PDZ interactions and proximal tubular phosphate reabsorption

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Biber, Jürg, Serge M. Gisler, Nati Hernando, Carsten A. Wagner, and Heini Murer. PDZ interactions and proximal tubular phosphate reabsorption. Am J Physiol Renal Physiol 287: F871–F875, 2004; doi:10.1152/ajprenal.00244.2004.—In adults, the extent of renal reabsorption of Pi, and consequently the extent of urinary excretion of phosphate are to a large extent determined by the abundance of the Na-Pi cotransporter NaPi-IIa (SLC34A1). Localization of this cotransporter is restricted to the apical membrane of proximal tubular cells, and its abundance is controlled by a number of factors and pathophysiological conditions. To guarantee a proper apical localization and specific regulated endocytosis of NaPi-IIa, an orchestrated pattern of protein interactions has to be envisaged. Attempts to screen for such interacting proteins resulted in the identification of a PDZ domain containing proteins. The purpose of this review is to discuss the roles of these PDZ proteins in proximal tubular Na-Pi cotransport.

INTERACTIONS OF PDZ PROTEINS WITH Na-Pi COTRANSPORTER NaPi-IIa

Initially, in a classic yeast two-hybrid screen using the intracellular COOH terminus of NaPi-IIa as bait, several PDZ domain-containing proteins were identified (9; for a definition of PDZ domains, see Refs. 6 and 27). No PDZ proteins were identified using the NH2 terminus of NaPi-IIa as bait, which is localized at the cytoplasmic site as well. COOH-terminal-associated PDZ proteins were identical as NHERF1/EBP50 (42), NHERF2/E3KARP (44), PDZK1 [17; formerly NaPi-Cap1 (9)], a protein similar to PDZK1, named NaPi-Cap2 (9), and a CFTR-associated ligand (CAL) (5). Importantly, all PDZ proteins mentioned were localized either in the brush borders or in the subapical compartment of proximal tubular cells (Fig. 1).

Results obtained with NHERF1-deficient mice indicated that a direct interaction of NaPi-IIa with NHERF2 is unlikely and may be conceived indirectly via NHERF1 (39). CAL was...
The interaction patterns of NHERF1 and PDZK1 to NaPi-IIa are as follows. 1) The interaction of the COOH terminus of NaPi-IIa with NHERF1 and PDZK1 depends on the last three amino acids, TRL, which represent a class I PDZ binding motif (27). 2) Although PDZK1 contains four and NHERF1 two PDZ domains, interaction with the COOH terminus of NaPi-IIa occurs specifically with single PDZ domains: PDZ-3 of PDZK1 and PDZ-1 of NHERF1 (8, 9). Whether the TRL motif confers enough specificity for interaction with the single PDZ domains mentioned remains uncertain. A contribution of more upstream amino acid residues has been postulated but not verified definitively.

It might be anticipated that the above-mentioned interactions with the soluble COOH terminus of NaPi-IIa do not reflect the circumstances of the entire and membrane-inserted protein. Recent progress allowed the expression of NaPi-IIa in yeast two-hybrid trap assays and confirmed by several biochemical studies, such as glutathione-S-transferase pull-downs and gel overlays (8, 9). The hallmarks of these studies are as follows. NaPi-IIa interacts with PDZK1, which were shown to interact with NHERF1 and PDZK1, which were shown to interact with NHERF1. However, recent studies demonstrated that NaPi-IIa-PDZ interactions are involved in the apical positioning of NaPi-IIa.

WHAT ARE NaPi-IIa-PDZ INTERACTIONS GOOD FOR?

Generally, PDZ proteins act as scaffolds of large, subcellular structures such as synapses and junctional complexes (6). Recent data supported a similar scaffolding role of PDZK1 and NHERF1 for the appropriate localization of a number of solute transport proteins in the apical membrane of proximal cells (8). In addition, those PDZ proteins provide a backbone for the spatial arrangements of receptors and other regulatory components necessary for the coordinate action of hormones. The current knowledge of how PDZK1 and NHERF1 influence NaPi-IIa-mediated Na-Pi cotransport is discussed below.

Avital Positioning

Whether NaPi-IIa-PDZ interactions are critical for the apical sorting of NaPi-IIa is not known. However, several studies demonstrated that NaPi-IIa-PDZ interactions are involved in the apical positioning of NaPi-IIa.

1) In opossum kidney (OK) cells, intrinsically expressed and green fluorescent protein-tagged NaPi-IIa colocalizes with the apical membrane with small microvillus-like and β-actin-rich structures, which are arranged in patches. After expression of truncated NaPi-IIa protein without TRL, the COOH-terminal PDZ binding motif, apical localization was disturbed and intracellular accumulation of NaPi-IIa was observed (15). Similarly, expression of single PDZ domains derived from NHERF1 and PDZK1, which were shown to interact with NaPi-IIa, impaired the apical localization of NaPi-IIa (11). It is noteworthy that NHERF1 is apically expressed in OK cells and colocalizes with NaPi-IIa in apical patches, whereas PDZK1 does not exhibit an exclusive apical localization but is distributed throughout the whole cell. Thus with respect to possible roles of PDZK1, results obtained with OK cells have to be considered as inconclusive.

2) OK-H cells, a clone which exhibits low endogenous expression of NHERF1, show an apical distribution of NaPi-IIa that is uniform and not organized in patches. After transfection of these cells with NHERF1, an apical patchy distribution of NaPi-IIa was observed similar to that in the original OK cells (22, 24). Obviously, these studies allocate to NHERF1 a direct or indirect function for the apical arrangement of NaPi-IIa.

3) Reduced apical abundance of NaPi-IIa was observed in kidneys of NHERF1-deficient mice. Compared with wild-type mice, the NaPi-IIa protein content in NHERF1−/− mice was reduced by ~50%. In accordance, these mice showed hypophosphatemia and an increased urinary loss of P. It is likely that under normal laboratory conditions (i.e., diet), the absence of PDZK1 may be compensated for by (a) redundant mechanism(s). However, after PDZK1−/− mice were fed a high-P diet, a moderate reduction of the amount of NaPi-IIa and an increase in urinary excretion of P compared with...
Regulation of NaPi-IIa

The abundance of NaPi-IIa protein in the apical membrane is controlled by a number of hormones and metabolic factors. The best-studied changes in NaPi-IIa abundance are by down-regulation initiated by PTH or alterations provoked by changes in dietary Pi content (20, 26, 33).

Hormonal regulation. Internalization of proximal tubular Na-Pi cotransport by PTH involves protein kinases that are activated on occupation of PTH receptors localized either at the apical membrane or at the basolateral membrane of renal proximal tubular cells; signaling via the basolateral PTH receptor is via PKA and PKC, whereas apically localized PTH receptors activate PKC only. Activation of either protein kinase results in a downregulation of NaPi-IIa, as evidenced from direct activations of PKA with cAMP or PKC with, for example, 1,2-dioctanoyl-glycerol (2, 25, 38). Both NHERF1 and PDZK1 may provide backbones for a correct apical and spatial arrangement of the components necessary for the regulation of NaPi-IIa by PTH.

Anchoring sites for PKA are indirectly provided by NHERF1 and PDZK1. Anchoring of PKA to NHERF1 is via ezrin, a member of the MERM (for merlin, ezrin, radixin, and moesin) protein family (4). This arrangement has been demonstrated to be important for the regulation of the Na/H exchanger NHE3 by cAMP (41). In contrast, in an in vitro study using kidney slices derived from NHERF-deficient mice, cAMP-stimulated internalization of NaPi-IIa was normal (Capuano P, Bacic D, Weinman EJ, Biber J, Murer H, and Wagner CA, unpublished observations). Therefore, it is concluded that the PKA/ezrin/NHERF1 complex is not required for the regulation of NaPi-IIa by PKA. Besides ezrin, AKAP79 (16) and D-AKAP2 (7, 12) were described as additional PKA-anchoring sites in the brush borders of proximal tubular cells. Direct association of AKAP79 has been suggested based on coprecipitations performed with OKT cells, and functional evidence was obtained by an uncoupling of the PKA binding to AKAP79, which prevented PTH-mediated inhibition (16). Based on a yeast two-hybrid screen, D-AKAP2 has been found to interact with PDZK1 (7). Whether the PDZK1/D-AKAP2 complex has any functional significance for the regulation of NaPi-IIa has recently been investigated by using kidney slices derived from PDZK1-deficient mice. In these studies, regulation of NaPi-IIa by activation of PKA via PTH receptors or with cAMP was normal (Capuano P, Bacic D, Stange G, Hernando N, Kaissling B, Kocher O, Biber J, Wagner CA, and Murer H, unpublished observations). Therefore, the possible relevance of the spatial arrangement of PKA via the D-AKAP2/PDZK1 complex with respect to the regulation of NaPi-IIa remains debatable.

The signaling mechanism of apical PTH receptors involves activation of phospholipase C-ß (2, 24). NHERF1 was shown to interact with both the apical PTH receptor and PLC-ß (23, 32). In studies performed with kidney slices from NHERF1-deficient mice, impaired regulation of NaPi-IIa after activation of apical PTH receptors was observed (Capuano P, Bacic D, Weinman EJ, Biber J, Murer H, and Wagner CA, unpublished observations), indicating the requirement of a precise spatial arrangement of NaPi-IIa together with the apical PTH receptor and PLC-ß.

Nevertheless, not much is known about the signaling mechanisms leading to the downregulation of NaPi-IIa. As the apical distributions of PDZK1 and NHERF1 on activation of PTH receptors are not altered (2), it is assumed that specific modifications of either NaPi-IIa itself, the PDZ proteins, or other proteins that may associate with the PDZ complexes are required to modify the affinity constants of the PDZ-NaPi-IIa associations. Lowering the affinities of these interactions could, for example, increase the mobility of NaPi-IIa along the microvillar axis, allowing an increased delivery of NaPi-IIa to the endocytic machinery. This hypothesis would be sustained by the observation that deletion of the TRL motif in CFTR, which binds to NHERF1, increases the diffusional mobility of CFTR (10).

The demonstration that a number of protein kinases are involved in the regulation of NaPi-IIa suggests that phosphorylation reactions can be envisaged as off signals for NaPi-IIa-PDZ interactions. Thus far, attempts to demonstrate that NaPi-IIa is phosphorylated on PTH action have not been successful (2). On the other hand, both NHERF1 and PDZK1 represent phosphoproteins (Ref. 40 and Deliot N, Hernando N, Liu Z, Capuano P, Bacic D, Wagner CA, O’Brien S, Biber J, and Murer H, unpublished observations). Whether changes in the phosphorylation of NHERF1 and PDZK1, e.g., provoked by PTH, may alter the interactions with NaPi-IIa is not known.

By means of yeast two-hybrid screens, two proteins that are supposed to be part of the NaPi-IIa/PDZ protein complexes have been further identified: 1) the peroxisomal protein PEX19 was identified in a screen performed against an intracellular loop of NaPi-IIa, which confers PTH responsiveness to NaPi-IIa (13, 14); and 2) the calcium binding protein Vilip-3 was identified in a screen done against the NHERF1 terminus of NaPi-IIa (29). It is of interest that both proteins are modified by farnesylation and myristoylation, respectively (13, 34). As calcium-dependent myristoylation of Vilip-3 was shown to alter its membrane association (34), it could be speculated that calcium-dependent lipid modifications are involved in the modulation (e.g., via the calcium-sensor; see Ref. 1) of the regulation of NaPi-IIa by PTH.

Adaptation. A diet of low Pi content results in a marked increase in the abundance of the NaPi-IIa protein that is not paralleled by an increase in NaPi-IIa mRNA (20, 21). The signaling mechanisms leading to this upregulation of NaPi-IIa are poorly understood. Under the same conditions, using different mouse strains and rats, work by our laboratory showed that the abundances of NHERF1 and PDZK1 (protein and mRNA) were not altered (22), which is in contrast to results reported by others (40).

Regulation of NaPi-IIa by dietary content of Pi has been investigated in NHERF1 as well as in PDZK1 knockout mice. In NHERF1-deficient mice, the chronic adaptation to a low-Pi diet leads to an increase in NaPi-IIa content that was quantitatively similar to that in wild-type mice (40). Interestingly, also in PDZK1−/− mice, no differences in the acute and chronic adaptive responses compared with wild-type animals were noticed (Capuano P, Bacic D, Stange G, Hernando N, Kaissling B, Kocher O, Biber J, Wagner CA, and Murer H, unpublished observations). These data therefore suggest that...
neither NHERF1 nor PDZK1 is essentially required for the adaptation of NaPi-IIa to a low-Pi diet.

SUMMARY AND OUTLOOK

Apical localization and regulation of NaPi-IIa in the brush-border membrane of proximal tubular cells require a network of protein interactions. Currently, the best-described members of such a protein network are the PDZ proteins NHERF1 and PDZK1. Interactions of NaPi-IIa with these PDZ proteins (notably NHERF1) appear to be important for apical stabilization of NaPi-IIa but also appear to provide anchoring sites for a number of regulatory elements, such as protein kinases and elements required for hormonal signaling mechanisms involved in the controlled endocytosis of NaPi-IIa. It is of interest that the anchoring of PKA to neither the ezrin/NHERF1 nor to the D-AKAP2/PDZK1 complex seems to be required for the regulation of NaPi-IIa by cAMP. In the context of a regulated endocytosis, which eventually also includes on-off reactions of PDZ interactions) and in the context of the routing of internalized NaPi-IIa to the lysosomes, a number of questions remain to be answered. Finally, it is of note that NHERF1 and PDZK1 not only interact with NaPi-IIa but also with a number of other solute transporters such as NHE3, the chloride/formate exchanger CEFR, the ureate transporter URAT1, and others (8). Whether such an arrangement of different transporters in microdomains of the brush-border membrane may affect Na-Pi cotransport activity by local changes (e.g., pH or ionic environment) remains to be clarified.

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