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Apicobasal Polarity in the Kidney

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Abstract

The apicobasal polarization of epithelia is critical for many aspects of kidney function. Over the last decade there have been major advances in our understanding of the mechanisms that underlie this polarity. Critical to this understanding has been the identification of protein complexes on the apical and basolateral sides of epithelial cells that act in a mutually antagonistic manner to define these domains. Concomitant with the creation of apical and basolateral domains is the formation of highly specialized cell-cell junctions including adherens junctions and tight junctions. Recent research points to variability in the polarity and junctional complexes amongst different species and between different cell types of the kidney. Defects in apicobasal polarity are prominent in several disorders including acute renal failure and polycystic kidney disease.

Keywords

Apical; Basolateral; Par; Crumbs; Lethal Giant Larvae; Podocyte

Introduction

Formation of epithelial tubes is central to the development of several organs including the kidney. Key in this process is the generation of polarized epithelia with the asymmetric distribution of biomolecules to apical or basolateral surfaces. In polarized tubules, the apical membrane faces the lumen while the basolateral membrane forms by cell-cell interactions (lateral) and cell-matrix interactions (basal). Cell matrix interactions are mediated by proteins such as integrins while cell-cell interactions are mediated by transmembrane proteins such as cadherins [1]. Cadherins are at the center of a region of compacted cell-cell interactions referred to as the adherens junction. This is in contrast to the tight junction formed by claudins that exist between the apical and basolateral membranes of most epithelia. At the tight junction, membranes from adjacent cells fuse providing both a fence and a barrier function. The fence function prevents the apical proteins from admixing with the basolateral proteins while the barrier function prevents larger molecules from moving between epithelial cells.

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Mediators of Cell Polarity

Work over the last ten years has pointed to apical and basolateral polarity complexes as important mediators of epithelial cell polarization [2]. These basolateral and apical complexes function in a mutually antagonistic manner to delineate distinct membrane surfaces. Much of our understanding of these polarity complexes comes from studies in model organisms. The Partitioning defective (Par) protein complexes were initially identified in the *C. elegans* zygote. Upon fertilization, the zygote polarizes into anterior and posterior compartments. Polarization creates a sharp boundary in an otherwise contiguous plasma membrane that encompasses the zygote. Par3, Par6 and atypical PKC (aPKC) are found anteriorly (akin to apical) while Par1 and Par2 form the posterior (akin to lateral) complex. In *Drosophila* several tissues have been used to study apicobasal polarity including the oocyte and the epithelia. In the *C. elegans* zygote, polarity is determined after fertilization whereas in the *Drosophila* oocyte, polarity is determined prior to fertilization. The anterior (apical) complex again involves the Par3, Par6 and aPKC proteins while the lateral complex contains Par1 in combination with the protein, Lethal Giant Larvae (Lgl). Later in *Drosophila* development, the primary *Drosophila* epithelium is formed [3]. This model of primary epithelia is somewhat different than a mammalian epithelial model because it forms initially from a syncytium. However, many of the proteins we know to be important in mammalian epithelial cells play an essential role in the formation of this epithelium, including the Crumbs complex, the Par complex and the Discs Large (Dlg), Lgl and Scribble complex. These complexes are discussed in detail below. Further insights are obtained from epithelia in other organisms including *C. elegans* and zebrafish although there are variations. For example junctional complexes that often provide the boundary between apical and basal lateral surfaces are much different [2]. In mammalian epithelia the tight junction sits above the adherens junction and separates apical and basolateral surfaces. In most *Drosophila* epithelia, the septate junction is the tight seal between cells but sits basal to the adherens junction. As it sits below the adherens junction, the septate junction does not play a role in separating apical and basolateral surfaces. In contrast in *C. elegans* epithelia there is a compound junction that combines adherens and tight junctions. Thus no one system is a perfect model for mammalian epithelia and in fact there is variation in the arrangement of junctions even in mammalian epithelial cells as will be discussed below.

Apical Polarity complexes

There are multiple protein complexes that form at the apical surface and these work to define the apical membrane and the tight junction. Two complexes felt to be primary in defining the apicobasal polarization of epithelia are the Par complex and the Crumbs complex.

The Apical Par Complex

The apical Par complex consists of the proteins Par3, Par6 and aPKC [4]. Par3 and Par6 act as scaffolds and a major role of these proteins is to localize and regulate aPKC which is a serine/threonine kinase and the core effector of this complex. These scaffold proteins also have numerous interactions with the small G proteins, Cdc42 and Rac, that are also important effectors in the apical polarity pathway.

Atypical PKCs (PKC ζ and PKC λ/τ) directly bind to Par6 via an amino-terminal Phox and Bem1 (PB1) domain and to Par3 via interactions involving the aPKC kinase domain [4]. There are multiple functions for aPKCs in cells but one of their primary roles is as a mediator of apicobasal polarity. aPKC is localized to the tight junction and functions to prevent lateral proteins from localizing to the apical domain. The best examples of this are the phosphorylation of the lateral proteins, Lgl and Par1 (Fig. 1A) [5,6].

Par3 is a multi-domain scaffold protein, with three Postsynaptic Density/Discs Large/Zonula Occludens (PDZ) domains that can interact with Par6 and aPKC [7]. Interestingly Par3 is also a target of aPKC phosphorylation [8]. Phosphorylation of Par3 at S827 by aPKC is required for proper tight junction localization of Par3. In addition to phosphorylation by aPKC, phosphorylation of S144 and S885 of Par3 by the Par1 Kinase leads to the binding of protein 14-3-3 (also known as Par5) to Par3 [9]. The binding of 14-3-3 keeps Par3 off the lateral membrane and restricts it to the apical membrane. Disruption of Par3 S144 phosphorylation prevents the Par3-14-3-3 interaction, resulting in polarity defects [10]. Par3 regulates and is regulated by Ras-family GTPases. Tiam1/2 is a Rac1 guanine nucleotide exchange factor (GEF) that binds the C-terminus of Par3A. The interaction of Tiam1/2 with Par3A spatially regulates activation of Rac1 at the cell periphery leading to a stabilization of epithelial junctions [4].

Par6 is an apical complex protein that contains a PB1 domain, followed by a semi-Cdc42/Rac Interactive Binding (Semi-CRIB) domain, and a carboxy-terminal PDZ domain. The interaction with aPKC occurs via PB1 domains on both proteins and is important in aPKC activation [11]. Par6 plays a pivotal role in polarity by also binding members of the lateral polarity complex. Par6 binds Lgl and the binding of Lgl to Par6 mediates phosphorylation of Lgl by aPKC [5]. The binding of Par3 and Lgl to Par6 is mutually exclusive and phosphorylation of Lgl by aPKC impairs its binding to Par6 and promotes Par3/Par6 interactions. Par6 also interacts with Cdc42 and Rac1 via the semi-CRIB domain [7]. The interaction of Par6 with Cdc42 renders a conformational change in Par6 altering the binding of Par6 to other apical polarity proteins [4].

The Apical Crumbs complex

The second major apical protein complex is referred to as the Crumbs complex (Fig. 1A). The Crumbs complex is composed of three proteins: Crumbs, Protein associated with Lin Seven 1 (PALS1) and PALS1-associated tight junction protein (PATJ). Crumbs was first identified in *Drosophila* and both PALS1 and PATJ have highly related *Drosophila* orthologues. The Crumbs complex plays an important role in targeting the apical Par complex and is also important in connecting the apical complex to the tight junction. There are three mammalian Crumbs genes and two of the protein products, Crumbs1 and Crumbs2, have large extracellular domains like their *Drosophila* orthologue. However the role of the extracellular domain in Crumbs protein function is unclear as the most common form of Crumbs in mammalian epithelia, Crumbs3, has a non conserved short extracellular domain [12]. More certain is the role of the highly conserved intracellular region that contains two domains, the 4.1/ezrin/radixin/moesin (FERM) binding domain and a PDZ domain binding motif. The best characterized binding partner for the FERM binding domain is the FERM protein known as Moe in Zebrafish, Yurt in *Drosophila* and Erythrocyte Membrane Protein Band 4.1 like 5 in mammals [13, 14]. This Band 4.1 family member appears to antagonize Crumbs signaling [15]. The ERLI motif on Crumbs is recognized by the PALS1 and Par6 scaffold proteins via their PDZ domains [12, 16]. These interactions are crucial to tie Crumbs to the Par polarity complex and ultimately to the proper localization of the effector, aPKC. Due to the series of interactions of the conserved Crumbs3 intracellular domain, the level of Crumbs 3 in a cell is crucial for proper cell polarity. Overexpression of Crumbs3 in MDCK cells leads to an expansion of the apical surface, an extended tight junction, and a reduced basolateral membrane [17] whereas knockdown of Crumbs3 in mammalian epithelia leads to an extensive loss of the tight junction best seen in three dimensional culture [18]. In addition to controlling polarity, recent work suggests that Crumbs regulates growth control pathways. This seems well established in *Drosophila* [19] and similar studies are beginning to emerge in mammalian cells [20].

PALS1 is a tight junction scaffold protein and a member of the Membrane Associated Guanylate Kinase (MAGUK) family of modular adaptor proteins [21]. PALS1 has orthologues in lower organisms including Stardust in *Drosophila* and Nagie Oko in Zebrafish. PALS1 contains at least six distinct protein interacting domains including a pair of L27 domains, a PDZ domain, a Src Homology 3 (SH3) domain, a Hook domain, and a Guanylate Kinase (GUK) domain [22]. Knockdown and knockout studies of PALS1 indicate that it is essential for cell polarity [23, 24]. The PDZ domain of PALS1 binds to Crumbs as does the PDZ domain of Par6. It is not clear if PALS1 and Par6 compete for binding to Crumbs but likely the interaction is cooperative as PALS1 also binds Par6. The GUK domain of PALS1, like other MAGUK proteins, lacks any discernable kinase activity and likely is involved in protein-protein interactions but the targets are not clear. The L27 domains of PALS1 each have a different binding partner. The more carboxy-terminal L27 domain binds the L27 domain of Lin-7, a small PDZ domain protein. This interaction is crucial for PALS1 stability [25]. Via its more amino-terminal L27 domain PALS1 binds an L27 domain on PATJ, a highly related multiple PDZ-domain containing proteins. PATJ contains multiple PDZ domains and functions as a molecular scaffold at the tight junction. PATJ contains 10 PDZ domains and can bind directly to ZO-3 and Claudin-1 via these domains [26]. The binding of the ZO and Claudin families to PATJ directly links the apical Crumbs and Par polarity complexes to tight junction structural proteins and has an important role in tight junction positioning.

The Basolateral Polarity Complex

Counteracting the apical complexes is a basolateral complex of proteins. The role of the basolateral proteins are confusing due to their varying composition and role in different species and in different tissues. In *Drosophila* epithelia, a basolateral signaling complex has been the best studied and includes the proteins Dlg, Lgl and Scribble. Loss of these proteins in *Drosophila* leads to a common defect including loss of polarity and tissue overgrowth [27].

The Lgl protein contains a series of WD-40 repeats in its N-terminus that function in binding to other proteins including Scribble [28]. Mammalian Lgl binds Par6/aPKC and this binding is found only in Par6 complexes missing Par3 [29]. Lgl is phosphorylated by aPKC and the phosphorylation of Lgl restricts its localization to the basolateral membrane (Fig.1A), as a non-phosphorylatable mutant of Lgl localizes with apical markers [5]. In the classic model of polarity in the *C. elegans* zygote, the anterior complex of aPKC, Par3 and Par 6 are countered by the posterior complex of Par1 and Par2. While there is no Par2 homologue in other polarity models, it is interesting that recent studies have indicated that Lgl is also active and is redundant to Par2 in the *C. Elegans* zygote polarity model [30].

Knockdown of Lgl1/2 in MDCK cells leads to only minor polarity effects when MDCK cells are grown on a monolayer but severely disrupts lumen formation in 3D culture [31]. Mouse knockdown of Lgl1 leads to dysplasia of the central nervous system and a possible polarity defect in neuronal progenitor cells [32]. In contrast knockout of Lgl2 leads to placental defects possibly due to polarity and branching morphogenesis defects [33]. However postnatally, *lgl2*^{-/-} animals develop normally without overgrowth phenotypes. This is in contrast to the *lgl2* mutation in Zebrafish, the *penner* mutation, that leads to loss of hemidesmosomes, cellular overgrowth and early death [34].

Dlg is one of the first identified MAGUK proteins and loss of Dlg leads to loss of polarity in larval epithelial cells and overgrowth of these cells in *Drosophila* [27]. In *C. elegans*, Dlg-1 is critical for epithelial junction formation [35] and knockdown studies of Dlg in mammalian cells suggested Dlg aids in adherens junction formation [36] although the effect is quite

small. Dlg knockout leads to kidney hydronephrosis but this is not due to defects in cell polarity or cell adhesion and may represent a smooth muscle defect [37,38]. There are multiple mammalian Dlg genes so redundancy may explain the lack of an epithelial phenotype.

Scribble is a large, cytoplasmic scaffold protein associated with the lateral membrane in polarized renal epithelial cells that contains 16 Leucine Rich Repeats in the N-terminus and 4 PDZ domains in the C-terminus [27]. The N-terminal 16 Leucine Rich Repeats of Scribble are necessary for binding to Lgl2 and targeting Scribble to the lateral membrane in polarized renal epithelia via interactions with E-Cadherin [28]. The association of Scribble with the intracellular domain of E-cadherin at the lateral membrane of polarized renal epithelia is necessary for proper cell-cell adhesion as Scribble knockdown alters adherens junction stability [39]. Although Scribble plays an important role in cell adhesion and binds Lgl2, its role in apicobasal polarity is not clear. Rather Scribble has been shown to have a larger role in planar polarity via its interaction with the planar polarity protein, Vangl2 [28]. Like many other polarity proteins, Scribble has a close connection with small G proteins. The PDZ domains of Scribble bind β Pix, a Rac/Cdc42 GEF involved in exocytosis [40]. Scribble has been shown to regulate directed migration and wound healing in association with Rac1 and Cdc42. In addition, the tumor suppressor function of Scribble is related to its ability to regulate the Ras-MAPK pathway, and overexpressed Scribble was able to effectively suppress oncogenic Ras-associated invasiveness [28].

Par1 kinase is an important polarity protein of the lateral membrane. It has been identified as being active in several polarity model systems notably the *C. elegans* zygote model and the *Drosophila* oocyte model. Par1 mediated phosphorylation of Par3 promotes binding of 14-3-3 to Par3 and keeps Par3 off the lateral membrane. [9, 10]. Reciprocally Par1 is phosphorylated by aPKC and this induces 14-3-3 binding to Par1 and reduces Par1 membrane targeting [6]. Another name for Par1 is Microtubule Affinity Regulating Kinase, which derives from the fact that this kinase can phosphorylate microtubule-associated proteins and lower their affinity for microtubules. Indeed several studies have now indicated that the Par1 protein controls microtubule organization and polarity in the *Drosophila* oocyte [41]. However interactions of Par1 with other members of the lateral protein complex and the mechanism targeting Par1 to the lateral membrane are still poorly understood [42].

Polarity in the Podocyte; a Specialized Kidney Epithelial Cell

As noted above many species have epithelia with variants of junctional structure and apicobasal polarity complexes. *Drosophila* epithelia have the septate (tight) junction on the lateral side where it does not act as a fence between apical and basolateral membranes. *C. elegans* epithelia have tight and adherens junctions in one structure. Similarly mammalian kidney has tubular epithelial cells that have classic adherens and tight junctions but also has other cells such as podocytes that are epithelia but have hybrid junctions. Podocytes are key cells of the glomeruli that are necessary for the filtration of blood to produce the primary urine. The glomerular filter is formed by a fenestrated endothelium, the glomerular basement membrane (GBM), and the slit diaphragm (SD) of the podocytes. Projecting from their cell bodies, podocytes form primary processes that branch out into secondary foot processes. In between these processes, the slit diaphragm is formed, a highly-selective part of the kidney filtration barrier [43].

Mature podocytes possess a large apical domain facing Bowman's space, comprising the podocytes' cell bodies, primary processes and the apical side of the podocytes' foot processes. The small basal domain spans the basal side of the foot processes that adheres to the GBM (Fig. 1B). Both domains possess sets of proteins reminiscent of classical epithelial

cells, e.g. Podocalyxin (termed GP135 in epithelial cells) on their apical side and basement membrane receptors like integrins on their basal side [43]. In between both domains, the foot-processes of neighboring podocytes form the slit diaphragm, their only intercellular junction, controlling paracellular permeability in a fashion similar to epithelial tight junctions. In order to establish its zipper-like structure, the SD possesses a unique set of proteins, among which the most prominent ones are Nephrin, Podocin, and the Neph proteins [43].

The nature of the slit diaphragm has been intensely disputed. On the one hand, tight junction proteins such as ZO-1 and Occludin are concentrated at the slit diaphragm [44]. On the other hand, adherens junction proteins p-Cadherin, β -Catenin and p120-Catenin have been found to contribute to the slit diaphragm structure as well [45]. Interestingly, the slit diaphragm component Nephrin co-localizes with tight as well as adherens junction components when overexpressed in MDCK cells [46].

Recent reports in distinct organisms indicate that podocytes express at least a subset of the epithelial polarity proteins in order to maintain their apicobasal polarization, and expression of polarity proteins seems to precede expression of slit diaphragm proteins Nephrin and Podocin during podocyte development. As for the Crumbs complex, it has been demonstrated that downregulation of Crb2b in Zebrafish results in foot-process effacement and disruption of the slit-diaphragm fence function [47]. Furthermore, all members of the Crumbs complex are expressed in human immortalized podocytes [48]. Down-regulation of KIBRA, a PATJ-interacting protein, results in impaired directional migration of these cells [48].

Furthermore, all members of the Par/aPKC complex are expressed in podocytes [48]. Knockdown of aPKC in mice causes absence or displacement of SD slit diaphragms. aPKC knockout mice develop a severe nephrotic syndrome, growth retardation, and die at a median age of approximately 6 weeks [49, 50]. Apart from maintaining podocyte polarity, polarity proteins may have a role in recruiting and stabilizing slit diaphragm components, since Neph and Nephrin proteins have been shown to interact with Par-3 and aPKC [51].

Polarity and kidney disease

One of the most common renal diseases that alter cell polarity is acute tubular necrosis [52] often seen after ischemia and sepsis. Prominent in this loss of polarity is defects in tight junction formation leading to back leak and a lowering of effective glomerular filtration [52]. In addition to loss of tight junctions, the polarized distribution of molecules is also perturbed in ischemic renal damage. For example integrins [53] and sodium potassium ATPase [54] redistribute from their basal location to apical location leading to abnormal cellular interactions and fluid secretion. The cause of loss of polarity during ischemia is likely multifactorial but ATP depletion is probably a prominent mechanism. Loss of ATP leads to breakdowns of the adherens junction and loss of lateral adhesion [55]. Loss of ATP also leads to defects in phosphorylation that directly affects the polarity complexes. For sample ATP depletion leads to aPKC malfunction and defects in Par3 phosphorylation. Lgl interactions also appear to be abnormal after ATP depletion [56].

Polarity is also defective in polycystic kidney disease. This review has focused on apicobasal polarity but defects in planar polarity are often considered as one of the causes of kidney cysts [57]. Planar polarity runs perpendicular to the apicobasal plane and defects in planar polarity may lead to abnormal cell divisions that widen the tubular lumen. This has been reviewed in other articles [58] and is not the focus of this review. However in addition to planar polarity there a well-known defects in apicobasal polarity leading to mislocalization of proteins [59]. One example of this mislocalization is the redistribution of

Epidermal Growth Factor (EGF) receptor from the basolateral domain to the apical domain [60]. In this localization, the EGF receptor can be exposed to EGF ligand and lead to abnormal proliferation that appears to be an important aspect in driving polycystic kidney disease [61]. Similar findings of abnormal localization has also been found for other members of the EGF receptor family [59]. In addition to growth factor receptors, abnormal distribution of channels from the basolateral to the apical have been observed including the sodium potassium ATPase [62]. Abnormal distribution of channels including sodium potassium ATPase can lead to secretion of fluid into cysts speeding kidney destruction [63].

The majority of defects in protein polarization have been studied in models of autosomal dominant polycystic kidney disease. In these diseases caused by mutations in Polycystin 1 and Polycystin 2, the molecular etiology of the polarity defect is not entirely clear although many theories have been proposed [59]. For example, the polycystin proteins, felt to primarily function in cilia, have also been localized to adherens junctions where they interact with and regulate E-cadherin [64]. Another form of juvenile cystic kidney disease is associated with more global cilia dysfunction and referred to as the nephronophthisis associated ciliopathies [65]. These diseases are characterized by cysts but also by fibrosis. The connection between these disorders and polarity protein complexes is more established. Knockout of one of the Lin-7 isoforms which disrupt several polarity complexes results in fibrotic cystic disease [66]. In addition the NPHP proteins have been found to associate with the Crumbs complex and knockout of NPHP proteins can lead to abnormal polarity in three-dimensional culture [67, 68].

In summary we have a basic understanding of how protein complexes at apical and basolateral surfaces function to carve out membrane domains that are the basis of apicobasal polarity in the kidney. This polarity is not one size fits all and has variations between different cell types in the kidney. We are just beginning to understand the roles of these proteins in disease but already several cellular defects can be ascribed to defects in these proteins.

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Abbreviations

Lgl	Lethal Giant Larvae
aPKC	Atypical Protein Kinase C
Dlg	Discs Large
Par	Partioning Defective
PATJ	PALS1 Associated Tight Junction Protein-
PALS1	Protein Associated with Lin-7
EGF	Epidermal Growth Factor Receptor
PDZ	Postsynaptic Density/Discs Large/Zonula Occludens
PB1	Phox and Bem1

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Fig. 1. Apicobasal polarity and intercellular junctions in epithelial cells and podocytes

A. The establishment of apicobasal polarity in epithelial cells depends on mutual phosphorylation-dependent exclusion of apical and basolateral polarity proteins. Crumbs3 (Crb3) is localized at the apical (green) membrane of epithelial cells and helps to recruit aPKC to the apical domain via Par6. aPKC-dependent phosphorylation of Par1 at the apical membrane results in exclusion of Par1 from this domain. The same mechanism has been described for Lgl. Vice versa, Par1-dependent phosphorylation of Par3 at the basolateral (blue) side leads to its dissociation from Par6 and aPKC, resulting in exclusion of the Par/aPKC complex from the basolateral domain. **B.** The basic apicobasal polarity mechanisms are conserved in the epithelium-derived kidney podocytes. Since all members of the Crumbs and Par/aPKC complex have been shown to be expressed in podocytes, although in different model systems, it can be assumed that all mechanisms required for epithelial cell polarity are conserved. Since podocytes possess a unique shape and only one specialized junction, these mechanisms may have been modified in order to acquire their specific architecture. Immunoprecipitation studies have demonstrated that slit diaphragm proteins interact with both tight junction and adherens junction proteins, thus the SD may have properties of both epithelial types of junctions. Furthermore, Neph1/Neph proteins have been shown to interact with members of the Par complex, providing a possible link between cell polarity and the establishment of the SD.