Structure and Function of PICK1

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Key Words

PICK1 • PDZ domain • BAR domain • Protein trafficking • AMPA receptor • Long-term potentiation • Long-term depression • Synaptic plasticity • Lipid binding • Phosphoinositides

Abstract

PICK1 is a peripheral membrane protein conserved from *Caenorhabditis elegans* to the human. It is expressed in many tissues with high levels in brain and testis. Inside cells, PICK1 is localized at the perinuclear region as well as specialized structures such as synapses of neurons. PICK1 contains a PDZ domain and a BAR domain. The PDZ domain of PICK1 binds to a large number of membrane proteins, especially proteins with C-terminal type II PDZ-binding motifs. The BAR domain of PICK1 binds to lipid molecules, mainly phosphoinositides. While the PDZ domain and the linker region of PICK1 enhance BAR domain's lipid binding, the C-terminal region of PICK1 inhibits its lipid binding. PICK1 regulates the subcellular localization and surface expression of its PDZ-binding partners. Lipid binding of PICK1's BAR domain is important for this regulation. With its PDZ domain interacting with membrane proteins and its BAR domain binding to lipids, the unique structure of PICK1 enables it to couple membrane proteins to protein-trafficking machinery.

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Overview

PICK1 (protein that interacts with C kinase 1) is a peripheral membrane protein initially cloned as one of the proteins interacting with protein kinase C- α (PKC α) from a yeast two-hybrid screening [1]. PICK1 exists in many animal species and is conserved all the way from *Caenorhabditis elegans* to humans. In fact, database analysis indicates that all of the animal species with full genome sequenced to date have PICK1. However, PICK1 has not been found in unicellular organisms such as yeast or bacteria, suggesting it may not be an essential gene for cell survival. This is supported by the finding that PICK1 knockout mice are viable [2, 3]. Surprisingly, we did not find anything close to PICK1 in plants from a database search. This suggests that PICK1 could be involved in functions unique to animals.

PICK1 was found to be expressed in many tissues [1, 4]. In immunoblot analysis using rat tissue homogenates, PICK1 was recognized as a band around 55 kDa, with the highest expression found in the brain, followed by testis [4]. A modest level of PICK1 was found in heart, lung, liver, spleen, kidney and muscle. Developmentally, PICK1 can be detected from rat brain as early as embryonic day 15 [4]. It gradually increases and reaches a plateau around 2 weeks after birth. In most cells, PICK1 was found to diffuse in the cytosol with a certain enrichment around the perinuclear region [1, 4–9], but it was also reported that PICK1 can be detected on mitochondria in NIH 3T3 cells [10]. In neurons, PICK1 was found to localize at syn-

apses, a specialized structure mediating neuronal communication [4, 5].

PICK1 protein is made of over 400 amino acid residues, ranging from 405 residues in *Xenopus*, 415 in humans to 504 in *Drosophila* (fig. 1). It contains two structurally known domains, a PDZ (PSD-95/Dlg/ZO-1) domain with about 90 amino acid residues close to its N-terminus, and a larger BAR (Bin/amphiphysin/Rvs) domain with about 200 residues spanning half of the protein (fig. 1). In addition, there are three regions that border these two domains: a short N-terminal region of ~18 residues before the PDZ domain enriched with acidic residues, a linker region of ~40 residues between the PDZ and BAR domains, and a C-terminal region characterized with a stretch of acidic residues. The two domains and three regions are conserved all the way from *C. elegans* to humans (fig. 1). The C-terminal region is the most divergent across species. The C-terminal region is about 60 amino acids long in most species, except in *C. elegans* and *Drosophila*, which have about 90 and 140 residues, respectively. Despite the diversity, the C-terminal regions from all species possess a common feature, which is a stretch of acidic amino acid residues. The domain structure of PICK1 is rather unique. There is no other gene in the NCBI database that has a similar structure. In fact, PICK1 is the only known gene which possesses both a PDZ domain and a BAR domain in the database.

PICK1 PDZ Domain Is a Protein-Protein Interaction Module

PDZ domains are well-characterized protein-protein interaction modules [11]. They normally consist of 80–100 amino acid residues that form six anti-parallel β -strands (β A– β F) and two α -helices (α A and α B) (fig. 2). The β B strand and α B helix form the binding pocket to PDZ-binding motifs. The classical PDZ-binding motifs are short peptides located at the extreme C-termini of proteins. An internal sequence that forms a hairpin-like structure mimicking the C-terminal peptide could also bind to the binding pocket [11]. In addition, the PDZ domain is capable of forming a dimer with the other PDZ domain [12]. More recently, the PDZ domain has been reported to bind to lipid molecules, such as phosphoinositides [13].

PDZ domains can be divided into three types, based on their selectivity on PDZ-binding motifs [11]. These types were mainly determined by the residue located at the -2 position of the PDZ-binding motif (the last amino

acid at the C-terminus was set as the 0 position). The type I PDZ domain recognizes -X-T/S-X- Φ , where X stands for any residues and Φ stands for hydrophobic residues. The type II PDZ domain recognizes -X- Φ -X- Φ and the type III PDZ domain recognizes -X-D/E-X- Φ . The selectivity to different types of PDZ-binding motifs is determined by the first residue of α -helix B (α B1) of the PDZ domain. In the type I PDZ domain, α B1 is a histidine, whose N3 nitrogen bond forms hydrogen bond with the hydroxyl group of the serine or threonine at the -2 position of PDZ-binding motifs. In the type II PDZ domain, the α B1 is usually a hydrophobic residue, which accommodates the hydrophobic residue at -2 position. The α B1 positions of type III PDZ domains were usually a tyrosine, whose hydroxyl group coordinates the carboxyl group of acidic residues [14, 15].

The PDZ domain was found to function in regulating subcellular targeting of proteins, especially membrane proteins. For example, the prototypical PDZ domain containing protein PSD-95 is highly enriched at synapses and believed to be responsible for bringing and regulating glutamate receptors at synapses [16]. Another important function of the PDZ domain is to serve as protein scaffolds for assembly of multimeric protein complexes. PDZ domains are often found to form tandem repeats. This arrangement will enable the PDZ domain-containing protein to bring different proteins together to form complexes. For example, InaD has five PDZ domains that bring different components of phototransduction cascade together to form a signaling complex [17]. In addition to tandem repeats, the PDZ domain could also team up with other protein interaction modules, such as SH3 and GK domains to assemble protein complexes [11].

PICK1 has a single PDZ domain. The crystal structure of the human PICK1 PDZ domain was recently resolved (unpublished structure in NCBI database). Similar to other PDZ domain proteins, PICK1's PDZ domain also consists of six β sheets and two α helices with α B and β B forming the binding pocket (fig. 2). Lysine 27 of PICK1 (unless otherwise specified, the residue numbers of PICK1 in this article refer to human, rat or mouse PICK1 sequence, which differ only in one position in the C-terminal acidic region) at the loop between β A and β B sheet forms the bottom of the pocket, with its positively charged side chain coordinates the carboxyl group of the C-terminus. Mutation of K27 together with the nearby D28 will completely disrupt PICK1's PDZ interaction [4, 18]. In the critical α B1 position, which determines the types of PDZ domain, PICK1 has a lysine (K83) (fig. 2). This

Fig. 1. Domain structure and sequence alignment of PICK1 protein. **a** Diagram of PICK1 domains showing the N-terminal acidic region (NAR), PDZ domain, linker region, BAR domain and the C-terminal acidic region (CAR). **b** Protein sequence alignment of PICK1 from different species. The alignment was generated by CLUSTW with slight modification. Sequences used in the alignment are: Human NP_036539, Rat NP_445912, Mouse NP_032863, *Xenopus* AAI08637, Sea urchin XP_788322, *Drosophila* NP_609582, and *C. elegans* NP_502796. The border of the PDZ domain was set based on a recent crystal structure of human PICK1 deposited in the NCBI database. The borders of the BAR domain were set based on secondary structure analysis and sequence alignment with arfaptin and amphiphysin BAR domains, whose structures had been determined by crystallography. All amino acid residue positions on the top row refer to the human sequence.

Table 1. Classification of PICK1 PDZ domain interacting proteins

PICK1 interacting protein	Binding sequence	Detection approach	Co-cluster	Surface change	Ref.
Type I: -X-S/T-X-Φ					
Aquaporin 2, water channel	LPRGSKA	Y2H	ND	ND	58
CAR, coxsackievirus and adenovirus receptor	SKDGSIV	In vitro	Yes	ND	59
ERBB4, receptor tyrosine-protein kinase	RHRNTVV	Y2H	ND	ND	8
GluR5, kainate receptor	QRKETVA	Y2H, in vitro	ND	ND	60
GluR6, kainate receptor	PGKETMA	Y2H, in vitro	ND	ND	60
<i>Kalirin-7, neuronal Rho-GEF</i>	DPFSTYV	Y2H	ND	N/A	61
mGluR8b, metabotropic glutamate receptor	VKSGSTS	Y2H	ND	ND	62
Neuroigin 1	SHSTTRV	Y2H	ND	ND	63
Neuroigin 2	PHSTTRV	Y2H	ND	ND	63
<i>PKCa</i>	PILQSAV	Y2H, in vitro	No	N/A	1, 18
Type II: -X-Φ-X-Φ					
Anionic exchanger 1	DEVAMPV	Y2H	ND	ND	58
Anionic exchanger 2	NEMPMPV	Y2H	ND	ND	58
Aquaporin 9, water channel	HELVSIM	Y2H	ND	ND	58
ASIC1, acid-sensing ion channel	TFEEIAC	Y2H, in vitro	Yes	ND	64–66
ASIC2, acid-sensing ion channel	TFEDFTC	Y2H, in vitro	Yes	ND	64, 65
DAT, dopamine transporter	LRHWLKV	Y2H, in vitro	Yes	Yes	6
EphA7, ephrin receptor	HGTGIQV	Y2H, in vitro	ND	ND	7
EphB2, ephrin receptor	QIQSVEV	Y2H, in vitro, in vivo	Yes	ND	7
Ephrin B1	ANIYYKV	Y2H, in vitro	Yes	ND	7
ERBB2, receptor tyrosine-protein kinase	LGLDVPV	Y2H, in vitro	ND	ND	8
GluR2, AMPA receptor subunit	GIESVKI	Y2H, in vitro, in vivo	Yes	Yes	4, 35
GluR3, AMPA receptor subunit	GTESVKI	Y2H	ND	ND	4, 35
GluR4c, AMPA receptor subunit	GTESIKI	Y2H	ND	ND	4, 35
JAM-A, junctional adhesion molecule	QTSSFLV	Y2H, in vitro	ND	ND	67
JAM-B, junctional adhesion molecule	HTKSFII	Y2H	ND	ND	67
JAM-C, junctional adhesion molecule	HKSSFVI	Y2H, in vitro	ND	ND	67
mGluR7a, metabotropic glutamate receptor	SYNNLVI	Y2H, in vitro, in vivo	Yes	No	62, 68, 69
mGluR7b, metabotropic glutamate receptor	YTIPPTV	Y2H, in vitro	ND	ND	62, 70, 71
MuSK, muscle-specific kinase receptor	AEGTVSV	Y2H	ND	ND	8
Nectin 1α, herpesvirus Ig-like receptor	SKKEWYV	Y2H, in vitro	Yes	ND	67
Nectin 2α, herpesvirus Ig-like receptor	SRRAVYV	Y2H	ND	ND	67
Nectin 2δ, herpesvirus Ig-like receptor	MSRAMYV	Y2H	ND	ND	67
Nectin 3α, herpesvirus Ig-like receptor	SRREWYV	Y2H	ND	ND	67
NET, norepinephrine transporter	LQHWLAI	Y2H	ND	ND	6
PrRP-R, prolactin-releasing peptide receptor	MTVSVVI	In vitro	Yes	ND	72
<i>Serine racemase</i>	SYQSVSV	Y2H, in vitro	No	N/A	54
UNC5H1, netrin receptor	TVSEAEC	Y2H, in vitro, in vivo	Yes	Yes	33
Others					
Aquaporin 1, water channel	RVEMKPK	Y2H, in vitro	ND	ND	58
<i>Arf1, ADP-ribosylation factor</i>	NQLRNQK	Y2H	ND	N/A	27
<i>Arf3, ADP-ribosylation factor</i>	NQLKNKK	Y2H	ND	N/A	27
mGluR4a, metabotropic glutamate receptor	TYTNHAI	Y2H	ND	ND	62
mGluR8a, metabotropic glutamate receptor	SYSDHSI	Y2H	ND	ND	62
Nectin 3β, herpesvirus Ig-like receptor	NPREHYV	Y2H	ND	ND	67
Nectin 4, herpesvirus Ig-like receptor	NGRGHLV	Y2H	ND	ND	67
<i>TIS21, NGF-inducible protein</i>	YVMAVSS ^a	Y2H, in vitro	ND	N/A	73
SER, serotonin transporter	DIRLNAV	Y2H	ND	ND	6

Proteins in italic are non-membrane proteins. Y2H = Yeast two-hybrid; ND = not done; N/A = not applicable. In vitro interaction characterization includes co-immunoprecipitation from overexpression in heterologous cells, GST pulldown and immuno-fluorescent experiments. In vivo interaction indicates detection of interaction from native tissues such as brain homogenate.

^a The exact interaction sequence has not been determined, the C-terminal sequence of TIS21 is shown.

ranging from protein phosphorylation, neurotransmission to cell adhesion. In the cases where the interaction sites were determined, it is usually the C-termini of these proteins that interact with PICK1. According to their C-termini, these proteins were classified to three categories: type I, type II PDZ-binding motif, and others (table 1). While PICK1's PDZ domain binds to both type I and type II PDZ-binding motifs, the majority of the PICK1's PDZ ligands can be classified as type II. Furthermore, while the interactions of PICK1 to several type II PDZ-binding motifs have been confirmed in vivo, no type I motif has been done so (table 1). This suggests that the PDZ domain of PICK1 does have a preference for type II motif. Indeed, it was found that the affinity of PICK1's PDZ domain to type II motif is much higher than to type I [19, 20].

BAR Domain of PICK1 Binds to Lipid

The BAR domain is a protein module of about 200 amino acid residues. It was frequently found in proteins involved in membrane trafficking, especially endocytosis. The prototypical BAR domain-containing protein amphiphysin plays important roles in endocytosis of synaptic vesicles [21, 22]. The BAR domain of amphiphysin could directly bind to liposomes, with binding affinity varying according to the diameters of liposomes. In addition, the BAR domain can deform liposomes to form tubules [23, 24]. This suggests that the BAR domain could serve as a membrane curvature sensor or actively bends the membrane to induce formation of trafficking vesicles.

A crystallographic study of the *Drosophila* amphiphysin BAR domain revealed that a complete BAR domain is a crescent-shaped dimer [24] (fig. 3). In other words, a BAR domain module in a protein is actually half of a BAR domain. It has to join with another BAR domain from the same protein (homodimer) or a different protein (heterodimer) to form a fully functional BAR domain. The full BAR domain is made of six helical bundles, with each monomer contributing three helices. There are four positively charged groups that are important for BAR domain's function. Two locate at both ends of the crescent dimer and two locate at the concave surface of the dimer (fig. 3). These positively charged residues are responsible for binding to negatively charged lipid molecules such as phosphoinositides. This provides the structure basis for BAR domain's lipid binding [24].

The central region of PICK1's was initially predicted to form coiled-coil repeats. These repeats have high se-

quence homology to proteins called arfaptins. Arfaptins, which include arfaptin 1 and arfaptin 2, are a group of proteins that bind to small G proteins such as Arf and Rac [25]. The sequence of PICK1 and arfaptin is not very similar to the prototypical amphiphysin BAR domains. As such, they were not recognized as BAR domains initially. However, when the crystal structure of the BAR domain was resolved [24], it was found to have a similar structure to the GTPase-binding domain of arfaptin 2 [25]. It was subsequently confirmed that similar to the amphiphysin BAR domain, the GTPase-binding domain of arfaptin 2 could also bind and tubulate liposomes [24]. Mutating the positively charged residues of arfaptin 2 also eliminates its lipid-binding capability. These results indicate that the GTPase domain of arfaptin 2 is a bona fide lipid-binding BAR domain.

The sequence homology of PICK1 with arfaptin 2 suggests that the coiled-coil repeats in the central region of PICK1 may also be a lipid-binding BAR domain. Sequence alignment of PICK1, arfaptin 2 and amphiphysin indicates that the positive charged residues responsible for lipid binding are conserved in PICK1's BAR domain (fig. 3). Using liposome sedimentation assay and lipid strip overlay assay, we recently demonstrated that the BAR domain of PICK1 indeed binds to lipid molecules [26]. The lipid molecules that interact with PICK1 are mainly negatively charged phosphoinositides. Similar to other BAR domains, PICK1's lipid binding is mainly mediated by two groups of positively charged residues. Lysine 266 and 268 (K266,268) are predicted to locate at the end of the crescent-shaped dimer and lysine 251, 252 and 257 (K251, 252, 257) are predicted to locate at the concave surface of the dimer. Mutations of either group significantly reduced PICK1's lipid-binding capability [26]. Furthermore, lipid binding of PICK1's BAR domain was found to be both positively and negatively regulated by other regions of PICK1. The PDZ domain and the linker region of PICK1 enhance BAR domain's lipid binding and the C-terminal region of PICK1 inhibits its lipid binding.

The BAR domain of PICK1 is most close to arfaptins. The BAR domains of arfaptins were shown to directly bind to small GTPase such as Arf1 and Rac1 [25]. This raises the question of whether PICK1's BAR domain could also bind to a small GTPase. This idea was explored using a yeast two-hybrid technique. While Arf1 and Arf3 were found to interact with PICK1, they do not bind to the BAR domain of PICK1 [27]. Instead, they were found to interact with the PDZ domain of PICK1. The interaction of Arfs with PICK1 has yet to be confirmed in vivo

and the physiological function of this interaction is not clear. It remains an open question whether and what GT-Pase binds to the BAR domain of PICK1.

Apart from interaction with lipid molecules and self-association, the BAR domain of PICK1 has been reported to interact with several other proteins. Using fusion protein *in vitro* binding assay, deleting the BAR domain of PICK1 was found to disrupt its interaction with GluR2 [28]. A region located 30–40 residues upstream of GluR2 C-terminus is believed to be important for this interaction. Using a similar *in vitro* binding assay, PICK1 was found to interact with both α -SNAP and β -SNAP (soluble NSF attachment protein), two proteins required for disassembly of SNARE complexes during membrane fusion [28]. NSF was found to disassemble the PICK1-GluR2 complex in the presence of α -SNAP. Intriguingly, GRIP (glutamate receptor interacting protein), another PDZ domain containing protein that interacts with GluR2 [29], was also found to interact with the BAR domain of PICK1 and this interaction is involved in AMPA receptor trafficking [30]. More recently, the membrane proximal region of GluR δ 2 was reported to interact with part of PICK1's BAR domain (aa 245–278) and this interaction is important for synaptic plasticity at cerebellar Purkinje neurons [31].

The N-Terminal Acidic Region, Linker Region and C-Terminal Acidic Region of PICK1

In addition to the PDZ and BAR domains, there are three short regions in PICK1. A short region with about 18 amino acid residues locates at the extreme N-terminus of PICK1. This short region is enriched with acidic amino acid residues. The N-terminal acidic region (NAR) of PICK1 was reported to directly bind to Ca^{2+} [32]. The binding of Ca^{2+} regulates the affinity of PICK1's interaction to GluR2. The optimal Ca^{2+} concentration for PICK1 and GluR2 interaction was determined to be around 15 μM . Deletion of the N-terminal region renders the interaction of PICK1 and GluR2 insensitive to Ca^{2+} . The Ca^{2+} binding of PICK1 was also found to be important to AMPA receptor trafficking regulated by activation of NMDA receptors.

Between the PDZ domain and BAR domain of PICK1, a linker region of about 40 amino acids is predicted to form a helical structure. It has been reported that in some BAR domains, a short sequence before the BAR domain could form an amphipathic helix. The helix could enhance BAR domain's lipid-binding capabilities

by inserting themselves into lipids [24]. This kind of BAR domain is called N-BAR. The linker region of PICK1 was predicted to form a helical structure and it is likely that the BAR domain of PICK1 is one of the N-BAR. Indeed, the linker region was found to enhance lipid binding of PICK1's BAR domain [26]. In addition to the linker region, the PDZ domain of PICK1 was also found to enhance its lipid binding. It is not clear at this moment how the PDZ domain enhances PICK1's lipid binding.

The C-terminal region of PICK1 contains a stretch of acidic residues that are highly conserved across species (4 in *C. elegans*, 9 in humans and 17 in sea urchins) (fig. 1). The C-terminal acid region (CAR) of PICK1 was found to inhibit lipid binding of PICK1's BAR domain [26]. Without the presence of the PDZ domain and the linker region, the C-terminal region of PICK1 could completely abolish PICK1 BAR domain's lipid-binding capability [26]. This autoinhibition is likely due to the negatively charged acidic residues of PICK1's C-terminal region fold back and bind to the positively charged residue of PICK1's BAR domain to inhibit its lipid binding. It has been reported that the C-terminal acidic region also has weak Ca^{2+} -binding affinity [32]. It would be interesting to examine if the autoinhibition of lipid binding could be regulated by Ca^{2+} or other mechanisms, which will make PICK1's lipid binding more dynamic. In fact, deletion of the C-terminal region has been reported to increase synaptic targeting of PICK1 in neurons [19], suggesting that the autoinhibitory effect of PICK1's C-terminal region may have physiological significance.

PICK1 Functions as a Protein-Trafficking Regulator

PICK1 binds to a large number of proteins, especially membrane proteins. In most of the cases, the binding of PICK1 regulates the trafficking of its binding partners by altering either their subcellular targeting and/or surface expression (table 1). For example, PICK1 induces clustering of ephrein-B1 and Eph receptor EphB2 when co-expressed in heterologous cells [7]. PICK1 was also found to alter the surface expression of netrin receptor UNC5H1 and regulate netrin-mediated axon outgrowth [33, 34]. Here we use the extensively studied interaction of PICK1 and AMPA receptors as an example to illustrate how PICK1 regulates trafficking and function of membrane proteins.

PICK1 was found to interact with AMPA receptor subunits GluR2, 3 and 4c in yeast two-hybrid assays [4, 35].

The interaction of PICK1 with GluR2 was subsequently verified by co-immunoprecipitation (co-IP) from heterologous cells and in vivo co-IP from rat brain homogenate. When co-expressed in heterologous cells, PICK1 forms many co-clusters with GluR2 in the cytosol. The nature of these clusters is unclear at this moment but they are unlikely to be just simple aggregation of proteins, as the formation of these clusters requires PICK1's lipid-binding capability [26]. The clustering of GluR2 requires the interaction of PICK1 and GluR2, as either mutation at the PDZ domain of PICK1 (K27D28 to AA) or deletion at the C-terminus of GluR2 disrupt the co-clusters [4]. In neurons, PICK1 was found to co-localize with GluR2 in synapses. PICK1 has the ability to bring AMPA receptors to synapses as overexpression of wild-type PICK1 increases the synaptic GluR1 and GluR2 [26]. This is also dependent on the interaction between PICK1 and GluR2, as PDZ mutant of PICK1 failed to target AMPA receptors to synapses. In addition to regulating the subcellular localization of AMPA receptors, PICK1 also alters the surface expression of GluR2. Overexpression of PICK1 reduced surface expression of GluR2, as measured by surface biotinylation assay and immunostaining of surface receptors [5, 26, 36].

More interestingly, PICK1 was found to regulate the dynamic trafficking of AMPA receptor during synaptic plasticity. Forms of synaptic plasticity such as long-term potentiation (LTP, where synaptic response is increased) and long-term depression (LTD, where synaptic response is decreased) are regarded as the cellular basis of learning and memory [37]. A great deal of evidence suggests that LTP and LTD could be the result of an increasing or decreasing number of AMPA receptors at the synaptic surface, respectively [38]. Disrupting the interaction between PICK1 and GluR2 using the last 20 amino acids of GluR2 or an antibody targeting the PDZ domain of PICK1 inhibited expression of LTD in cerebellar Purkinje neurons [39, 40] and hippocampal pyramidal neurons [41]. A PDZ mutant of PICK1 was also found to inhibit LTD in Purkinje neurons, indicating the requirement of PICK1-GluR2 interaction for LTD [40]. This is further confirmed in PICK1 knockout mice, where LTD was found to be completely abolished in cerebellar Purkinje neurons [3]. Transfecting wild-type PICK1 rescued the LTD but the PDZ mutant of PICK1 failed to do so. These results provided strong evidence to support the role of PICK1 in the removal of AMPA receptors from the synaptic surface during LTD in cerebellar Purkinje neurons.

Protein phosphorylation was found to play important roles in PICK1-mediated AMPA receptor trafficking and synaptic plasticity. The serine 880 of GluR2, which locates at the -3 position of GluR2 C-terminus, could be phosphorylated by protein kinase C [42, 43]. The phosphorylation of S880 does not change PICK1's interaction with GluR2 but significantly reduces binding of GluR2 to GRIP. By disrupting the interaction with GRIP, phosphorylation of S880 by PKC was found to promote interaction of GluR2 with PICK1 and facilitate PICK1-mediated internalization of AMPA receptors during LTD [5, 39, 43–45].

PICK1's lipid-binding capability was found to be important for its function in AMPA receptor trafficking as well. The lipid-binding deficient BAR domain mutant PICK1 2K-E (K266,268 mutated to E) could no longer cluster GluR2 when co-expressed in the heterologous system [26]. The BAR domain mutant of PICK1 also lost its ability to regulate surface expression of GluR2. Furthermore, the BAR domain mutant of PICK1 lost its ability to target to synapses in neurons. Consequently, the BAR domain mutant of PICK1 could not bring AMPA receptors to synapses [26]. In an electrophysiological study, the BAR domain mutant of PICK1 was found to inhibit expression of LTD [26]. The role of PICK1's BAR domain in LTD was further verified in PICK1 knockout mice. While expression of wild-type PICK1 could rescue PICK1 knockout mice's deficiency in cerebellar LTD, the BAR domain mutant PICK1 was not able to do so [3]. Taken together, this suggests a mechanism for PICK1-mediated AMPA receptor trafficking: while PICK1's PDZ domain binds to the C-termini of AMPA receptors, its BAR domain binds to negatively charged lipids and tethers the AMPA receptors as cargo to curved-membrane microdomains that are designated to form trafficking vesicles (fig. 4). As PICK1 interacts with a number of other membrane proteins, it could be a general mechanism for PICK1 to regulate the trafficking of membrane proteins via the lipid-binding capability of its BAR domain.

While most studies suggest that PICK1 is involved in endocytosis of AMPA receptors, evidence has started to emerge that PICK1 could also be involved in the insertion of AMPA receptors to cell surface. In the cerebellar stellate cells, repetitive stimulation of parallel fiber could induce insertion of GluR2-containing receptors to the surface of synapses [46]. The interaction of PICK1 with GluR2 was found to be important in this process, as blocking the interaction between GluR2 and PICK1 inhibits the insertion of GluR2 to surface [2, 47]. Furthermore, the delivery of GluR2 to surface was also impaired

in PICK1 knockout mice [2]. These results support a role of PICK1 in insertion or exocytosis of AMPA receptors. To reconcile the role of PICK1 in both endocytosis and exocytosis, we propose that PICK1 may maintain an intracellular pool of AMPA receptors at synapses and serve as a shuttle between the intracellular and surface pool of AMPA receptors (fig. 4).

Most current studies of PICK1 focus on its cellular function, especially its roles in membrane protein trafficking. The function of PICK1 in whole organisms has not been adequately addressed. While PICK1 knockout mice were found to have a deficiency in trafficking of AMPA receptor and synaptic plasticity [2, 3], it remains to be tested if these cellular deficiencies would manifest in animal behavior such as learning and memory. It is interesting to note that PICK1 has been implicated in mental disorders. In humans, PICK1 is localized at chromosome 22q13.1. This region was found to have a significant association with schizophrenia and bipolar disorder [48–53]. Using an association study, polymorphisms

of PICK1 were found to correlate with schizophrenia [54, 55]. It was also reported that schizophrenia patients had a decreased PICK1 mRNA level in the cortex by in situ hybridization [56], but in a separate report, no significant change on PICK1's mRNA level was detected by real-time PCR from the cortex of elderly schizophrenia patients [57]. It is not clear what role PICK1 may play in schizophrenia, but PICK1's interaction with monoamine transporters, serine racemase, glutamate receptors and other neuronal proteins could contribute to the disorder. Further investigations are needed to clarify the role of PICK1 in schizophrenia and other mental disorders.

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