PDZ DOMAIN PROTEINS OF SYNAPSES

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Abstract | PDZ domains are protein-interaction domains that are often found in multi-domain scaffolding proteins. PDZ-containing scaffolds assemble specific proteins into large molecular complexes at defined locations in the cell. In the postsynaptic density of neuronal excitatory synapses, PDZ proteins such as PSD-95 organize glutamate receptors and their associated signalling proteins and determine the size and strength of synapses. PDZ scaffolds also function in the dynamic trafficking of synaptic proteins by assembling cargo complexes for transport by molecular motors. As key organizers that control synaptic protein composition and structure, PDZ scaffolds are themselves highly regulated by synthesis and degradation, subcellular distribution and post-translational modification.

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PDZ DOMAINS are modular protein-interaction domains that are specialized for binding to short peptide motifs at the extreme carboxy (C) termini of other proteins, athough they can also have other modes of interaction[1]. Found in many varied proteins (more than 400 in humans or mice), PDZ domains are classified on the basis of the sequence of their preferred C-terminal ligands. The structural basis of their binding specificity is well understood (FIG. 1).

PDZ domains are often arranged in tandem arrays and/or associated with other interaction domains to form multidomain scaffold proteins (FIG. 2). By binding to specific polypeptides through each domain, such PDZ-containing scaffold proteins can assemble large molecular complexes. Typically, the PDZ scaffold and its associated multiprotein complex are targeted to a specific subcellular site to perform a specialized local function.

In the nervous system, excitatory synapses, and in particular their POSTSYNAPTIC DENSITIES (PSDs), contain many PDZ proteins and provide outstanding examples of PDZ-domain-based functions (TABLES 1,2). The best characterized of the synaptic PDZ proteins is post-synaptic density protein 95 (PSD-95), an abundant component of the PSD. As the archetypal PDZ-based scaffold, it is discussed in most detail in this review. Although PSD-95 is exemplary of PDZ scaffold proteins, its properties are not common to all PDZ proteins. Even close relatives of PSD-95 exhibit distinct cell-biological behaviours, as described below. To illustrate the diverse structure and function of PDZ proteins, we compare the PSD-95 family of proteins, which bind directly to NMDA (*N*-methyl-D-aspartate) receptors (NMDARs), with the PDZ proteins that interact with AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors (AMPARs).

We regret that, owing to space restrictions, many excellent publications could not be cited. In many cases we have cited just the latest publication or a review to help the reader to navigate the recent literature. Related information can also be found in other reviews[2–5].

The PSD-95 family of PDZ scaffolds

Structure of PSD-95. The PSD-95 family of PDZ scaffold proteins is encoded by four genes (*PSD-95*/*SAP90* (synapse-associated protein 90), *PSD-93*/*chapsyn-110*, *SAP102* and *SAP97*). These proteins are characterized by three PDZ domains, an SRC HOMOLOGY 3 (SH3) DOMAIN, and a GUANYLATE KINASE-LIKE (GK) DOMAIN (FIG. 2). The SH3 and GK domains interact in an intramolecular fashion, but the functional significance of this interaction is unclear[6,7]. Electron microscopy images indicate that purified, full-length PSD-95 monomers are folded into compact C-shaped particles of around 110 × 60 Å

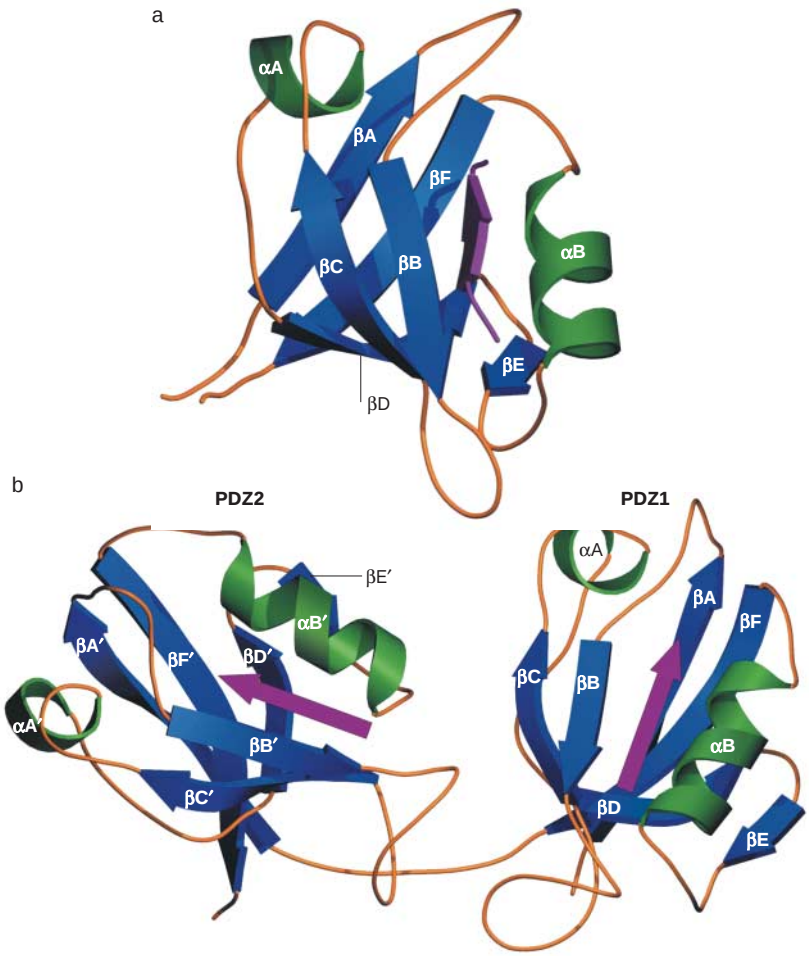


Figure 1 | **Three-dimensional structure of PDZ domains. a** | Ribbon diagram of the structure of the third PDZ domain of PSD-95 (α-helices in green, β-strands in blue) complexed with its target C-terminal peptide (purple). For details of the structural basis of specific interactions see REFS 1,130. **b** | Structural model of the PDZ1–PDZ2 domains of PSD-95. The tandem PDZ1 and PDZ2 domains, which bind to NMDA (*N*-methyl-D-aspartate) receptor (NMDAR) NR2 subunits and Kv1 channels, are arranged in similar orientations[8]. The structure provides a mechanistic explanation for the synergistic binding of two cytoplasmic tails extending from oligomeric membrane proteins such as receptors and channels.

PDZ DOMAIN
A peptide-binding domain that is important for the organization of membrane proteins, particularly at cell–cell junctions, including synapses. It can bind to the carboxyl termini of proteins or can form dimers with other PDZ domains. PDZ domains are named after the proteins in which these sequence motifs were originally identified (PSD-95, discs large, zona occludens 1).

POSTSYNAPTIC DENSITY
An electron-dense specialization of excitatory postsynaptic membranes that contains a high concentration of glutamate receptors and associated signalling and cytoskeletal proteins.

(T. Nakagawa, T. Walz and M.S., unpublished observations), implying that PSD-95 is not a flexible set of protein-interaction domains linked like 'beads on a string'. Reinforcing this idea, NMR studies have provided evidence that the first two PDZ domains of PSD-95 are specifically oriented relative to each other in a way that would allow them to interact with C termini coming from the same direction (REF. 8; FIG. 1).

PSD-95 forms multimers, and this process seems to be mediated by amino (N)-terminal 'head-to-head' interactions[9,10]. Self-association is a common feature of many PDZ scaffold proteins, and is sometimes mediated by direct interactions between PDZ domains (as in the case of glutamate-receptor-interacting protein (GRIP), described below[11]). Multimerization of PDZ scaffolds might enhance the clustering of partner proteins in large multimolecular assemblies at specific sites, such as the PSD.

Ion channels, receptors and membrane proteins. Electron microscopy immunolocalization and tomography studies indicate that PSD-95 is located close to the postsynaptic membrane (at a mean distance of 12 nm from the postsynaptic membrane), and that it can be labelled by antibodies from both the extracellular and cytoplasmic faces of purified PSDs[12,13]. It is therefore in a good position to interact with postsynaptic membrane proteins such as receptors, ion channels and cell-adhesion molecules, as well as with cytoplasmic proteins (FIG. 3; TABLE 1). Such interactions are proposed to be important for the localization and clustering of these proteins at the postsynaptic membrane. In support of this idea, PSD-95 can cluster NMDARs and Shaker-type K^+ channels on the surface of heterologous cells[14]. The best *in vivo* evidence that PSD-95 clusters postsynaptic proteins comes from *Drosophila melanogaster*, in which mutations in discs large (Dlg), the *D. melanogaster* homologue of PSD-95, abolish synaptic clustering of Shaker K^+ channels, which bind to the PDZ domains of Dlg[15].

When studied using light microscopy, the clustering of NMDARs at synapses seems to be independent of PDZ interactions, as clustering of these receptors is not altered by mutations of the cytoplasmic tails of NMDAR subunits, by genetic disruption of PSD-95 or by interfering peptides that disperse synaptic clusters of PSD-95 (REFS 16–18). On the other hand, functional localization of NMDARs in synapses might depend on PSD-95 (REFS 19–22). Clustering at the cell surface might be related to inhibition of receptor internalization. Removing the C-terminal PDZ-binding motif of NR2B, a NMDAR subunit, enhances its internalization in cultured neurons[23]. Similarly, PSD-95 suppresses the internalization of the Kv1.4 K^+ channel[24] and attenuates agonist-induced internalization of β1-adrenergic receptors in non-neuronal cells[25].

PSD-95 also interacts with neuroligin, a postsynaptic membrane protein that interacts trans-synaptically with β-neurexins, which in turn bind to the PDZ domain of CASK/LIN2[26]. CASK, another scaffold of the membrane-associated guanylate kinase (MAGUK) superfamily of proteins (FIG. 2), is enriched on both sides of the synapse and interacts with synaptic membrane proteins such as β-neurexin, syndecan and SynCAM[27–29]. As well as mediating cell adhesion, the trans-synaptic neuroligin–β-neurexin interaction seems to induce presynaptic differentiation[28,30]. So, the PSD-95-based scaffold is probably involved in synaptic adhesion and synapse development, a role that resembles that of fasciclin II (FasII), a *D. melanogaster* cell adhesion molecule that interacts with Dlg in the development of the fly neuromuscular junction[31].

Although the emphasis has always been on cell-biological functions of PSD-95 (for example, surface delivery or stabilization, synaptic targeting or clustering of other proteins), it is possible that PSD-95 also functionally modulates the activities of membrane proteins to which it binds. For instance, PSD-95 can suppress the activity of the inward rectifier K^+ channel (Kir2.3), mainly by reducing its single-channel conductance[32].

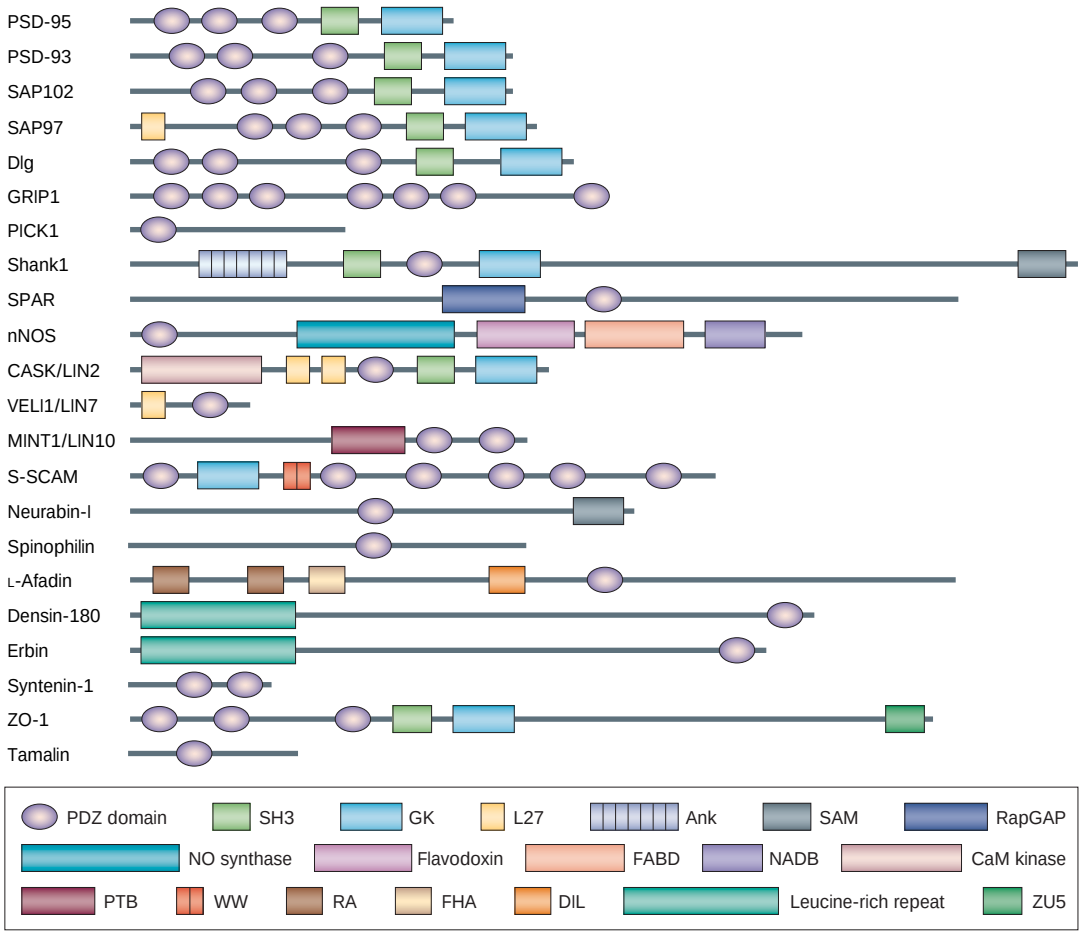


Figure 2 | **Schematic diagram of PDZ proteins.** PDZ domains are often found in scaffold proteins as multiple tandem arrays and/or linked to other kinds of modular protein-interaction domain. PDZ domains are shown as purple ellipses. Other domains are indicated: Ank, ankyrin repeats; CaM kinase, calmodulin-dependent kinase (CaMK)-like domain; DIL, dilute domain; FABD, FAD-binding domain; FHA, forkhead-associated domain; GK, guanylate kinase-like domain; L27, domain initially found in LIN2 and LIN7; NADB, NAD-binding domain; NO, nitric oxide; PTB, phosphotyrosine-binding domain; RA, RAS association domain; RapGAP, Rap GTPase-activating protein; SAM, sterile α motif; SH3, Src homology 3 domain; WW, domain with two conserved Trp (W) residues; ZU5, domain present in ZO-1 and UNC5-like netrin receptors. Proteins: Dlg; discs large; GRIP1, glutamate-receptor-interacting protein 1; LIN7, lin7 homologue; LIN10, lin10 homologue; nNOS, neuronal nitric oxide synthase; PICK1, protein interacting with C-kinase 1; PSD-93, postsynaptic density protein 93; PSD-95, postsynaptic density protein 95; SAP97, synapse-associated protein 97; SAP102, synapse-associated protein 102; Shank, SH3 and ankyrin repeat-containing protein; SPAR, spine-associated RapGAP; S-SCAM, synaptic scaffolding molecule; ZO-1, zona occludens protein 1.

SRC HOMOLOGY 3 DOMAIN (SH3 domain). A protein–protein interaction domain that binds to PXXP or related peptide sequences.

GUANYLATE KINASE-LIKE DOMAIN (GK domain). A protein–protein interaction domain found in the membrane-associated guanylate kinase (MAGUK) superfamily of proteins, which includes PSD-95 and related proteins.

RAS, RAP AND RAC Small monomeric G-proteins that, in their activated GTP-bound state, interact with and stimulate their downstream effectors. Hydrolysis of bound GTP by the intrinsic GTPase activity of these proteins terminates their activity. Guanine nucleotide exchange factors (GEFs) stimulate GTP loading and activate these small G-proteins; GTPase-activating proteins (GAPs) inhibit their activity.

So, PSD-95 can regulate the activity of interacting membrane proteins by influencing their surface delivery, endocytosis, subcellular location, subunit composition and even intrinsic functional properties, such as channel conductance.

Organization of postsynaptic signalling by PSD-95. Perhaps the most important biochemical function of PSD-95 is to organize signalling complexes at the post-synaptic membrane. In addition to membrane proteins, PSD-95 interacts with a wide variety of cytoplasmic signalling molecules (FIG. 3 and TABLE 1). By physically bringing together cytoplasmic signal-transducing enzymes and surface receptors (such as NMDARs), PSD-95 is thought to facilitate signal coupling in the PSD. An example is the association of PSD-95 with neuronal nitric oxide synthase (nNOS)[3,33], a Ca^{2+}/calmodulin-activated enzyme that produces nitric oxide, a diffusible 'transmitter' that has been implicated in the regulation of neurotransmission and excitotoxicity. As NMDARs are permeable to Ca^{2+}, the ternary NMDAR–PSD-95–nNOS complex might functionally couple NMDAR gating to nNOS activation. This is supported by evidence that disrupting the NMDAR–PSD-95 interaction by introducing a synthetic peptide that mimics the last nine residues of NR2B reduces NMDAR-induced excitotoxicity, without affecting NMDAR function[34].

An abundant PSD protein that binds to PSD-95 is synaptic RAS GTPase-activating protein (SynGAP), a GTPase-activating protein (GAP) for the Ras small GTPase[35,36] (TABLE 1). SynGAP is activated by Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII)[37] and suppresses the Ras–extracellular signal-regulated kinase (ERK) pathway, which regulates synaptic plasticity[38]. Mice that are heterozygous for a mutant version of

Table 1 | Proteins that interact with PSD-95 family scaffolds

Interacting protein	Comments on the interacting proteins	References
PDZ domains		
NR2A–D	Subunits of NMDA receptors	131
GluR6	Subunit of kainate receptors	132
δ2 GluR	Subunit of δ-ionotropic glutamate receptors	133
β1-adrenergic receptor	G-protein-coupled receptor	25
nAChRc	Subunit of neuronal nicotinic acetylcholine receptor	76,77
5-HT2A and 5-HT2C Rc	Subunits of 5-HT (serotonin) receptors	134
ErbB4	A receptor tyrosine kinase for neuregulin	135,136
Kv1	Voltage-gated potassium channel	14
Kir2, Kir3, Kir4 and Kir5	Inward-rectifying potassium channels	32,124
Neuroligin	A postsynaptic membrane protein that binds to β-neurexins and regulates synaptic adhesion and development	26,28,30
Stargazin family proteins	Tetra-spanning transmembrane proteins required for surface and synaptic expression of AMPA receptors	64
nNOS	Neuronal nitric oxide synthase	3,33
SynGAP	An abundant RasGAP of the PSD that regulates synaptic plasticity	35,36,39,40
Kalirin-7	A guanine nucleotide exchange factor for Rac1 that regulates spine morphogenesis	53
Fyn, Lyn, Src and Yes	Src family non-receptor protein tyrosine kinases; might also interact with the SH3 domain of PSD-95	48,49
Cypin	A cytosolic protein that regulates dendrite patterning by promoting microtubule assembly	137
CRIPT	A microtubule-binding protein	18
Sec8	A subunit of the exocyst complex involved in protein and vesicle trafficking	115
KIF1Bα	A motor of the kinesin superfamily	111
SH3 domain		
Pyk2	A non-receptor tyrosine kinase regulated by calcium and PKC and required for LTP induction	50,138
GK domain		
GKAP/SAPAP	An abundant multi-domain scaffold of PSD that links PSD-95 with Shank	139
SPAR	A postsynaptic RapGAP that regulates spine morphogenesis	57
SH3 and GK domains		
KA2 GluR	Subunit of kainate receptors	132
AKAP79/150	An anchoring protein that binds to protein kinase A and protein phosphatase 1	41
L27 domain		
CASK	Mammalian homologue of LIN2	140,141
Myosin VI	A minus-end-directed actin-based motor	113

Only proteins that interact directly with PSD-95 family scaffolds are listed. These interactions might not apply to all members of the PSD-95 family. Owing to space limitations, this list is not comprehensive and not all relevant references are cited. AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; GK, guanylate kinase-like domain; LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartate; PKC, protein kinase C; PSD-95, postsynaptic density protein 95; Rac, Rap and Ras, small monomeric G-proteins; RapGAP, Rap GTPase-activating protein; RasGAP, Ras GTPase-activating protein; SH3 domain, Src homology 3 domain; Shank, SH3 and ankyrin repeat-containing protein.

SynGAP show elevated basal activity of ERK in the hippocampus, increased synaptic AMPAR clustering in cortical cultures, reduced LONG-TERM POTENTIATION (LTP) in the CA1 region of the hippocampus and impaired spatial learning[39,40].

PSD-95 and SAP97, another member of the PSD-95 family of proteins, interact with A-kinase-anchoring protein 79/150 (AKAP79/150) (REF. 41), a scaffold for protein kinase A (PKA), PKC and the Ca^{2+}/calmodulin-dependent protein phosphatase calcineurin (also known as PP2B). The interaction between PSD-95 and AKAP might bring these kinases and phosphatases close to their specific substrates in the synapse. For instance, the SAP97–AKAP complex facilitates the phosphorylation by PKA of the glutamate receptor GluR1 (REF. 41), a subunit of AMPARs that binds to SAP97 (see below), and is required for the downregulation of AMPAR currents by Ca^{2+} and PP2B[42]. As PKA-dependent phosphorylation of GluR1 at Ser845 is involved in the regulation of AMPAR recycling[43] and synaptic plasticity[44,45], the SAP97–AKAP79 complex might be important for the recruitment of kinases and phosphatases to synaptic AMPARs.

Tyrosine phosphorylation regulates NMDAR activity and NMDAR-dependent synaptic plasticity[46], including the trafficking of AMPARs[47]. PSD-95 associates with non-receptor tyrosine kinases of the Src family[48,49] and their upstream activator, proline-rich tyrosine kinase 2 (Pyk2)[50], both of which are thought to be important for synaptic plasticity. So PSD-95 might localize the Pyk2–Src signalling cascade close to NMDARs; however, the importance of PSD-95 scaffolds in synaptic regulation by tyrosine phosphorylation has not been directly investigated.

Another group of signalling molecules that is attracting growing interest is the group that regulates the assembly and dynamics of F-actin, which is the predominant cytoskeletal element in DENDRITIC SPINES and is important for synaptic morphogenesis and plasticity[51,52]. PSD-95 binds directly to kalirin-7, a guanine nucleotide exchange factor (GEF) for RAC1 that promotes spine formation[53]. However, the molecular mechanisms that link activated Rac1 to the postsynaptic actin cytoskeleton are not clear. Kalirin functions downstream of EphB receptors, which have been implicated in the regulation of NMDARs and spine development[54,55].

In addition to kalirin-7 (which activates Rac), the PSD also contains many regulators of other small GTPases[56]. Spine-associated RapGAP (SPAR), an inhibitory GAP for RAP, binds to PSD-95 and promotes the growth of dendritic spines. This function depends on SPAR's GAP domain. SPAR itself contains a PDZ domain (TABLE 1). Degradation of SPAR by the ubiquitin–proteasome pathway leads to loss of PSD-95 and depletion of synapses[57]. Overexpression of PSD-95 promotes spine growth[58], although whether this depends on its interactions with kalirin and SPAR remains to be determined.

As well as interacting directly with various signalling enzymes, PSD-95 is also linked by protein interactions to other scaffolds in the PSD, including guanylate kinase-associated protein (GKAP/SAPAP), SH3 and ankyrin repeat-containing protein (Shank/ProSAP) and Homer[5,59] (FIG. 2 and TABLE 1). These proteins, which are

Table 2 | Other synaptic PDZ proteins

PDZ protein	Interacting protein(s)	References
Neurabin, spinophilin/neurabin-II		
Localized in spines; modulates synaptic transmission and spine morphology	Protein phosphatase 1 F-actin	142
Afadin		
Involved in synapse adhesion and development	Nectin (cell adhesion molecule) F-actin Eph receptors (receptor tyrosine kinases)	143
Densin-180		
Abundant PSD protein; member of LAP (leucine-rich repeat and PDZ) family of proteins	CaM kinase IIα α-Actinin (F-actin-binding protein) δ-Catenin (N-cadherin-interacting protein)	144
Erbin		
LAP protein; suppresses the Ras–MAPK signalling pathway	ErbB2 (receptor tyrosine kinase for neuregulin) PSD-95 δ-Catenin	145
S-SCAM		
Synaptic multi-PDZ scaffold; might regulate assembly and trafficking of synaptic proteins	NMDAR Neuroligin KIF1Bα β-Catenin (cadherin-associated protein) nRapGEF (guanine nucleotide exchange factor for Rap1)	2
Shank		
Important scaffold protein of the PSD; promotes morphological and functional maturation of synapse and dendritic spine	GKAP Homer Cortactin (actin regulatory protein) CIRL (calcium-independent receptor for α-latrotoxin) IRSp53 (actin regulatory protein that binds Rac1 and Cdc42) ABP1 (F-actin-binding protein) βPIX (guanine nucleotide exchange factor for Rac1 and Cdc42) Sharpin (multimeric PSD protein)	59,146
Syntenin		
Small scaffold protein that binds to phosphatidylinositol 4,5-bisphosphate	AMPA, kainate and metabotropic glutamate receptor subunits Syndecan (transmembrane proteoglycan) Neurexin (neuronal surface proteins) SynCAM (synaptic cell adhesion molecule) Ephrin B Neurofascin (neural cell adhesion molecule) Merlin (product of the causal gene for neurofibromatosis type II)	94
Tamalin		
Possibly involved in trafficking of mGLURs	Group I metabotropic glutamate receptor subunits Cytohesin (guanine nucleotide exchange factor for ARF small GTPases) GKAP S-SCAM	147

Only proteins that directly interact with the indicated PDZ proteins are described. Owing to space limitations, this list is not comprehensive and not all relevant references are cited. AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; Cdc42, Rac, Rap and Ras, small monomeric G-proteins; GKAP, guanylate kinase-associated protein; KIF1Bα, kinesin family member 1Bα; NMDAR, N-methyl-D-aspartate receptor; PSD-95, postsynaptic density protein 95; Ras–MAPK, Ras mitogen activated protein kinase; S-SCAM, synaptic scaffolding molecule.

found in the deeper (cytoplasmic) part of the PSD[12,13], bind to additional signalling and cytoskeletal proteins (TABLE 2). So, PSD-95 is integrated in a large network of signalling and adaptor proteins, many of which also contain PDZ domains. Although PSD-95 interacts directly with only a subset of PSD proteins, the central importance of PSD-95 scaffolding in the PSD is reflected by its stoichiometric abundance[60]. Mass spectrometry analysis indicates that in the PSD, PSD-95 is an order of magnitude more abundant in molar terms than NMDARs, and several times more abundant than GKAP and Shank[56].

PSD-95 regulates synaptic transmission. As befits an important scaffold of the PSD, PSD-95 has a strong influence on synaptic transmission and plasticity. Overexpression of PSD-95 potentiates AMPAR-mediated excitatory postsynaptic currents (EPSCs), an effect that depends on two palmitoylated N-terminal cysteines in PSD-95 (REFS 58,61–63). Conversely, if PSD-95 is knocked down by RNA INTERFERENCE (RNAi), AMPAR-mediated EPSCs are suppressed (K. Futai, T. Nakagawa, Y. Hayashi & M. S., unpublished observations). NMDAR-mediated EPSCs are unaffected by either gain- or loss-of-function of PSD-95. How does PSD-95 affect AMPAR-mediated EPSCs, given that it does not interact directly with AMPARs? In one current model, PSD-95 recruits the tetraspanning membrane protein stargazin to synapses, where it binds directly to AMPAR subunits. Stargazin and its relatives are essential for the surface expression and synaptic accumulation of AMPARs, and the latter activity depends on an interaction of the stargazin C terminus with the PDZ domains of PSD-95 (REFS 64,65).

Synaptic potentiation induced by the overexpression of PSD-95 seems to mimic LTP, in that it converts silent synapses into functional synapses, drives GluR1 into synapses, occludes LTP and enhances LONG-TERM DEPRESSION (LTD)[61–63]. Moreover, dominant-negative forms of PSD-95 can block LTP and experience-driven synaptic potentiation in the barrel cortex[63]. These overexpression studies indicate that PSD-95 has a central role in the expression of LTP. However, this conclusion needs to be reconciled with the phenotype of PSD-95-deficient mice, which show enhanced LTP and reduced LTD[16,66].

Dynamic regulation of synaptic PSD-95. If PSD-95 acts as a physiologically important regulator of synaptic strength and structure, then it might be expected that its activity or abundance would be controlled by neural activity. Synaptic accumulation of PSD-95 requires the palmitoylation of two N-terminal cysteines (Cys3 and Cys5)[67]. Neuronal activity promotes the dispersal of PSD-95 from synapses, in part by depalmitoylating these two residues[68]. Synaptic stimulation also causes loss of synaptic PSD-95 through the ubiquitin–proteasome pathway[57,69]. The latter mechanism could involve direct ubiquitylation of PSD-95 (REF. 69) or could be indirect, through the ubiquitylation and degradation of other postsynaptic regulatory proteins such as SPAR[57]. Activity-dependent dispersal or degradation of PSD-95 is known to correlate with a loss of AMPARs and weakening of synapses, but beyond this its physiological importance is not well understood.

The function of PSD-95 is regulated more acutely by phosphorylation. Cyclin-dependent kinase 5 (CDK5), a serine–threonine kinase that is essential for brain

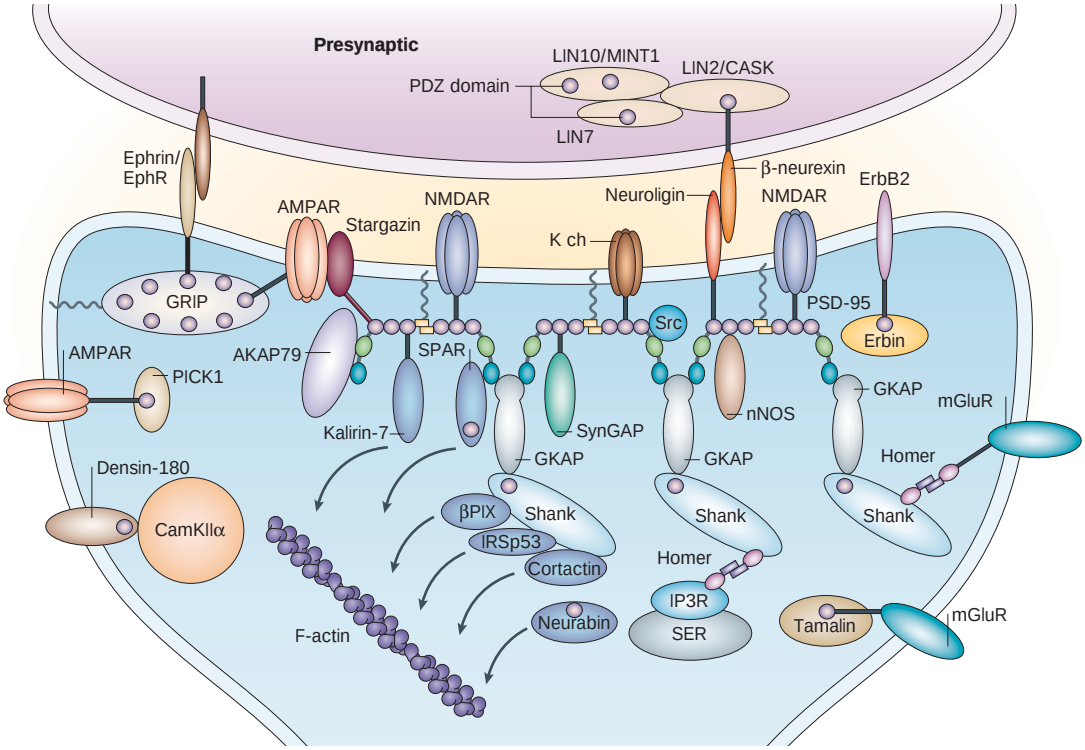


Figure 3 | **A schematic diagram of the organization of PDZ proteins at a mammalian excitatory synapse.** The main PDZ-containing proteins of a glutamatergic synapse are shown, focusing on the postsynaptic density. PDZ domains are indicated by purple circles. The C-terminal cytoplasmic tails of membrane proteins are indicated by black lines. Specific protein–protein interactions are indicated by the overlap of proteins. Only a subset of known protein interactions is illustrated. Although not shown, LIN2, LIN7 and LIN10 are also present postsynaptically, and many of the proteins of the postsynaptic domain are also present in the presynaptic terminal. Green and blue ellipses in PSD-95 represent SH3 and GK domains, respectively. Crooked lines indicate palmitoylation of PSD-95 and GRIP. Grey arrows indicate binding and/or regulatory actions of proteins on the actin cytoskeleton. AKAP79, A-kinase anchor protein 79; AMPAR, AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor; βPIX, PAAK-interactive exchange factor; CaMKIIα, α-subunit of Ca^{2+}/calmodulin-dependent protein kinase II; GK, guanylate kinase-like domain; EphR, ephrin receptor; ErbB2, EGF-related peptide receptor; GKAP, guanylate kinase-associated protein; GRIP, glutamate-receptor-interacting protein; IP3R, IP3 receptor; IRSp53, insulin-receptor substrate p53; K ch, potassium channel; LIN7, lin7 homologue; LIN10, lin10 homologue; mGluR, metabotropic glutamate receptor; NMDAR, NMDA (*N*-methyl-D-aspartate) receptor; nNOS, neuronal nitric oxide synthase; PICK1, protein interacting with C kinase 1; PSD-95, postsynaptic density protein 95; SER, smooth endoplasmic reticulum; SH3, Src homology 3 domain; Shank, SH3 and ankyrin repeat-containing potein; SPAR, spine-associated RapGAP; SynGAP, synaptic Ras GTPase-activating protein.

development, phosphorylates the N-terminal region of PSD-95, inhibiting its multimerization, channel clustering activity and possibly its synaptic localization[70]. By contrast, phosphorylation by CaMKII of SAP97 in the N-terminal L27 domain promotes synaptic targeting of SAP97, and of its binding partner GluR1 (REF. 71). In *D. melanogaster*, CaMKII-dependent phosphorylation of the first PDZ domain of Dlg decreases the synaptic localization of Dlg[72]. Time-lapse imaging has confirmed that PSD-95 tagged with green fluorescent protein undergoes dynamic turnover, although at a slower rate than several other synaptic proteins[73]. PDZ scaffolds are probably all regulated in a dynamic fashion by subcellular redistribution and protein phosphorylation and degradation.

In vivo functions of PSD-95 proteins. Although the PSD-95 family of proteins has been extensively studied in cultured neurons, their functions *in vivo* are not well established. PSD-95 mutant mice have impaired spatial learning despite enhanced LTP (these animals also have defective LTD)[16]. PSD-95 deficiency in knock-out mice prevents the maturation of orientation preference in the visual cortex[74] and eliminates behavioural sensitization induced by chronic cocaine administration[66]. These *in vivo* results indicate that PSD-95 is involved in learning and memory, maturation of cortical circuits and behavioural responses to drugs of abuse, all of which presumably reflect the importance of PSD-95 in synaptic plasticity.

Genetic disruption of PSD-93 reduces NMDAR-mediated postsynaptic responses and blunts NMDAR-dependent persistent pain[75]. In addition, PSD-93 associates with neuronal nicotinic acetylcholine receptors and is required for the normal function and stability of neuronal cholinergic synapses[76,77]. So, PSD-93 might function as a key scaffold in cholinergic as well as glutamatergic synapses. The *in vivo* importance of SAP97 and SAP102 for brain function is unclear.

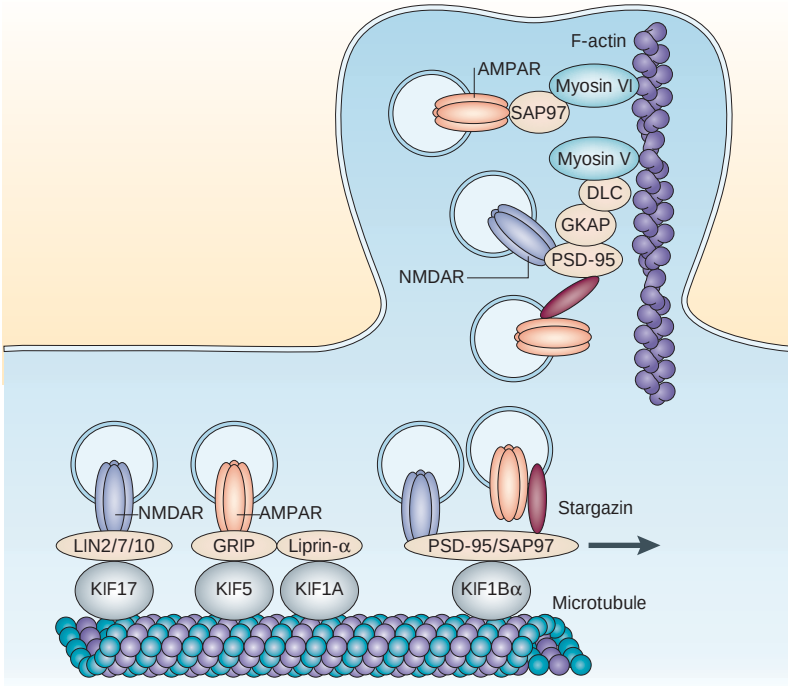
LONG-TERM POTENTIATION (LTP). A long-lasting enhancement of synaptic strength that is elicited by specific patterns of synaptic stimulation (for example, high frequency tetanus). Typically dependent on NMDA-receptor activation, and widely believed to be a means of information storage in the brain.

DENDRITIC SPINES
Tiny actin-rich protrusions from the dendrite that form the postsynaptic compartment for most excitatory synapses in the brain.

RNA INTERFERENCE (RNAi). A method for suppressing the expression of a specific protein based on targeted hybridization of small interfering RNAs to the mRNA encoding that protein.

LONG-TERM DEPRESSION (LTD). A long-lasting suppression of synaptic strength that is elicited by specific patterns of synaptic stimulation (for example, low frequency stimulation). Typically dependent on NMDA-receptor activation, and widely believed to be a means of information storage in the brain.

Box 1 | PDZ scaffolds in trafficking of protein complexes



PDZ-based membrane protein complexes can be moved around the cell as pre-assembled packages. Transport along microtubule tracks is mediated by motor proteins of the KINESIN superfamily (KIFs), whereas transport along actin tracks is carried out by motors of the MYOSIN family. PDZ scaffolds on the surface of cargo vesicles can act as 'receptors' for molecular motors by binding to specific kinesins and myosins. For instance, the PDZ domains of PSD-95 (postsynaptic density protein 95), SAP97 (synapse-associated protein 97) and S-SCAM (synaptic scaffolding molecule) interact directly with the C terminus of KIF1Bα (kinesin family member 1Bα), a kinesin motor[111]. SAP97 can also bind, through its GK (guanylate kinase-like) domain, to KIF13B/GAKIN (kinesin family member 13B)[112], and through its N-terminal L27 domain to myosin-VI[113]. PSD-95 family proteins can also associate indirectly with myosin-V through the PSD-95-binding protein GKAP (guanylate kinase-associated protein)[114]. Although it is not a motor protein, the Sec8 subunit of the 'exocyst' complex (which targets secretory vesicles to the cell surface) also interacts with PSD-95 family members, particularly SAP102 (REF. 115). Dominant-negative Sec8 inhibits NMDA (*N*-methyl-D-aspartate) receptor (NMDAR) currents in neurons, supporting the involvement of the exocyst complex in synaptic trafficking of an NMDAR–SAP102 complex[115].

In *Caenorhabditis elegans*, a complex of PDZ proteins (CASK/LIN2–LIN7–LIN10) (FIGS 2, 3) is important for basolateral targeting of a receptor tyrosine kinase in epithelia[116]. LIN10 is also required for the synaptic localization of glutamate receptors[117]. Homologous proteins exist in mammalian neurons on both pre- and postsynaptic sides of the synapse and are probably involved in subcellular targeting of the proteins with which they interact[2]. The kinesin family motor KIF17 binds directly to the PDZ1 domain of LIN10 and might transport a CASK–LIN7–LIN10–NMDAR complex to synapses[118,119]. Remarkably, mammalian CASK can redistribute from the plasma membrane to the nucleus to regulate transcription[120,121].

The GluR2/3-binding protein GRIP interacts directly with conventional kinesin (KIF5) and this association is important for the targeting of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors (AMPARs) to dendrites[122]. The GRIP-interacting protein liprin-α also binds to KIF1A, another kinesin-like motor[123]. As for NMDARs, it is possible that multiple motor proteins contribute to the transport of AMPARs, each interacting in different ways with the AMPAR protein complex.

So, beyond their well-known function as organizers of protein complexes at the plasma membrane, there is mounting evidence that PDZ scaffolds have an important role in intracellular protein trafficking in neurons. Indeed, PDZ proteins can act as the 'motor receptor', enabling specific motor proteins to bind to and transport the complex. DLC, dynein light chain.

Differences between PSD-95 family proteins. PSD-95 family proteins are distributed differently from each other both in the brain and in neurons. PSD-95 and PSD-93 are most alike in being highly enriched in the PSD, and this enrichment might be related to the selective palmitoylation of these proteins[78]. On the other hand, SAP102 and SAP97 are found in dendrites and axons and are abundant in the cytoplasm as well as at synapses[79–81]. SAP102 is highly expressed early in postnatal development, whereas PSD-95 and PSD-93 predominate at later stages[82].

Although they show similar specificities of protein interaction *in vitro*, PSD-95 family members interact with different (but overlapping) sets of proteins *in vivo*. For instance, PSD-95 is preferentially associated with NR2A *in vivo*, whereas SAP102 is more associated with NR2B[82]. The NR2B–SAP102 complex in immature synapses tends to be replaced by the NR2A–PSD-95/PSD-93 complex in mature synapses[82,83]. Overexpression of PSD-95 promotes synaptic insertion of NR2A rather than NR2B, thereby modifying the subunit composition and functional properties of synaptic NMDARs[84]. Stargazin family proteins are selectively associated with PSD-95 and PSD-93 in the brain[85]. Perhaps most strikingly, SAP97 interacts directly with the AMPAR subunit GluR1 (REF. 86), whereas the other members of the family bind directly to NMDAR NR2 subunits. The SAP97–GluR1 association can be detected early in the secretory pathway, indicating that SAP97 might be involved in the trafficking of GluR1 (REF. 80). Overall, it seems that PSD-95 and PSD-93 are more specifically associated with synaptic functions, whereas SAP97 and SAP102 might be more important in trafficking (BOX 1).

PDZ scaffolds associated with AMPARs

The PSD-95 family is mainly associated with NMDARs and the PSD, with the exception of SAP97 (see above). AMPAR subunits interact directly with different PDZ proteins, which might account for the more dynamic cell-biological behaviour of AMPARs. The C termini of the AMPAR subunits GluR2 and GluR3 bind to glutamate-receptor-interacting protein/AMPAR-binding protein (GRIP/ABP; encoded by two distinct genes, *GRIP1* and *ABP*/*GRIP2*) and to protein interacting with C kinase 1 (PICK1). These PDZ-based interactions are important for the synaptic targeting and regulated trafficking of AMPARs (for recent reviews on this subject, see REFS 87,88). Here, we focus on recent progress in this area.

GRIP. The GluR2/3 subunit binds specifically to the PDZ5 domain of GRIP, but the PDZ4 domain is also required for a strong interaction. The structure of the tandem PDZ4 and 5 domains reveals that PDZ4 is unlikely to bind to C-terminal peptides but instead stabilizes PDZ5 through interdomain interactions[89]. GRIP has up to seven PDZ domains (FIG. 2), through which it can interact with many proteins, including Eph receptors and their ephrin ligands[90]; a RAS guanine nucleotide exchange factor (RasGEF)[91]; liprin-α[92]; the transmembrane protein Fraser syndrome 1 (FRAS1)[93]; and, perhaps, also metabotropic and kainite-type

Box 2 | Regulation of PDZ interactions by protein phosphorylation

The protein interactions of PDZ-based scaffolds should be regulated to allow controlled assembly and disassembly of protein complexes at the synapse. There is widespread evidence that PDZ-ligand interactions are disrupted by phosphorylation, typically on the C-terminal peptide of the ligand. For instance, phosphorylation of the C termini of potassium channels[124], β1-adrenergic receptors[125] and stargazin[126,127] prevents them from binding to the PDZ domains of PSD-95 (postsynaptic density protein 95). Accordingly, phosphorylated stargazin is poorly enriched in PSD fractions[126], and phosphorylation-mimicking stargazin mutants fail to cluster at synaptic sites and attenuate synaptic AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor (AMPAR) currents[127]. For most of these regulated PDZ interactions, the specific protein kinase that phosphorylates the PDZ-binding C terminus *in vivo* is unknown.

A specific protein kinase has been implicated in regulating interactions between AMPARs and PDZ domains. Phosphorylation of Ser880 in the C terminus of GluR2 (AMPA glutamate receptor 2) by PKC (protein kinase C) prevents it from interacting with GRIP/ABP (glutamate-receptor-interacting protein/AMPAR-binding protein) but not with PICK1 (protein interacting with C-kinase 1), indicating that the phosphorylation might displace AMPARs from GRIP in favour of PICK1. Phosphorylation of GluR2 at Ser880 is correlated with the internalization of AMPARs from synapses and is important for long-term depression (LTD), this mechanism being particularly well-established for cerebellar LTD[107,110,128].

PDZ–peptide interactions can also be regulated by phosphorylation of the PDZ domain, although this is less common than phosphorylation of the ligand's C-terminal motif. For example, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII)-dependent phosphorylation of the PDZ1 domain of SAP97 (synapse-associated protein 97) disrupts its interaction with the NR2A subunit of NMDA (*N*-methyl-D-aspartate) receptors (NMDARs), but not with the GLUR1 (glutamate receptor 1) subunit of AMPARs[129].

glutamate receptors[94]. GRIP can also dimerize through an interaction between the PDZ6 domains of two monomers[11]. GRIP is widely expressed in body tissues and in neurons — it is present in both axons and dendrites[92]. Therefore, the function of GRIP must extend beyond the regulation of AMPARs. This is supported by evidence from knockout mice in which the *Grip1* gene is disrupted, which show haemorrhagic blisters and embryonic lethality[93,95].

Certain splice variants of GRIP can be palmitoylated, like PSD-95 and PSD-93, and palmitoylation of the protein results in it being associated with the plasma membrane and localized at synapses[96]. Non-palmitoylated GRIP mostly associates with intracellular membranes[97]. These differentially modified subpopulations of GRIP might stabilize synaptic and intracellular pools of AMPARs, respectively.

GRIP is believed to be involved in synaptic trafficking and/or synaptic stabilization of AMPARs and other interacting proteins. The widespread distribution of GRIP in cells, and its interactions with motor proteins (BOX 1), support the hypothesis that it is involved in trafficking[98]. GRIP can participate in synaptic function not only by interacting with AMPARs, but also by associating with Eph receptors and their ephrin ligands, which have been implicated in dendritic spine morphogenesis and hippocampal synaptic plasticity[99,100].

PICK1. PICK1 is present at synaptic and non-synaptic sites in neurons, and its PDZ domain shows relatively promiscuous binding. In addition to PKCα and GluR2/3, it has many other binding partners (both presynaptic and postsynaptic), including the netrin receptor UNC5H[101], various metabotropic glutamate-receptor subtypes[102,103], the dopamine plasma-membrane transporter[104] and the erythroblastic leukaemia viral oncogene homologue 2 (ErbB2) receptor tyrosine kinase[105]. In many of these cases, the interaction with PICK1 seems to regulate the subcellular localization and/or surface expression of its protein partners.

Phosphorylation of the C terminus of GluR2 alters its binding specificity for GRIP and PICK1, and contributes to synaptic plasticity by altering the trafficking of AMPARs (BOX 2). There is some controversy regarding the respective roles of GRIP and PICK1 in the stabilization of synaptic versus intracellular AMPARs[106–110]. This could be related to the existence of different subpopulations of GRIP and/or PICK1 at synaptic and intracellular locations[96], as well as to difficulties in interpreting the results of experimental perturbations of AMPAR trafficking. For instance, increased intracellular accumulation of AMPARs could arise from increased endocytosis of surface receptors or from reduced recycling of intracellular receptors. Moreover, because of overlapping specificities of PDZ–C-terminal interactions, the peptides that are typically used to interfere with the PDZ interactions of GRIP and PICK1 are probably not highly specific for these proteins or for GluR2/3 interactions.

Genetic loss-of-function experiments would be helpful to dissect out the functions of GRIP and PICK1. Unfortunately, a generalized GRIP1 knockout is lethal in mice, and ABP/GRIP2 mutants lack an obvious phenotype[93,95]. PICK1-knockout mice are viable and show normal synaptic transmission in several brain areas. However, cerebellar LTD is abolished, and can be rescued by transient transfection of PICK1-deficient Purkinje cells with wild type PICK1, but not mutants of PICK1 with mutations in the PDZ domains (J. Steinberg, J. Xia, K. Takamiya, D. Linden and R. Huganir, unpublished observations).

In addition to AMPARs, GRIP and PICK1 have been reported to bind kainate receptors (KARs). Disrupting these PDZ-based interactions with fusion proteins and peptides decreases KAR-mediated synaptic transmission, indicating that GRIP and PICK1 interactions might be required to maintain synaptic KAR function[94].

Conclusions

PDZ domains were characterized as protein-interaction modules only a decade ago, but they have now come of age. Nowhere is the diversity and function of PDZ proteins better illustrated than at excitatory glutamatergic synapses. Our view of PDZ proteins has evolved from one of static adaptors for clustering interacting proteins to a more dynamic picture in which PDZ scaffolds organize heterogeneous ensembles of proteins, the composition of which changes at different locations in the cell, both during development and in response to neuronal activity. Moreover, PDZ proteins themselves can be mobile within neurons, and their activity and expression levels are regulated by phosphorylation, lipid modification and ubiquityation–degradation. As befits their central role in the organization of glutamate-receptor

KINESINS
A large family of structurally related motor proteins that use ATP to transport specific cargoes along microtubules.

MYOSINS
A large family of structurally related motor proteins that use ATP to transport specific cargoes along actin filaments.

complexes, PDZ domain scaffolds have been shown by genetic, electrophysiological and morphological studies to be essential for controlling the structure, strength and plasticity of synapses. The next stage of investigations promises to reveal more insights into the *in vivo* significance of synaptic PDZ proteins, beyond their protein interactions and cell-biological functions. With increasing knowledge of their structure and function, PDZ interactions could become plausible targets for pharmaceutical intervention, thereby opening up a wealth of possibilities for the treatment of brain diseases.

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Competing interests statement

The authors declare no competing financial interests.

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