lead to the same general finding as in mammalian cells that phosphorylation of Par3 promotes definition of the apical complexes. The main difference is that while Par3 is functional for zonula adherens development in Drosophila, in mammalian cells it is more important for ZO/tight junction formation. The necessity of the Par3A-aPKC interaction has been further demonstrated as essential by knockdown experiments in both two- and three-dimensional mammalian tissue culture models. In a Par3A knockdown system, MDCK cells formed intercellular lumens in monolayer culture and exhibited a multi-lumen phenotype in three dimensions (52). In complementation assays, these Par3A knockdown phenotypes were only rescued by introduction of a Par3A construct that could specifically interact with aPKC (52).

In addition to phosphorylation by aPKC, phosphorylation of Par3A by other kinases also regulates the function of the protein in polarized epithelia. Phosphorylation of S144 and S885 of Par3A by the polarity kinase Par1 (also known as EMK1/MARK2) leads to the binding of protein 14-3-3 (also known as Par5) to Par3A in both canine and murine models (9). Disruption of Par3A S144 phosphorylation prevents a Par3A-14-3-3 interaction, resulting in polarity defects in three-dimensional culture (56). In MDCK cells, Y1127 of Par3A is phosphorylated by c-Src or c-Yes through an epidermal growth factor (EGF)-dependent mechanism, and the phosphorylation event is necessary for EGF-induced tight junction formation (168). In addition, dephosphorylation of serines in Par3A can be regulated. Par3 dephosphorylation is mediated, in part, by protein phosphatase 1-α (PP1α), and repression of PP1α expression in MDCK cells causes delays in tight junction formation (160).

Par3 regulates and is regulated by Ras family GTPases (59). Ras family GTases such as Rho and Rac are known for regulating a multitude of cellular functions, including modulating cytoskeleton and epithelial architecture through cytoskeleton dynamics. Tiam1/2 is a Rac1 guanine nucleotide exchange factor (GEF) that binds the C terminus of Par3A. The interaction of Tiam1/2 to Par3A spatially regulates activation of Rac1 at the cell periphery, leading to a stabilization of epithelial junctions (21). Another Ras family member, RhoA, is a potent antagonist of Rac1 activity. RhoA activates Rho kinase (ROCK), leading to phosphorylation of yet another site on Par3A, T833 (115). The phosphorylation of Par3 at T833 has been recently demonstrated to prevent formation of the Par polarity complex, leading to the inactivation of Rac1, presumably by impairing the function of Tiam1/2 bound to Par3A (115). It has been demonstrated that a functional Par polarity complex (consisting of Par3A, Par6, and aPKC) enhances Rac1 activity and is antagonized by Rho activity (115).

Par3 also interacts with several additional proteins that regulate cell polarity. Par3A has been shown to interact with cytoplasmic keratin via the keratin binding protein Albatross. The association of Par3A with Albatross enforces the identity of the basolateral surfaces (148). In an Albatross knockdown cell, lateral membrane components are mislocalized in the cell

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(148). The second and third PDZ domains of Par3A have been suggested to anchor the Par complex to the cell membrane and to be associated with phosphoinositide signaling. PDZ2 of Par3A binds phospholipids found in the inner leaflet of the plasma membrane, while PDZ3 of Par3A has been shown to bind the phosphoinositide phosphatase, phosphatase and tensin homolog (PTEN) (33, 172). Par3A also directly interacts with junction adhesion molecule (JAM) via PDZ domains 1 and 3 (30, 64). The binding of JAMs to Par3A occurs at the tight junction, suggesting that JAMs are an anchor for the Par polarity complex.

Par6. In mammals, there are three identified Par6 genes, Par6A (also known as Par6C and Par6α), Par6B, and Par6G (also known as Par6D or Par6γ), and all appear to be ubiquitously expressed, albeit with slightly different temporal-spatial and subcellular localizations (37). Regardless of the isoform, it appears that Par6 is a multifunctional protein in epithelial cells, being a key adapter protein that allows the Par complex to interact with both the Crumbs and Scribble polarity complexes (57, 87, 167). Par6 contains an N-terminal Phox and Bem1 (PB1) domain, followed by a semi-Cdc42/Rac interactive binding (semi-CRB) domain, and a C-terminal PDZ domain (Fig. 3). Par6A directly interacts with Crumbl via Par6A’s PDZ domain, and this binding is important for proper tight junction formation (Fig. 2) (57, 87). The PDZ domain of Par6 also binds PALS1 with the PDZ domain of Par6B, binding PALS1 with highest affinity (37). Par6B binding to PALS1 can interrupt the binding of PALS1 to PATJ (167). The difference in binding preference between Par6A and Par6B may be explained by the conformation of the protein when bound with another binding partner, Cdc42 (37).

The Par6 isoforms interact with members of the aPKC family in a multitude of tissues (151). The function of Par6 binding to aPKC is to link Par6 and Par3 into a complex that may be dependent on the phosphorylation status of Par3 and to control the activity of the complex. The semi-CRIB domain and the PDZ domain of Par6 binds the N terminus of Par3 forming a stable tripartite complex that promotes junction formation (69). Par6 is thought to activate aPKC through direct interaction, allowing aPKC to phosphorylate Par3 (176). Par6 interacts with Cdc42 and Rac1 via the semi-CRIB domain (69, 70). The interaction of Par6 with Cdc42 renders a conformational change in Par6, altering the binding of Par6 to PALS1 and Crb3 (37, 40). When activated, GTP-bound Cdc42 binds Par6; the PDZ domain of Par6 most likely becomes hidden through conformational change, resulting in the masking of the PDZ domain of Par6, leaving Par6 with weakened interactions with PALS1 and possibly Crb3 (37).

Par6 also plays a pivotal role in polarity by binding members of the lateral polarity complex (Fig. 2). Par6 binds Lgl, and the binding of Lgl to Par6 excludes the binding of Par3 and PALS1 to Par6 (128, 175). The binding of Lgl by Par6 also mediates phosphorylation of Lgl by aPKC (more details below). With the binding between Par3 and Lgl to Par6 being mutually exclusive, Par6 is likely a regulator of the delicate balance between the formations of distinct apical vs. basolateral domains in polarized epithelial cells. In this case, Par6 reinforces both basolateral and apical identities through binding to both apical and basolateral polarity complexes. It is not surprising then that overexpression of Par6 in MDCK cells inhibits tight junction formation, as the dosage of Par6 is critical in maintaining proper protein interactions and subsequent polarity (36). On the other hand, Par6 overexpression has also been linked to cell hyperproliferation without loss of apicobasal polarity, suggesting that Par6 effects may be Par6 isoform as well as tissue specific (122).

aPKC. There are two major forms of aPKC, PKCζ and PKCα/β. Members of the aPKC family lack most of the C1 and all of the C2 domains, distinguishing themselves from typical PKCs (Fig. 3) (4). aPKCs directly bind to Par6 via an N-terminal PB1 domain and to Par3 via interactions involving the aPKC kinase domain (Fig. 2) (66, 69, 90). While aPKC has many functions in cells, in polarized epithelia it acts as a molecular switch controlling the identity of apical and lateral domains. This is accomplished by the selective phosphorylation of known targets of aPKC in mammals, including the previously mentioned Par3A and Lgl (49, 66, 90, 128, 175). The scaffold Par6 is critical in regulating aPKC and placing polarity substrates in close proximity.

aPKC phosphorylation of Par3 and Lgl is believed to modulate the identity of the Par complex in mammalian cells, where the phosphorylated species of either Par3A or Lgl are excluded or included in a particular complex based on conformational change. As discussed above, phosphorylation of Par3A by aPKC results in Par3-Par6 dissociation, beginning a cascade resulting in the stabilization of tight junctions and the development of apical-basal polarity (49, 109, 111, 165). In a similar way, aPKC phosphorylates Lgl, inducing Lgl dissociation from Par6 and the inclusion of Lgl in the Scribble complex (128, 174, 175). The dissociation of Lgl from Par6 leads to Par complex reformation and results in cytoskeletal rearrangements that reinforce surface identity through Ras family GTPases (see above). The Par complex with aPKC also regulates the basolateral membrane by promoting endocytosis of E-cadherin. This process, observed in Drosophila, also requires CDC42 and Par6 as well as the actin cytoskeleton (41, 85). Similar mechanisms may function in mammalian cells to modulate adherens junctions and are important in early polarity and junctional remodeling (138). Similar endocytic regulation may also have relevance for apical membrane regulation (140).

Recent studies have examined mice with knockouts of aPKC. Knockout mice of PKCA are lethal at an early embryonic stage (141), while PKCζ knockouts have primarily immunological defects (86). In neuronal development, selective loss of PKCA leads to a defect in adherens junctions but ultimately neurogenesis is not affected (62). Interestingly, tissue-specific knockout of PKCA has been examined in podocytes by two groups (50, 54). The loss of aPKC leads to polarity and slit diaphragm defects in podocytes, resulting in severe proteinuria. Recent work indicates that the crucial slit diaphragm proteins nephrin and nephril interact with the Par3/Par6/aPKC complex (48).

The Scribble Complex

The mammalian Scribble complex comprises the genetically linked conserved polarity proteins Scribble (SCRIB), discs large (Dlg), and lethal giant larvae (Lgl). Although the evidence for physical interaction between these proteins is somewhat limited, studies in lower organisms suggest that they function in the same genetic pathway. The Scribble complex localizes to the lateral membrane in polarized epithelia (Fig. 2),
a location consistent with a role in defining the lateral vs. the apical surface of these cells.

**Scribble.** SCRIB is a large cytoplasmic scaffold protein associated with the lateral membrane in polarized renal epithelial cells (25, 114). Originally described in *Drosophila*, SCRIB’s name is derived from the disorganized phenotype described in the developing embryo, imaginal wing discs, and follicles of the fly (11, 12). SCRIB is a member of the leucine-rich repeat (LRR) and PDZ domain (LAP) family of proteins with 16 LRRs in the N terminus and 4 PDZ domains in the C terminus (Fig. 3) (10).

The N-terminal 16 LRRs of SCRIB are necessary for binding to Lgl2 and targeting SCRIB to the lateral membrane in polarized renal epithelia (71, 117). The association of Scribble with the lateral membrane, however, appears to be cell type specific. In intestinal epithelia, Scribble associates with the tight junction structural protein ZO-1 and regulates tight junction stability, a phenomenon not seen in renal epithelia (65). The lateral targeting of SCRIB depends on the presence of E-cadherin, and in MCF10A cells lacking E-cadherin SCRIB is no longer associated with the membrane (117). The association of SCRIB with the intracellular domain of E-cadherin at the lateral membrane of polarized renal epithelia is necessary for proper cell-cell adhesion, as SCRIB knockdown alters adherens junction stability (129). In this study, knockdown of SCRIB in MDCK cells was shown to lead to increased motility and reduced adhesion, a phenotype similar to that seen with knockdown of E-cadherin. However, it is interesting to note that overall cell polarity was not affected. The PDZ domains of SCRIB bind βPix, a Rac/Cdc42 GEF involved in exocytosis (7). SCRIB has been shown to regulate directed migration and wound healing in association with Rac1, Cdc42, and presumably the Par complex (27, 121). Also, PDZ domains 3 and 4 of SCRIB bind the tight junction structural protein ZO-2 in contact naive epithelial cells, but not fully polarized epithelia, further suggesting a role for SCRIB in cell migration (106). Similarly, mice displaying mutated variants of SCRIB display impaired directed epithelial migration phenotypes (27).

PDZ domains 3 and 4 of SCRIB bind to the planar cell polarity factor and noncanonical Wnt-signaling molecule Vangl2, and this interaction is responsible for proper orientation of polarized structures in mammals (71, 108). Abrogation of Vangl2 in mouse reproductive tract epithelium, in turn, resulted in a mislocalization of SCRIB and defects in apical surface of Vangl2 in mouse reproductive tract epithelium, in turn, resulting in a mislocalization of SCRIB and defects in apical surface (71, 117). The association of SCRIB with the lateral membrane in polarized renal epithelia is necessary for proper polarity (174, 175). As mentioned previously, mLgl is phosphorylated by aPKC, and the phosphorylation of Lgl restricts its localization to the basolateral membrane, as a nonphosphorylatable mutant of mLgl localizes with apical markers (110, 128). In human cancers, overexpression of aPKC at the membrane results in a cytosolic accumulation of Lgl, suggesting that membrane-bound Lgl is necessary for tissue homeostasis (45, 92). In terms of vertebrate development, it has been demonstrated that Lgl gives identity to the basolateral membrane and the interaction between Lgl and aPKC results in the definition of apical vs. basolateral surfaces (20). Similarly, in depolarization models, knockdown of Lgl prevented the disassociation of the Par polarity complex, resulting in cellular overgrowth (174). Knockout of Lgl1 in mice leads to a central nervous system hyperproliferation phenotype possibly due to defects in asymmetric cell division (77). These data suggest that there exists a coordinated series of events where the action of Lgl and aPKC function to define the apical and basolateral surfaces by mutual inhibition and scaffolding.

Lgl has also been demonstrated to interact with trafficking machinery. Lgl interacts with myosin II heavy chain and the basolateral targeted syntaxin 4, suggesting it plays a role in the trafficking of components to the lateral membrane (110). More studies are needed on this phenomenon in mammalian systems; however, work on *Drosophila* has illustrated that Lgl may be involved with the regulated endocytosis and exocytosis of Crumbs (14, 95). In particular, disrupting exocytosis of Crumbs resulted in an expanded lateral membrane, but suppression of Lgl in this system resulted in a rescue of Crumbs presentation on the apical surface (14).

**Dlg.** Dlg is the mammalian homolog of the *Drosophila* gene *Discs large*, of which there are five mammalian family members (Dlg1–5), with Dlg1 being the most thoroughly studied in the kidney (104). Dlg1 (also known as SAP97) localizes to the lateral membrane in polarized epithelia (96). Dlg1 contains, in order, from N terminus to C terminus, an L27 domain, three PDZ domains, and SH3 domain, a 4.1 binding domain, and a MAGUK domain (Fig. 3) (96).

The Scribble complex consisting of SCRIB, Lgl, and Dlg1 have a strong genetic interaction; however, physical interaction between Dlg1 and the other members of the complex in mammalian cells has remained elusive. However, multiple other binding partners for Dlg1 have been identified. Proteins that bind the L27 domain of Dlg1 include membrane palmitoylated protein (MPP) family members MPP2, MPP3, and MPP7 (73). The tripartite complex consisting of Dlg1, MPP7, and Lin-7 is important for tight junction formation and stability of Dlg1 (15, 146). Dlg1 also interacts with Lin2/CASK(120), another MAGUK polarity protein, via an L27-L27 domain dimerization interaction (84), and this interaction is important for Dlg1 recruitment to the lateral membrane (84, 94). This interaction may be most important in the nervous system, where Lin-2/CASK has a role in synaptic function and gene
expression (6, 53). Lin-2/CASK is mutated in a human disorder characterized by microcephaly and mental retardation (113).

In addition, Dlg1 interacts with APC via its PDZ domains (102). APC is a tumor suppressor that binds β-catenin in polarized epithelial cells and negatively regulates Wnt signaling, suggesting that Dlg1 may play a role in planar cell polarity (79). The interaction of Dlg1 and APC has been shown to affect cell cycle progression, as overexpressed Dlg1 leads to overproliferation most likely due to an increase in Wnt signaling (63). Adey and colleagues (2) also identified Dlg1 as a binding partner for the tumor suppressor PTEN via a yeast two-hybrid screen and demonstrated that unphosphorylated PTEN interacts with the second PDZ domain of Dlg1. Although the functional significance of this lipid phosphatase binding to Dlg1 is not known at this time, it is possible that Dlg1 acts to stabilize PTEN. Dlg1 would then facilitate PTEN phosphorylation, affecting PTEN interactions with other PDZ domain-containing proteins including Par3 (162).

Posttranslational modifications to Dlg1 protein have also given insight into the role of Dlg1 in epithelial polarity. Dlg1 is phosphorylated in epithelial cells, and phosphorylation appears to have a significant role in maintaining the structural integrity of the adherens junction (83). Additionally, the phosphorylation of Dlg1 may confer its subcellular localization and stability during the cell cycle and be regulated by cyclin-dependent kinases (CDK) 1 and 2 (116). The stability and half-life of Dlg1 in epithelial cells is regulated by ubiquitination (99, 100). Ubiquitination of Dlg1 can be a result of viral infection, where oncogenic viruses coding for ubiquitin ligases selectively target PDZ polarity proteins for degradation. Virtually mediated ubiquitination of polarity proteins like Dlg1 or SCRIB results in a breakdown of the polarity program (39, 100, 158). A Dlg1 knockout mouse has been generated, and the phenotype includes hypoplastic kidneys associated with the lack of epithelial development (60). The C-terminal SH3, Hook, and GUK domains alone of Dlg1 are known to be important in Dlg1 function. A gene trap mouse that leads to a C-terminal truncation of Dlg1 displays an array of developmental phenotypes, including defects in nephrogenesis and cleft palate (18, 112).

An additional comment is required when the function of the lateral complex proteins including Dlg is considered. Lgl proteins are known to have a role in polarity due to their interaction with Par6, but the role of Dlg and SCRIB in apicobasal polarity is less clear. In fact, these proteins appear to play a larger role in planar polarity and cancer. Also missing from this complex are effectors such as kinases although SCRIB interacts with βPix to control small G proteins (7). Prominently absent from this discussion of the SCRIB complex is the Par1 kinase that was first identified in C. elegans as a critical effector in zygote polarity (42). Par1 has the ability to phosphorylate Par3 and in this fashion antagonize the apical complexes (9, 56). Reciprocally, Par1 is phosphorylated by aPKC, and this induces 14-3-3 binding to Par1 and reduces Par1 membrane targeting (58, 149). However, the factors that target Par1 to the lateral membrane are poorly understood (161) and do not involve the SCRIB complex although Par1 can phosphorylate Dlg (177). In general, our knowledge of the function of apical polarity complexes exceeds our knowledge of the lateral complexes in mammalian apicobasal polarity.

**Polarity Proteins and Mesenchymal-Epithelial Transitions**

This review catalogues the polarity complexes and their multiple interactions. Together via multiple interactions and protein modifications, these complexes work to define the apical and basolateral surfaces. Modulation of these polarity complexes and their function in physiology is best demonstrated in EMT and the converse mesenchymal-to-epithelial transition (MET) (19, 157). EMT is characterized by the loss of epithelial morphology where cells first lose junctional architecture and then move toward a fibroblastic morphology. To exhibit such a morphological change, the cell undergoes massive alterations in its transcription and translation networks, along with a vast reorganization of its cytoskeletal network. The EMT process is a normal part of development during gastrulation where cells need to move away from the epithelial sheet and migrate into the embryo. In contrast to EMT, renal tubules are initially formed by the reverse process of MET (19). During MET in kidney development, the ureteric bud induces the metanephric mesenchyme to become tubular epithelia. (28). In the process of MET when mesenchyme is converted to epithelia, expression of polarity complexes and tight junction proteins is increased (137). This is due to a reversal of transcriptional pathways that are discussed below. Our work and the work of others have described how these polarity proteins once induced in MET can lead to primordial tubular structures (135).

Regulation of EMT or MET primarily rests in transcriptional regulation. A series of transcriptional repressors including the Snail family, the ZEBs, Twist, E47, and other transcription factors induce EMT by directly or indirectly repressing epithelial gene expression and inducing mesenchymal genes (126). Many of the EMT-inducing transcription factors bind E-boxes in the promoter region of E-cadherin, downregulating cell-cell adhesion and promoting cell migration. Another major target of Snail and related proteins are tight junction proteins such as occludin and claudins (61). Most relevant to this review is the effect of EMT on polarity complexes. If epithelial cells are to move to the nonpolarized mesenchymal phenotype, then the main polarity complexes must be neutralized. The major effects are seen with the Crumbs3 protein with Snail and ZEB transcriptional repressors binding directly to the promoter of Crumbs3 (3, 171). Other members of the Crumbs complex are also repressed but mainly at a protein level (171). This could be due to instability secondary to repression of Crumbs3 expression. Expression of lateral polarity proteins such as Lgl2 are also repressed in EMT likely through direct transcriptional repression by ZEB1 (142).

EMT is induced by a variety of upstream factors including transforming growth factor (TGF)-β, Wnts, growth factors, and hypoxia (157). Many of these factors can lead to the upregulation of Snail and promote EMT via transcriptional repression of polarity proteins. However, there are other non-transcriptional mechanisms to modulate polarity proteins. One of the best described is the effect of TGF-β on the Par polarity complex. The TGF-β receptor interacts with Par6 and, upon stimulation with TGF-β, Par6 is phosphorylated. This phosphorylation serves to destabilize tight junction proteins (125). A similar effect has been seen with the growth factor receptor ErbB2, which interacts with Par6 and disrupts the Par6/Par3/aPKC complex (5). Taken together, these upstream signaling...
pathways can perturb polarity and tight junctions by immediate disruption of polarity complexes as well as by long-term transcriptional repression of component proteins. In pathophysiology, the effects of TGF-β and other immune modulators during renal inflammation is of interest. One school of thought is that renal fibrosis is due to EMT with epithelia transforming to fibroblasts (118). However, this is not completely accepted (93). In contrast, incomplete EMT may be common when tight junctions are loosened but complete mesenchymal transformation does not occur. This incomplete EMT is likely to occur in renal inflammation and would lead to worsening of inflammation due to edema and increased ingress of inflammatory cells. Thus therapies that maintain epithelial polarity and tight junctions may have some efficacy in inflammatory and fibrotic disorders.

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We regret that we could not reference all work in this area due to space limitations.

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REFERENCES


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APICOBASAL POLARITY COMPLEXES  


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