PDZ proteins retain and regulate membrane transporters in polarized epithelial cell membranes

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Brône, Bert, and Jan Eggermont. PDZ proteins retain and regulate membrane transporters in polarized epithelial cell membranes. Am J Physiol Cell Physiol 288: C20–C29, 2005; doi:10.1152/ajpcell.00368.2004.—The plasma membrane of epithelial cells is subdivided into two physically separated compartments known as the apical and basolateral membranes. To obtain directional transepithelial solute transport, membrane transporters (i.e., ion channels, cotransporters, exchangers, and ion pumps) need to be targeted selectively to either of these membrane domains. In addition, the transport properties of an epithelial cell will be maintained only if these membrane transporters are retained and properly regulated in their specific membrane compartments. Recent reports have indicated that PDZ domain-containing proteins play a dual role in these processes and, in addition, that different apical and basolateral PDZ proteins perform similar tasks in their respective membrane domains. First, although PDZ-based interactions are dispensable for the biosynthetic targeting to the proper membrane domain, the PDZ network ensures that the membrane proteins are efficiently retained at the cell surface. Second, the close spatial positioning of functionally related proteins (e.g., receptors, kinases, channels) into a signal transduction complex (transducisome) allows fast and efficient control of membrane transport processes.

Differential of epithelial cells consists of two major steps: apicobasal polarization and asymmetrical protein expression. The first process leads to the delineation of apical and basolateral membrane domains that are separated by tight junctions (63). The second step entails the asymmetrical sorting of proteins to the polarized membrane domains (45). During the past few years, it has emerged that PDZ proteins—their name derives from the first three proteins in which PDZ domains were identified: the postsynaptic density protein PSD-95/SAP-90, the Drosophila septate junction protein Disc-large DLG, and the epithelial tight junction protein zonula occludens (ZO)-1 (30)—are crucial components in both processes (for review, see Refs. 63, 64). In this review, we focus on the role of PDZ proteins in the polarized expression and function of membrane transporters (e.g., ion channels, exchangers, cotransporters) in epithelial cells, mainly of renal origin. Interestingly, the apicobasal differentiation process in epithelial cells shares some striking similarities with the axodendritic polarization in neurons, among which is the involvement of a common set of PDZ proteins (26, 64).

Correct surface expression of membrane proteins in a polarized epithelial cell is a multistep process (45) involving 1) biosynthetic sorting from the trans-Golgi network to the correct plasma membrane domain (apical or basolateral), 2) anchoring and/or retention at the plasma membrane, and 3) endocytosis followed by either recycling to the plasma membrane or targeting for lysosomal degradation or delivery to the opposing membrane domain (i.e., transcytosis). As discussed below, PDZ proteins have been implied in one or more of these steps for both apical and basolateral membrane proteins.

PDZ domains, proteins, and ligands

The cellular structure and function strongly depend on the coordinated cooperation of proteins. At the molecular level, protein-protein interactions are driven by structurally conserved domains that specifically recognize peptide ligands in other proteins (55). The most abundant protein interaction domains in metazoan organisms are the PDZ domains (58). The primary function of PDZ domains is to recognize and bind to specific approximately five-amino acid motifs that occur at the COOH terminus of target proteins. Depending on the target sequence, PDZ domains are traditionally classified as class I domains, which recognize the consensus sequence $X_{-3}^{3}$ (S/T)$_{-2}X_{-1}^{1}-\phi_{0}$ ($X$ = any amino acid; $\phi$ = hydrophobic amino acid; $-3$, $-2$, $-1$, and 0, the position relative to the COOH-terminal residue) and in class II domains (target sequence: $X_{-3}^{3}-\phi_{2}X_{-1}^{1}-\phi_{0}$) (22). Thus amino acids at positions 0 (the COOH-terminal amino acid) and −2 are critical determinants for PDZ domain binding. The relatively simple architecture of the ligand is such that a single PDZ domain can bind to proteins with (slightly) different PDZ ligands. In addition, a growing number of PDZ domains have been shown to recognize internal PDZ-binding motifs, so-called β-fingers that structurally mimic a COOH-terminal end (21). The structural basis of peptide binding and the classification of PDZ domain-
containing proteins (simply called “PDZ proteins” in the following text) have been reviewed in two publications (22, 72). On the basis of their structure, PDZ proteins can be divided into three major groups: 1) PDZ-only proteins containing one or more PDZ domains; 2) membrane-associated guanylate kinase (MAGUK) domain-containing PDZ proteins; and 3) the largest group, harboring PDZ proteins that contain other protein-interacting domains, e.g., SH3 domains, PH domains, and L27 domains, in addition to their PDZ domains. Examples of every group can be found in Fig. 1. The functional importance of this structural classification is that the latter two classes of PDZ proteins can easily participate in higher-order protein networks because of their multiple binding sites.

**PDZ Proteins Involved in the Apical Expression and Function of Membrane Proteins**

In the past few years, a complex network of PDZ interactions involved in the apical expression of membrane proteins has emerged (47). Much of this work has been performed in kidney (e.g., proximal tubule), airways, and intestine and has heavily focused on the effects of PDZ proteins on the expression and function of \( \text{Na}^{+}/\text{H}^{+} \) exchanger type 3 (NHE3), cystic fibrosis transmembrane conductance regulator (CFTR), and \( \text{Na}^{+}/\text{H}^{+} \)/\( \text{Pi}^{2-} \) cotransporter IIa (Npt2 or NaPi IIa). NHE3 (82) and Npt2 (48) are ion transporters in the apical membrane (brush border) of the renal proximal tubule, whereas CFTR is an apical \( \text{Cl}^{-} \) channel in multiple exocrine tissues (e.g., airway, intestine, pancreas, sweat gland) (67). All three transporters contain a prototype COOH-terminal class I PDZ ligand (NHE3: \(-S_{3-T_{2-H_{1}-M_{0}}-0};\) Npt2: \(-A_{3-T_{2-R_{1}-L_{0}}-0};\) CFTR: \(-D_{3-T_{2-R_{1}-L_{0}}-0};\)), which allows them to interact with a variety of PDZ proteins. As reviewed below, these PDZ interactions drastically influence the localization and function of the transporters.

**GOPC favors the intracellular retention and degradation of CFTR.** GOPC (Golgi-associated PDZ coiled-coil protein (78), also called PIST [PDZ domain protein interacting specifically with TC10 (50)], CAL [CFTR-associated ligand (8)] and FIG (fused in glioblastoma (7))] is a scaffolding protein with two predicted coiled-coil domains and one PDZ domain (Fig. 1) that binds to the COOH terminus of several membrane proteins, including CFTR (8), frizzled (a plasma membrane receptor) (78), and CIC-3B (an intracellular \( \text{Cl}^{-} \) channel) (15).

Endogenous and heterologously expressed GOPC is primarily located at the Golgi apparatus and the trans-Golgi network (TGN) (7, 8). Apparently, GOPC associates indirectly with the Golgi by binding to syntaxin 6 via its second coiled-coil domain (7). Syntaxin 6 is a SNARE (soluble \( N \)-ethylmaleate-
Imide-sensitive factor-activating protein receptor) protein localized in TGN and endosomes and is involved in vesicular transport between TGN and endosomes (2, 34). However, GOPC is also found in the plasma membrane when coexpressed in COS-7 cells with the plasma membrane protein frizzled (78) or with a dominant-negative dynamin 2 that blocks clathrin-mediated endocytosis (9). This suggests that GOPC undergoes dynamic trafficking between the Golgi apparatus and the plasma membrane.

There is strong evidence that overexpression of GOPC interferes with the surface expression of CFTR. Indeed, overexpression of GOPC (or CAL) in CFTR-expressing COS-7 cells reduces cAMP-activated Cl− currents by decreasing the number of channels in the plasma membrane. The reduced surface expression of CFTR is caused both by a decreased rate of insertion in the plasma membrane and by a decrease in plasma membrane half-life of CFTR (8). Of note, CFTR surface expression is restored to normal when GOPC and NHERF-1, an apical PDZ protein (see NHERF-1 and -2 are involved in signaling complex assembly anchoring in the apical membrane), are coexpressed. Furthermore, Cheng et al. (9) showed that GOPC does not affect biosynthetic maturation of CFTR in the Golgi apparatus but that it targets mature CFTR for lysosomal degradation. Interestingly, the negative effects of GOPC on CFTR are counteracted by coexpression with dominant-negative dynamin 2, in which case GOPC is trapped at the cell periphery together with the dominant-negative dynamin 2. More evidence of a role for GOPC in modulating protein expression stems from work on CIC-3, an intracellular Cl− channel that probably serves as an electrical shunt facilitating acidification of intracellular organelles by V-type H+ pumps. Different splice isoforms of the CIC-3 have been identified (51). CIC-3A does not exhibit a PDZ ligand and is located in late endosomes. In contrast, CIC-3B primarily resides in the Golgi apparatus and is identical to CIC-3A, except for its COOH terminus, which contains a class I PDZ ligand (S3T2T1L0) that interacts with GOPC as well as NHERF-1 (15, 51). Overexpression of GOPC has a dual effect on CIC-3B (15). First, CIC-3B becomes heavily concentrated in the Golgi region colocalizing with GOPC. Second, it drastically reduces the expression level of CIC-3B, suggesting that it promotes degradation of CIC-3B.

To conclude, GOPC is a Golgi/TGN-based PDZ protein that reduces the apical membrane expression of CFTR. Although its mode of action is not fully understood, one can think of two mechanisms that are not mutually exclusive (9). One possibility is that GOPC interferes with the biosynthetic sorting of CFTR at the level of TGN. By binding to CFTR, it may prevent CFTR from being included in secretory transport vesicles destined for the plasma membrane and redirect CFTR toward the endosomal/lysosomal compartment. Alternatively, GOPC may affect the endocytic recycling of CFTR by promoting the lysosomal targeting of endocytosed CFTR. Regardless of the mechanism, the net outcome would be enhanced lysosomal degradation of CFTR and, consequently, reduced plasma membrane expression. However, it remains to be established that the GOPC effects on CFTR are physiologically relevant rather than an artifact due to heterologous overexpression. An intriguing question in the case of physiological relevance is how CFTR normally escapes the negative regulation by GOPC. One explanation could be competition between GOPC and other PDZ proteins that favor plasma membrane expression of CFTR (e.g., NHERF-1); another possibility is a dynamic interaction between GOPC and CFTR due to regulation of the PDZ binding (e.g., phosphorylation).

NHERF-1 and -2 are involved in signaling complex assembly and anchoring in the apical membrane. Na+/H+ exchanger regulatory factors (NHERF) are PDZ proteins that associate with the apical brush-border membrane in polarized epithelial cells. NHERF-1 was originally cloned as an essential cofactor for the protein kinase A (PKA)-mediated inhibition of NHE3 in renal proximal tubule cells (66, 77). The human ortholog of NHERF-1 is also known as EBP50 (ezrin-radixin-moesin-binding phosphoprotein-50) (62). NHERF-2, also known as E3KARP, is a structurally and functionally related isoform (81). In mouse proximal tubule cells, NHERF-1 is strongly expressed in microvilli, whereas NHERF-2 can be detected weakly in the microvilli but is predominantly expressed at the microvillar basis in the vesicle-rich domain (73). NHERF-1 and -2 contain two tandem PDZ domains (Fig. 1) that can bind to a long list of ion channels (CFTR, ROMK K+ channels), ion transporters (NHE3, Npt2), and receptors (β2-adrenergic receptor; see Fig. 2) with a relative degree of specificity for binding to the first or the second PDZ domain (75). NHERF proteins also contain at their COOH terminus an ezrin-radixin-moesin (ERM)-binding domain that interacts with ezrin, a scaffolding protein associated with the actin cytoskeleton. In addition, ezrin functions as an A kinase anchoring protein (AKAP); it interacts with the regulatory subunit (RII) of PKA, thereby recruiting PKA to the PDZ complex. The multivalent properties of NHERF (PDZ1, PDZ2, ERM-binding domain) allow the formation of macromolecular complexes that anchor membrane proteins to the apical actin cytoskeleton. Typical examples include the NHE3-NHERF-1-erzir and Npt2-NHERF-1-erzir complexes attached to the actin cytoskeleton in the microvilli of proximal tubule cells in the kidney (73). Another example of the scaffolding properties of NHERF is found in airway and intestine epithelial cells with the formation of apical CFTR-NHERF-1-erzir or CFTR-NHERF-2-erzir complexes (49, 69). Finally, it should be mentioned that although NHERF-1 and -2 share overlapping expression and binding profiles, they do seem to fulfill unique properties with respect to various membrane proteins, possibly due to the partly different subcellular localization (73).

How do these NHERF-based complexes affect function and expression of apical membrane transporters? In general, one can discern three modes of action: 1) formation of transducersome complexes for efficient signal transduction, 2) enhancement of apical membrane expression, and 3) direct modulation of the transporters.

First, because NHERF are multivalent scaffolds, they can assemble functionally connected proteins (receptors, kinases, transporters) into a spatially restricted unit (so-called transducersome), which allows tight control and efficient activation/inhibition of membrane transport processes. For example, the basal Na+/H+ exchange activity in brush-border membranes of renal proximal tubule cells is indiscernible between wild-type and NHERF-1-deficient mice. However, the cAMP-dependent inhibition of NHE3 and the concomitant cAMP-stimulated phosphorylation of NHE3 are observed only in NHERF-1-expressing preparations (76). In addition, the stimulating effect of glucocorticoids on NHE3 activity is seen only in the pres-
Intracellular retention, degradation
Surface expression

Fig. 2. Putative interaction pattern of PDZ proteins involved in apical expression of membrane proteins in epithelia. The PDZ domain-containing proteins (shown in ovals) interact with various membrane transporters (rounded rectangles) and membrane receptors (rectangles). Additional links to signaling or cytoskeletal molecules are shown. The dashed lines show mutual interactions between PDZ proteins. The shaded circles and the corresponding arrow indicate a putative common role for PDZ interactions in the subcellular location of the transporters. β2AR, β2-adrenergic receptor (20, 49, 69); CFEX, Cl− formate exchanger (17); ClC3B, Cl− channel type 3 splice form B (15); CFTR, cystic fibrosis transmembrane conductance regulator (8, 49, 69); D-AKAP2, dual specific A-kinase anchoring protein 2 (16); ezrin (see Refs. 49, 69); GOPC, Golgi-associated PDZ and coiled-coil motif-containing protein (9, 15); MAP17, membrane-associated protein (59); NHE3, Na+/H+ exchanger (17, 73, 77); NHERF, Na+/H+ exchanger regulatory factor (17); OCTN1, organic cation transporter (17); P2Y1, purinergic receptor (20); PDZK1, PDZ containing 1 (17); PKA, protein kinase A; PTH1R, parathyroid hormone receptor (20, 41); ROMK, renal outer medullary K channel (79); SGK1, serum- and glucocorticoid-induced protein kinase 1 (80); SR-BI, scavenger receptor type B1 (33); URAT1, renal specific urate anion exchanger (17).

ence of NHERF-2 (80), which recruits the serum- and glucocorticoid-induced protein kinase 1 (SGK1) in a PDZ-dependent interaction to the apical surface in close proximity to NHE3. Remarkably, this activation could not be duplicated by NHERF-1 (80). These results indicate that phosphorylation of NHE3 occurs in a local complex in which the NHERF scaffold efficiently approximates the kinases and their substrate NHE3. Similarly, the CFTR-NHERF complex facilitates channel activation by cAMP/PKA. Indeed, the CFTR-NHERF complexes contain not only PKA (recruited via ezrin) but also, in airway epithelial cells, the β2-adrenergic receptor (β2AR), which binds to NHERF-1 via its COOH-terminal PDZ ligand (49, 69). Taken together, the NHERF-ezrin scaffold assembles a macromolecular complex comprising β2AR, PKA, and CFTR in which the close proximity between receptor, kinase, and ion channel promotes efficient regulation of CFTR Cl− currents by β2-agonists. Thus one function of NHERF-based protein complexes is the formation of multiprotein signaling complexes (transducisomes) in apical membranes.

Second, NHERF proteins also affect membrane expression and localization of apical proteins. This is most clearly illustrated for Npt2, which normally resides in the apical brush-border membrane of renal proximal tubule cells. However, in kidneys from NHERF-1−/− mice, Npt2 is redistributed to intracellular vesicles, thereby reducing Npt2 expression in the apical membrane (65). Consistent with the altered Npt2 localization, NHERF-1−/− mice display a renal phosphate wasting phenotype. Interestingly, NHERF-1 is not required for apical localization of NHE3 in the proximal tubule, because NHE3 was expressed at normal levels in the apical brush-border membrane of NHERF-1−/− kidneys (65). In contrast, NHERF-2 seems to affect the surface expression of NHE3 in a dual way. NHERF-2 mediates the glucocorticoid and lysophosphatic acid-induced increase in NHE3 activity by stimulating exocytosis and plasma membrane delivery of NHE3 (37, 80). On the other hand, elevated intracellular Ca2+ concentrations diminish NHE3 activity and membrane expression through oligomerization and endocytosis of NHE3, which requires the formation of an NHERF-2-NHE3-α-actinin-4 complex (31). These data indicate that NHERF isoforms in epithelial cells play a crucial role in the apical positioning of some (but clearly not all) PDZ ligand-containing membrane proteins. However, many of the underlying mechanisms remain to be resolved. Is NHERF-1 required for apical sorting of Npt2, or does it interfere with the anchoring or endocytic turnover of Npt2 at the apical membrane? The latter possibility is an attractive hypothesis, because a major regulatory step for Npt2 expression in the apical membrane is endocytosis followed by either recycling to the plasma membrane or sorting to lysosomes for degradation (48). In addition, such a mechanism would be consistent with the evidence that NHERF-1 promotes endocytic recycling of CFTR. Another question prompted by these studies is what determines the apical localization of NHE3 in the absence of NHERF-1. Is there functional redundancy between PDZ proteins, and can NHERF-2 and/or PDZK1 substitute for NHERF-1? Consistent with this possibility is the recent observation that in mouse proximal tubules, both NHERF-1 and NHERF-2 interact with NHE3, whereas only
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NHERF-1 associates with Npt2 (73). Alternatively, PDZ-independent mechanisms, e.g., direct association with the apical cytoskeleton, could be involved in the apical sorting and/or retention of NHE3.

Whether PDZ-based interactions are also required for the apical expression of CFTR is a controversial issue. The PDZ ligand of CFTR was initially proposed by Stanton and colleagues (46, 47) to be an apical targeting signal because a truncation mutant of CFTR lacking the three COOH-terminal amino acids (CFTR-ΔTRL) had lost its apical positioning in Madin-Darby canine kidney (MDCK) cells and human airway epithelial cells and distributed nearly equally in the apical and basolateral membrane. Because deletion of the PDZ ligand also abolishes CFTR binding to NHERF-1, it was concluded that apical polarization of CFTR requires an interaction with NHERF-1 (47). In a subsequent study, Stanton and colleagues (70) showed that there is no difference in the initial polarized targeting between newly synthesized wild-type CFTR and CFTR-ΔTRL when stably transfected in MDCK cells. In contrast, Lukacs and colleagues (1) showed that disruption of the CFTR-NHERF complex (by COOH-terminal epitope tagging of CFTR, COOH-terminal deletion of CFTR, or overexpression of a dominant-negative NHERF mutant) does not interfere at all with the apical localization of CFTR in polarized epithelia derived from the trachea, pancreatic duct, intestine, and distal tubule of the kidney. However, a common point in the observations of both groups is that the COOH-terminal PDZ ligand does not function as a sorting signal for the (initial) biosynthetic targeting of CFTR from the trans-Golgi network to the apical membrane.

Still, controversy exists about the role of PDZ proteins in the apical expression of CFTR. Deletion of the PDZ ligand reduced the half-life of CFTR in the apical membrane, and it also diminished the recycling of endocytosed CFTR back to the plasma membrane (70). It was therefore concluded that the PDZ ligand in CFTR functions as an apical endocytic recycling motif and that PDZ-based interactions, e.g., with NHERF-1, affect the endocytic turnover of CFTR by promoting the reinsertion of internalized CFTR in the plasma membrane (70).

In contrast, truncation of the PDZ ligand in the COOH terminus of CFTR did not affect the apical expression when membrane half-life was measured in pancreatic duct cells and baby hamster kidney (BHK) cells (1). However, a role for PDZ interactions in the “recycling” mechanism would also explain the functional antagonism between NHERF-1 and GOPC, with the former increasing apical positioning of CFTR by promoting endocytic recycling and the latter decreasing apical positioning by promoting lysosomal targeting of endocytosed CFTR (8).

Furthermore, similar experiments on the endocytic turnover of G-protein-coupled receptors suggest that the postendocytic sorting step could be a key action point for NHERF-1. Recycling to the plasma membrane of endocytosed β2AR depends on an intact PDZ ligand in the β2AR COOH terminus (-D-3-S-2-S-1-L0) and requires an interaction between the receptor and NHERF-1 (5). Moreover, transfection of the COOH-terminal PDZ ligand of β2AR (-D-3-S-2-S-1-L0) to the δ-opioid receptor changes the postendocytic sorting of the opioid receptor. The endocytosed mutant δ-opioid receptor is rapidly recycled to the plasma membrane instead of being targeted to the lysosomal compartment for degradation as found in the wild-type δ-opioid receptor (14).

Finally, the accumulation of Npt2 in intracellular vesicles in NHERF-1-deficient proximal tubule cells (65) is also consistent with a role for NHERF-1 in the recycling of internalized Npt2 to the brush-border membrane. To conclude, there are several lines of evidence indicating that PDZ-based interactions with NHERF-1 promote surface expression of a variety of membrane proteins (CFTR, Npt2, β2AR), possibly by affecting the postendocytic sorting step. However, it should be kept in mind that in several experimental systems, NHERF-1 plays only a minor role or no role in the apical localization of CFTR (1).

A third mode of interaction between NHERF proteins and apical transport proteins is direct modulation of the transport process. For example, addition of recombinant NHERF-1 containing the two PDZ domains (PDZ1-PDZ2) to excised membrane patches from a lung submucosal gland cell stimulates the open probability of CFTR single channels (61). This effect requires the presence of two intact PDZ domains in a single peptide and can be competed by the addition of single PDZ domains. In addition, PKC phosphorylation of the second PDZ domain in NHERF-1 disrupts the PDZ2-CFTR interaction and precludes the stimulatory effect of NHERF-1 on CFTR open probability (60). One interpretation of these experiments is that the functional CFTR channel is a homodimeric complex and that multivalent PDZ proteins such as NHERF-1 promote or stabilize the homodimeric conformation by capturing the COOH termini of two CFTR molecules.

PDZK1 is a docking station for apical membrane proteins. PDZK1 (also called CAP70 and NaPi-Cap1) is a PDZ-only protein containing four PDZ domains and is expressed in kidney, intestine, and liver (18, 32). Renal expression of PDZ1 is restricted to the proximal tubule, where it localizes to the apical brush-border membrane (microvilli and intermicrovillar clefts) (18). Interestingly, PDZK1 seems to be devoid of an intrinsic apical sorting signal because apical positioning requires the interaction with other proteins such as MAP17, an apical membrane protein that forms a PDZ-dependent complex with PDZK1 (59).

Murer and colleagues (16, 17, 25) have proposed that in proximal tubule cells, PDZK1 and NHERF-1 form an extended network underneath the brush-border membrane that serves as a docking station for apical proteins. Because of its multivalent binding properties, PDZK1 is a crucial stabilizer of this protein network. First, because of its four PDZ domains, PDZK1 can simultaneously bind several membrane transporters via their COOH-terminal PDZ ligands: Npt2 (NaPi, IIa), the solute carrier SLC17A1 (NaPi, I), NHE3, the organic cation transporter (OCTN1), the Cl- formate exchanger (CFEX), and the urate anion exchanger (URAT1) (17). Second, PDZK1 forms heteromeric complexes with NHERF-1 (17), which anchors the network to the apical cytoskeleton. In addition, several types of signaling proteins are recruited by the PDZK1/NHERF-1 scaffold. G-protein-coupled receptors, such as the purinergic P2Y1 receptor or the parathyroid hormone I receptor (PTHIR) receptor, bind to NHERF via a PDZ-based interaction (20, 41). Furthermore, PKA is positioned in the apical network via various AKAP such as ezrin and D-AKAP2, which binds to PDZK1 (16). So, PDZK1, in combination with NHERF, establishes an extensive network of transport and signaling proteins beneath the brush-border membrane of renal proximal tubule cells, but further exploration of the precise physiological relevance of the NHERF-1/PDZK1 is required.
Finally, it should be mentioned that other functions have been attributed to PDZK1. First, similar to what has been described for NHERF-1, addition of recombinant PDZK1 increases the open probability of CFTR single channels (74). This stimulation depends on an intact PDZ ligand in CFTR and on the presence of two functional PDZ domains (PDZ3-PDZ4) in PDZK1, which is consistent with the hypothesis that multivalent PDZ proteins such as PDZK1 and NHERF-1 favor a more active dimer configuration of CFTR (61, 74). Second, expression of the scavenger receptor class B type I (SR-BI) in hepatocytes critically depends on PDZK1 (33). PDZK1–/– mice show strongly reduced levels of hepatic SR-BI and consequently have increased plasma levels of high-density lipoprotein particles.

**PDZ Proteins Affecting Basolateral Expression of Membrane Proteins**

An evolutionary conserved PDZ protein complex associates with the basolateral membrane. Research on the LET-23 epithelial growth factor receptor (EGFR) of the nematode *Caenorhabditis elegans* revealed an important role of the heterotrimeric PDZ complex Lin-10-Lin-2-Lin-7 in the basolateral localization of the LET-23 receptor in vulval precursor cells. In these processes, Lin-7 accounts for the binding to LET-23 via a PDZ-based interaction (for review, see Refs. 29, 64). Three laboratories (3, 4, 29) independently discovered that the mammalian homologs of Lin-10, Lin-2 and Lin-7 interact with each other and can be found in an evolutionary conserved, tripartite complex. The three mammalian homologs of Lin-10 are called Munc-interacting proteins (Mint1–3) for their interaction with the synaptic protein Munc18-1 or X11 (X11α-γ) (52, 53). The mammalian Lin-2 homolog CASK is a MAGUK protein and was found to bind to neurexins, a family of neuronal cell surface molecules (24). Three mammalian Lin-7 counterparts were identified and were given various names: mLin7a, mLin7b, and mLin7c for mammalian Lin-7 (3, 27); Veli-1, Veli-2, and Veli-3 for vertebrate Lin-7 (4); and MAL1S1, 2, and 3 for mammalian Lin-7 (28, 43). For simplicity, we refer to them as Veli-1, Veli-2, and Veli-3.

At the core of the heterotrimeric PDZ complex is CASK, a MAGUK protein with multiple protein interaction domains (see Fig. 1), among which are two L27 domains, one PDZ domain, a calmodulin kinase (CaMK)-like domain, a protein 4.1-binding or Hook domain, and a guanylate kinase domain. L27 domains are conserved interaction modules that form heterodimers (and dimers of dimers) with L27 domains in other proteins, thereby providing the molecular basis for the formation of an extended protein network (13, 40). It has been shown for both the *C. elegans* and the rat proteins that the second L27 domain (L27C) in CASK heterodimerizes with the L27 domain in Veli, whereas the first L27 domain (L27N) forms a complex with the L27 domain of SAP97, another MAGUK PDZ protein (23, 36). In addition, CASK can bind to Mint1 via its NH2-terminal CaMK-like domain. However, CASK is unable to form complexes with either Mint2 or Mint3 because both Mint isoforms lack the critical interaction domain (42). In view of the different expression profiles of Mint1 (neuronal) and Mint3 (ubiquitous), this implies that heterotrimeric Veli-CASK-Mint complexes are found in the brain, where they are involved in synapse formation (64). In contrast, renal epithelial cells contain dimeric Veli-CASK complexes that are localized to the basolateral membrane (42, 68). Finally, the Hook domain connects CASK to protein 4.1, an actin-binding protein in the cortical actin cytoskeleton (10). In contrast, Velis are small proteins with a relatively simple structure; in addition to one L27 domain, with which they bind to CASK, they also contain one PDZ domain. Basolateral location of Veli in renal epithelia depends on an intact L27 domain, indicating that Veli is recruited to the basolateral membrane via binding to CASK (68). Thus, in epithelial cells, the Veli-CASK core complex forms a large basolateral protein network that is anchored to the actin cytoskeleton and that exposes protruding PDZ domains that are free to interact with other proteins. In addition, other PDZ proteins such as SAP97, a MAGUK protein, can be recruited to this complex (36).

Velis retain transporters in the basolateral membrane in renal epithelial cells. Velis are reported to bind via a PDZ-based interaction to several basolateral membrane transporters, such as the strong inward rectifier K+ channel (Kir2.3) and the epithelial γ-aminobutyric acid (GABA) transporter (BGT-1) (54, 57). Both proteins contain a bona fide class I PDZ ligand: -E−3-S−2-A−1-I0 (Kir2.3), -E−3-T−2-H−1-L0 (BGT-1). As in the apical expression process of membrane transporters, the PDZ ligand of these basolateral transporters is required for efficient retention in the basolateral membrane rather than for the initial sorting process to the basolateral membrane. First, BGT-1 with a truncated PDZ motif (deletion of the 5 COOH-terminal amino acids) is still targeted to the basolateral membrane of MDCK cells, but its surface expression is reduced because of increased internalization in an endosomal recycling compartment (57). Moreover, injection of a small synthetic peptide (17-mer) that corresponds to the BGT-1 COOH terminus and binds in vitro to Veli-1 also relocates BGT-1 from the basolateral surface to an intracellular vesicular compartment. These observations are therefore consistent with a Veli-1-dependent retention in the basolateral membrane, although a role for other PDZ proteins cannot be formally excluded. Second, Veli-2-mediated plasma membrane retention has also been reported for Kir2.3 channels expressed in MDCK cells (54). The cytosolic COOH terminus of the Kir2.3 protein contains two partially overlapping motifs: a biosynthetic sorting signal (amino acids 431–442) and a class I PDZ ligand (amino acids 442–445) (38). Deletion of the COOH-terminal 15 amino acids (431–445) or of amino acids 431–441 results in apical missorting of the channel (38). In contrast, selective deletion of the PDZ ligand (amino acids 442–445) causes channels to accumulate in intracellular vesicles that partially overlap with recycling endosomes (54). Furthermore, it was shown that Veli-2 and Kir2.3 interact in a PDZ-dependent way and that Kir2.3, Veli-2, and CASK colocalize at the basolateral membrane of the renal cortical collecting duct, which is consistent with the basolateral presence of a Kir2.3-Veli-2-CASK complex. A functional correlate of such a complex was obtained by coexpression of Kir2.3 and Veli-2 in *Xenopus* oocytes, which results in an increased K+ current due to a Veli-2-promoted membrane retention of Kir2.3 (54). A third line of evidence in favor of Veli-mediated basolateral membrane retention stems from work on the *Drosophila ShakerB* K+ channel (44). *ShakerB* contains a COOH-terminal class I
PDZ ligand (-E$_3$T$_2$D$_{1}$V$_{0}$) and, after expression in MDCK cells, is sorted to the basolateral membrane, where it forms a complex with Veli and CASK (44). Deletion of the COOH-terminal 45 amino acids disrupts the basolateral sorting. However, a mutant in which only the PDZ ligand is removed (deletion of the COOH-terminal 3 amino acids) is still sorted to the basolateral membrane, but this mutant again accumulates in intracellular vesicles. From these results, one can conclude that 1) the COOH-terminal PDZ ligands in BGT-1, Kir2.3, and ShakerB do not function as biosynthetic sorting signals for the basolateral membrane; 2) these membrane transporters are incorporated in a basolateral Veli/CASK complex; and 3) interaction with Veli promotes basolateral membrane retention of BGT-1, Kir2.3, and ShakerB.

Whether the basolateral Veli-CASK network is also required for the spatial assembly of signal transduction complexes is less clear. In neurons, PKA is recruited to the Veli-CASK complex via SAP97, a MAGUK protein that binds AKAP79/FRAP and, if so, what the functional consequences are.

OUTLOOK

The available data indicate that PDZ proteins play a crucial role in the localization and function of apical and basolateral membrane ion channels and transporters in epithelial cells. However, to fully appreciate the role of PDZ proteins, one should also consider the aspects discussed next.

Dynamics of PDZ Interactions and PDZ Complexes

As described above, Npt2 is part of an apical PDZK1/NHERF-1-based protein network that extends all along the microvillus of proximal tubule cells. Yet, after PTH stimulation, Npt2 is endocytosed via invaginations at the base of the microvilli (71). Thus, in PTH-stimulated proximal tubule cells, Npt2 proteins that are located at the microvillar tip must travel down the microvillus to the intermicrovillar cleft at the base. However, there is evidence that a PDZ network restricts the lateral mobility of bound membrane proteins (6). This prompts the question of whether Npt2 endocytosis requires prior disruption of the Npt2-PDZK1 and Npt2-NHERF-1 complexes so that Npt2 is free to move laterally in the brush-border membrane and, if so, how this disruption is triggered, e.g., by phosphorylation (see Regulation of PDZ Interactions). Alternatively, it has recently been shown for CFTR that PDZ interactions are dynamic and do not result in immobilization of CFTR in the plasma membrane (19).

Second, in MDCK cells, there is evidence for a dynamic interaction between Veli-1 and β-catenin that contains a class I PDZ ligand (-D$_3$T$_2$D$_{1}$L$_{0}$) (56). The progressive translocation of Veli-1 from the cytosol to the junctional domain during polarization of MDCK cells requires the presence of β-catenin in the junctional domain and depends on a PDZ-based interaction between Veli-1 and β-catenin. Furthermore, only Veli-1 proteins that have been translocated to the junctional domain are able to retain BGT-1 transporters in the basolateral membrane (also a PDZ-dependent effect). Because Veli-1 has only one PDZ domain, this implies that Veli-1 must first disengage from β-catenin before it can interact with basolateral membrane proteins such as BGT-1 or Kir2.3, but at present it is not known what regulates the Veli-1-β-catenin interaction.

Recent work on Veli-CASK-SAP97 complexes in brain also hints at a dynamic rearrangement of these PDZ complexes (39). The Kir2.2 K$^+$ channel has a COOH-terminal class I PDZ ligand (-E$_3$S$_2$E$_{1}$L$_{0}$), which binds to PDZ domains in Veli and SAP97. In neurons, Kir2.2 seems to be incorporated in two different PDZ complexes dependent on the partnering PDZ protein. Kir2.2 is bound in the first complex to SAP97, which also recruits CASK and the CASK-associated proteins Veli and Mint1. Kir2.2 is in the second complex attached to Veli-1 or -3, which then associates with CASK and the CASK-associated SAP97. It has been proposed that these two complexes serve different functions: the Kir2.2-SAP97-based complex would be involved in membrane trafficking of the channel, whereas the Kir2.2-Veli complex would be involved in membrane anchoring. This would require a rearrangement of the PDZ interactions whereby Kir2.2, upon arrival in the plasma membrane, is transferred from the PDZ domain of SAP97 to the PDZ domain of Veli-1 or -3 (39). However, at this time, it is not clear what regulates or triggers such a dynamic rearrangement. It remains to be established whether dynamic rearrangement of CASK/Veli complexes is also involved in the basolateral expression of membrane proteins in epithelial cells.

Regulation of PDZ Interactions

Because typical class I PDZ ligands contain a serine or threonine at position −2, PDZ ligands can in principle be phosphorylated, which opens the perspective for regulation of PDZ interactions. This was clearly shown for the interaction between β$_2$AR and NHERF-1, which is under the control of G protein-regulated kinase (GRK). GRK-mediated phosphorylation of serine at position −2 (Ser$^{411}$) in the COOH terminus of β$_2$AR precluded the NHERF-1-dependent recycling of the receptor to the plasma membrane (5). Moreover, a serine to aspartate mutation at position −2, which mimics the effect of phosphorylation, inhibited both complex formation with NHERF-1 and recycling of internalized mutant receptors. Similarly, serine phosphorylation of the Kir2.3 PDZ ligand (-ESAI) by PKA has been shown to disrupt Kir2.3 interaction with the neuronal PDZ protein PSD-95, which could potentially alter the postsynaptic anchoring of Kir2.3 or change the gating characteristics of the channel (11). Whether phosphorylation of the Kir2.3 PDZ ligand also occurs in renal epithelial cells and whether this would affect basolateral expression is still an open question.

Furthermore, phosphorylation outside the PDZ ligand has also been reported to modulate PDZ complex composition. For example, PKA phosphorylation of the CFTR R domain inhibited binding of CFTR to NHERF-1, but it had no effect on the β$_2$AR-NHERF-1 interaction (49). Clearly, phosphorylation-dependent PDZ interactions allow for variations in PDZ complexes as a function of specific extracellular stimuli or developmental stage. Consequently, regulation of PDZ interactions should be kept in mind when addressing the physiological modulation of transport proteins incorporated into a PDZ network.

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Spatial Limitations of a PDZ Network

A PDZ scaffold such as the NHERF-1-PDZK1 network can in principle bind a whole array of ion channels, transporters, receptors, and kinases. However, this does not necessarily imply that all of these proteins are close neighbors in the cell and therefore can functionally interact. Indeed, biochemical and yeast two-hybrid experiments indicate that Npt2, NHE3, and D-AKAP2 all bind to PDZK1 (17). However, NHE3 and Npt2 are localized in the microvilli (brush border), whereas D-AKAP2 is found at the base of microvilli and in the subapical compartment (16). The different subcellular localization therefore renders it unlikely that D-AKAP2 is involved in the regulation of transport activity of either Npt2 or NHE3. However, because Npt2 endocytosis is stimulated by PKA and endocytosis occurs at the base of microvilli, it may well be that D-AKAP2 plays a role either in the endocytosis at the microvillar base or in the sorting from the subapical compartment to the lysosomes. Thus the biochemical analysis of complex composition should always be complemented by a morphological analysis to find out whether the different PDZ-bound proteins have the same spatial distribution within the cell.

Functional Competition Between PDZ Proteins

As mentioned above, the surface expression of CFTR is reduced by overexpression of GOPC in COS-7 cells and can be rescued again by the coexpression of GOPC and NHERF-1, suggesting a functional competition between the Golgi-associated GOPC and the plasma membrane-associated NHERF-1, with the outcome of the competition being determined by the relative amounts of either protein (8). A relevant question therefore is whether such a situation also occurs in vivo, i.e., whether antagonizing pairs of PDZ proteins can form an on-off switch for PDZ-bound proteins that could be regulated by changes in expression level or phosphorylation of one of the PDZ proteins. Another potential mechanism for functional competition stems from the multimeric composition of PDZ complexes, which renders them sensitive to dominant-negative effects. Indeed, overexpression of a dominant-negative CASK isoform in MDCK cells results in the mislocalization of endogenous SAP97 (35). Again, it remains to be clarified whether dominant-negative isoforms of PDZ proteins occur in vivo (e.g., as splice variants or because of gene mutations) and what their physiological or pathophysiological role would be.

CONCLUSIONS

PDZ proteins play a crucial role in the correct positioning of membrane proteins in polarized epithelial cells. On the basis of the above evidence discussed in this review, we propose that PDZ proteins form an extended protein network underneath the apical and basolateral membrane that is linked to both the membrane and the cytoskeleton. The apical network consists of a NHERF1-PDZK1 scaffold that associates with the brush-border membrane of proximal tubule cells. At the basolateral side, there is evidence for a Veli-CASK scaffold in MDCK cells and collecting duct cells. Such a protein network would then constitute a large “Velcro strip” because of the protruding PDZ domains. A variety of membrane proteins (e.g., ion channels, transporters, receptors) is then recruited to this scaffold via PDZ-based interactions with two functional consequences. First, although PDZ-based interactions are dispensable for the biosynthetic targeting to the proper membrane domain, the PDZ network ensures that the membrane proteins are efficiently retained at the cell surface, either by downregulating the endocytosis or by promoting surface recycling of endocytosed transporters. Second, the close spatial positioning of functionally related proteins (e.g., receptors, kinases, channels) into a signal transduction complex allows fast and efficient control of membrane transport processes. In this respect, PDZ scaffolds function as social networks in humans: You need an invitation ticket (i.e., a biosynthetic sorting signal) to get in, but once entered, you are firmly anchored in the network (via PDZ-based interactions), and you get a lot of functional bonuses (i.e., transducisomes).

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