PDZ-Binding and Di-Hydrophobic Motifs Regulate Distribution of Kir4.1 Channels in Renal Cells

Masayuki Tanemoto,* Takaaki Abe,*† and Sadayoshi Ito*

*Division of Nephrology, Hypertension and Endocrinology, Department of Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan; and †PRESTO, Japan Science and Technology Agency, Kawaguchi, Japan

It was shown previously that the carboxyl-terminal cytoplasmic portion of Kir4.1 determines the localization of basolateral K+ channel in renal distal tubules, which is composed from the assembly of Kir4.1 and Kir5.1. For clarifying the signals for this localization, specific sequence motifs of Kir4.1 were sought. In HEK293T cells, where Kir4.1 showed linear expression on the cell surface, disruption of the carboxyl-terminal PDZ-binding motif induced mostly clustered distribution but did not reduce whole-cell channel activity. Point mutation analysis revealed that serine377 in this motif was responsible for the surface vicinity expression. Disruption of the di-hydrophobic array of valine333/valine334 induced diffuse cytoplasmic distribution and diminished channel activity. Both valine333 and valine334 contributed to this effect. In contrast to the di-hydrophobic motifs of other membrane proteins that facilitate the sorting, valine333/valine334 supported the cell-surface retention. Because both the PDZ-binding and di-hydrophobic motifs participated in the basolateral expression of both Kir4.1 homomer and Kir5.1/Kir4.1 heteromer in MDCK cells, they are thought to be responsible for the localization of basolateral K+ channel in renal distal tubules.

A s members of the inwardly rectifying K+ channel (Kir) superfamily, Kir1.0 and Kir4.0 are expressed in several tissues, including brain and kidney, and function as key molecules for ion handling (1). In the brain, they are expressed in the astrocyte as pathways for K+ siphoning and maintain ionic homeostasis of the perineuronal environment (2). In the kidney, they are expressed in renal tubules as pathways for K+ recycling and play an important role for maintenance of the systemic fluid and electrolyte homeostasis (3–6). Supporting this notion, mutations in the gene coding a member of Kir1.0/4.0, Kir1.1, induces sodium loss in renal distal tubules and causes Bartter’s syndrome, a genetic disorder characterized by hypokalemic alkalosis (7).

Kir4.1, a member of Kir1.0/4.0, forms a basolateral K+ channel in renal distal tubules by heteromeric assembly with Kir5.1, another member of Kir (4,8). The channel activity of Kir5.1/Kir4.1 heteromer is regulated by intracellular pH, and this channel is also thought to take part in physiologic regulation of the fluid and acid-base homeostasis as a pathway for K+ recycling (8). For proper function of K+ recycling, expression of K+ transporters in the appropriate sites of renal tubules (localization) is necessary. Renal epithelium is considered to have machinery for this localization, and clarification of this machinery would contribute to our understanding of the fluid and acid-base homeostasis.

The machinery of the localization is thought to recognize specific sequence motifs on proteins and sort them by several steps (9–11). Synthesized membrane proteins are sorted to the final destination through the Golgi apparatus and then anchored there to show their function. PDZ (PSD-95/Dlg/ZO-1, also known as disc-large homology repeats or GLGF repeats)-binding motif, di-hydrophobic motif, tyrosine-based motif, and acidic cluster motif are thought to belong to these motifs and would contribute to our understanding of the fluid and acid-base homeostasis.

In previous reports, we have shown that the carboxyl-terminal (CT) portion of Kir4.1 determines the localization of basolateral K+ channels in renal distal tubules (4). We found that PDZ-binding motif of Kir4.1 contributed to the proper localization of the channels, but signals in the CT portion of Kir4.1 other than PDZ-binding motif were also responsible for the localization. To clarify these signals, we explored sequence motifs in the CT portion of Kir4.1 and then examined the role of these motifs for the localization and functional expression of basolateral K+ channels in renal distal tubules.

Materials and Methods

Construct of Deletion and Point Mutants

Kir4.1 and Kir5.1 (GenBank accession nos. NM 031602 and NM 053314) with and without amino-terminal green fluorescence protein (GFP) tag were constructed by subcloning each coding region into mammalian cell expression vectors as described previously (4). Several deletion and point mutants of Kir4.1 (as shown in Figure 1) were constructed by the PCR amplification method, inducing stop codon in its CT end, and using QuickChange Site-Directed Mutagenesis Kits (Stratagene, La Jolla, CA), respectively, according to the manufacturer’s instructions.
Figure 1. Carboxyl terminal (CT) amino acid sequence of Kir4.1 channels used in this study. Each amino acid is indicated in single-letter code and only the CT portion of each mutant is shown. Amino acids characteristic of so-called sorting motifs are indicated as bold letters in the sequence of wild-type (WT): di-hydrophobic motif (J), tyrosine-based motif (2), acidic cluster with aliphatic cluster (3), and PDZ-binding motif (4). Amino acids that mutated in this study are also indicated as bold letters.

Transient Expression of Kir in HEK293T Cells and MDCK Cells
HEK293T and MDCK cells were plated on poly-L-lysine-coated (Sigma, St. Louis, MO) coverslips and polycarbonate Millicell transwell filters (Millipore, Bedford, MA), respectively. Both cells were cultured in DMEM that contained 10% FBS and were transfected with vectors that were constructed as above by using LipofectAMINE 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. For co-transfection of Kir4.1 with GFP-Kir5.1, five times more Kir4.1 cDNA was co-transfected with GFP-Kir5.1 cDNA. Cytologic and electrophysiological analyses were usually conducted 48 to 72 h after transfection.

Cytologic Observation
After transfection, cytologic observation was performed as described previously (4). Especially in experiments with MDCK cells, the confluent growth of cells was confirmed before observation. For the staining with ZO-1, cells were pretreated with PBS that contained 0.2% Triton-X100 before fixation in 4% paraformaldehyde (pH 7.4 with sodium phosphate). The immunostaining was performed in PBS that contained 0.1% Triton-X100, 5% BSA, and 1% normal goat serum. Mouse anti-ZO1 (Zymed Lab. Inc., South San Francisco, CA) was used as the first antibody at a concentration of 10 μg/ml followed by incubation with 1:100 diluted Alexa Fluor 594 anti-mouse IgG. For the membrane labeling, wheat germ agglutinin (WGA)-conjugated Texas Red-X (Molecular Probes, Eugene, OR) was used at a concentration of 10 μg/ml. For the treatment with trypsin, HEK293T cells were rinsed with PBS and incubated with Trypsin-EDTA (Invitrogen) for 5 min at 37°C before fixation. Fluorescence observation was performed with a confocal microscope, model LSM 5 PASCAL (Carl Zeiss Co., Ltd., Jena, Germany).

Electrophysiologic Recordings
Channel activity was analyzed with patch-clamp method in whole-cell configurations as described previously (8). In brief, the resistance of patch electrodes, when filled with the pipette solution, was adjusted to 1 to 2 MΩm. The pipette solution contained (in mM) 140 KCl, 1 MgCl2, 1 CaCl2, and 5 HEPES-KOH (pH 7.4). The recording was performed in the bath solution that contained (in mM) 120 NaCl, 20 KCl, 5 EGTA, 2 MgCl2, and 5 HEPES-KOH (pH 7.3) using a patch-clamp amplifier (EPC-7, List Electronics, Darmstadt, Germany). Recorded data were low pass filtered at 1 kHz (~3 dB), sampled at 5 kHz, and analyzed. All of the experiments were performed at room temperature (22 to 24°C).

The amplitude of the currents was shown as mean ± SD, and comparison was done by an unpaired t test.

Cell-Surface Biotinylation
Biotinylation of proteins that were expressed on the extracellular surface was performed as described previously (13). In brief, transfected cells were washed with ice-cold phosphate buffer (PB) that contained (in mM) 120 NaCl, 4.4 KH2PO4, and 20.6 Na2HPO4. Then cell-surface proteins were biotinylated with 2 mg/ml Sulfo-NHS-LC-Biotin (Pierce, Rockford, IL) in PB for 20 min at room temperature or 4°C. Unreacted biotin reagent was blocked by addition of an equal volume of 10 mM glycine in PB for >10 min at room temperature.

Immunoprecipitation Analysis
Immunoprecipitation analysis was performed as described previously using BD Living Colors A.v. peptide antibody (BD Biosciences Clontech, Palo Alto, CA) (8). In brief, membrane proteins were solubilized in a lysis buffer that contained 150 mM NaCl, 50 mM Tris-HCl (pH 7.4), 1 μg/ml aprotinin, 100 μg/ml PMSF, 0.02% sodium azide, 0.1% SDS, 0.5% sodium deoxycholate, and 1% Triton X-100. Solubilized proteins were immunoprecipitated by antibody-pretreated protein A-Sepharose (Amersham Biosciences, Piscataway, NJ). The precipitated proteins were analyzed by immunoblot. For detecting biotinylated proteins, streptavidin-biotinylated horseradish peroxidase complex (Amersham Biosciences) was used at 1:1000 dilution for 12 h at 4°C, and then immunoreactive bands were detected by enhanced chemiluminescence kit (Amersham Biosciences). The intensity of detected proteins was calculated using NIH Image software (National Institutes of Health, Bethesda, MD). The relative intensity was shown as mean ± SD, and comparison was done by an unpaired t test.

Results
CT 47 Amino Acids Determine Cell-Surface Expression of Kir4.1 in HEK293T Cells
Amino acid sequences of mutants that were used in this study are summarized in Figure 1. Each amino acid is indicated in single-letter code, and only the CT portion of each mutant is shown. Among the amino acids deleted in this study, several motifs have been reported to take part in the localization, such as (I) di-hydrophobic, (2) tyrosine-based, (3) acidic cluster, and (4) PDZ-binding motifs. Characteristic amino acids of these motifs are indicated as bold letters in the lane of the wild-type (WT) Kir4.1.

Figure 2A shows intracellular distribution of GFP-tagged channels in HEK293T cells. The distribution on the surface of the ruffled edge is obscure in the cells that adhered to the glass surface. The trypsin treatment makes the cell surface smoothly round and the distribution on the cell surface seen easier. Therefore, the distribution of the channels was also observed after the trypsin treatment. Upper and lower panels show the distribution in trypsin-untreated and -treated cells, respectively. Whereas WT was expressed linearly on the cell surface in trypsin-untreated cells, the trypsin treatment revealed that the distribution was mostly in a clustered manner. The mutants with deletion of 3, 31, and 41 amino acids from CT (Δ3, Δ31, and Δ41, respectively) showed clustered distribution. The trypsin treatment revealed that several clusters of these deletion mutants were localized on the cell surface. In contrast to these deletion mutants, deletion of six more amino acids (deletion of...
47 amino acids from CT; Δ47) induced diffuse cytoplasmic distribution. The trypsin treatment showed no apparent distribution on the cell surface. The vertical views of trypsin-treated cells more clearly show the localizations.

Motifs of Kir4.1 That Determine Intracellular Localization

As shown in Figure 1, Kir4.1 contains a so-called PDZ-binding motif at its CT end and a di-hydrophobic motif in the region from the 333rd to the 338th amino acid (between Δ41 and Δ47).

To evaluate the significance of these motifs for the localization of Kir4.1, we made mutants by replacing the critical residues (serine377 or valine333/valine334) of these motifs to alanine (S377A or V333A/V334A). S377A showed clustered distribution as Δ3, Δ31, and Δ41 (Figure 2B, left), and V333A/V334A distributed diffusely in the cytoplasm as Δ47 (Figure 2B, right). The localization of these mutants was also more clearly observed after the trypsin treatment (Figure 2B, bottom).

Channel Activity of Mutants

To clarify the effect of the mutations on the function of Kir4.1, we examined the channel activity of the mutants. The Ba2+-sensitive (1 mM Ba2+) components of whole-cell currents from HEK293T cells that were transfected with each channel are summarized in the bar graph in Figure 3. The amplitude of the currents recorded at −120 mV are indicated. Voltage steps were delivered from the holding potential of −50 mV to potentials between −120 mV and 20 mV in 20-mV increments. Arrowheads indicate 0 current level. Current amplitude recorded at a holding potential of −120 mV is summarized in the bar graph. Current is normalized to cell-surface capacitance. Data are shown as means ± SD (n = 3 to 5).

Di-Hydrophobic Motif Determines Cell-Surface Expression

To clarify further the underlying mechanism for the functional channel formation, we performed cell-surface biotinylation experiments by using the mutants that showed dramatic change in the intracellular distribution (Δ3, S377A) or the function (Δ47, V333A/V334A). We performed the experiment at two different conditions: Room temperature and 4°C. At room temperature, protein sorting would take place continuously, and any proteins that are exposed to the extracellular space even transiently during the biotinylation procedure could be biotinylated. At 4°C, sorting would be considerably decelerated, and the proteins that are stably expressed on the cell surface during the procedure would be mostly biotinylated.

As shown in Figure 4A, the expression of each channel was equivalent (top), and all channels were sufficiently biotinylated at room temperature (middle). The amount of biotinylated mutants was not significantly different from that of WT. The relative values with reference to WT were 0.90 ± 0.04, 0.84 ± 0.09, 0.88 ± 0.07, and 0.83 ± 0.13 for Δ3, Δ47, S377A, and V333A/V334A, respectively (n = 3; filled columns in the bar graph). At 4°C, however, whereas WT was sufficiently biotinylated, biotinylation was negligible in the mutants with diminished channel activity (Δ47 and V333A/V334A).
values with reference to WT were significantly low for Δ47 and V333A/V334A: 0.30 ± 0.04 and 0.30 ± 0.10, respectively (n = 3; P < 0.001 for each). During the same period, the mutants that showed clustered distribution (Δ3 and S377A) were substantially biotinylated even at 4°C (Figure 4, bottom). The values for Δ3 and S377A were 0.76 ± 0.19 and 0.74 ± 0.27, respectively (n = 3; open columns in the bar graph). These results indicate that Kir4.1 could be transported to the cell surface without di-hydrophobic and PDZ-binding motifs and that di-hydrophobic but not PDZ-binding motif facilitated the cell-surface retention of Kir4.1.

The expression of channels on the cell surface was analyzed further by staining with a marker of membrane, Texas Red–conjugated WGA. WGA selectively binds to N-acetylglucosamine and N-acetylneuraminic acid residues of the membrane proteins. Therefore, the membrane of cells can be shown clearly as red images (Figure 4B). The higher magnification image revealed that the green image of most of WT channels did not co-localize with the red image of the cell surface, indicating that WT channels distributed just beneath the cell surface, although these channels seemed to be expressed linearly on the cell surface.

**Critical Residue in Di-Hydrophobic Motif for Localization**

The responsibility of each amino acid of the di-hydrophobic motif for the intracellular localization was analyzed further by inducing single amino acid mutations (Figure 5). Both single mutants of the di-hydrophobic motif (V333A and V334A) disrupted the linear expression of Kir4.1. However, V334A showed some fine clustered distribution, whereas V333A distributed diffusely in the cytoplasm. The trypsin treatment revealed that the fine clusters of V334A mainly distributed around the nucleus.

**Di-Hydrophobic and PDZ-Binding Motifs Determine Localization of Kir4.1 Homomer and Kir5.1/Kir4.1 Heteromer in MDCK Cells**

The role of the di-hydrophobic and PDZ-binding motifs for the localization of K⁺ channels in renal tubules was examined further by using MDCK cell as a model of renal epithelium, because it is a mammalian cell line derived from renal epithelium (Figure 6A). The formation of tight junction among MDCK cells after the sheet formation was confirmed by the expression of a typical protein of tight junction, ZO-1, on the top of the lateral wall of the cells (Figure 6A, bottom, red images, WT). In these tight junction–formed MDCK cells, Kir4.1 showed mostly lateral distribution (WT), but the disruption of PDZ-binding motif (S377A) or di-hydrophobic motif (V333A/V334A) dimin-

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**Figure 4.** Cell-surface expression of Kir4.1 channels. (A) Channels (indicated at the top of panels) were surface-biotinylated for 20 min at room temperature (RT) and 4°C. Mutations did not alter the amount of channels expressed in HEK293T cells (top). At RT, a significant amount of each channel protein was biotinylated (middle). At 4°C, whereas WT, Δ3, and S377A were biotinylated, few Δ47 and V333A/V334A were biotinylated (bottom). The bar graph summarizes the relative values of biotinylated mutants with reference to WT (means ± SD, n = 3). *Mutants that showed statistically significant difference from WT (P < 0.001). (B) Cells expressing each mutant of green fluorescence protein (GFP)-tagged Kir4.1 channel were stained with Texas Red–conjugated wheat germ agglutinin (WGA). Even WT Kir4.1, which seemed to locate linearly on the cell surface, was mostly expressed just beneath the cell surface as shown in the higher magnification view (inset in overlay view of WT). Bar = 10 μm.

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**Figure 5.** Disruption of linear expression of Kir4.1 by each single amino acid mutation in the di-hydrophobic motif. Both point mutants, V333A and V334A, disrupted the linear expression of Kir4.1 and induced diffuse cytoplasmic distribution (top). Although V334A showed tendency of cluster formation, the cluster distributed mainly around the nucleus as shown in the images after trypsin treatment (bottom). Bar = 10 μm.
Scaffolding proteins (16–18). In Kir4.1, serine377 was also re-
ponsible for the localization of Kir4.1. A single mutation of
S377A showed the similar effect as a whole deletion of PDZ-
binding motif, indicating that PDZ-binding motif–dependent
interaction to some scaffolding protein(s) affected the localiza-
tion of Kir4.1.

Characteristic sequence of the binding domains of scaffold-
ing proteins that can interact with PDZ-binding motifs (so-
called PDZ domains) has been clarified (16,17,19). These do-
main forms the specific interactive structure for PDZ-binding
motifs, and the domain of α-syntrophin, a scaffolding protein of
dystrophin-associated protein complex (DPC), can interact with
the PDZ-binding motif of Kir4.1 (20). Because DPC that con-
tains α-syntrophin is reported to exist on the basolateral side of
both MDCK cells and mouse renal distal tubules, α-syntrophin
is a candidate that contributes to the basolateral localization of
Kir4.1 in these cells (21,22). However, several other scaffolding
proteins with PDZ domain(s) are known to be involved in DPC,
and these proteins might contribute to the basolateral localiza-
tion of Kir4.1 (22). It remains to be clarified which scaffolding
protein determines the localization of Kir4.1 in MDCK cell or
renal distal tubules.

For functional expression of channels, two processes are nec-
essary: (1) expression of channel subunits and (2) formation of
active channel with the subunits. We have previously shown
that the PDZ-binding motif takes part in both processes for
Kir5.1 (13). In contrast, Kir4.1 could form functional channel
independent of the PDZ-binding motif, and the di-hydrophobic
motif rather than the PDZ-binding motif determined the func-
tional channel formation. Because disruption of the di-hydro-
phobic motif of Kir4.1 decreased the amount of channels that
are stably expressed on the cell surface but not that transported
to there, this motif is thought to take part in the process of
cell-surface retention rather than sorting.

Previous studies have shown that the di-hydrophobic motifs
of other membrane proteins participate in the process of sorting
or endocytosis rather than retention (23–25). These previously
identified di-hydrophobic motifs are generally composed from
leucine and isoleucine and have been shown to interact with
clathrin adaptor protein (AP) complexes. Because clathrin is a
key molecule to form cargo vesicles, a capability to interact
with AP complexes is thought to contribute to the process of
sorting and endocytosis (26). The di-hydrophobic motif of
Kir4.1 shows different sequence property (an array of two
valines), and this different sequence property might contribute
to the process of retention. It is speculated that the di-hy-
drophobic motif of Kir4.1 interacts with some anchoring proteins
and thereby retains Kir4.1 on the cell surface. Because there are
no precedent anchoring components that interact with di-hy-
drophobic motif, the proteins that retain Kir4.1 on the cell
surface remain to be elucidated.

The different contribution as a signal among the array of
amino acids is recognized in other di-hydrophobic motifs (27).
In the di-hydrophobic motif of Kir4.1, both single mutations of
V333A and V334A disrupted the linear cell-surface expression
of Kir4.1, although a single mutant V334A showed some fine
clustered distribution in the cytoplasm. Among the members of
the Kir superfamily, some (Kir1.1, Kir5.1, Kir6.1, and Kir6.2)
also contain valine at the residue corresponding to valine334 of

Discussion

In this study, we identified two motifs, PDZ-binding motif
and di-hydrophobic motif, in Kir4.1 that regulate the localiza-
tion of basolateral K+ channels in renal distal tubules. Al-
though Kir4.1 also contains tyrosine-based and acidic cluster
motifs that can regulate intracellular distribution in other mem-
brane proteins, neither of them affected localization of Kir4.1
(14,15).

In PDZ-binding motif of other proteins, the third residue
from CT is known to be a critical residue, and its single muta-
tion to alanine disrupts the interaction of this motif to so-called
scaffolding proteins (16–18). In Kir4.1, serine377 was also re-
ponsible for the localization of Kir4.1. A single mutation of

Figure 6. Intracellular distribution of Kir4.1 and Kir5.1 channels
in MDCK cells. (A) Localization of GFP-Kir4.1 mutants. As a
marker of tight junction, ZO-1 was expressed at the apical top
of the lateral wall of cells (red signals in the bottom panel of WT
[white arrowheads]). WT Kir4.1 mostly distributed on the lat-
eral surface of cells. In MDCK cells as in HEK293T cells, point
mutant S377A and V333A/V334A induced clustered and dif-
fuse cytoplasmic distribution, respectively. Bar = 10 μm. (B)
Localization of GFP-Kir5.1 co-expressed with untagged Kir4.1
mutants. Although Kir5.1 distributed diffusely in cytoplasm
(mock), co-expression with WT Kir4.1 induced clustered distri-
bution, and some of these clusters distributed on lateral surface
(WT). Co-expression of Kir4.1 mutants of S377A and V333A/
V334A did not induce apparent change in intracellular local-
ization of Kir5.1.

ished this lateral distribution. Mutation of S377A induced clus-
tered distribution, and mutation of V333A/V334A induced
diffuse cytoplasmic distribution in the same manner as shown in
HEK293T cells.

The role of PDZ-binding and di-hydrophobic motifs of Kir4.1
on the localization of Kir5.1/Kir4.1 heteromer was examined
further (Figure 6B). Although Kir5.1 distributed diffusely in
cytoplasm without the aid of Kir4.1 (mock), co-expression with
WT Kir4.1 induced clustered and lateral expression of Kir5.1
(WT). However, co-expression of the mutants of Kir4.1, which
contain either the disrupted PDZ-binding motif or di-hy-
drophobic motif (S377A or V333A/V334A), could not induce the
clustered and lateral expression of Kir5.1.
Kir4.1, but none contains hydrophobic amino acid at the residue corresponding to valine^{333} (I). This amino acid sequence property of Kir4.1 among the superfamily probably supports the notion that the array of hydrophobic residues (valine^{333} / valine^{334}) and especially the former one (valine^{333}) at the position of cytoplasmic tail of channel subunit is responsible for the intracellular localization of Kir4.1.

In HEK293T cells, Kir4.1WT seemed to be expressed mostly on the cell surface. However, both the whole-cell channel activity and the cell-surface biotinylation experiments revealed that the amount of channels expressed on the cell surface were not significantly different from the mutants with disrupted PDZ-binding motif, which showed clustered distribution in the cell. Therefore, most of Kir4.1WT is thought to be distributed to the intracellular compartments just beneath the cell surface, being demonstrated as clustered distribution on the inner surface of cells in trypsins-treated cells. The co-staining with a membrane marker also demonstrated that Kir4.1WT that shows linear expression on the plasma membrane was distributed not on the extracellular surface but just beneath the cell surface. The localization of Kir4.1 channels on the intracellular compartments just beneath the cell surface, which is easy to be expressed on demand, is thought to be appropriate for the in vivo dynamic regulation of their function by extracellular signals (28,29).

Although the lateral expression of Kir4.1 in a layer of MDCK cells made it technically difficult to record channel activity, the similar pattern of channel distribution in MDCK as in HEK293T cells suggests that the di-hydrophobic motif also contributes to functional expression of Kir4.1 in MDCK cells. Co-expression of the Kir4.1 mutant that contains the disrupted PDZ-binding motif as well as the disrupted di-hydrophobic motif could not change the intracellular distribution of Kir4.1. Therefore, in the case of Kir5.1/Kir4.1 heteromer, the PDZ-binding motif of Kir4.1 is thought to contribute not only to the intracellular localization but also to the functional expression in MDCK cells. Because Kir5.1/Kir4.1 heteromer is considered to be the principal basolateral K\(^+\) channels in renal distal tubules, both the di-hydrophobic and PDZ-binding motifs are necessary for the functional basolateral expression of K\(^+\) channels in renal distal tubules (4).

In MDCK cells, we could also observe expression of WT Kir4.1 on the apical side, but its amount is few compared with that on the basolateral side. This is thought to indicate that Kir4.1 could be transported to the apical side but did not stay there. According to the results of our study, the localization of basolateral K\(^+\) channel in renal epithelium is summarized in Figure 7. Channels are transported to the cell surface by the machinery that recognizes some signal motifs other than the di-hydrophobic and PDZ-binding motifs (Figure 7, left). The anchoring machinery that interacts with the di-hydrophobic motif could retain channels on the cell surface, but this retention process can not anchor channels on the intracellular compartments near the cell surface, and most of the channels are transported to some other intracellular compartments (Figure 7, middle). With the PDZ-binding motif, channels are efficiently retained on the compartments just beneath the basolateral cell surface (Figure 7, right).

In summary, the di-hydrophobic and PDZ-binding motifs of Kir4.1 regulated the functional expression of the basolateral K\(^+\) channels in renal distal tubules, Kir4.1 homomer, and Kir5.1/Kir4.1 heteromer. To form functional basolateral K\(^+\) channels, the di-hydrophobic and PDZ-binding motifs facilitated the cell-surface retention and the basolateral-side distribution, respectively. As the signals to facilitate functional expression of basolateral K\(^+\) channels in renal distal tubules, the two motifs of Kir4.1 clarified in this study are considered to be responsible for the fluid and acid-base homeostasis.

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