Composition and function of PDZ protein complexes during cell polarization

Michael H. Roh and Ben Margolis

1Department of Biological Chemistry, 2Howard Hughes Medical Institute, and 3Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan 48109-0650

Roh, Michael H., and Ben Margolis. Composition and function of PDZ protein complexes during cell polarization. Am J Physiol Renal Physiol 285: F377–F387, 2003; 10.1152/ajprenal.00086.2003.—Complexes consisting of PDZ proteins have been implicated in a variety of cellular processes. In recent years, it has become increasingly clear that PDZ proteins play essential roles during the establishment of spatial asymmetry in various metazoan cell types such as epithelial cells. Epithelial cells possess asymmetry with respect to the apicobasal axis reflected by the differential distribution of proteins and lipids in the apical and basolateral surfaces. In Drosophila, three PDZ protein complexes have been shown to play crucial functions during the establishment of cell-cell adhesions and epithelial cell polarity: Bazooka/Dm-Par6/DaPKC, Crumbs/Stardust/Discs Lost, and Scribble/Discs Large/Lethal Giant Larvae. In this review, we focus primarily on our current knowledge of the localization and function of these complexes in Drosophila epithelia. We also discuss recent data that enhance our understanding of the homologous protein complexes and their roles during junctional assembly and polarization of mammalian epithelial cells.

PSD-95/Discs Large/Zonula occludens-1 domain; polarity; epithelium; cell junctions

THE ESTABLISHMENT AND MAINTENANCE of cell polarity within various organs are crucial for the proper development of both invertebrates and vertebrates. Two classic cell types for which polarization is essential for function are neurons and epithelial cells. The asymmetry of neurons is manifested by the formation of axon terminals (presynaptic side) and dendrites (postsynaptic side) at opposite aspects (68). Presynaptic and postsynaptic membranes contain distinct collections of proteins, ultimately allowing for directional transmission of action potentials and nerve impulses. Epithelial cells, which will be the topic of focus throughout this review, are polarized along the apicobasal axis. The apical surface faces a lumen or external environment, and the basolateral membrane contacts the substra-

Address for reprint requests and other correspondence: B. Margoli, Howard Hughes Medical Institute, Univ. of Michigan Medical Ctr, 4570 MSRB II, Box 0650, 1150 W. Medical Center Dr., Ann Arbor, MI 48109-0650 (E-mail: bmargoli@umich.edu).

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proteins at distinct junctional complexes along the lateral membrane represent an important aspect of polarity establishment and maintenance (78, 86). Recently, an increasing number of studies have emphasized the role of proteins containing the PSD-95/Discs Large/ZO-1 (PDZ) domain during cell polarization. PDZ proteins typically associate with the extreme COOH-terminal residues of their ligands (71). However, this mode of binding is not absolute as some PDZ domains heterodimerize with other PDZ domains (26, 69). PDZ domains are often found in scaffolding proteins along with one or more PDZ and/or other protein interaction domains. For example, the membrane-associated guanylate kinase (MAGUK) proteins consist of a signature arrangement of at least one PDZ domain plus an Src homology 3 (SH3) and a catalytically inactive guanylate kinase (GUK) domain (1). The previously mentioned ZO-1, ZO-2, and ZO-3 proteins belong to this protein family. In this review, we will focus on our current understanding of the variety of PDZ proteins and PDZ protein complexes that are essential during the morphogenesis of polarized epithelia.

THE ROLES OF PDZ PROTEIN COMPLEXES IN INVERTEBRATE CELL POLARITY

Three polarity complexes, each consisting of one or more PDZ proteins in Drosophila, have been topics of intense study during recent years (6, 78). The first complex consists of Bazooka (Baz), Dm-Par6, and Drosophila atypical PKC (DaPKC). The transmembrane protein Crumbs (Crb), along with its associated cytoplasmic proteins, Stardust (Sdt) and Discs Lost (Dlt), represents components of the second complex. The third consists of Scribble (Scrib), Discs Large (Dlg), and Lethal Giant Larvae (Lgl). Of these nine proteins, six contain one or more PDZ domains, reinforcing the notion that PDZ proteins can assemble protein scaffolds that serve essential functions.

The Baz/Dm-Par6/DaPKC complex has been shown to be essential during Drosophila epithelial morphogenesis. In these cells, this complex localizes to the subapical region which resides just above the most apical site of cell contact, the ZA (37). The position of the subapical region corresponds to that of the TJ in vertebrate epithelia (Fig. 1). Nonetheless, it must be noted that homologues of claudins, occludin, or JAM have yet to be elucidated at the subapical region. Thus whether this region represents a region of cell-cell adhesion remains unknown. It has been shown that the localization of Dm-Par6 and DaPKC to the subapical region is dependent on that of Baz (62, 84). The ability of Baz to directly interact with Dm-Par6 and DaPKC suggests that Baz is responsible for properly targeting both proteins. Interestingly, Baz is mislocalized in the absence of either Dm-Par6 or DaPKC, suggesting that only the heterotrimeric complex is stably associated with the subapical region (62, 84). Fly embryos lacking expression of Baz, Dm-Par6, or DaPKC exhibit a disruption of apicobasal polarity in epithelia (52, 62, 84). These epithelia also exhibit gross structural defects and lose their regular monolayer arrangement. Furthermore, Baz null flies also exhibit defects in ZA formation. In wild-type embryos, the ZA forms a continuous belt at the apicobasal aspect of epithelial cells as a result of the fusion of spot adherens junctions (sAJ). In flies missing Baz, sAJ material localizes irregularly along the lateral membrane and fails to coalesce into a beltlike ZA during early gastrulation (52). Furthermore, the proper localization of apical polarity markers (e.g., Crb) is compromised. These observations suggest that Baz along with DaPKC and Dm-Par6 are important players during ZA formation and epithelial cell polarization.

The Baz/Dm-Par6/DaPKC complex is also important during neuroblast polarization. Neuroblasts delaminate from the neuroectodermal epithelium and then proceed to divide asymmetrically, resulting in the generation of a neuroblast and ganglion mother cell (5). The polarized targeting of proteins to the apical and basal cortex is a prerequisite for asymmetrical neuroblast division. The Baz/Dm-Par6/DaPKC complex is localized to the apical pole and is excluded from the basal cortex (5). As in the case of epithelia, the localization of one member of the complex relies on the correct targeting of the other two proteins. Ectopically expressing one of these proteins at the basal cortex causes mistargeting of the other components (62, 84). Furthermore, a deficiency in any one of three proteins leads to abnormal mitotic spindle orientation during cell division. Asymmetrical neuroblast division is reminiscent of asymmetrical cell division in the Caenorhabditis elegans zygote, where the Baz/Dm-Par6/DaPKC complex is conserved (16). The homologous Par3/Par6/PKC3 complex is essential in orchestrating asymmetrical cell division in the worm zygote (60). Finally, Baz and DaPKC have been shown to be crucial during the polarization and differentiation of Drosophila oocytes (13, 18). Thus Baz/Dm-Par6/DaPKC functions in establishing asymmetry in a variety of cellular contexts.

In epithelia, the Crb/Sdt/Dlt complex colocalizes with Baz/Dm-Par6/DaPKC at the subapical region (Fig. 1) and is also important for the establishment of epithelial cell polarity (37, 49, 77). Sdt likely represents an adapter protein that mediates the indirect interaction between Crb and Dlt (66). In the absence of Sdt, Crb and Dlt are mislocalized, suggesting that the intact complex is stably retained at the subapical region (3, 27). Overexpression of Crb leads to apical surface expansion, ZA disruption, and multilayering of epithelia; however, overall apicobasal polarity seems to be preserved (36, 83). Thus Crb is an important apical surface determinant (31, 37, 77), and this correlates with its ability to organize the apical spectrin/actin cytocortex through β-moesin, a member of the band 4.1 superfamily of actin-associated proteins (50, 61). Drosophila embryos lacking expression of Crb, Sdt, or Dlt exhibit apicobasal polarity defects (4, 38). Furthermore, in the absence of Crb or Sdt, a continuous ZA also does not form from sAJs, similar to the case in baz null embryos (24, 52). Interestingly, compared with
Fig. 1. Protein-protein interaction domains involved in stabilizing the polarity complexes in vertebrate and Drosophila epithelia. A: vertebrate epithelial cells possess tight junctions (TJs) and adherens junctions (AJs) at cell-cell adhesions. TJs represent the most apical site of cell contact. The Crumbs (Crb)/Pals1/Pals1-associated tight junction (PATJ) protein and Par3/Par6/atypical PKC (aPKC) complexes associate via direct Par6-Pals1 interaction and colocalize to TJs. The zonula adherens (ZA) in Drosophila is the counterpart to the AJ and represents a beltlike junction that encircles the apex of fly epithelial cells. The septate junctions (SJs) exist at the lateral membrane below ZA. The subapical region (SAR) exists to the apical border of the ZA, reminiscent of the localization of TJs in epithelia. Strikingly, the Crumbs/Stardust/Discs Lost and Bazooka/Dm-Par6/DaPKC complexes colocalize to the subapical region. Protein-protein interaction domains were identified by using the Simple Modular Architecture Research Tool (SMART) database (http://smart.embl-heidelberg.de/). PDZ, PSD-95/Discs Large/zonula occludens-1; aPKC, atypical PKC; DaPKC, Drosophila aPKC; GUK, catalytically inactive guanylate kinase; CRIB, CDC42/Rac interactive binding; FERM, band 4.1/ezrin/radixin/moesin.
Baz null flies, ZA defects in *crb* or *sdt* null mutants are observed later during gastrulation (9, 52). This is consistent with the observation that Crb is required to maintain Baz at the subapical region but is dispensable for the initial localization of Baz in early gastrulae (9). Furthermore, the early ZA defects seen in *baz sdt* double-mutant embryos resemble those observed in *baz* null flies, suggesting that the Baz complex functions upstream of the Crb complex (52). However, in all of these mutants, the overall architecture of ectodermally derived epithelia is disrupted by the end of gastrulation. Collectively, these results suggest that the Baz/Dm-Par6/DaPKC and Crb/Sdt/Dlt complexes function cooperatively during ZA formation. It should be noted that whereas the former orchestrates polarization in various cellular contexts, the function of the latter could be restricted to epithelial cells. This is evident in *sdt* mutant embryos, where the polarity and asymmetrical cell division of neuroblasts remain unaffected (27).

The third PDZ protein polarity complex consists of Scrib, Dlg, and Lgl. It has been shown that all three proteins colocalize in epithelia and that they function in a common pathway (7). In the absence of Lgl or Dlg, Scrib is mislocalized. In *dlg* or *scrib* mutant epithelia, Lgl is mistargeted as well, suggesting that Scrib, Dlg, and Lgl could exist in a complex. Nonetheless, there is a deficiency in biochemical data to confirm this notion. Loss of Scrib, Dlg, or Lgl leads to apical surface expansion and mislocalization of ZA proteins to more lateral positions along the basolateral membrane during late gastrulation, which is reminiscent of the Crb overexpression phenotype (7, 8). The opposite but common temporal characteristics of the Crb/Sdt and Scrib/Dlg/Lgl mutant phenotypes suggest that the functions of these complexes are delicately balanced, allowing for the proper positioning of the ZA and determination of apical and basolateral membranes (9, 76). The functional importance of Scrib and Dlg is supported by studies performed in *C. elegans*. In worms, Dlg and Let-413, the Scrib homologue, are also essential for epithelial morphogenesis and formation of *C. elegans* apical junctions (10, 39, 43, 48). However, adhesion and polarity defects seem to be more severe in *Let-413* deficient embryos than *Dlg*-deficient embryos (48).

The majority of the *Drosophila* genetic studies on cell polarity have focused on the activities of individual members of a single PDZ polarity complex at a time. However, recent work from the Bilder (9) and Tepass (76) laboratories has addressed the important issue of how the functions of these individual complexes are coordinated to yield polarized epithelia by analyzing double mutant embryos, respectively. For instance, these studies have confirmed the antagonistic relationship between the Crb and Scrib complexes. Specifically, *dlg* or *scrib* mutations exaggerate the Crb overexpression phenotype and suppress the *crb* null phenotype (76). Interestingly, in the absence of the activities of both the Crb and Scrib complexes, ZA and polarity defects are rescued to an extent. This suggests that Crb is not absolutely required for epithelial polarity (51). It has been speculated that the Baz complex could compensate for the lack of the Crb complex in *crb scrib* and *sdt dlg* double mutant embryos (76).

Analysis of double mutants also facilitates the elucidation of epistatic relationships between the three polarity complexes. These studies support the notion that the Baz/Dm-Par6/DaPKC complex is epistatic to both the Crb and Scrib complexes. This is consistent with the fact that Baz seems to function earlier in development (9, 76). Furthermore, Scrib/Dlg/Lgl is epistatic to the Crb complex, in agreement with the observation that Scrib localization is unaffected in the absence of Crb. Given these epistatic relationships, the nature of polarity defects exhibited by the mutants, and the temporal differences in phenotypic onsets, a hierarchical model has been proposed (9). The initial event of epithelial polarization involves the assembly of sAJs material and establishment of apical membrane identity by Baz/Dm-Par6/DaPKC. This complex subsequently targets Crb/Sdt/Dlt to the apical surface. Meanwhile, Scrib/Dlg/Lgl is localized to the lateral membrane and counteracts the apicizing effects of the Crb complex at the basolateral membrane domain. Because Crb is required for the maintenance of Baz localization, it seems likely that Crb/Sdt/Dlt and Baz/Dm-Par6/DaPKC function cooperatively in establishing apical surface identity. As the activities of these complexes coordinate with that of the laterally localized Scrib complex, sAJs fuse at the apical aspect of cell contacts to form a continuous, beltlike ZA, ultimately leading to the proper establishment and maintenance of apicobasal polarity.

**THE ROLES OF EVOLUTIONARILY CONSERVED BAZ, CRB, AND SCRIB COMPLEXES IN VERTEBRATE EPITHELIA**

The Baz/Dm-Par6/DaPKC, Crb/Sdt/Dlt, and Scrib/Dlg/Lgl polarity complexes are conserved in vertebrate epithelial cells: Par3/Par6/aPKC, Crb/Pals1/Pals1-associated tight junction protein (PATJ), and Scrib/Vatul/mammalian Dlg (mDlg)/mammalian Lgl (mLgl), respectively. This suggests that these homologues also serve important polarity functions in vertebrates (49, 78). The majority of studies that examined the localization, protein-protein interactions, and functions of these complexes have been performed using mammalian epithelial cell lines. Studies utilizing zebrafish mutants have also clarified the importance of some of these polarity proteins during the development of epithelium-rich tissues during embryogenesis. In contrast to *Drosophila* epithelia, the TJ represents the most apical site of cell contact in mammalian epithelial cells; it is at the TJ where the Par3/Par6/aPKC and Crb/Pals1/PATJ complexes reside (Fig. 1). On the other hand, mDlg and mLgl have been demonstrated to localize along the lateral membrane below the TJ (42, 53). Scrib/Vatul also localizes to the lateral membrane; however, the precise localization with respect to the TJ and AJ has yet to be determined (55).
Par3/Par6/aPKC has been shown by several groups to be important during TJ formation and epithelial polarization. Biochemical data suggest that aPKC serves as an adapter protein as Par3 and Par6 bind to the kinase domain and NH2 terminus of aPKC, respectively (32, 33, 45, 73). Par6 contains a single PDZ domain, whereas Par3 bears three PDZ domains, the first of which binds to the extreme COOH terminus of JAM (17, 30). The localization of Par3, Par6, and aPKC to TJs is interdependent (59). At least in mammalian epithelia, members of the small GTPase family associate with this complex via Par6 (33, 45). Specifically, activated CDC42 and Rac directly interact with the CDC42/Rac interactive binding domain of Par6, which lies just NH2 terminal to the PDZ domain. Overexpression of Par3, Par6, aPKC, and CDC42 mutants negatively regulates the initial formation of TJs but does not affect TJ maintenance (22, 33, 54, 67, 73, 85). For instance, overexpressing the Par6ΔN protein, which lacks the ability to bind aPKC, delays TJ formation. Conversely, overexpression of a kinase null aPKC mutant negatively regulates TJ biogenesis and causes basolateral markers to mislocalize to the apical surface. Furthermore, expression of a truncated Par3 (1–371), which is able to bind JAM but not aPKC or Par6, delays TJ formation. This suggests that aPKC is an important player during assembly of TJ strands and apicobasal polarization (59). The importance of aPKC activity has also been demonstrated by analyzing zebrafish that carry mutations in heart and soul (has), the gene encoding an aPKC (28). Clearly, the regulated phosphorylation of proteins by aPKC plays significant roles in these processes as protein phosphatase 2A has been recently shown to regulate aPKC activity and also TJ assembly (56). Currently, Par3 is the only known substrate for aPKC at the TJ. Interestingly, aPKC phosphorylates Ser827 of Par3, which lies within the aPKC binding site, and this modification destabilizes the Par3/aPKC association (54). The dynamic nature of this interaction plays a role in TJ formation as the Par3S827A mutant, but not wild-type Par3, acts as a dominant negative protein that disrupts TJ assembly. The identification of other downstream targets of aPKC should shed more light on the mechanisms by which Par3/Par6/aPKC orchestrates TJ biogenesis and epithelial cell polarization. It should also be noted that, as in invertebrates, the function of this complex is not confined to epithelia. Par3/Par6/aPKC is involved in establishing polarity in astrocytes and hippocampal neurons (19, 70). In Drosophila epithelia, Baz/Dm-Par6/DaPKC and Crb/Sdt/Dlt colocalize to the subapical region. Similarly, the Par3/Par6/aPKC and Crb/Pals1/PATJ complexes target to TJs in mammalian epithelial cells (Fig. 1). It should be noted that whereas only one Crb isoform is expressed in Drosophila, three Crb isoforms (CRB1, CRB2, and CRB3) are predicted to exist based on the recently sequenced human genome (44, 78). CRB1 is primarily expressed in the brain and retina and, when exogenously expressed in Madin-Darby canine kidney (MDCK) cells, localizes to TJs (14, 15, 66). However, attempts to detect endogenous CRB1 protein in epithelia have not been successful, suggesting that CRB1 is not the predominant Crb protein in epithelia. In contrast, CRB3 protein is found predominantly in epithelium-rich tissues and in the MDCK epithelial cell line (46). Unlike CRB1, however, CRB3 targets not only to TJs but also to the apical surface. The expression pattern of CRB2 remains unknown. While CRB1 and CRB2 contain numerous extracellular EGF-like repeats and laminin A G-like domains, CRB3 bears a short extracellular region that is N-glycosylated (46). Nevertheless, all Crb proteins contain a conserved transmembrane segment and an intracellular 37-amino acid tail (49).

There are two recognized domains contained within the cytoplasmic tail of Crb isoforms: a juxtamembrane consensus motif predicted to bind proteins of the band 4.1/ezrin/radixin/moesin (FERM) superfAMILY and a PDZ binding motif at the extreme COOH terminus (31, 49). Drosophila Crb binds to d-moesin via the FERM binding motif and the PDZ domain of Sdt via the COOH-terminal ERLI sequence (3, 27, 50). Similarly, we have shown that the ERLI motifs of CRB1 and CRB3 are capable of binding to Pals1, the PDZ domain of the mammalian Sdt homologue (46, 66). Pals1 consists of multiple protein-protein interaction domains besides its PDZ domain (34, 66). The NH2-terminal unknown 1 and two L27 domains precede the PDZ domain, whereas the SH3, 4.1B, and GUK domains follow it. The NH2-terminal L27 (L27N) domain of Pals1 interacts with the NH2-terminal MAGUK recruitment (MRE) domain of PATJ (Fig. 1). We have also observed that Pals1 and multiple PDZ domain protein 1 (MUPP1), the PATJ parologue, also associate in a similar manner (66). The L27 and MRE domains are also conserved in Sdt and Dlt, respectively. We were able to demonstrate that the L27-MRE mode of interaction could mediate the direct association between Sdt and Dlt (66). Interestingly, on closer examination of the sequences and predicted secondary structures of the MRE domains, it has become clear that MRE domains belong to the L27 family of protein interaction domains (12). In fact, according to the Simple Modular Architecture Research Tool database (http://smart.embl-heidelberg.de/), MRE domains are considered to be L27 domains.

The Pals1/PATJ interaction is required for localizing Pals1 to TJs as Pals1 lacking its L27N domain mislocalizes apically (66). On the other hand, this interaction is not sufficient for the targeting of PATJ itself (65). These data are consistent with our observations that an NH2-terminal fragment of PATJ (residues 1–238) displays a diffuse localization pattern, and endogenous Pals1 is mislocalized in these cells (66). Furthermore, the extreme COOH termini of ZO-3 and claudin 1 associate with the sixth and eighth PDZ domains of PATJ, respectively. However, this ZO-3-PATJ interaction is crucial for targeting PATJ to TJs, suggesting that ZO-3 could serve to tether the CRB3/Pals1/PATJ complex to TJs (65). Coincidentally, MUPP1, a PATJ parologue that can also bind Pals1, has also been...
shown to localize to TJs (25, 63). The ninth and tenth PDZ domains of MUPP1 bind to the COOH termini of JAM and claudin 1, respectively (25). Thus it is conceivable that a CRB3/Pals1/MUPP1 complex could exist at TJs; however, this has yet to be directly examined to date.

The role of the Crb complex in Drosophila epithelial polarization has been intensively studied during the past decade. The similarities of localization patterns between the fly Crb/Sdt/Dlt and mammalian CRB3/Pals1/PATJ complexes suggest that they serve similar functions. However, what exactly are the functions of the recently identified CRB3/Pals1/PATJ complex in vertebrate epithelia? Studies using zebrafish demonstrate the importance of Pals1-containing complexes during epithelial polarization. Recently, the gene product of *nagie oko* (*nok*) has been identified as a Pals1 homologue that plays an essential role during the morphogenesis of two polarized cell types in the retina, neuroepithelial and photoreceptor cells (81). Polarization and junctional assembly can also be conveniently monitored in mammalian cell lines using the calcium-switch method (64). When grown in low-calcium media, MDCK cells do not form adhesive contacts. However, when calcium is replenished in the culture medium, MDCK cells do not form adhesive contacts. Moreover, overexpression of CRB3 at the apicolateral membrane. This is reinforced by our recent finding that the CRB3/Pals1/PATJ and Par3/Par6/aPKC complexes physically interact via the direct association of Par6 with Pals1 (29).

MDCK monolayers composed of cells expressing PATJ (1–238) or overexpressing CRB3 exhibit delayed TJ biogenesis although continuous TJs eventually form. This is likely attributable to the mislocalization of Pals1 and members of the Par3/Par6/aPKC complex in these cells (29). The importance of Pals1 in TJ assembly has also been investigated with respect to CRB3. Specifically, overexpression of CRB3 at the plasma membrane negatively regulates TJ formation in a manner dependent on the Pals1 PDZ-binding ERLI motif (Roh and Margolis, unpublished observations). Furthermore, disruption of endogenous Pals1-CRB3 interaction also leads to similar effects on TJ assembly (Fan S and Margolis, unpublished observations). Thus our data seem to suggest that this complex, similar to Par3/Par6/aPKC, not only resides at TJs but also regulates TJ assembly at the apicolateral membrane. This is reinforced by our recent finding that the CRB3/Pals1/PATJ and Par3/Par6/aPKC complexes physically interact via the direct association of Par6 with Pals1 (29).

MDCK monolayers composed of cells expressing PATJ (1–238) or overexpressing CRB3 exhibit delayed TJ biogenesis. Furthermore, in CRB3-overexpressing MDCK cells subjected to the calcium-switch protocol, the localization of CRB3 and two apical markers, ezrin and gp135, is seen to extend into the lateral plasma membrane domain during early polarization (Roh and Margolis, unpublished observations). Nonetheless, there were no significant disruptions in the overall apicobasal polarity axis in these monolayers. Monolayers of cells expressing PATJ (1–238) also exhibit normal polarity as CRB3 and E-cadherin are localized to the apical and lateral membranes, respectively (29). This may be attributed to the presence of a free surface and cell contacts representing cues sufficient to establish polarity in monolayers.

An alternative method used to examine MDCK cell polarization involves culturing cells in collagen matrix. Under these conditions, the entire cell surface is in contact with an extracellular matrix. However, as successive rounds of cell division ensue, polarity is established in a stepwise fashion that results in the de novo formation of a continuous apical membrane (58). Specifically, parental MDCK cells develop into three-dimensional cysts, in which cells surround a central lumen surrounded by the apical surface (Fig. 2). Meanwhile, the basolateral membrane is engaged in cell-cell and cell-matrix contacts. MDCK cells overexpressing PATJ (residues 1–238) do not develop into cysts but instead form multicellular aggregates where apical markers exhibit an abnormal distribution (Fig. 2). Similarly, cysts arising from CRB3 overexpressing cells also exhibit abnormal polarity; this phenotype requires the presence of the Pals1 PDZ binding motif (M. Roh. and B. Margolis, unpublished observations). Therefore, the mammalian Crb complex represents an evolutionarily conserved complex that plays an essential role during epithelial polarization.

Finally, homologues of Scrib, Dlg, and Lgl are also expressed in mammalian epithelia: Scrib/Vartul, mDlg (also referred to as SAP97), and mLgl, respectively. Recently, it was shown that mDlg/SAP97 directly associates with mLin-2/CASK, Dlg2, and Dlg3 and that these interactions influence the targeting of mLgl/SAP97 to the lateral membrane (35, 42). Furthermore, mLgl localizes in a similar manner (53). Interestingly, mLgl is capable of being serine phosphorylated, and this seems to be essential for preventing its localization to the apical surface (53). Consistent with this, mLgl coimmunoprecipitates with syntaxin-4, a N-ethylmaleimide-sensitive factor attachment receptor protein involved in trafficking of vesicles to the basolateral surface. However, it is not currently known whether mLgl interacts directly with either Scrib/Vartul or SAP97. Regardless, the evolutionarily conservation of these proteins in mammalian epithelia suggest that these proteins function cooperatively during the establishment of basolateral membrane identity during apico-basal polarization.

PERSPECTIVES

Here, we have discussed the genetic studies highlighting the functional importance of three complexes, each containing PDZ proteins during the polarization of invertebrate cells: Baz/Dm-Par6/DaPKC, Crb/Sdt/Dlt, and Scrib/Dlg/Lgl. Homologues of all nine proteins have been identified in vertebrate epithelial cells. Studies using the mammalian MDCK cell line as a model system have facilitated the biochemical characterization of the protein-protein interactions that stabilize the Par3/Par6/aPKC and CRB3/Pals1/PATJ complexes. Furthermore, recent work from our laboratory has demonstrated that these two protein complexes are conserved in regard to their subcellular localization.

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and that, at least in mammalian epithelia, they can exist as a single complex (29). Scrib, Dlg, and Lgl as well as their homologues colocalize to the lateral membrane in invertebrate and vertebrate epithelial cells, suggesting that these respective groups of proteins may form ternary complexes.

Studies using Drosophila as a model organism have shed light on the coordinated function of these three polarity complexes during invertebrate epithelial polarization (9, 76). In parallel, experiments using MDCK cells have provided insight into the functions of these complexes during the establishment of polarity in mammalian epithelia. The majority of these reports have focused on the role of Par3/Par6/aPKC during TJ assembly at the apical pole of the lateral surface. We have also demonstrated that the CRB3/Pals1/PATJ complex also regulates TJ assembly. This is highly reminiscent of the situation in Drosophila, where Baz/Dm-Par6/DaPKC and Crb/Sdt/Dlt are essential for the biogenesis of the ZA at the apex of cell contacts.

Models describing junction formation and apicobasal polarization in fly epithelia have been recently reported (9, 76). Based on those and our current knowledge of the three conserved PDZ protein polarity com-
plexes in mammals, we now propose a model regarding TJ assembly and mammalian epithelial polarization (Fig. 3). Establishment of polarity commences when two epithelial cells form initial cell contacts via E-cadherin and nectin (74). Nectin is involved in the recruitment of JAM to these early adhesive contacts (21). In turn, the Par3/Par6/aPKC can interact with JAM and be subsequently recruited to these early AJs (17, 30, 47, 72, 75). We have observed that Pals1 and PATJ are recruited to these premature AJs (Roh M, Fan S, and Margolis B, unpublished observations), consistent with our recent finding that Pals1 can bind directly to Par6. Therefore, the initial phase of polarization likely involves the recruitment of a variety of polarity proteins to initial sites of epithelial cell contacts. Intriguingly, CRB3 is predominantly intracellular during the formation of premature AJs and starts to appear later at the apical surface as Par3/Par6/aPKC and Pals1/PATJ are being recruited to early cell contacts in MDCK cells (Roh M, Fan S, and Margolis B, unpublished observations). This is consistent with Drosophila studies demonstrating that the Baz complex recruits Crb to the apical membrane.

As premature AJs become beltlike AJs, TJ proteins polymerize to form a continuous TJ at the apicolateral plane of the monolayer. This suggests that some molecular cue exists to ensure that TJs lie subjacent to the apical membrane. CRB3 could serve as a possible candidate because it binds directly to Pals1, which, in turn, associates with Par3/Par6/aPKC. The resulting supramolecular complex could then function to drive assembly of TJ strands in a single plane under the apical membrane. In support of this hypothesis, Drosophila geneticists have observed that the Baz and Crb proteins associate with each other along with Pals1 and PATJ, forming a supramolecular complex that is critical for TJ formation.

Table 1. Structure and function of mammalian and Drosophila polarity proteins

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<th>Protein</th>
<th>Drosophila Homologue</th>
<th>Structure/Function</th>
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<tr>
<td>Crumbs3 (CRB3)</td>
<td>Crumbs (Crb)</td>
<td>Transmembrane protein</td>
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<td>Pals1</td>
<td>Stardust (Sdt)</td>
<td>Apical determinant</td>
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<tr>
<td>PATJ</td>
<td>Discs Lost (Dlt)</td>
<td>Adapter protein</td>
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<tr>
<td>Par6</td>
<td>Dm-Par6</td>
<td>Multiple PDZ protein</td>
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<td>aPKC</td>
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<td>Par3</td>
<td>Bazooka (Baz)</td>
<td>Signaling molecule</td>
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<td>Scribble/Vartul</td>
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<td>Scaffolding protein</td>
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<td>mDlg</td>
<td>Discs Large (Dlg)</td>
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<td>Larvae (Lgl)</td>
<td>Scaffolding protein</td>
</tr>
</tbody>
</table>

PATJ, Pals1-associated tight junction; aPKC, atypical PKC; MAGUK, membrane-associated guanylate kinase; PDZ, PSD-95/Discs Large/zonula occludens-1.
complexes are important for the formation of a continuous ZA at the apicalolateral aspect of epithelial cell contacts. Furthermore, we have observed that disrupting the endogenous CRB3/Pals1 association significantly delays the formation of continuous TJs (Fan S and Margolis B, unpublished observations). Thus it is tempting to speculate about the role of CRB3 as a guide for the polymerization of TJ strands to form TJs at the apicalolateral plane of the epithelial monolayer. Finally, the formation and stabilization of TJs are essential for the maintenance of apicobasal polarity by preventing the intermixing of apical and basolateral membrane proteins and lipids.

In summary, significant progress has been made during the past several years in identifying the molecules essential for establishment of cellular asymmetry. However, this increased knowledge has spurred more questions, especially those centering on the actual mechanisms by which these proteins orchestrate cell polarization. Epithelial cells represent one system where the intricate mechanisms of PDZ protein function still remain largely elusive. Future studies will hopefully provide more insight into numerous issues, such as the signaling events that occur during the various phases of polarization; the way in which signaling influences the dynamic nature of protein-protein interactions within these polarity complexes; the identities of additional molecules that associate with these polarity complexes; and the exact roles of mammalian homologues of Scrib, Dlg, and Lgl and their role during junctional assembly and establishment of epithelial polarity. Answering some of these questions will make possible a more refined understanding of cell polarization.

We thank members of the Margolis laboratory for helpful discussions on this work. We apologize to those whose work is not described here due to space limitations.

DISCLOSURES

This work was partially supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-58208. M. Roh was supported by Medical Scientist Training Program Grant T32 GM-07863 and Genetics Predoctoral Training Program Grant T32 GM-0754024 to the University of Michigan. B. Margolis is an investigator of the Howard Hughes Medical Institute.

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AJP-Renal Physiol • VOL 285 • SEPTEMBER 2003 • www.ajprenal.org


