Pdz adaptors: Their regulation of epithelial transporters and involvement in human diseases

PDZ adaptors: Their regulation of epithelial transporters and involvement in human diseases

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Abbreviations: PDZ, PSD95/Dlg/ZO1; GLUT, glucose transporter; MRP, multidrug resistance-associated protein; CFTR, cystic fibrosis transmembrane conductance regulator, OCTN, organic cation/carnitine transporter, NaPi-II, type-II sodium-phosphate cotransporter; EAAC, excitatory amino acid carrier; NHERF, sodium/proton exchanger regulatory factor; ERM, ezrin/radixin/moesin; NHE, sodium/proton exchanger; URAT, uric acid/anion exchanger; OATP, organic anion-transporting polypeptide; PEPT, proton/oligopeptide cotransporter; PTH, parathyroid hormone; SR-BI, scavenger receptor class B type I; SNP, single nucleotide polymorphism

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ABSTRACT

Homeostasis in the body is at least partially maintained by mechanisms that control membrane permeability and thereby serve to control the uptake of essential substances (e.g., nutrients) and the efflux of unwanted substances (e.g., xenobiotics and metabolites) in epithelial cells. Various transporters play fundamental roles in such bidirectional transport, but little is known about how they are organized on plasma membranes. Protein-protein interactions may play a key role: several transporters in epithelial cells interact with so-called adaptor proteins, which are membrane-anchored and interact with both transporters and other membranous proteins. Although most evidence for transporter-adaptor interaction has been obtained *in vitro*, recent studies suggest that adaptor-mediated transporter regulation does occur *in vivo* and could be relevant to human diseases. Thus, protein-protein interaction is not only associated with the formation of macromolecular complexes, but is also involved in various cellular events, and may provide transporters with additional functionality by forming transporter-networks on plasma membranes. Interactions between xenobiotic transporters and PDZ adaptors were previously reviewed by Kato and Tsuji (Eur J Pharm Sci 27, 487, 2006); the present review focuses on the latest findings about PDZ adaptors as regulators of transporter-networks and their potential role in human diseases.

1. Turnover of transporters

After membrane proteins have been biosynthesized, they are generally translocated from endoplasmic reticulum into Golgi apparatus, where they undergo post-translational processing. These proteins are then sorted to apical or basal membranes in epithelial cells, to exert their transport activity (Fig. 1). The membrane proteins are subsequently internalized and sequestrated into lysosomes, followed by degradation, or recycled back to the cell surface (Fig. 1). Cell-surface expression of transporters is therefore determined by the balance among sorting, internalization and recycling (Fig. 1). This implies that there could be some molecular mechanisms that allow functional transporters to remain stably localized on plasma membranes. The interaction of transporters with adaptor (scaffold) proteins is a candidate for such a mechanism[1]. Thousands of adaptor proteins including PDZ adaptors are known in the human proteome, and these are classified into more than 70 distinct families [2]. Apart from PDZ adaptors, ERM (ezrin/radixin/moesin) proteins are known to directly interact with xenobiotics transporters [3,4]. These adaptor proteins are involved in the assembly of various intracellular complexes and regulation of cellular functions.

Translocation of transporters between plasma membranes and the intracellular compartment in peripheral epithelial cells has been most extensively studied for glucose transporter GLUT4, which plays a pivotal role in homeostasis of blood glucose [5,6], but has also recently been described for several other transporters, including multidrug resistance-associated protein (MRP) 2, cystic fibrosis transmembrane conductance regulator (CFTR), type-IIa sodium-phosphate cotransporter (NaPi-IIa), excitatory amino acid carrier (EAAC) 1 and organic cation/carnitine transporter (OCTN) 2 [7-13]. Most of these transporters are localized on apical membranes of epithelial cells. For example, MRP2 is expressed on canalicular membranes of hepatocytes, and its internalization is stimulated by oxidative stress [7,8]. This internalization could occur to block MRP2-mediated efflux of a major antioxidant, glutathione, thus serving to protect hepatocytes [7,8]. NaPi-II is expressed on apical membranes of renal tubular epithelial cells. NaPi-II is internalized and undergoes degradation in the presence of excessive phosphate [14], and these events could be associated with the regulation of phosphate reabsorption in the kidney [14-16]. In both cases, the degradation process of the transporters occurs relatively slowly, within

4-6 hrs [7,14], whereas the internalization of MRP2 occurs at a much higher rate, within 10 min [7].

2. PDZ adaptor-mediated regulatory mechanisms for transporters

This section summarizes the regulatory mechanisms for transporters by PDZ (PSD95/Dlg/ZO1) adaptors, most of which are based on *in vitro* evidence. The regulatory mechanisms by PDZ adaptors are summarized in Table 1.

2-1. PDZ domain and binding motif: Relevant to sorting? (Fig. 2A)

Most transporters expressed on apical membranes of epithelial cells have a class I PDZ binding motif (-S/T-X-Φ, Φ is a hydrophobic acid) at their C-terminus. This is the reason why this motif has been thought to play a role in the sorting of transporters to the apical membranes. Recent studies have clarified that some of the motifs can directly bind to PDZ domains [17-21], which are structural regions generally consisting of 80-90 amino acids. In humans, there are over 250 PDZ domains, which are present in over 100 PDZ domain-containing proteins [22]. Some of these proteins act as scaffolds for

membranous proteins, so they are called PDZ adaptors.

Among them, four PDZ adaptors, PDZK1 (also known as diphor-1, NaPiCap1, CLAMP, CAP70 and NHERF3), PDZK2 (also named NaPiCap2, IKEPP and NHERF4), sodium/proton exchanger regulatory factor (NHERF) 1 (also named EBP50, SLC9A3R1) and NHERF2 (also known as E3KARP, SIP-1, TKA-1 and SLC9A3R2) interact with the C-terminus of transporters expressed in intestinal, renal and hepatic epithelial cells. PDZKs have four PDZ domains, whereas NHERFs have two PDZ domains at their N-terminus and an ERM (ezrin/radixin/moesin) binding domain at their C-terminus. ERM proteins can interact with actin, so NHERFs may also interact with the cytoskeleton. These four PDZ adaptors are mostly localized on apical membranes in small intestine and kidney, although there are some exceptions in liver. For example, NHERF1 is expressed on apical membranes of epithelial cells in intestine, kidney and liver [23-26]. On the other hand, PDZK1 is localized on apical membrane and intermicrovillar clefts in renal proximal tubules [27,28], on apical membrane in intestinal epithelial cells [29], but on sinusoidal membrane of hepatocytes [30]. Therefore, transporter-PDZ domain interaction alone cannot fully explain the localization of

transporters on apical membranes.

Mutation in the PDZ binding motif at the C-terminus results in down-regulation of several transporters from the apical membranes of epithelial cells *in vitro* [9-12,20]. Thus, the PDZ binding motif could be essential for the apical membrane localization of certain transporters (Fig. 2A). However, limited information is available on how this PDZ binding motif affects the sorting of transporters.

2-2. Stabilization of transporters on plasma membranes (Fig. 2B)

The interaction of transporters with PDZ adaptors may affect their stable expression on the cell-surface. In fact, when transporters are cotransfected with PDZ adaptors such as PDZK1 and NHERF1 in cultured cell lines, expression levels of the transporters on the cell-surface are higher than in the case without cotransfection with the adaptors [13,19,~~20~~,31-35]. On the other hand, mutants of these transporters lacking the PDZ binding motif do not interact with PDZ adaptors, and the expression level of the mutants is only minimally affected by cotransfection with PDZ adaptors [13,19,20,31-34]. These results suggest that PDZ adaptors can stabilize the transporters on the cell-surface

(Fig. 2B). D'Amico et al. reported that the stable localization of EAAC1 endogenously expressed on plasma membranes of MDCK cells is controlled by the interaction with endogenous PDZK1 [12]. Deletion of the PDZ binding motif in EAAC1 promotes internalization of EAAC1 via the interaction with another adaptor for internalization, adaptor protein 2 complex [12], indicating the role of the interaction with PDZK1 in stabilization of EAAC1. Many of these studies, however, were performed by over-expressing exogenous proteins (e.g., transporters and/or PDZ adaptors). Therefore, it should be carefully considered whether or not these results reflect *in vivo* phenomena. On the other hand, recent studies using gene knockout mice for the PDZ adaptors also support the existence of the stabilizing effect (see section 3).

Over-expression of a single PDZ domain, which interacts with NaPi-IIa, stimulates internalization and degradation of this transporter [10]. This phenomenon is probably caused by a dominant-negative effect, which means that the exogenously transfected single PDZ domain competitively inhibits the interaction between transporters and endogenous PDZ adaptors. Thus, the interaction of this transporter with PDZ adaptor can enhance the residence time of the transporter on the cell-surface,

probably by stabilization in the plasma membrane. Recently, LaLonde and Bretscher have proposed a possible explanation of the stable localization mechanism by PDZ adaptors on cell-surface membranes, as follows. All ERM proteins, NHERF1 and PDZK1 undergo a "head-to-tail" intramolecular interaction (N-terminal domain interacts with C-terminal tail region), and this represents an inactive form having minimal interaction with other proteins, such as transporters. When a certain membrane protein, such as sodium/proton exchanger (NHE) 3, interacts with the first PDZ domain of PDZK1, the intramolecular interaction is broken, and PDZK1 can interact with the first PDZ domain of NHERF1 upon release of the C-terminal tail of PDZK1, leading to loss of intramolecular interaction in NHERF1. NHERF1 can then interact with ERM proteins, which can bind to filamentous actin. Thus, such a "domino-effect" in conformational change from inactive to active form could be associated with the stability of these large structural complexes [36]. This hypothesis can explain how ERM, NHERF1 and PDZK1 are localized on plasma membranes, although further studies are needed to examine whether the same mechanism works *in vivo*.

2-3. Signal Transduction (Fig. 2C)

PDZ adaptors, such as PDZKs and NHERFs, have multiple PDZ domains in their structure, and each domain alone can interact with the C-terminus of transporters. Therefore, it is speculated that these adaptors can serve to cluster various interacting proteins at a specific region of plasma membrane. PDZ adaptors are thought not only to play a static role as a scaffold, but also a dynamic role by gathering functionally associated proteins at a certain microdomain on the cell-surface. For example, parathyroid hormone (PTH) regulates the expression level of transporters, such as NaPi-IIa [37,38], sodium/proton exchanger (NHE) 3 [25,39] and Na^+/K^+-ATPase [40]. This regulation is associated with the recruitment of PTH receptor, protein kinases and phosphorylated NHERF1 [41,42]. Similar regulation is also reported for scavenger receptor class B type I (SR-BI) by protein kinase A (PKA) and phosphorylated PDZK1 [43]. These reports suggest that PDZ adaptors are involved in the signal transduction (Fig. 2C).

PDZ adaptor-mediated clustering of protein complexes would be advantageous in minimizing unwanted diffusion of signal messenger(s). Li et al. have reported that the concentration of second messenger cAMP in close proximity to plasma

membrane is regulated by MRP4 [44], which pumps out cAMP as a substrate. cAMP signaling is associated with the activity of several transporters, including CFTR. The dramatic elevation of cellular cAMP leads to an increase in CFTR-mediated Cl^- secretion and thereby causes diarrhea. Therefore, the concentration of cAMP at the region close to CFTR should be tightly regulated. PDZK1 interacts with both CFTR and MRP4, and *mrp4* gene knockout mice are more prone to CFTR-mediated secretory diarrhea [44], suggesting that PDZK1 regulates the local concentration of cAMP by bridging between MRP4 and CFTR. Similarly, NHERF1 is required for phosphorylation and functional regulation of NHE3 in response to intracellular cAMP [45-47]. The PDZ adaptors are thus involved in homeostasis of signal transduction by clustering various proteins to prevent abnormal response (Fig. 2C).

2-4. Activation and functional coupling (Fig. 2D)

PDZ adaptors increase the function of various transporters on cotransfection in cultured cell lines. Many of these cases, however, can simply be explained by the increase in expression levels of the transporters on plasma membrane (Fig. 2D)

[9,19,20,32,34,48,49]. In contrast, OCTN2-mediated uptake of the substrate carnitine is increased 6-fold in the presence of PDZK1, despite a minimal effect of PDZK1 on cell-surface expression of OCTN2 [50]. Such stimulation of transport activity is not observed for OCTN2 mutant with the PDZ binding motif deleted, suggesting that PDZK1 direct regulates the functional activity of OCTN2[50]. In *in vivo* experiments, however, expression of OCTN2 on apical membranes of intestinal epithelial cells was reduced in *pdzk1*$^{-/-}$ mice, compared with wild-type mice, with a concomitant delay in gastrointestinal absorption of carnitine [51], indicating that PDZK1 is involved in stabilization of OCTN2 on the apical membrane. The *pdzk1*$^{-/-}$ mice thus exhibit reduced expression of interacting transporters, and this leads to difficulty in demonstrating the stimulatory effect on transporter function *in vivo*.

Clustering of multiple transporters by PDZ adaptors may allow the driving force for a certain transporter to be provided by another one located nearby, thereby leading to efficient transport activity. For example, the driving force of proton/oligopeptide cotransporter (PEPT) 1 is an H^+ gradient, a part of which is supplied by NHE3 [52]. Both of them are expressed on the apical membranes of intestinal

epithelial cells and interact with PDZK1 [51,53], possibly resulting in localization of NHE3 adjacent to PEPT1 and effective rotation of H^+ into or out of the cell (Fig. 2D) [54].

3. Roles of PDZ adaptors *in vivo*: Influence of deficiency and mutation

Concerning the four PDZ adaptors, gene knockout mice for PDZK1, NHERF1 and NHERF2 (*pdzk1*$^{-/-}$, *nherf1*$^{-/-}$ and *nherf2*$^{-/-}$, respectively) have already been constructed with the aim of establishing the functions of PDZ adaptors *in vivo*. In addition, gene knockin mice for PDZ adaptors or a single PDZ domain alone have also been constructed using transgenic technology. This section summarizes *in vivo* evidence, mainly obtained in such transgenic animals, concerning the pharmacological and physiological roles of PDZ adaptors (Fig. 3).

3-1. Roles of PDZ adaptors in the small intestine

Roles of PDZK1 as an adaptor have been demonstrated for various transporters in the small intestine (Fig. 3). For example, expression of PEPT1, OCTN2, and OATP1A was reduced on apical membranes in *pdzk1*$^{-/-}$ mice [51,55]. This reduction

was accompanied by a reduction in gastrointestinal absorption of cephalexin and carnitine, typical substrates of PEPT1 and OCTN2, respectively [51]. The absorption rate constant of cephalexin was much lower in *pdzk1*$^{-/-}$ mice (0.0654 and 0.0172 min^{-1} in wild-type and *pdzk1*$^{-/-}$ mice, respectively) [51]. Intestinal accumulation of carnitine in *pdzk1*$^{-/-}$ mice was approximately 50% of that in wild-type mice [51]. Similarly, the fraction of intestinal absorption of [^{3}H]estrone-3-sulfate, a substrate of OATP1A, was also lower in *pdzk1*$^{-/-}$ mice (14.5 and 0.5% in wild-type and *pdzk1*$^{-/-}$ mice, respectively) [55]. CFTR-dependent duodenal HCO_3^- secretion was also reduced in *pdzk1*$^{-/-}$ mice [56,57]. Interestingly, PEPT1 is localized at multivesicular bodies (MVBs) in *pdzk1*$^{-/-}$ mice [51]. Because MVBs represent a compartment for degradation of plasma membrane proteins following their internalization (Fig. 1), the localization in MVBs may implies that PEPT1 is unstable on plasma membrane due to loss of PDZK1. On the other hand, forskolin-responsive intestinal net Na^+ absorption was significantly reduced in *pdzk1*$^{-/-}$ mice, even though the expression level and localization of NHE3 were not significantly different from those of wild-type mice [56].

In *nherf1*$^{-/-}$ mice, expression of NHE3 and CFTR on brush-border membranes

of epithelial cells and in crypt cells, respectively, is reduced [26,58]. Both of these transporters are involved in the membrane permeation of water and inorganic ions. Absorption of fluid and Na^+ [58], and secretion of HCO_3^- [57] are also reduced in small intestine of *nherf1*$^{-/-}$ mice. Levels of NHE3 and NHERF1 were significantly lower in mucosal biopsies from patients with inflammatory bowel disease (IBD), as well as from acute murine IBD models, suggesting that down-regulation of NHERF1 induces IBD-associated diarrhea, possibly caused by unbalanced ion concentrations [59].

Regulation by PDZ adaptors of small intestinal transporters could be very complex. For example, a certain transporter can be regulated by multiple PDZ adaptors in opposite directions. For example, cAMP-dependent stimulation of HCO_3^- secretion is reduced in *nherf1*$^{-/-}$ mice, but increased in *nherf2*$^{-/-}$ mice [57]. This may imply that these two adaptors differentially regulate inorganic ion transporters, such as NHE3 and CFTR. In addition, the regulation by PDZ adaptors could be different between proximal and distal regions in the small intestine. For example, function of NHE3 and CFTR was reduced in proximal regions, but not in ileum of *nherf1*$^{-/-}$ mice [26,60]. Similarly, we have recently found that expression on apical membrane of PEPT1 and OCTN2 is tightly

regulated by PDZK1 in proximal regions, but such regulation is only partial in distal regions (unpublished observation) [51]. It is also noteworthy that no effect of PDZK1 and NHERF2 gene knockout on CFTR function can be observed in isolated tissues or Ussing-type chambers [26,56], but an effect was observed in an *in situ* perfusion system [57]. Thus, the function of PDZ adaptors could highly depend on the experimental systems, and might be more easily observed under physiologically relevant conditions.

Both NHERF1 and NHERF2 can also interact with cytoskeleton proteins, such as actin filament, via the interaction with ERM proteins. This may be associated with morphological change in the gene knockout mice, and indeed, the length of microville in small and large intestines was reduced in *nherf1*$^{-/-}$ mice [58,61]. The length of microvilli was also reduced, though not significantly so, in *pdzk1*$^{-/-}$ mice [51]. PDZK1 is localized not only on plasma membranes of microvilli, but also in the base of microvilli, which probably represents an intracellular subapical compartment with abundant actin filaments [29]. Thus, PDZK1 may also be involved in linking membrane proteins and cytoskeleton components.

3-2. Roles of PDZ adaptors in the kidney

On the apical membranes, uric acid/anion exchanger (URAT) 1 plays an important role in reabsorption of uric acid. URAT1 has a PDZ binding motif at its extreme C-terminus, and mutation in this motif leads to hypouricemia in humans, probably because of deficiency in its interaction potential with PDZ adaptors, such as PDZK1 and/or NHERF1 [19,62,63]. In *nherf1*$^{-/-}$ mice, URAT1 is mislocalized in the intracellular compartment of proximal tubules with a concomitant reduction in expression of the gene product in membrane fractions, leading to an increase in urinary excretion of uric acid [62]. Concomitantly, inhibitory effect of probenecid on uptake of uric acid in isolated proximal convoluted tubules was reduced in *nherf1*$^{-/-}$ mice (percentage of inhibition from control was 47 and 26 in wild-type and *nherf1*$^{-/-}$ mice, respectively) [62]. Similarly, NaPi-IIa is involved in reabsorption of phosphate at proximal tubules and interacts with the four PDZ adaptors (PDZK1, PDZK2, NHERF1 and NHERF2) [18,27]. In *nherf1*$^{-/-}$ mice, NaPi-IIa is mislocalized intracellularly with a concomitant reduction in expression of the gene product on plasma membranes [38,64], leading to hypophosphaturia [64]. Serum phosphate concentration in *nherf1*$^{-/-}$ mice was

reduced to 72% of wild-type mice whereas urinary phosphate excretion in *nherf1*$^{-/-}$ mice was ~3-fold higher compared with wild-type mice [64]. Thus, NHERF1 plays pivotal roles in homeostasis of these solute ions via direct interaction with tubular transporters.

On the other hand, limited information is available on the roles of PDZ adaptors other than NHERF1 in relation to NaPi-IIa. Minimal change was observed in the expression level of NaPi-IIa or the urinary excretion of phosphates in *nherf2*$^{-/-}$ mice [65], while down-regulation of NaPi-IIa in *pdzk1*$^{-/-}$ mice and a concomitant increase in urinary excretion of phosphate were observed only under a high phosphate diet condition, but not under a low phosphate diet [66].

In kidneys, PDZ adaptors are suggested to be involved in receptor-mediated signal transduction: parathyroid hormone binds to the G-protein-coupled receptor (PTH1R), thereby stimulating intracellular signaling through phospholipase C and protein kinase C. This signal transduction is associated with the reduction of phosphate reabsorption by inducing internalization of NaPi-IIa. Both NHERF1 and NHERF2 play a pivotal role in the PTH-mediated activation of phospholipase C by directly interacting with the C-terminus of PTH1R and subsequently assembling signal complexes [67,68]. In

addition, the internalization of NaPi-IIa is stimulated by the dissociation of NaPi-IIa from its adaptor NHERF1, and such dissociation follows phosphorylation of a serine residue in the first PDZ domain in NHERF1 by protein kinase C [41]. Actually, the PTH-stimulated down-regulation of NaPi-IIa is minimally observed in *nherf1*$^{-/-}$ mice [38]. Thus, homeostasis of phosphate is governed by macromolecular complex formation mediated by NHERF1.

A similar story was also reported for another G-protein-coupled receptor (dopamine D1-like receptor), which also binds to NHERF1 and is involved in the regulation of NaPi-IIa [69]. The regulation of phosphate homeostasis by dopamine involves the second messenger cAMP and an intracellular signaling cascade mediated by PKA and PKC, finally leading to reduction of NaPi-IIa expression [70]. In *nherf1*$^{-/-}$ mice, production of cAMP and subsequent PKC activation by dopamine is much impaired [69]. Thus, NHERF1 is involved in phosphate homeostasis through at least two different receptor-mediated signal cascades.

3-3. Roles of PDZ adaptors in the liver

In *pdzk1*$^{-/-}$ mice, expression of SR-BI, which plays a role as high-density lipoprotein receptor, is almost completely (by ~95%) down-regulated with a concomitant increase in plasma cholesterol level [71]. In addition, sinusoidal organic anion transporting polypeptide OATP1A1 is internalized in hepatocytes of *pdzk1*$^{-/-}$ mice, and consequently elimination rate constant of its typical substrate bromosulphophthalein is reduced (0.78 and 0.59 min^{-1} in wild-type and *pdzk1*$^{-/-}$ mice, respectively) with minimal change in distribution volume [30]. These results indicate that PDZK1 is an adaptor for certain types of receptor and transporter on sinusoidal membranes. On the other hand, NHERF1 interacts with MRP2 on canalicular membranes [72]. In *nherf1*$^{-/-}$, localization of MRP2 gene product on apical membranes is reduced, whereas the mRNA level for MRP2 is close to that in wild-type mice, demonstrating that NHERF1 is an adaptor protein for post-translational regulation of MRP2.

Both OATP1A1 and MRP2 widely accept various types of organic anions, including bilirubin, bile acids and anionic therapeutic agents [73,74], implying that both PDZK1 and NHERF1 may affect the disposition of various types of endogenous and

exogenous compounds. However, PDZK1 does not interact with OATP1A4, another sinusoidal OATP transporter [30]. In addition, expression of MRP2 on canalicular membrane is also regulated by an ERM protein, radixin, and is reduced in *radixin*$^{-/-}$ mice [75]. Therefore, there could be other adaptors than PDZK1 and NHERF1 in the liver. The bile flow rate is much lower in *nherf1*$^{-/-}$ mice compared with wild-type mice, probably because of the down-regulation of MRP2, which is involved in bile acid-independent bile flow through the excretion of glutathione.

In addition to gene knockout mice, so-called gene knockin mice have also been used to clarify the role of endogenous PDZ adaptors *in vivo*. Such knockin mice include transgenic mice, in which a single PDZ domain is incorporated into the genome. Overexpression of the PDZ domain alone may interfere with the function of endogenous PDZ adaptor by competitively inhibiting binding to transporters and/or other interacting proteins (dominant-negative effect). Knockin mice of the first PDZ domain of PDZK1 exhibit a 75% reduction of expression of the gene product for SR-BI with internalization of a substantial amount of SR-BI inside hepatocytes [76], probably because the first PDZ domain can bind to the C-terminal PDZ binding motif of SR-BI

and competitively inhibit the binding of SR-BI with endogenous PDZ adaptors. On the other hand, knockin mice for full-length PDZK1 in *pdzk1*$^{-/-}$ mice show recovery of the expression of SR-BI on sinusoidal membrane to a level comparable with that of wild-type mice [77], supporting a pivotal role of PDZK1 in cell-surface expression of SR-BI in the liver.

3-4. Roles of PDZ adaptors in other organs

Compared with the above three organs, information on the roles of the four PDZ adaptors in other organs is limited. Nevertheless, NHERF1 is also expressed in neurons of the nucleus raphe magnus and is involved in signal transduction of G-protein coupled δ-opioid receptor (DOPr) [78], which is constitutively localized in intracellular compartments, and translocation of which into plasma membrane is stimulated by the substrate, morphine, present in the extracellular space. NHERF1 is essential for this translocation of DOPr, and in *nherf1*$^{-/-}$ mice the activation of DOPr by morphine is only minimally observed [78].

In addition to its expression in various peripheral epithelial cells, PDZK1 was

recently identified in endothelium [79]. Endothelial PDZK1 is not involved in expression, localization or cholesterol binding of SR-BI, but is required for intracellular signaling in response to HDL. HDL has various actions on the endothelium, including inhibition of apoptosis and promotion of cellular growth, so endothelial PDZK1 could also be involved in ensuring the integrity of the endothelial monolayer. Indeed, carotid artery reendothelialization after perivascular electric injury is hindered in *pdzk1*$^{-/-}$ mice [79].

PDZK1 is also suggested to be associated with susceptibility to adult diseases, based on findings in *pdzk1*$^{-/-}$ mice. High-fat/high-cholesterol ('western') diet-fed apolipoprotein E gene knockout mice (*apoE*$^{-/-}$) are a model of atherosclerosis. In western diet-fed *pdzk1*$^{-/-}$*apoE*$^{-/-}$ mice, atherosclerosis is increased compared with *apoE*$^{-/-}$ mice [80]. In a study with another atherogenic diet, high-fat, high-cholesterol, cholate-containing ('paigen') diet-fed *pdzk1*$^{-/-}$*apoE*$^{-/-}$ mice exhibited severe hypercholesterolemia and aortic root atherosclerosis, leading to occlusive coronary arterial atherosclerosis and myocardial infarction [81]. These results suggest that deficiency of PDZK1 may increase the risk for coronary heart diseases.

4. PDZ adaptors potentially relevant to pathogenesis of common diseases

Recent genome-wide study has identified single nucleotide polymorphisms (SNPs) in human genes for PDZK1 and NHERF1. The role of PDZ adaptors in humans is gradually being clarified by investigations of the relationship between genotype and phenotype in subjects with SNPs (Table 2). Gene knockout mice completely lack the protein and may provide an example of the phenotype likely to be seen in certain human SNPs. In this section, we summarized the SNPs of PDZ adaptors and the corresponding phenotypes in humans (Table 2). The findings overall are compatible with the hypothesis that these PDZ adaptors control various types of transporters, and dysfunction of these adaptors is likely to play a role in the pathogenesis of multifactorial disorders, such as metabolic syndrome.

4-1. PDZK1

All SNPs so far identified in the *PDZK1* gene are localized in the untranslated region (Table 2); no SNP involving amino acid mutation has yet been reported. However, one clone (AF012281) with an amino acid mutation of E195K is listed in the

NCBI database, whereas other two clones (BC006496, BC006518) have the same amino acid sequence as the reference sequence (wild-type, NM_002614). The mutation E195K involves a change in the side chain charge in the second PDZ domain in PDZK1. Co-transfection with E195K construct of PDZK1 with its interacting transporters, such as PEPT2, OCTN1 or OCTN2, in cultured cell lines, only partially increased the transport activity and resulted in activity intermediate between those of wild-type PDZK1 and transporter alone [49]. Thus, genetic variability of PDZ adaptors affects the extent of increase of various transporter activities. It should be noted that mutation of PDZK1 may affect the membrane permeation of many compounds, because PDZK1 is known to bind to various transporters, and some of them have broad substrate specificity.

For humans who have SNPs of PDZK1 (rs3912316, rs11576685), higher plasma concentrations of triglyceride (TG) and VLDL, and increased risk for metabolic syndrome or abdominal obesity are observed [82], suggesting the possible association of PDZK1 with lipid metabolism. PDZK1 regulates the expression of HDL receptor (SR-BI) in the liver [71,83]. Moreover, an increased plasma concentration of VLDL is also

seen in SR-BI knockout mice [84]. Therefore, it is possible that the phenotype seen with rs3912316 and rs11576685 is due to a decrease in SR-BI regulation by PDZK1. However, despite this possible influence of PDZK1 on cholesterol homeostasis, the frequency of another SNP (rs12129861) of PDZK1 is not associated with coronary artery disease [85]. Rather, this SNP (rs12129861) tends to be related to higher systolic blood pressure [86]. It is thus possible that PDZK1 mutation may be a risk factor for certain vascular diseases.

The increase in plasma concentration of TG and VLDL by SNP-containing PDZK1 may also be explained by PDZK1-mediated regulation of carnitine transporter OCTN2. Carnitine is involved in ß-oxidation of fatty acids. OCTN2 mutant (*jvs*) mice exhibit carnitine deficiency and have a remarkably high TG concentration in plasma [87]. Carnitine treatment significantly decreases the serum concentration of TG in humans [88], also supporting a negative correlation between carnitine and TG. Similarly, carnitine treatment decreases the plasma concentration of VLDL in rabbits fed a high fat diet [89]. Thus, carnitine and OCTN2 could be highly relevant to plasma levels of both TG and VLDL. On the other hand, the carnitine transport activity of OCTN2 is highly

stimulated by PDZK1 and moderately stimulated by PDZK1-E195K [49,50]. Therefore, it is possible that SNPs of PDZK1 decrease OCTN2 regulation by PDZK1, resulting in increased TG and VLDL concentrations.

There are several reports concerning the association of PDZK1 SNPs with serum uric acid. Serum uric acid is lower in subjects who have one PDZK1 SNP (rs12129861), but higher in subjects who have another PDZK1 SNP (rs1471633), as compared with that in subjects with the wild-type [86,90]. Other SNPs (rs1797052, rs1298954 and rs12129861) have no effect on serum uric acid [85,91]. PDZK1 interacts with URAT1, which is involved in reabsorption of uric acid in renal proximal tubules [19]. PDZK1 also interacts with apical phosphate transporter NaPi-I, which in turn secretes uric acid in proximal tubules [18,92]. Thus, PDZK1 is involved in uric acid transport in both directions (reabsorption and secretion). Consequently, complicated phenotypes may be associated with SNPs in PDZK1. For example, if a certain SNP leads to more potent regulation by PDZK1 of the reabsorption transporter rather than the secretory transporter, this SNP may be associated with higher reabsorption of uric acid. Alternatively, if a certain SNP equally contributes to the regulation of both influx and

efflux transporters, essentially no change in phenotype may be observed. In short, PDZ adaptors might control multiple transporters involved in both influx and efflux, and the net flux of the substrate across membranes might reflect the sum of the regulatory effects of the PDZ adaptors on every individual transporter.

4-2. NHERF1

Subjects who have certain SNPs of NHERF1 (Table 2) exhibit several phenotypes, including hypophosphatemia, possibly due to impaired reabsorption of phosphate in renal tubules, increase in cAMP excretion and increase in serum calcitriol level, compared with the wild-type [93]. Hypophosphatemia is a symptom observed in various diseases, including osteomalacia, and SNPs of NHERF1 could increase the risk of these diseases. Hypophosphatemia due to SNPs of NHERF1 can be explained by a decrease in reabsorption of phosphate owing to a decreased expression level of NaPi-IIa, which reabsorbs phosphoric acid in renal tubules. NHERF1 regulates apical localization of this transporter, and loss of NHERF1 down-regulates the expression of NaPi-IIa in the renal tubule [38].

On the other hand, NaPi-IIa expression is also under control by PTH: via the PTH receptor, PTH1 down-regulates the uptake of phosphoric acid in renal tubules through down-regulation of NaPi-IIa expression [38]. In cultured cell lines, this PTH-induced down-regulation of phosphate uptake is not observed in the case of SNPs of NHERF1 [93]. Therefore, certain SNPs of NHERF1 may result in loss of such down-regulation of phosphate transporter, thereby possibly leading to hyperphosphatemia. Thus, NHERF1 might regulate not only the phosphate transporter, but also the receptor that is colocalized adjacent to the transporter and is involved in the regulation of transporter expression.

5. Conclusion and Prospects

PDZ adaptors directly or indirectly interact with various types of transporters, receptors and intracellular signaling molecules (see sections 2 and 3). On the other hand, genetic variation of PDZ adaptors is associated with metabolic syndrome and other human diseases (see section 4). The association of PDZ adaptors with such multifactorial disorders could be explained in terms of the regulation of multiple

proteins by the PDZ adaptors (Fig. 4). Thus, PDZ adaptors are not just regulators of certain proteins, but appear to function as pleiotropic factors involved in various cellular events and diseases (Fig. 4).

However, there is still a gap between the regulation of proteins and association with diseases: the molecular mechanism(s) involved in PDZ adaptor-mediated human disorders remain poorly understood (Fig. 4). To clarify how PDZ adaptor dysfunction is associated with failure of homeostasis, further genome-wide studies in humans and gene knockin/knockout studies in experimental animals seem to be necessary. An example of a road map to fill this scientific gap could be deficiency in small GTP-binding protein Rab8, which plays a pivotal role in the targeting of various transporters and receptors to apical membranes of small intestine. Deficiency of Rab8 in humans and mice results in missorting of transporters, such as PEPT1 and SGLT1. Such a reduction in multiple transporter functions could be associated with impairment of gastrointestinal absorption of multiple nutrients, including oligopeptides and glucose, leading to lethality just after weaning in *rab8*$^{-/-}$ mice and microvillus inclusion disease in humans [94,95]. On the other hand, *pept1*$^{-/-}$ mice exhibit reduced intestinal absorption of

oligopeptides, but develop normally [96], while *sglt1*$^{-/-}$ mice exhibit hereditary malabsorption of glucose and galactose, but without lethality [97]. This may imply that deficiency in a single nutrient transporter can be compensated, but deficiency in multiple transporters provoked by the knock-down of an adaptor protein cannot be compensated, possibly due to the malfunction of other compensating proteins, leading to irreversible imbalance of homeostasis. Research on the transporter network may thus clarify the mechanisms of multifactorial disorders and perhaps provide target molecules for pharmacotherapy of those diseases (Fig. 4). On the other hand, since PDZ adaptors are involved in various membrane permeation processes, they could be useful targets to clarify the importance of transporters in pharmacokinetics. Research on the transporter-network may also contribute to elucidation of unknown membrane permeation mechanisms.

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LEGENDS TO FIGURES

Fig. 1 Schematic diagram representing intracellular trafficking of transporters

Fig. 2 Schematic diagram representing regulatory mechanisms for transporters exerted by PDZ adaptors

(A) PDZ adaptors are involved in sorting of transporters to specific cell-surface regions of plasma membrane.

(B) PDZ adaptors stabilize transporters on the cell-surface, thereby increasing residence time in plasma membrane.

(C) PDZ adaptors facilitate signal transduction by clustering various interacting proteins.

(D) PDZ adaptors stimulate the functional activity of transporters. Loss of the interaction renders the effect of the adaptors on transporters less facilitative.

Fig. 3 Effect of PDZ adaptor gene knockout on transporters in the small intestine, kidney and liver

Fig. 4 Possible association of impairment of the transporter network with increased risk of developing common diseases

Loss or mutation in PDZ adaptors diminishes the expression and/or function of various interacting transporters. Simultaneous malfunction of various transporters may increase the risk of common diseases.

Table 1 Regulatory Mechanisms for PDZ Adaptors (*in vitro* studies)

PDZ Adaptor	Binding Proteins	Effect of Interaction	Cell line(s) or other[a)]	Reference
PDZK1	MRP2	Sorting and/or Stabilization	Overexpression (HepG2)	[98]
	DRA	Signaling	Overexpression (Caco2BBe and HEK293)	[99]
	EAAC1	Sorting Stabilization Activation	Overexpression (MDCKII)	[12]
	MAP17/NaPi-IIa	Sorting and/or Stabilization	Overexpression (OK)	[100]
	MRP2/CFTR	Activation	Endogenous (Calu-3)	[101]
	MRP4/CFTR	Signaling	Endogenous (HT29-CL19A and T84)	[44]
	NHE3	Signaling Activation	Overexpression (PS120 and Caco2BBe)	[102]
	NOS2	Activation	Endogenous (MDCKII)	[103]
	OAT4	Sorting and/or Stabilization Activation	Overexpression (HEK293)	[31]
			Overexpression (LLC-PK1)	[34]
	OCTN1	Sorting and/or Stabilization Activation	Overexpression (HEK293)	[49]
	OCTN2	Activation	Overexpression (HEK293)	[50]
	PEPT1	Sorting and/or Stabilization Activation	Overexpression (HEK293)	[51]
	PEPT2	Sorting and/or Stabilization Activation	Overexpression (HEK293)	[20], [32]
	SR-BI	Signaling	Endogenous (mouse bovine aortic endothelial cells)	[79]

Table 1. (continued)

PDZ Adaptor	Binding Proteins	Effect of Interaction	Cell line(s) or other[a)]	Reference
NHERF1	β_2-AR	Sorting and/or Stabilization	Overexpression (CHO-N10)	[104]
	PTHR (PTH1R)	Signaling	Overexpression (PS120-R, CHO-R and HEK293R)	[105]
		Sorting and/or Stabilization Signaling	Overexpression (A10)	[106]
		Sorting and/or Stabilization	Overexpression (CHO-N10 and MC4) Endogenous (HEK293)	[107]
		Sorting and/or Stabilization Signaling	Overexpression (ROS 17/2.8)	[108]
		Sorting and/or Stabilization	Overexpression (CHO-N10)	[104]
	PTEN	Stabilization Signaling	Endogenous and overexpression (GBM)	[109]
	FzdR	Stabilization Signaling	Endogenous (MCF7) Overexpression (CHO-N10)	[110]
	CCR5	Stabilization Signaling	Overexpression (HEK293)	[111]
	MRP4	Stabilization Activation	Endogenous (HeLa)	[112]
		Sorting	Overexpression (MDCKI and LLC-PK1)	[113]
	OAT4	Sorting and/or Stabilization Activation	Overexpression (LLC-PK1)	[34]
		Stabilization	Overexpression (COS7)	[35]
	CFTR	Stabilization	Overexpression (COS7)	[114]
		Stabilization	Overexpression (CFBE41o-)	[115]
		Sorting and/or Stabilization Activation	Overexpression (16HBE14o- and CFBE41o-)	[116]
	NaPi-IIa	Stabilization	Overexpression (OK and OK-H)	[117]
		Sorting and/or Stabilization Activation	Endogenous (OK and OK-H)	[118]
	NaS1	Activation	Overexpression (*Xenopus* oocyte)	[119]
	iNOS	Sorting Activation	Endogenous and overexpression (RAW 264.7)	[120]
	ERK1/2	Signaling	Overexpression (CHO-N10)	[121]

a) Endogenous: for proteins endogenously expressed in the cells; Overexpression: for proteins exogenously transfected into the cells

Table 2. SNPs reported for *PDZK1* and *NHERF1* genes

Gene	dbSNP rs# cluster id	Nucleotide change[a)]	Amino acid substitution	Allele frequency	Population	Phenotype		Possible disorder	Reference
PDZK1	rs1797052	g.1055270C>T c.-69C>T	-	0.039	Croatian	Serum uric acid	→	-	[91]
	rs1298954	g.1057747A>G c.-3+2411A>G	-	0.689	Croatian			-	[91]
	rs12129861	g.1053276G>A	-	0.472[b)] 0.500[c)]	German	Allele frequency in gout patients	→	-	[85]
				0.492[d)] 0.483[e)]	German	Allele frequency in coronary artery disease patients	→	-	[85]
				0.46	European	Serum uric acid	↓	Hypouricemia	[90]
				0.48	Caucasian	Serum uric acid Systolic blood pressure	↓ ↑	Hypouricemia Hypertension	[86]
	rs1471633	g.1051326A>C	-	N.D.	European	Serum uric acid	↑	Hyperuricemia	[90]
	rs3912316	N.D.	-	0.131-0.261[f)]	Caucasian	Risk of metabolic syndrome Plasma triglycerides A trend toward abdominal obesity A trend toward hypertension Large and medium VLDL level	↑ ↑ ↑ ↑ ↑	Metabolic syndrome Hypertension Hyperlipemia	[82]
	rs11576685	g.1070683A>G c.-73-167A>G	-	0.035-0.070[f)]	Caucasian	A trend toward abdominal obesity	↑	Metabolic syndrome	[82]
	rs1284300	g.1075050C>T c.210+210C>T	-	0.078-0.155[f)]	Caucasian	-	-	-	[82]

Table 2. (continued)

Gene	dbSNP rs# cluster id	Nucleotide change[a)]	Amino acid substitution	Allele frequency	Population	Phenotype		Possible disorder	Reference
NHERF1	rs35910969	g.38019465C>G c.328C>G	L110V	0.006-0.012[f)]	Caucasian	Tubular maximum for phosphate reabsorption per glomerular filtration rate (TmP/GFR)	↓	Hypophosphatemia	[93]
	rs41282065	g.38032319G>A c.458G>A	R153Q	0.003-0.006[f)]	Caucasian	Urinary cAMP excretion	↑	Osteomalacia Renal tubular acidosis	[93]
	-	c.673G>A	E225K	0.003-0.006[f)]	Caucasian	Serum 1,25-dihydroxyvitamin D (calcitriol)	↑		[93]

a) c, coding DNA reference sequence; g, genomic reference sequence

b) in gout

c) gout-free

d) in coronary artery disease case

e) coronary artery disease-free

f) Estimated from the data shown in references

Fig. 1

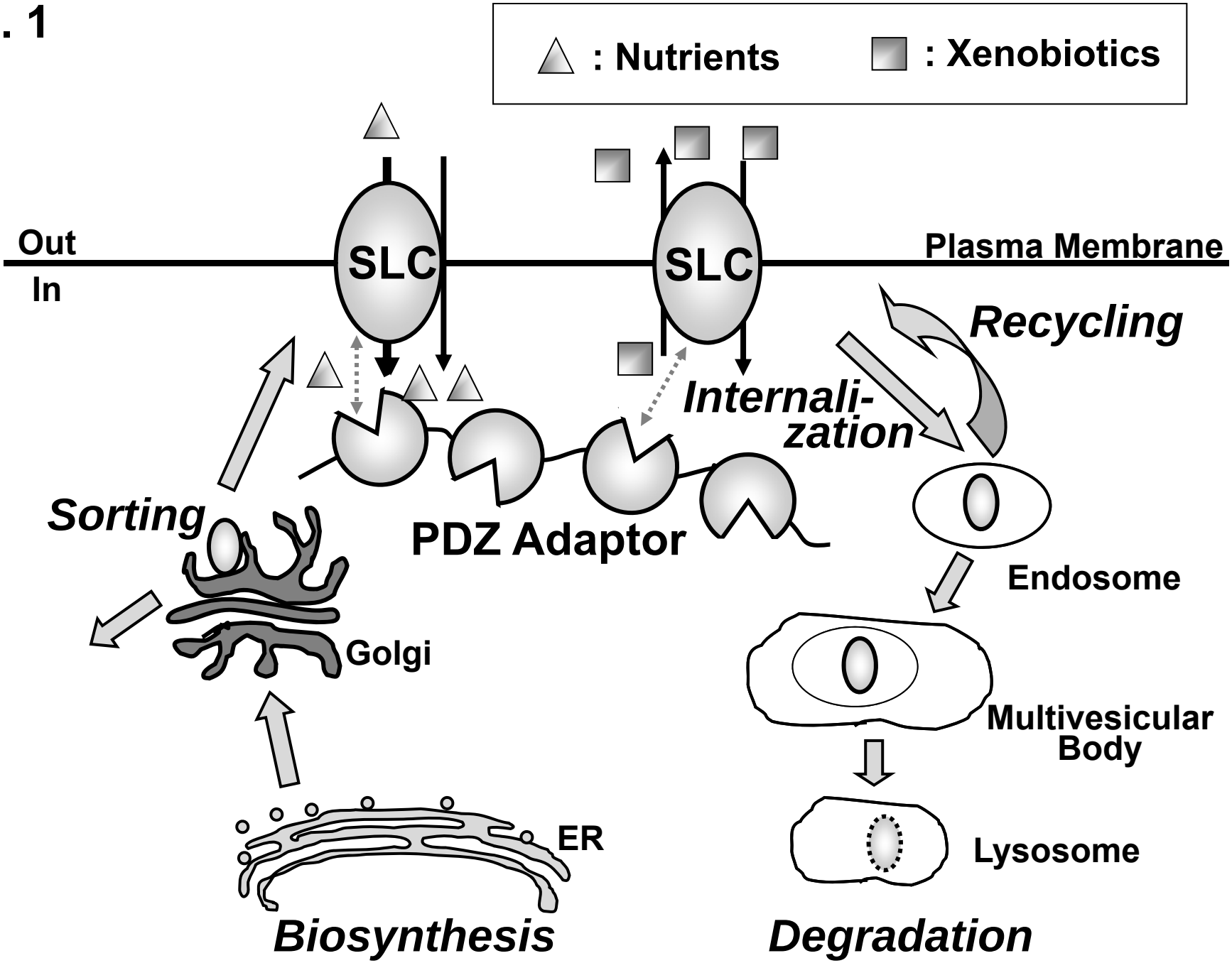
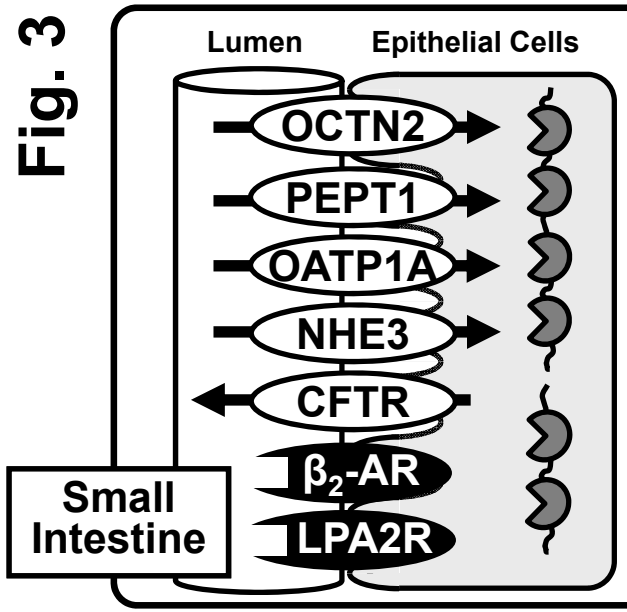
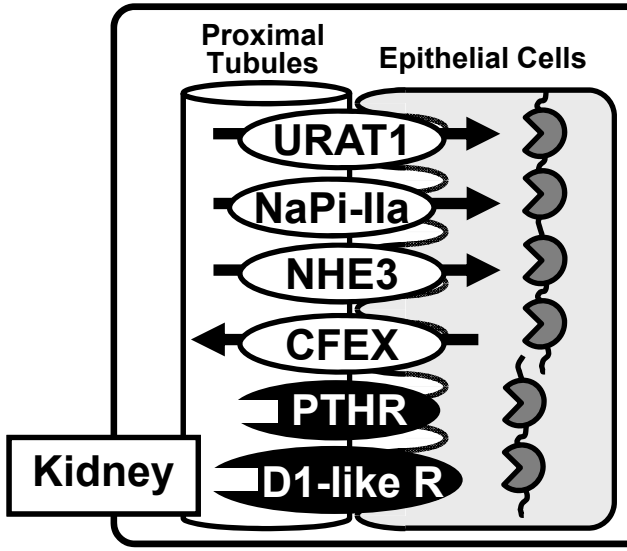

: Nutrients
: Xenobiotics
Out
In
SLC
SLC
Plasma Membrane
Recycling
Internali-
zation
Sorting
PDZ Adaptor
Endosome
Golgi
Multivesicular
Body
ER
Lysosome
Biosynthesis
Degradation

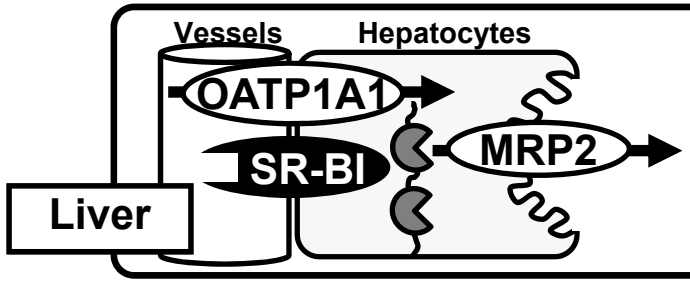
Fig. 3



Transporters Receptors	PDZ Adaptors	Change in Expression	Change in Function	Reference
OCTN2	PDZK1	↓	↓ (Transport)	[51]
PEPT1	PDZK1	↓	↓ (Transport)	[51]
OATP1A	PDZK1	↓	↓ (Transport)	[55]
NHE3	NHERF1 PDZK1	↓ / → →	↓ / → (Transport) ↓ (Transport)	[58], [60] [56]
CFTR	NHERF1 NHERF2 PDZK1	↓ (Crypts) → (Crypts) —	↓ (Transport, Signaling) ↑ (Transport) / ↓ (Signaling) ↓ (Transport)	[26], [57] [26], [57] [57]
β2-AR	NHERF1	—	↓ (Signaling)	[57]
LPA2R	NHERF2	—	↓ (Signaling)	[57]

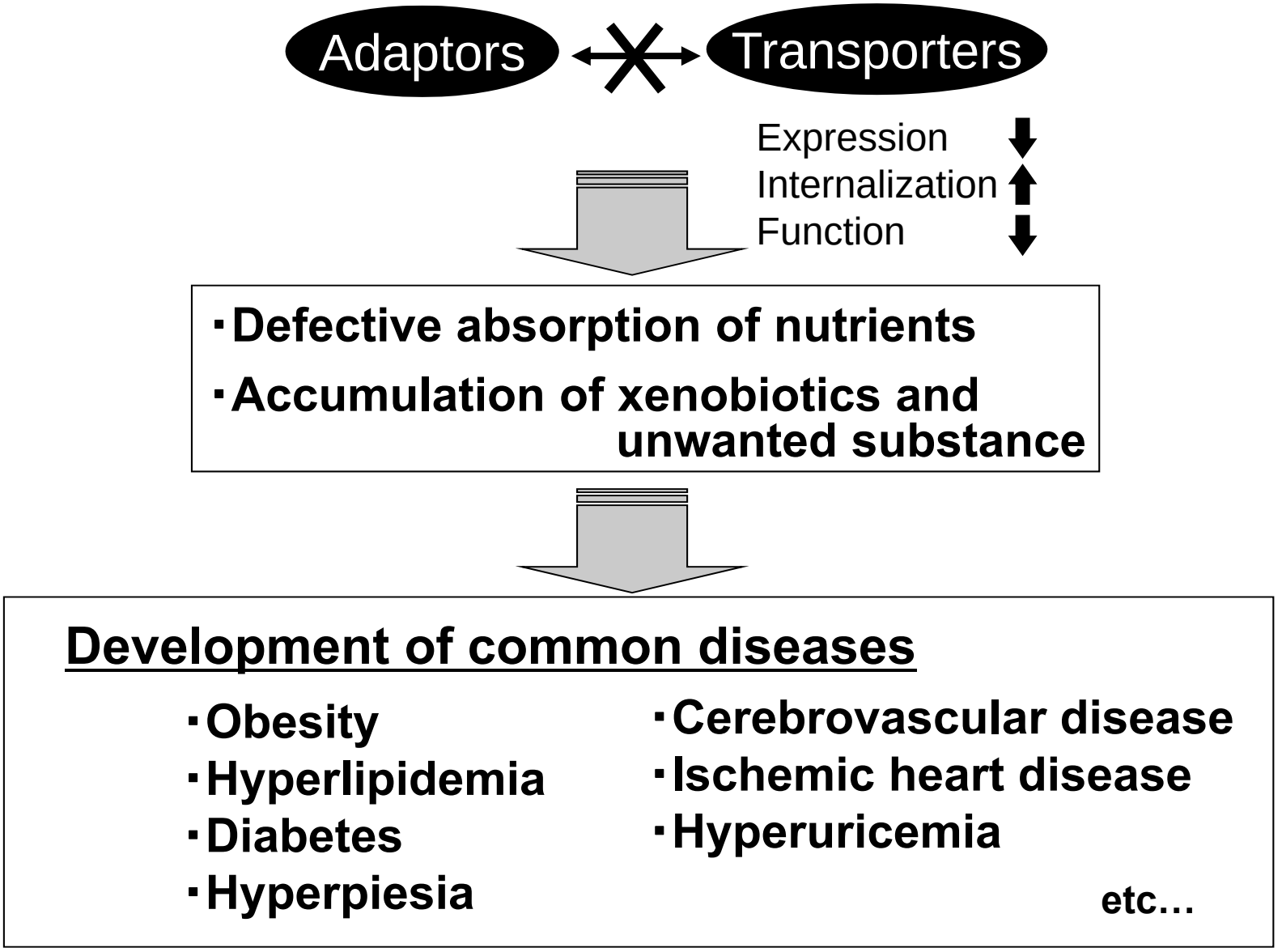


Transporters Receptors	PDZ Adaptors	Change in Expression	Change in Function	Reference
URAT1	NHERF1	↓	↓ (Transport)	[62]
NaPi-IIa	NHERF1 NHERF2 PDZK1	↓ → →	↓ (Transport, Signaling) → (Transport) →	[38], [64] [26], [65] [66]
NHE3	NHERF1 PDZK1	→ →	↓ (Transport) —	[60] [122]
CFEX	PDZK1	→	↓ (Transport)	[122]
PTHR	NHERF1	→	↓ (Signaling)	[38]
D1-like R	NHERF1	→	↓ (Signaling)	[69]



Transporters Receptors	PDZ Adaptors	Change in Expression	Change in Function	Reference
OATP1A1	PDZK1	↓	↓ (Transport)	[30]
MRP2	NHERF1	↓	↓ (Transport)	[72]
SR-BI	PDZK1	↓	↓ (Uptake)	[71]

Fig. 4


Adaptors
Transporters
Expression
Internalization
Function
・Defective absorption of nutrients
・Accumulation of xenobiotics and unwanted substance
Development of common diseases
・Obesity
・Hyperlipidemia
・Diabetes
・Hyperpiesia
・Cerebrovascular disease
・Ischemic heart disease
・Hyperuricemia
etc…

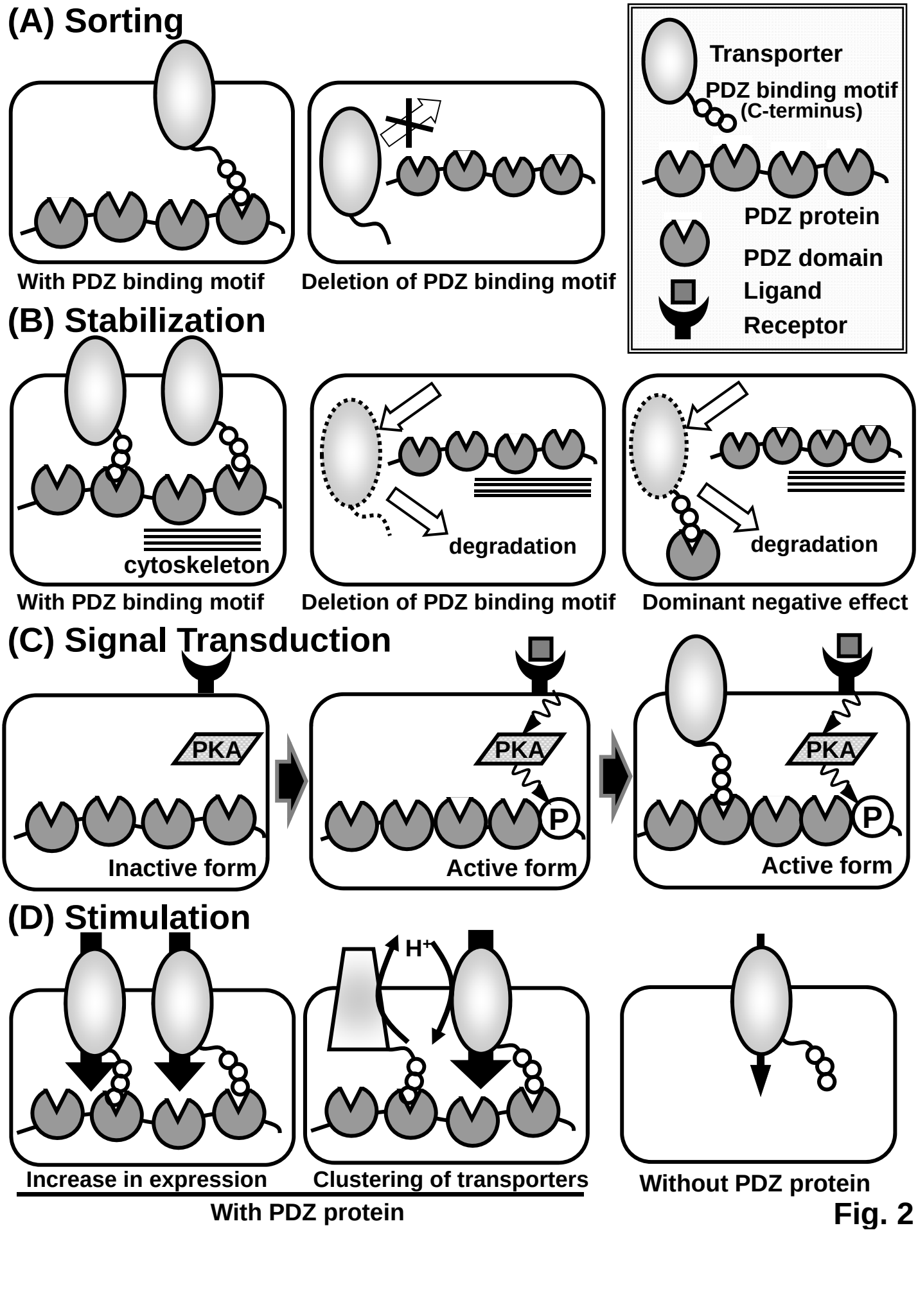

(A) Sorting
With PDZ binding motif
Deletion of PDZ binding motif
Transporter
PDZ binding motif
(C-terminus)
PDZ protein
PDZ domain
Ligand
Receptor
(B) Stabilization
cytoskeleton
degradation
degradation
With PDZ binding motif
Deletion of PDZ binding motif
Dominant negative effect
(C) Signal Transduction
PKA
PKA
PKA
P
P
Inactive form
Active form
Active form
(D) Stimulation
H+
Increase in expression
Clustering of transporters
Without PDZ protein
With PDZ protein

Fig. 2