Acta Biochimica Polonica

Vol. 50 No. 4/2003

985–1017

QUARTERLY

Review

PDZ domains – common players in the cell signaling

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Received: 14 June, 2003; revised: 06 October, 2003; accepted: 09 October, 2003

Key words: PDZ domain, signaling, protein–protein interaction, module

PDZ domains are ubiquitous protein interaction modules that play a key role in cellular signaling. Their binding specificity involves recognition of the carboxyl-terminus of various proteins, often belonging to receptor and ion channel families. PDZ domains also mediate more complicated molecular networks through PDZ–PDZ interactions, recognition of internal protein sequences or phosphatidylinositol moieties. The domains often form a tandem of multiple copies, but equally often such tandems or single PDZ domain occur in combination with other signaling domains (for example SH3, DH/PH, GUK, LIM, CaMK). Common occurrence of PDZ domains in Metazoans strongly suggests that their evolutionary appearance results from the complication of signaling mechanisms in multicellular organisms. Here, we focus on their structure, specificity and role in signaling pathways.

❁Jacek Otlewski was supported by a scholarship from the Foundation for Polish Science.

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Abbreviations: β_2AR, β_2-adrenergic receptor; NPRAP, δ-catenin/neural plakophilin-related armadillo repeat protein; AF-6, ALL-1 fusion partner from chromosome 6; AIPC, activated in prostate cancer; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; APC, adenomatous polyposis coli; aPKC, atypical protein kinase C; ASICs, acid-sensing ion channels; BP75, bromodomain-containing protein; CAMGUK, calcium/calmodulin-dependent serine protein kinase membrane-associated guanylate kinase; CaMK, calcium/calmodulin-dependent protein kinase domain; CASK, calcium/calmodulin-dependent serine protein kinase; Cdc42, cell division control protein 42; CFTR, cystic fibrosis transmembrane conductance regulator; CIPP, channel-interacting PDZ domain protein; Clik1, CLP-36 interacting kinase; CLP-36, C-terminal LIM domain protein 1; CFTR, cystic fibrosis transmembrane conductance regulator; DAX, domain present in Dishevelled and axin; DEP, Dishevelled, Egl-10, and pleckstrin; DH/PH, Dbl homology/pleckstrin homology; Dlg, Disc-large; Dlt, Discs Lost; DAT, dopamine transporter; E3KARP, NHE3 kinase A regulatory protein or NHERF-2; EBP50, ezrin-radixin-moesin binding phosphoprotein-50; ERM, ezrin-radixin-moesin; FAP-1, Fas-associated phosphatase-1; FERM, 4.1, ezrin, radixin, moesin; FH, forming homology domains;

Continued overleaf

PDZ domains are the most common protein interaction modules representing 0.2 to 0.5% of open reading frames in three currently sequenced metazoan genomes (Schultz *et al.*, 1998b; 2000). Originally PDZ domains were recognized in the postsynaptic density protein PSD-95/SAP90 (Tsunoda *et al.*, 1998), *Drosophila* septate junction protein Discs-large and the epithelial tight junction protein ZO-1 (Kennedy, 1995), hence the acronym PDZ. PDZ domains are also known as the Discs-large homology regions (DHRs) or GLGF repeats (after the highly conserved four-residue motif within the domain).

PDZ domains are built of 80–100 amino-acid residues, specialized for binding of C-termini in partner proteins, most often transmembrane receptors and channel proteins, and/or other PDZ domains. Such interactions localize membrane proteins to specific subcellular domains, thus enabling assembly of supramolecular complexes. This is supported by the fact that overwhelming majority of the PDZ-containing proteins is associated with the plasma membrane (Fanning & Anderson, 1999). The role of PDZ domains in clustering and localization of proteins at the plasma membrane has important biological implications, e.g., in signaling, mediating the adhesive properties of particular cells, ion transport, and formation of the paracellular barriers also known as tight junctions.

PDZ domains often occur in multiple copies within a single polypeptide chain, for example, MUPP1 (multi-PDZ domain protein 1) is a tandem of 13 PDZ domains. The multiplicity of PDZ domains suggests their role as "glue" combining many different proteins in a form of supramolecular complexes (Schultz *et al.*, 1998b; 2000).

OCCURRENCE OF PDZ DOMAINS

All the putative biological functions of PDZ domain containing proteins — signaling, adhesion, transport, etc. — are of crucial significance to multicellular organisms. It is possible that PDZ domains coevolved with multicellularity and development of intercellular

protein; GluR2, glutamate receptor; GRASP-1, GRIP1-associated scaffold protein; GRIP, glutamate receptor-interacting protein; GRK-5, G-protein-coupled receptor kinase 5; GUK, guanylate kinase homology domain; htrA, high temperature requirement A; IKEPP, intestinal and kidney enriched PDZ protein; ILR5α, IL5 receptor alpha; INAD, inactivation no afterpotential D; IRS, insulin receptor substrates; JAMs, junctional adhesion molecules; KIF17, kinesin family member 17; LAP, leucine-rich repeats and PDZ; LARG, leukemia-associated Rho guanine-nucleotide exchange factor; LRRs, leucine repeats; LIM, Zinc-binding domain present in Lin-11, Isl-1, Mec-3; MAGUIN-1, membrane-associated guanylate kinase-interacting protein 1; MAGUK, membrane-associated guanylate kinase; mGluRs, metabotropic glutamate receptors; Mint1-1, Msx2 interacting nuclear target; MRE, Maguk recruitment; MUPP1, multi-PDZ domain protein 1; NHE3, type 3 Na^+/H exchanger; NHERF-1, Na^+/H^+ exchanger regulatory factor; nNOS, neuronal nitric oxide synthase; NorpA, no receptor potential A; Par, partition-defective protein; PATJ, Pals-1 associated tight junction protein; PDGFR, platelet-derived growth factor receptor; PDZK1, PDZ domain containing-protein; PICK1, protein interacting with C-kinase; PIP_2, phosphatidylinositol 4,5-bisphosphate; PMCA, plasma membrane Ca^{2+}-ATPase; PSD, postsynaptic density; PRK2, protein kinase C-related kinase 2; PTP-BL, protein tyrosine phosphatase BL; RIL, reversion-induced LIM protein; CRIB, Cdc42 and Rac interactive binding motif; SH, Src homology domain; Shank, SH3 and multiple ankyrin repeat domains protein; Shc, Src homology 2 domain-containing protein; SOC, store-operated calcium channels; SSTR2, somatostatin receptor type 2; SAM, sterile alpha motif domain; TAZ, transcriptional co-activator with PDZ-binding motif; Tiam-1, T-lymphoma invasion and metastasis inducing protein 1; TRP, transient receptor potential channel; TRIP-6, thyroid receptor interacting protein 6; Tsp, tail-specific protease; YAP, Yes-associated protein; ZO, zonula occludens.

signaling. This structural motif is widespread among metazoans, but rare in single cellular organisms – SMART (a Simple Modular Architecture Research Tool) database lists 1163 PDZ domains in 484 human proteins, 259 domains in 153 proteins of *Drosophila melanogaster* and 130 PDZ domains in 95 *Caenorhabditis elegans* proteins, 26 in 23 proteins of *Arabidopsis thaliana* while only 3 in *Saccharomyces cerevisiae* and 5 in *Escherichia coli* (Schultz *et al.*, 1998b; 2000). Estimated occurence values vary significantly depending on the tools used for the calculations, nevertheless PDZ domains are always abundant in animals, yet scarce in yeast and bacteria (Ponting, 1997). Interestingly, as indicated above, PDZ domains are also rare in plants. Since the plant cell wall is a barrier in the cell–to–cell communication, plants may have developed other signaling mechanism (Venter *et al.*, 2001). Using database searching tools Ponting found 19 bacterial protein segments of significant similarity to previously described metazoan PDZ domains (Ponting, 1997). Each of them was homologous to either of the two *Escherichia coli* periplasmic proteases: high temperature requirement A (htrA or protease Do) (Lipinska *et al.*, 1989) and Tsp (tail-specific) protease (Silber *et al.*, 1992). HtrA and Tsp homologues were previously shown to occur in humans and in higher plants (Oelmuller *et al.*, 1996), respectively. Further searches revealed three additional 'PDZ-like' families: the yeast htrA-like hypothetical protein (N1897), *Escherichia coli* Yael proteins, and the *Bacillus subtilis* stage IV sporulation protein B (spoIVB) (Ponting, 1997). A PDZ-like domain was also found in the photosystem II D1 C-terminal protease (Liao *et al.*, 2000). A strong similarity between bacterial and mammalian PDZ domains suggests a horizontal mode of transmission, since primordial PDZs arose probably relatively late in the eukaryotic evolution (Ponting, 1997).

STRUCTURAL BASIS OF LIGAND RECOGNITION

The structure of PDZ domain comprises six β-strands (βA–βF) and two α-helices (αA and αB), which fold into a six-stranded β-sandwich domain (Fig. 1A). The amino- and car-

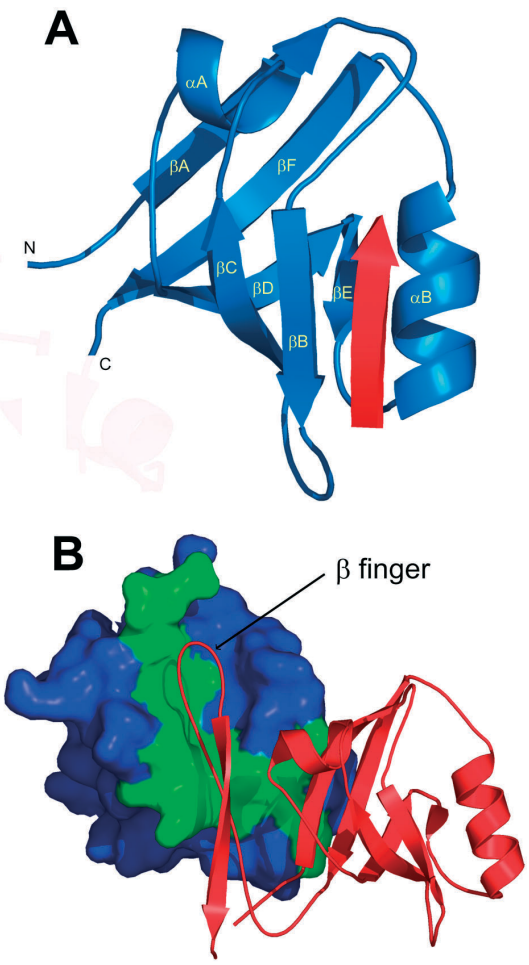


Figure 1. Structure of the PDZ domain bound to peptide and internal peptide motif.

A. Ribbon representation of the third PDZ domain of PSD95 (blue) with KQTSV peptide forming antiparallel β-sheet with βB strand (red arrow) (PDB code 1be9). Numering of β-strands and α-helices is shown. **B.** Complex of the syntrophin PDZ domain (shown as blue and green solvent-accessible surface representation) and nNOS PDZ domain (shown as red ribbon representation with β-finger indicated) (PDB code 1qav). The figure was made using program PyMOL (DeLano).

boxyl-termini of PDZ domains are close together, facilitating incorporation of the domain into different multi-domain proteins (Harris & Lim, 2001).

PDZ domains specifically recognize short (typically about five residues long) carboxyl-terminal peptide motifs. These sequences are often found in the cytoplasmic tails of transmembrane receptors and channels (Kornau *et al.*, 1995). Peptide ligands bind in an extended groove between strand βB and helix αB thus serving as an additional antiparallel β-strand within the PDZ domain (Figs. 1, 3). This mechanism is referred to as

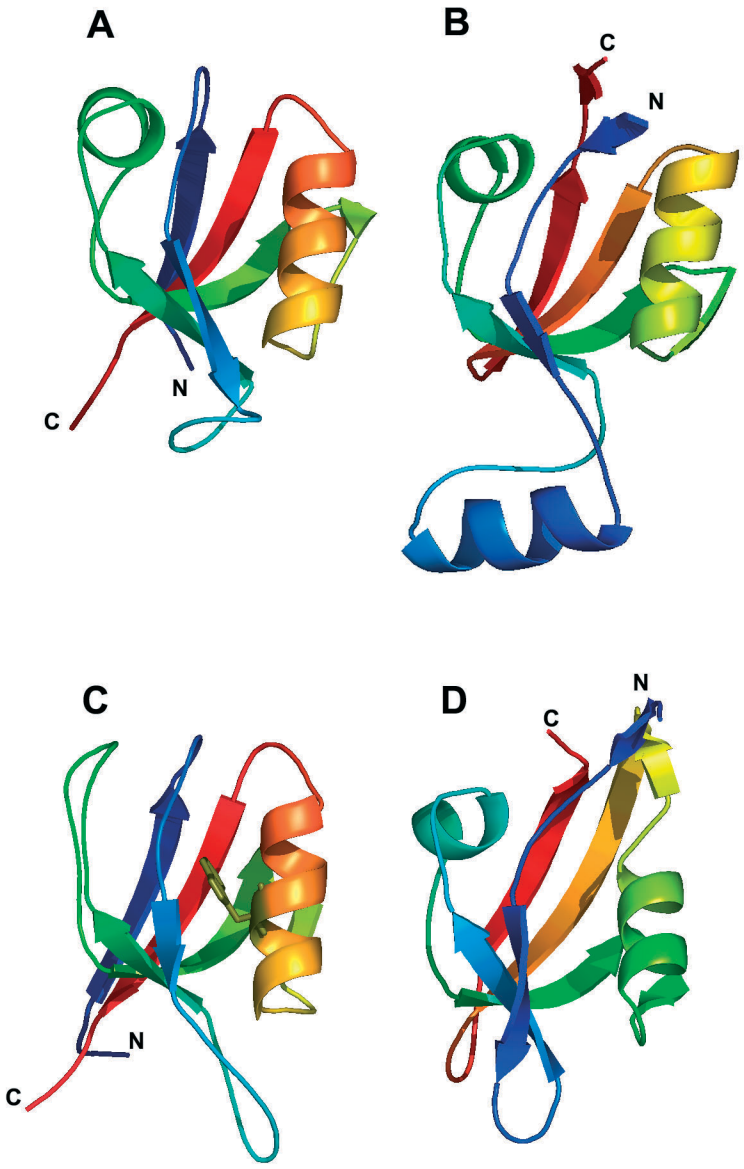


Figure 2. Ribbon representation of PDZ and PDZ-like domains with six β-strands and two α-helices demonstrated.

The N and C termini are depicted to highlight their proximity. **A.** The structure of the second PDZ domain of PSD95 (PDB code 1qlc). **B.** PDZ domain of Htr protease with the extra α-helical fragment visible (PDB code 1lcy). **C.** The interleukin 16 lacking one α-helix and Trp99 blocking the peptide binding pocket (olive) (PDB code 1i16). **D.** The photosystem II D1 C-terminal processing protease, the structure is perturbed, when compared to other PDZ domains but fold remains the same (PDB code 1fc9). The figure was made using program PyMOL (DeLano).

β-strand addition (Harrison, 1996). The structure of the PDZ domain does not change significantly upon ligand binding. The crystal structures of complexed and peptide-free third PDZ domain of PSD-95 are almost identical, showing RMSD between the α carbon atoms of 0.9 Å (Doyle *et al.*, 1996).

Studies on the molecular basis of ligand recognition demonstrate that valine residue at the C-terminal (0) position of the peptide is important for binding (Doyle *et al.*, 1996), but peptides carrying isoleucine or leucine at this position can also be tolerated by certain PDZ domains (Brakeman *et al.*, 1997; Dong *et al.*, 1997). This could be explained by a relatively small size of the hydrophobic pocket, which is generally not appropriate for aromatic side chains accommodation (Doyle *et al.*, 1996). A conserved "carboxylate-binding loop" (R/K-XXX-G-ΦGΦ or GLGF motif) is found within a loop connecting strands βA and βB, creating a hydrophobic cavity surrounding the typically hydrophobic C-termini of partner proteins. The terminal carboxylate of the ligand forms hydrogen bonds with main chain amides of the last three residues in the GLGF motif. The negatively charged carboxylate group of the binding partner is neutralized by the interaction with a conserved arginine (or lysine) residue found 3–4 residues upstream of the GLGF motif (Fig. 3), although the significance of this electrostatic interaction has recently been questioned (Harris *et al.*, 2003). In some PDZ domains, the first Gly in the GLGF motif can be substituted by Pro, Thr or Ser, whereas the second Gly is absolutely conserved.

The position (–1) of the partner peptide was predicted by site-directed mutagenesis to be non-essential for the interaction (Kim *et al.*, 1995). Substitutions at this site usually do not affect binding and, if they do, the effect is much smaller than of the adjacent amino acids (Songyang *et al.*, 1997). Residues (–2) and (–3) of the binding peptide are stabilized by hydrogen bonds with specific amino acids in the strand βB and the helix αB of the PDZ domain. These residues are crucial for the specificity of different PDZ domains (Doyle *et al.*, 1996; Songyang *et al.*, 1997). Crystallographic data indicate that side chain of the (–3) residue directly contacts the binding groove (Doyle *et al.*, 1996; Karthikeyan *et al.*, 2001), and this amino acid is important in determining the binding of ligands selected from the peptide library (Songyang *et al.*, 1997). It has also been demonstrated that ligand residues more distant from the C-terminus, up to position (–8), can influence the binding energy (Songyang *et al.*, 1997; Niethammer *et al.*, 1998; Kozlov *et al.*, 2000).

PDZ-like domains found in plants and bacteria display similar secondary and tertiary structures, but of somewhat different topology. In both photosystem II D1 C-terminal protease (Liao *et al.*, 2000) and Tsp protease from *Escherichia coli* (Beebe *et al.*, 2000) the strand βA is derived from the carboxyl-terminus of the domain instead of the N-terminal sequence, like in conventional PDZ domains. Despite this difference, the fold retains the ability to recognize the C-terminal sequences of target proteins (Beebe *et al.*, 2000).

CLASSIFICATION OF PDZ-CONTAINING PROTEINS

Rapidly growing number of known PDZ domains and their recognized physiological ligands led to classification problems. Initially three specificity classes were proposed (Table 1). In class I, including PSD-95, Dlg and ZO1 proteins, a serine or threonine residue is found at the (–2) position of the peptide ligand (Songyang *et al.*, 1997). Its side chain hydroxyl group forms a hydrogen bond with the N-3 nitrogen of the histidine residue at position αB1 that is conserved among class I PDZ domains (Doyle *et al.*, 1996). The second class of PDZ domains, characterized by hydrophobic residues occupying both the (–2) position of the partner protein and the αB1 position of the PDZ domain, was identified by

analysis of ligand specificity of CASK (calcium/calmodulin-dependent serine protein kinase) PDZ-containing protein (Songyang *et al.*, 1997). The third class of PDZ domains includes nNOS (neuronal nitric oxide synthase) and has a preference for negatively charged amino acids at the (–2) position and a tyrosine residue at the position αB1 of the PDZ domain (Stricker *et al.*, 1997). The specificity in this group is determined by a hydrogen bond between the hydroxyl group of tyrosine from the PDZ domain and side chain carboxylate of the peptide (–2) residue (Stricker *et al.*, 1997; Tochio *et al.*, 1999). There is some confusion regarding the third specificity class, since it was proposed that it comprises also ligand sequence E/D-X-W-C/S-COOH (or X-X-C-COOH) present in N-type Ca^{2+} channel bound by Mint1-1 (Msx2 interacting nuclear target) PDZ domain (Maximov *et al.*, 1999). Other authors suggest that the third class includes the recognition of an internal peptide sequence only (Fuh *et al.*, 2000). Still there are PDZ domains showing specificity other than those of particular classes, like MAGI (membrane-associated guanylate kinase-related) PDZ-2 which binds S/T-W-V-COOH consensus sequence, with Trp(–1) being the affinity determining position (Fuh *et al.*, 2000). The specificity of PDZ domains can be engineered and some of the novel ligands are different from those representing the three classes, like the K/R-Y-V-COOH (Schneider *et al.*, 1999).

The second approach to classify PDZ domains is based on the nature of amino acids in the two critical positions of the PDZ domain – αB1 and residue that immediately follows βB strand. Using this principle PDZ domains were divided into 25 groups based on their two amino acids polarity and/or bulkiness (Bezprozvanny & Maximov, 2001). This

Table 1. Classification of PDZ domains based on the C-terminal sequence of their binding partners.

Class	C-terminus of the partner protein	Interacting partner	Example of PDZ domain
Class I –X-[S/T]-X-Φ	-ETDV -ESDV -TTRV -TSVF -ESLV -QSAV -DSSL -DTRL -QTRL -SSTL - PTRL	- Shaker K^+ channel - NMDA receptor subunits NR2A/B - neuroligin - ILR-5α - PMCA4b - voltage-gated Na^+ channel - protein kinase C-α - β_2-adrenergic receptor - CFTR - GKAP - metabotropic glutamate receptor subunit mGluR5 - GRK6A	- PSD-95 (PDZ2) - PSD-95 (PDZ2) - PSD-95 (PDZ3) - syntenin (PDZ1, PDZ2) - PSD-95 (PDZ1,2 and 3) - syntrophin - PICK1 - NHERF (PDZ1) - NHERF (PDZ1) - Shank/ProSAP - Shank/ProSAP -
Class II -X-Φ-X-Φ	- SVKI - EYFI - EYYV - EFYA - YYKV - DVPV	- AMPA receptor subunit GluR2 - glycophorin C - neurexin - syndecan-2 - ephrin B1 - receptor tyrosine kinase ErbB2	- PICK2, GRIP (PDZ5) - erythrocyte p55 - CASK - CASK, syntenin (PDZ2) - PICK1, GRIP (PDZ6), syntenin (PDZ2) - erbin
Class III -X-[D/E/K/R]-X-Φ	- VDSV - GEPL - FEEL - K/RYV	- melatonin receptor - KIF17 - merlin - synthesized peptide	- nNOS - mLIN10/Mint1/X11 - syntenin (PDZ1) - engineered from AF6
Other -X-X-C -X-Ψ-[D/E]	- DHWC - YXC	- N-type Ca^{2+}channel - L6 antigen	- Mint1 - SITAC

(Φ-hydrophobic, X-unspecified)

classification provides a method for predicting specificity of all PDZ domains and relies on a close connection between ligand preference and the amino-acid residues at given positions in PDZ domain. However, Vaccaro and Dente (2002) pointed out that within these 25 groups, the first group covers PDZ domains that bind class I peptides and the remaining groups are less clearly determined. Two of them do not correspond to any known PDZ domains; 14 are not correlated with any ligand sequence, four groups can be unified into canonical class II domains, and one group includes PDZ domains that are known to have dual specificity (Vaccaro & Dente, 2002). Most probably, the classification of PDZ domains will be revised in the future, as number of sequences and binding data increases.

PDZ DOMAIN SPECIFICITY

The binding affinities for PDZ domains and their ligands are moderate – dissociation constants (K_ds) range is typically in low nanomolar to high micromolar (Harris & Lim, 2001). The average K_d is low micromolar, similarly like those of SH2 and SH3 (Src homology 2 and 3) domains. Such moderate values are suitable for regulatory functions, since binding can be reversible and dependent on intracellular conditions.

Interactions of various PDZ domains with their ligands were typically observed using yeast two-hybrid system (Xia *et al.*, 1997; Chetkovich *et al.*, 2002; Hirbec *et al.*, 2002; Miyagi *et al.*, 2002; Mok *et al.*, 2002), pull-down assay (Hirbec *et al.*, 2002; Mok *et al.*, 2002), coimmunoprecipitation experiments (Poulat *et al.*, 1997; Chetkovich *et al.*, 2002; Miyagi *et al.*, 2002; Mok *et al.*, 2002; Pupo & Minneman, 2002), biochemical analyses (Xia *et al.*, 1997; Mok *et al.*, 2002), functional approaches (Pupo & Minneman, 2002), competition experiments and overlay assays (Zimmermann *et al.*, 2002), target-assisted iterative screening (Kurakin & Bredesen, 2002), proteomic approach based on a peptide affinity chromatography followed by mass spectrometry and immunoblotting (Becamel *et al.*, 2002), phage display (Fuh *et al.*, 2000), *in situ* hybridization and post-embedding immunogold technique (Miyagi *et al.*, 2002), surface plasmon resonance (Grootjans *et al.*, 2000; Koroll *et al.*, 2001; Miyagi *et al.*, 2002), Western blotting (Grootjans *et al.*, 2000), NMR experiments (Kozlov *et al.*, 2002), and isothermal titration calorimetry (Grootjans *et al.*, 2000; Kang *et al.*, 2003). These experiments show that PDZ domains bind a variety of ligands, however, the role of these numerous interactions often remains to be revealed.

It is possible that *in vivo* the binding affinity can be much higher due to a presence of many PDZ domains within one polypeptide chain and simultanous interactions of other protein–protein interaction domains. Similarly, the excluded volume effects resulting from the highly crowded nature of the cytosol (300 to 400 g/liter of proteins and other macromolecules in *Escherichia coli*) (Ellis, 2001) should lead to a stronger association in a cell, compared with *in vitro* assays. Crowding generally provides a nonspecific force for macromolecular compaction and association (Minton, 2000), which may be crucial to the formation of large protein complexes.

PDZ domains can also recognize an internal sequence that structurally mimics the C-terminus. Such interactions, most intesively studied for the binding of nNOS PDZ domain by either the PDZ domain from α1-syntrophin or the second PDZ domain from PSD-95 (Christopherson *et al.*, 1999) are biologically important in localizing nNOS to the neuromuscular junction or the postsynaptic density (Brenman *et al.*, 1995). In order to interact with other PDZ domains, the nNOS PDZ domain has a 30-amino-acid extension folded into a stable β-hairpin (called the β-finger) immediately followed by a sharp type II β-turn. This unusual motif was shown to bind on the same surface groove of the syntrophin PDZ domain

as the C-terminal peptide ligand, with its β-turn positioned directly in place of the peptide's carboxyl-terminus (Hillier *et al.*, 1999). Closer insight into this interaction reveals that the β-finger of nNOS contains an internal peptide whose sequence and binding orientation are very similar to those of canonical C-terminal peptide ligands (Fig. 1B). In addition, there is an extensive area of contacts between the core PDZ domains of syntrophin and nNOS (Harris *et al.*, 2001). Thus, binding of the two different regions of nNOS by syntrophin is far more specific than recognition through a short C-terminal sequence.

The multi-domain scaffolding protein INAD (inactivation no after-potential D) contains five PDZ domains which independently bind various proteins including NorpA (no receptor potential A) and the phospholipase C-β isoenzyme. These interactions are required for the proper intracellular targeting and spatial arrangement of proteins involved in the fly phototransduction. The structure of the N-terminal PDZ domain of INAD with the C-terminal heptapeptide (GKTEFCA) derived from NorpA reveals an intermolecular disulfide bond necessary for the interaction (Kimple *et al.*, 2001). Since other proteins also possess similar, cysteine-containing consensus sequences adequate for binding to the PDZ domains, this disulfide-mediated interaction may be a common mode of interaction between PDZ domains and their target proteins. Moreover, there are also other important differences in INAD(PDZ1)-NorpA interaction. The NorpA peptide contains an abrupt turn at Phe(–2), while all other peptides are in an extended conformation (Doyle *et al.*, 1996; Daniels *et al.*, 1998; Schultz *et al.*, 1998a). Furthermore, even though PDZ1 of INAD possesses a characteristic hydrophobic cleft that normally buries the side chain of the terminal residue of the peptide, position (0) of the NorpA derived peptide is exposed to a solvent (Kimple *et al.*, 2001).

Erbin interacts with the receptor tyrosine kinase ErbB2 and plays a role in its localization at the basolateral membrane of epithelial cells (Borg *et al.*, 2000). The protein is also highly concentrated at neuronal postsynaptic membranes and neuromuscular junctions. The crystal structure of the Erbin and ErbB2-derived peptide reveals an interaction of the peptidic Tyr(–7) with the extended β2–β3 loop of the Erbin PDZ (Birrane *et al.*, 2003). The second crystal structure of this domain bound to the phosphotyrosine-containing ErbB2 peptide shows that phosphorylation of Tyr(–7) abolishes its interaction with the β2–β3 loop. Phosphorylation of the Tyr(–7) residue reduces 2.5-fold the affinity of the Erbin-ErbB2 interaction (Birrane *et al.*, 2003).

IL-16 has no significant sequence homology to other interleukins or any other member of the chemokine family and is the first known extracellular protein with the PDZ-domain-like fold. However, the protein does not exhibit any peptide binding properties of PDZ domains (Muhlhahn *et al.*, 1998), since its GLGF cleft is smaller and blocked with a bulky Trp side chain at its center.

Recently solved NMR structure of the second PDZ domain of PTP-BL (protein tyrosine phosphatase BL) shows a unique feature, compared to the canonical PDZ fold. An extended flexible loop at the base of the binding pocket, called L1, folds back onto the protein backbone and modulates the domain selectivity (Walma *et al.*, 2002).

The specificity of a PDZ domain can be easily altered by substituting residues in or directly adjacent to the strand βB and the helix αB. Stricker *et al.* (1997) changed the specificity of nNOS PDZ domain from D-X-V-COOH to T-X-V- COOH by introducing only two mutations – Tyr77His and Asp78Glu. Moreover, it was reported that PDZ domains could be engineered to specifically recognize a large number of proteins by combining different backbone templates with a computer-aided protein design (Reina *et al.*, 2002). Phage display approach was also used to alter the specificities of PDZ domains. Schneider *et al.*

(1999) selected from phage library different mutants of the AF-6 (ALL-1 fusion partner from chromosome 6) PDZ domain that bound a variety of peptides. They showed that no more than two residue substitutions localized to either αB, βB or the carboxylate binding loop were necessary to change the domain's binding specificity. Changing just a single amino-acid residue, however, was in many cases sufficient to alter the specificity and affinity of PDZ domains (Gee *et al.*, 2000).

There is also a growing number of PDZ domains with a mixed class specificity. Erbin contains a single class I PDZ domain that binds with a high affinity to the carboxyl terminus sequence DSWV of δ-catenin, ARVCF, and p0071 (Jaulin-Bastard *et al.*, 2002; Laura *et al.*, 2002). However, Erbin PDZ domain also recognizes ErbB2 sequence EYLGLDVPV, the class II ligand (Jaulin-Bastard *et al.*, 2001; Laura *et al.*, 2002). Syntenin consists of two PDZ domains and the N-terminal fragment of unknown properties. Each domain is able to bind peptides belonging to two different canonical classes: PDZ1 binds peptides from the class I and III (LEDSVF – the C-terminal fragment of IL-5 receptor α chain and AFFEEL of merlin, respectively), while PDZ2 interacts with the class I and II (IL-5αR peptide and TNEFYA of syndecan 4, respectively). Additionally, the N-terminal fragment of syntenin appears to function as a regulatory domain, interfering in solution with the peptide binding by PDZ2 (Kang *et al.*, 2003).

PDZ domains can not only serve as protein–protein interaction modules, but also are capable of binding phosphatidylinositol 4,5-bisphosphates (PIP2), as Zimmermann *et al.* (2002) showed for PDZ domains of syntenin, CASK, Tiam-1 (T-lymphoma invasion and metastasis inducing protein 1) and PTP-BL. Competition and mutagenesis experiments revealed that the peptide and the PIP2 binding sites in the PDZ domains overlap. Moreover, living cell studies suggest that PDZ domain containing protein can bind to plasma membrane in both the PIP2-dependent and peptide-dependent manner (Zimmermann *et al.*, 2002). Garrard *et al.* (2003) proposed a new type of function for the PDZ domains as observed in Par6 (partition-defective) protein, composed of aPKC (atypical protein kinase C) binding domain, semi-CRIB (Cdc42 and Rac interactive binding) motif and a PDZ domain. The CRIB motif in Par6 is uncapable of binding to Cdc42 (Cell division control protein 42) in the absence of the adjacent PDZ domain that provides structural stability to the motif (Garrard *et al.*, 2003).

POSSIBLE REGULATION MECHANISM INVOLVING PHOSPHORYLATION OF C-TERMINUS

There are several examples showing that phosphorylation can regulate interaction of PDZ domain with the C-terminus of binding partners. Interestingly, most of the C-terminal peptides from a variety of proteins possess serine, threonine or tyrosine residue at (–2) or (–3) position, which are critical for the interaction with the binding pocket of PDZ domain. For example, it was demonstrated that the C-terminus of inward rectifier K^+ channel (Kir 2.3), which is a specific target for PDZ domain of PSD-95 protein, contains a consensus sequence for protein kinase A (PKA). Phosphorylation of the (–2) position at the C-terminus of Kir 2.3 channel by PKA disrupts its interaction with PSD-95 PDZ domain (Cohen *et al.*, 1996). Another paper reported the phosphorylation-dependent modulation of interaction between the β2-adrenergic receptor and the PDZ domain of NHERF (Na^+/H^+ exchanger regulatory factor) protein. In this case, the interaction is abolished by GRK-5 (G-protein-coupled receptor kinase 5) specific phosphorylation (Cohen *et al.*, 1996; Cao *et al.*, 1999). Protein kinase C (PKC) was able to prevent binding of GRIP (glutamate receptor-interacting protein) PDZ domain to the GluR2 (glutamate receptor) subunit of

AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor by phosphorylation of serine residue at the (–3) position within the C-terminus of GluR2 (Matsuda *et al.*, 1999). On the other hand, recent studies demonstrated that phosphorylation can also increase the strength of the interaction – the phosphorylated form of the C-terminal peptide of MRP2 (mitochondrial ribosomal protein) protein was bound stronger to the three tested PDZ-containing proteins PDZK1 (PDZ domain containing-protein), IKEPP (intestinal and kidney enriched PDZ protein) and EBP50 (ezrin-radixin-moesin binding phosphoprotein-50) than the dephosphorylated form (Hegedus *et al.*, 2003).

MULTIMERIZATION OF PDZ-CONTAINING PROTEINS

The arrangement of PDZ domains within a multidomain protein determines the unique function of these proteins in an assembly of macromolecular complexes. To generate more complex signaling scaffolds, PDZ proteins can self-associate to form multimers and there are several examples showing that the multimerization is mediated by PDZ domains. For example, GRIP1 (multi-PDZ protein containing seven PDZ domains) form homo- and heteromultimers *via* association of its PDZ4, PDZ5 and PDZ6 domains (Srivastava *et al.*, 1998; Dong *et al.*, 1999). The second example is INAD protein (composed of five PDZ domains) which plays a key role in *Drosophila* vision mechanism. This mechanism is facilitated by homomultimerization of INAD through the PDZ3 and PDZ4 domains. This multimerization does not prevent the binding of PDZ3 and PDZ4 domains targets suggesting that the two types of interactions (PDZ–ligand and PDZ–PDZ) occur through different binding interfaces of PDZ domains (Xu *et al.*, 1998). PDZ proteins can also form multimers in the PDZ-independent mechanisms, like in case of PSD-95 protein, where the dimer formation is mediated by the N-terminal region (Hsueh *et al.*, 1997).

COMPARISON WITH PTB DOMAINS

There is a structural and ligand binding similarity between PDZ and PTB (phosphotyrosine binding) domains. PTB domains are regions of 100–150 residues in the insulin receptor substrates 1 and 2 (IRS-1 and IRS-2) and in the adaptor protein Shc (Src homology 2 domain-containing protein) (Kavanaugh & Williams, 1994). As shown by NMR (Zhou *et al.*, 1995; 1996) and crystallographic (Eck *et al.*, 1996) studies, both the Shc and IRS-1 domains have essentially identical seven-stranded β-sandwich framework, capped by the C-terminal helices. Both Shc and IRS-1 PTB domains recognize peptides containing phosphotyrosine at the end of an NPXpY sequence (Wolf *et al.*, 1995). In addition, the IRS-1 PTB domain requires a hydrophobic residue at position (–8), and the Shc domain, a hydrophobic side chain at position (–5). As in the PDZ complexes, the peptides bound to Shc and IRS-1PTB domains form antiparallel β-strand with the β-sheet and on the other side pack against the α-helix. The NPXpY motif at the C-terminal end of the PTB-bound peptide is much more extensive than the simple carboxylate at the C-terminus of the PDZ-bound peptide. In the PTB complexes, residues in this loop participate in an elaborate network of hydrogen bonds that anchor a β-turn formed by the NPXpY residues. Differential specificity appears to depend on the presence of pockets for hydrophobic residue binding at position (–5) in Shc or at position (–8) in IRS-1.

ARRANGEMENTS OF PDZ DOMAINS IN SIGNALING PROTEINS

PDZ domains seem to be crucial organizers of protein complexes at the plasma mem-

brane. They are important in transport and targeting of different proteins to the sites of cellular signaling thus assuring localization and organization of both relevant receptors and downstream effectors to proper regions of the cell. PDZ-containing proteins create scaffolds for the assembly of supramolecular signaling complexes, thereby coordinating and guiding the flow of regulatory information. This is possible due to the ability of PDZ-containing proteins to both bind an array of target proteins and oligomerize into branched networks.

According to arrangement type, all known PDZ-containing proteins can be divided into two groups. The first group comprises proteins containing only PDZ domains, typically several PDZ domains with different specificities within a single polypeptide chain called multi-PDZ proteins. Examples include single-PDZ domain proteins PICK1 (protein interacting with C-kinase), Par6 and multi-PDZ domain proteins NHERF (2 PDZ domains), CIPP (channel-interacting PDZ domain protein, 4 PDZ domains), INAD (5 PDZ domains), GRIP (7 PDZ domains), PATJ (Pals-1 associated tight junction protein, 10 PDZ domains) and MUPP1 (contains remarkable 13 PDZ domains). Proteins possessing single or multiple PDZ domains in combination with other functional domains form the second group. Among these, the MAGUK (membrane-associated guanylate kinase) proteins represent a very common class containing invariably one or three PDZ domains, a SH3 domain and a guanylate kinase homology (GUK) domain. Other PDZ-containing proteins present more diversified combinations with a variety of interaction domains such WW, LIM (zinc-binding domain present in Lin-11, Isl-1, Mec-3), CaMK (calcium/calmodulin-dependent protein kinase domain), DH/PH (Dbl homology/pleckstrin homology), ankyrin or leucine-rich repeats.

PDZ-only proteins

Single-PDZ domain proteins. Despite the presence of only a single PDZ domain, some of them can effectively multimerize and in consequence link partner proteins. This is illustrated by PICK1, a single-PDZ domain protein expressed at synapses and originally isolated due to its ability to bind the C-terminus of protein kinase C (Staudinger *et al.*, 1995; 1997). It was shown that PICK1 can homooligomerize through its coiled-coil region and this self-association is essential for clustering of the synaptic metabotropic glutamate receptors (mGluR7a) (Staudinger *et al.*, 1997). Besides this interaction, PICK1-binding partners include the GluR2 subunit of AMPA receptors (Dev *et al.*, 1999; Daw *et al.*, 2000; Osten *et al.*, 2000; Xia *et al.*, 1999; 2000; Iwakura *et al.*, 2001; Kim *et al.*, 2001; Perez *et al.*, 2001; Braithwaite *et al.*, 2002), the dopamine transporter (DAT) (Torres *et al.*, 2001), the ERBB2/HER2 receptor (Jaulin-Bastard *et al.*, 2001), the mitogen-stimulated TIS21 protein (Lin *et al.*, 2001) and the ASICs (acid-sensing ion channels) (Baron *et al.*, 2002). Taken together, it seems that PICK1 may serve as adaptor protein that links variety of synaptic transmembrane receptors and channels to protein kinase C.

Another single-PDZ domain containing protein, Par6, first identified in *C. elegans,* plays a critical role in the asymmetric cell division and the polarized cell growth (Hung & Kemphues, 1999). Later studies revealed a family of mammalian Par6 proteins, similar to *C. elegans* forms (Joberty *et al.*, 2000). Besides PDZ domain, Par6 protein contains a semi-CRIB motif, which can bind to Cdc42 GTPase but only in the presence of an adjacent PDZ domain. Moreover, it was shown that Par6 PDZ domain effects structural stability of the CRIB motif (Garrard *et al.*, 2003). Both PDZ and semi-CRIB motif are also necessary for binding to Par3, another protein containing three copies of PDZ domain. Functional complex of Par6 with Cdc42-GTP, Par3 and with the regulatory domains of atypical protein kinase C is implicated in the formation of tight junctions at epithelial cell–cell

contacts (Joberty *et al.*, 2000). This suggests that Par6 is an adaptor protein responsible for cross-talk between activated Cdc42 or Rac GTPases and apical protein kinase signaling.

Multi PDZ-domain proteins. NHERF-1 and its second isoform, NHERF-2, highly expressed in epithelial cells, serve as specialized adaptors for broad range of signaling proteins. Both isoforms contain two highly homologous PDZ domains and the C-terminal region that associates with members of the ERM (ezrin-radixin-moesin) family of membrane-cytoskeletal adapters. NHERF was first identified as a regulator of NHE3 (type 3 Na^+/H^+ exchanger) activity (Weinman *et al.*, 2000). However, the list of functions of NHERF protein in epithelial cell physiology can be extended. For example, regulation of membrane proteins such as β_2AR (β_2-adrenergic receptor) (Hall *et al.*, 1998) and CFTR (cystic fibrosis transmembrane conductance regulator) (Raghuram *et al.*, 2001) is mediated by PDZ1 domain of NHERF. Other proteins identified as potential partners for NHERF PDZ1 domain include PDGFR (platelet-derived growth factor receptor) (Maudsley *et al.*, 2000), GRK6A (an isoform of G-protein-coupled receptor kinase) (Hall *et al.*, 1999), SOC (store-operated calcium) channels, such as Trp4 and Trp5, as well as the phospholipases Cβ1 and Cβ2 (Tang *et al.*, 2000). On the other hand, besides the binding of NHE3, the PDZ2 domain of NHERF is reported to bind two additional targets: YAP-65 (Yes-associated protein) in YAP-65/c-Yes complex (Mohler *et al.*, 1999) and phospholipase Cβ3 (Hwang *et al.*, 2000). Thus, both isoforms of two PDZ-domain protein NHERF are involved in regulation of multiple signaling pathways such as growth regulation, phosphoinositide metabolism, receptor modulation and targeting non-receptor kinases (Voltz *et al.*, 2001).

The CIPP is an example of multi-PDZ domain protein, which was found to interact selectively with the C-termini of signaling receptors in synaptic membranes. CIPP is composed of four PDZ domains possessing different specificities; PDZ2 domain binds to the C-terminus of the inward rectifier K^+ (Kir) channel, Kir4.1, and neuroligin, PDZ3 interacts with the NR2C subunit of NMDA receptors and neurexin (Kurschner *et al.*, 1998), whereas PDZ4 domain was recently reported to bind the ASIC3 (acid-sensing ion channel 3) (Anzai *et al.*, 2002). Additionally, the C-termini of NR2B subunit of NMDA and Kir4.2 are specific ligands for both PDZ2 and PDZ3 domains of CIPP (Kurschner *et al.*, 1998). In contrast, the binding partners for PDZ1 domain have not yet been identified. Thus, the CIPP protein appears to be a typical scaffolding protein that links different types of neuronal cell surface molecules to intercellular signaling network in neurons.

INAD – *Drosophila* protein composed of five PDZ modules plays a central role in organization of supramolecular signaling complex in the phototransduction cascade. All five PDZ domains of INAD have been shown to interact with various phototransduction proteins. PDZ1 and PDZ5 domains of INAD were shown to bind the phospholipase C (PLC) (Tsunoda *et al.*, 1997; van Huizen *et al.*, 1998; Xu *et al.*, 1998), whereas PDZ2 and PDZ4 domains, the C-terminus of eye-specific protein kinase C (Huber *et al.*, 1996b; Tsunoda *et al.*, 1997; Adamski *et al.*, 1998; Xu *et al.*, 1998). Additionally, light-responsive, transient receptor potential (TRP) channel is a target for PDZ3 of INAD (Huber *et al.*, 1996a; Shieh & Zhu, 1996).

Three multi-PDZ domain proteins, Dlt (Discs Lost), PATJ (Pals-1 associated tight junction protein) and MUPP1 (multi-PDZ domain protein 1) are examples of proteins essential in organizing protein complexes crucial to maintaining polarity of epithelial and neuronal cells. First of them, the *Drosophila* Dlt contains four PDZ domains and its PDZ1 domain can interact with the C-terminal four amino acids of the dCrumbs protein – apical polarity determinant responsible also for positioning of the zonula adherens in *Drosophila*

epithelial cells (Klebes & Knust, 2000). PATJ and MUPP1 are mammalian homologues of Dlt protein. PATJ was originally found as a close human homologue of *Drosophila* INAD protein – hINAD, containing seven PDZ domains (Vaccaro *et al.*, 2001). Later studies indicated that the protein possesses eight (Lemmers *et al.*, 2002), and finally, ten PDZ domains (Roh *et al.*, 2002), suggesting that previously described shorter hINAD represents an incomplete version of PATJ protein. Moreover, it has been found that its similarity to Dlt is higher than to INAD protein. In mammalian cells, PATJ together with Pals-1 and CRB1 proteins, colocalize to tight junctions, where all these proteins play a critical role in establishment of epithelial cell polarity (Roh *et al.*, 2002). MUPP1 definitely holds a top position among other PDZ proteins in respect to the number of PDZ domains. This extraordinary long protein is composed of 13 PDZ domains and concentrated at tight junctions (TJs) of epithelial cells. Initially, MUPP1 was identified as a protein that interacts with the C-terminus of the serotonin 5-hydroxytryptamine type 2C (5-HT2C) receptor (Ullmer *et al.*, 1998). Later studies showed that MUPP1 is also a cytoplasmic ligand for the membrane-spanning proteoglycan NG2 (Barritt *et al.*, 2000), human mast/stem cell growth factor receptor c-Kit (Mancini *et al.*, 2000), and PDZ domain-binding motifs of human adenovirus type 9 and high-risk human papillovirus (HPV) oncoproteins – E4-ORF1 and E6 (Lee *et al.*, 2000). Moreover, PDZ10 domain of MUPP1 binds the C-terminal sequences of claudins and junctional adhesion molecules (JAMs) (Hamazaki *et al.*, 2002).

The PDZ domains of Disc Lost, PATJ and MUPP1 are highly conserved. Interestingly, domains PDZ2–PDZ5 of PATJ and MUPP1 can be aligned on PDZ1–PDZ4 domains of Disc Lost. The similar domain organization suggests that both proteins may have evolved from the same ancestor. Recent studies reported the presence of additional conserved region at the N-termini of all these proteins, called MRE (Maguk recruitment) domain which enables the interactions with other proteins essential for maintaining epithelial polarity (Roh *et al.*, 2002).

Another interesting multi-PDZ domain protein, GRIP1 containing seven PDZ domains is abundant in synaptic junctions of neurons. GRIP1 is a typical scaffold protein which plays an important role in the synaptic targeting of AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors. The C-termini of the GluR2/3 subunits of these receptors are targets for PDZ4 and PDZ5 domains of GRIP1 (Dong *et al.*, 1997; Srivastava *et al.*, 1998; Wyszynski *et al.*, 1998). Interestingly, it was shown that these two PDZ domains cooperate as an integral tandem and that covalent linkage of both domains is critical for its proper folding and binding to GluR2/3 (Dong *et al.*, 1999; Srivastava *et al.*, 1998; Zhang *et al.*, 2001). Moreover, PDZ6 domain of GRIP1 was shown to interact with EphB2/EphA7 receptor tyrosine kinases, ephrinB1 ligand (Torres *et al.*, 1998; Bruckner *et al.*, 1999; Lin *et al.*, 1999) and liprins-α family of multidomain proteins (Wyszynski *et al.*, 2002). Additional studies of PDZ4, PDZ5 and PDZ6 from GRIP1 showed that these domains also mediate homo- and heterodimerization of GRIPs, confirming a double function of PDZ domain as peptide recognition and multimerization modules (Dong *et al.*, 1999). Very unusual interaction mode presents PDZ7 domain of GRIP1 which binds GRASP-1 (GRIP1-associated scaffold protein 1), a Ras guanine exchange factor that regulates the synaptic distribution of AMPA receptors.

Proteins containing PDZ domains in combination with other signaling domains

MAGUK family. The MAGUKs are a large family of proteins involved in sequestering protein complexes at the plasma membrane and formation of different cell junctions. Members of this family occur in all multi-

cellular organisms and have specific domain organization – one or three PDZ domains, a SH3 domain and GUK (guanylate kinase) domain. Despite partially preserved domain architecture, MAGUKs can be divided into four subfamilies: 1) Dlg-MAGUKs; 2) ZO-1-MAGUKs; 3) p55-MAGUKs and 4) Lin-2-MAGUKs.

The first subfamily (Dlg-MAGUKs) includes proteins with a domain structure similar to *Drosophila* tumor suppressor protein – Dlg (Disc Large) composed of three copies of PDZ domain, an SH3 domain and a GUK domain. *Drosophila* Dlg colocalizes with one of its subcellular ligands, fasciclin-III at septate junctions and is required for proper localization of this protein (Woods *et al.*, 1996). Additionally, yeast two-hybrid experiments showed that Dlg PDZ1 and PDZ2 domains bind the C-terminus of Shaker K^+ channel and deletion of this C-terminal PDZ-binding motif eliminates channel clustering (Tejedor *et al.*, 1997). Another member of Dlg-MAGUKs subfamily, PSD-95, is specifically localized to the postsynaptic density (PSD) of excitatory synapses. The postsynaptic membrane is enriched in a variety of receptor proteins; several of them bind to the PDZ domains of PSD-95 *via* their cytoplasmic C-termini. For example, the cytoplasmic tails of NMDA receptors contain a conserved motif which mediates binding to the first two PDZ domains of PSD-95 (Kornau *et al.*, 1995). Additional membrane proteins that can bind to PSD-95 include neuroligin (binds to PDZ3 domain of PSD-95) (Irie *et al.*, 1997), ErbB4 (tyrosine kinase receptor) which interacts with PDZ1/2 of PSD-95 (Garcia *et al.*, 2000) and voltage-gated K^+ channels (Kim *et al.*, 1995).

Mammalian ZO-1, ZO-2 and ZO-3 (zonula occludens) represent the ZO-1-MAGUKs subfamily and are usually localized at sites of intercellular junctions (septate junctions in *Drosophila* and tight junctions in mammals). They organize these junctions by forming heterodimeric complexes with each other, creating a bridge between the actin cytoskeleton and transmembrane proteins of TJs. Besides SH3 and GUK domains, ZO proteins contain three PDZ domains. Moreover, they also contain characteristic C-terminal proline-rich extension. TJ strands are mainly composed of two distinct types of transmembrane proteins: occludins and claudins. PDZ-1 domains of ZO-1, ZO-2 and ZO-3 directly bind to the C-terminal sequence of claudins (Itoh *et al.*, 1999a). It was shown that PDZ2 domains of all members mediate heterodimeric interactions between ZO-1/ZO-2 and ZO-1/ZO-3 (Itoh *et al.*, 1999b). On the other hand, the binding partners for the PDZ3 domain of ZO proteins have not yet been identified.

The human p55, which represents the p55-MAGUKs subfamily, is a peripheral membrane protein of the erythrocyte membrane. p55 plays an important role in maintaining erythrocyte shape and its membrane properties. The protein contains a single copy of PDZ domain, together with SH3 and GUK domains. It has been reported that PDZ domain of p55 binds to the C-terminus of glycophorin C (Marfatia *et al.*, 1997). Additionally, abnormality of PDZ domain of p55 in chronic myeloid leukemia (CML) has been reported (Ruff *et al.*, 1999).

Like p55 subfamily, Lin-2-MAGUKs possess a single PDZ domain aside from SH3 and GUK domains. Additionally, they have characteristic N-terminal calcium/calmodulin-dependent protein (CaM) kinase domain. In *C. elegans*, Lin-2 protein is involved in localization of Let-23, an EGF receptor-like protein (Hoskins *et al.*, 1996). Other members of this subfamily, *Drosophila* CAMGUK (calcium/calmodulin-dependent serine protein kinase membrane-associated guanylate kinase), rat CASK/Lin-2 (calcium/calmodulin-dependent serine protein kinase) and human CASK (hCASK) are homologous to *C. elegans* Lin-2 and are localized to synapses, where, as scaffolding proteins, participate in multiple interactions. The PDZ domain of these proteins bind to cytoplasmic tails of several cell-sur-

face proteins. For example, the C-terminus of neurexin I is a high affinity binding partner for PDZ domain of CASK protein (Hata *et al.*, 1996) and the cytoplasmic tails of junctional adhesion molecules are targets for CASK/Lin2 PDZ domain (Martinez-Estrada *et al.*, 2001). Moreover, yeast two-hybrid screening showed an interaction between the human homolog hCASK and the C-terminal sequence of the membrane protein syndecan-2 (Cohen *et al.*, 1998). In another example, the C terminus of Parkin protein, selectively truncated by a Parkinson's disease-causing mutation, can selectively bind to the PDZ domain of CASK (Fallon *et al.*, 2002). Recent studies reported a new interaction partner for PDZ domain of CASK protein – plasma membrane Ca^{2+}-ATPase (PMCA), which is major regulator of Ca^{2+} homeostasis (Schuh *et al.*, 2003).

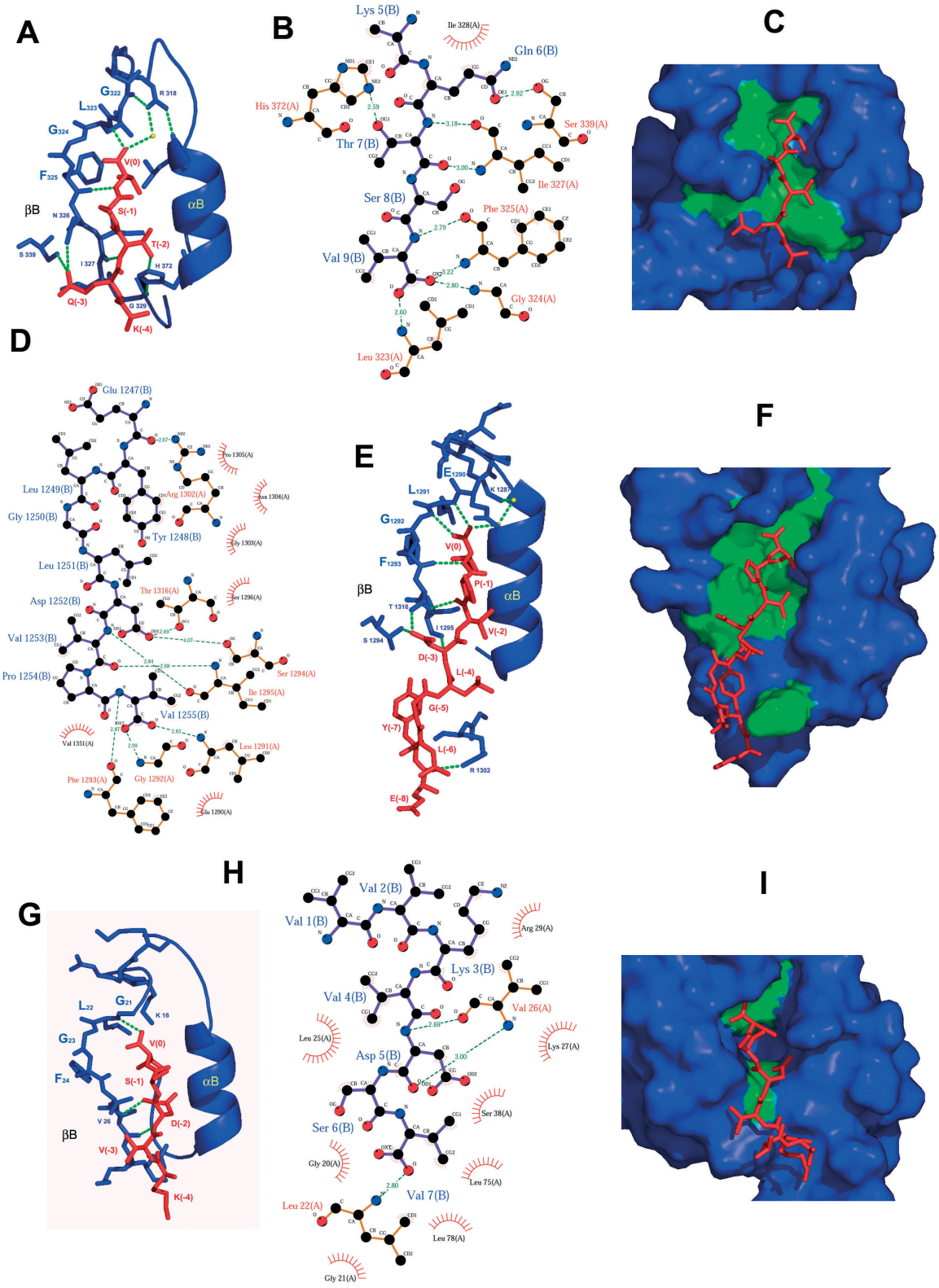
PDZ-LIM family. It has been suggested that cytoskeletal proteins belonging to the PDZ-LIM family serve as adapters for direct LIM-binding proteins to the cytoskeleton (Vallenius *et al.*, 2000). They contain a PDZ domain at the N-terminus followed by one or three LIM domains. All six members of the family associate with the cytoskeleton, five of them *via* interactions with α-actinin and/or β-tropomyosin. CLP-36 (C-terminal LIM domain protein 1) protein is expressed in epithelial cells and localizes to actin stress fibers. This localization is mediated *via* the PDZ domain of CLP-36 that associates with the spectrin-like repeats of α-actinin. Yeast two-hybrid analysis indicated a highly specific association of CLP-36 and Clik1 (CLP-36 interacting kinase) (Vallenius *et al.*, 2000). The association is mediated by the C-terminal part of CLP-36 containing LIM domain and leads to relocalization of the otherwise nuclear Clik1 kinase to actin stress fibers (Vallenius & Makela, 2002).

Cypher, a striated muscle-restricted protein, has two mRNA splice variants designated Cypher1 and Cypher2. Both proteins contain PDZ domain at the N-terminus. Cypher1, but not Cypher2, contains three LIM domains close to the C-terminus. Cypher1 and Cypher2 bind to β-actinin *via* their PDZ domains at the Z-lines of cardiac muscle. These data suggest that Cypher functions as an adaptor in striated muscle to link protein kinase C-mediated signaling to the cytoskeleton (Zhou *et al.*, 1999). In turn, PDZ domain of Enigma, another member of PDZ-LIM family, is present at the Z-line in skeletal muscle and its PDZ domain binds to the actin-binding protein tropomyosin (skeletal β-TM). The interaction suggests a role for Enigma as an adapter protein that directs LIM-binding proteins to actin filaments of muscle cells (Guy *et al.*, 1999).

LAP family. The LAP (leucine-rich repeats and PDZ) family of PDZ proteins plays a role in establishment of cell polarity, and mutation of these proteins can have oncogenic consequences. Sixteen leucine-rich repeats (LRRs) at the N-terminus and single PDZ domain at the C-terminus presents a characteristic architecture of all members of LAP family.

The LAP protein, Erbin was identified as an adaptor protein present in the basolateral epithelia and involved in proper localization of ERBB2/HER2 receptors to the basolateral membrane of epithelial cells. This process is mediated by Erbin PDZ domain, which was shown to bind the C-terminus of the receptor both *in vitro* and *in vivo* (Borg *et al.*, 2000). It was also reported that δ-catenin and ARVCF serve as interaction partners for Erbin PDZ domain (Laura *et al.*, 2002).

Another LAP family member, Densin, was identified as a transmembrane specific adhesion molecule mediating adhesion between pre- and postsynaptic membranes at glutamatergic synapses (Apperson *et al.*, 1996). Screening of human brain cDNA library resulted in identification of δ-catenin/neural plakophilin-related armadillo repeat protein (NPRAP) as a potential binding partner of PDZ domain of Densin. Colocalization of densin with δ-catenin/NPRAP at synapses suggested an important role in organization of the synaptic cell–cell junction (Izawa *et al.*, 2002). Later studies, showed binding of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T(-2)
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K(-4)
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Ser 339(A)
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Ile 327(A)
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Phe 325(A)
Val 9(B)
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Leu 323(A)
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Leu 1249(B)
Gly 1250(B)
Arg 1302(A)
Tyr 1248(B)
Leu 1251(B)
Thr 1316(A)
Asp 1252(B)
Val 1253(B)
Pro 1254(B)
Ser 1294(A)
Ile 1295(A)
Val 1255(B)
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Val 1(B)
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Lys 3(B)
Val 4(B)
Val 26(A)
Leu 25(A)
Lys 27(A)
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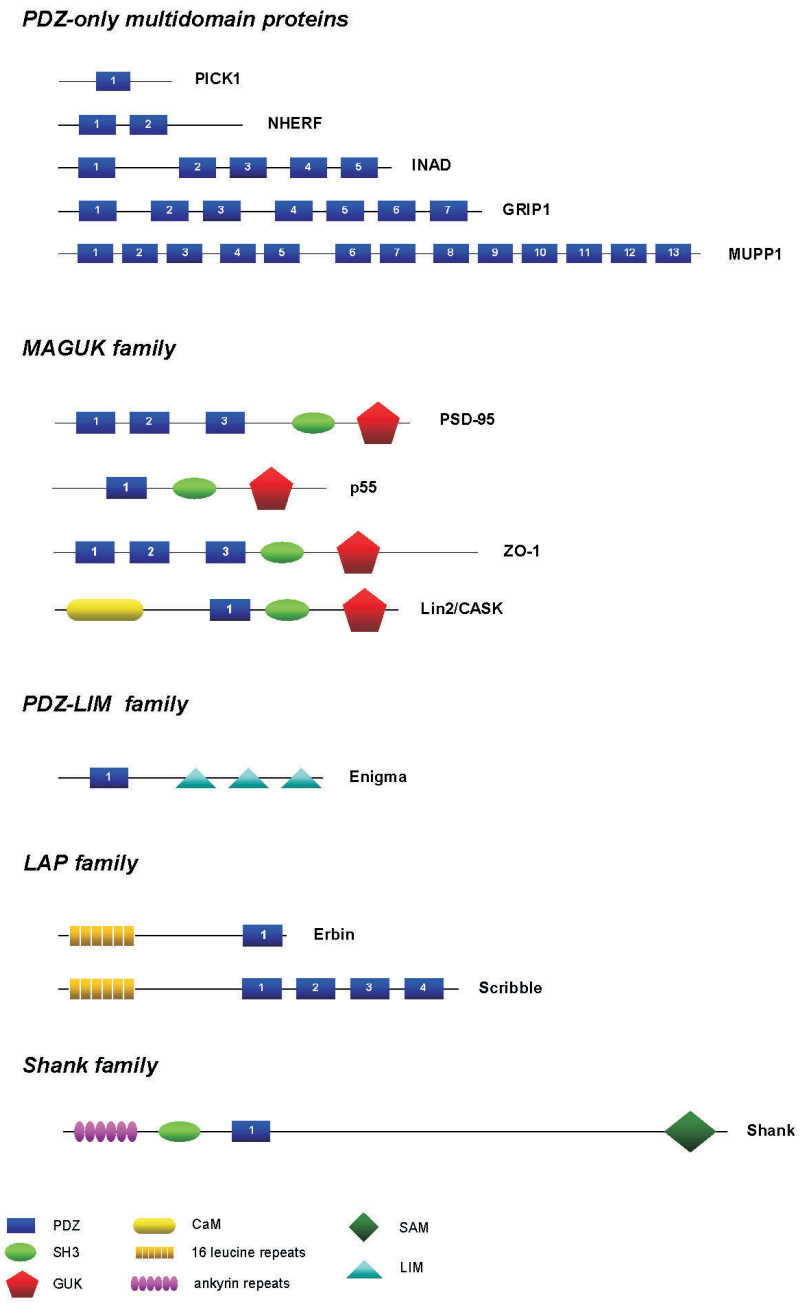

Figure 4. Modular organization of PDZ-containing proteins exemplified by representative members of described PDZ families.

Numbers within *blue squares* describe sequential PDZ domains.

Figure 3. Ligand binding pockets of class I, II and III PDZ domains.

A. The third PDZ domain from the synaptic protein PSD95 in complex with a C-terminal peptide derived from CRIPT (KQTSV). **E.** Erbin PDZ domain bound to the C-terminal tail of the ErbB2 receptor (EYLGLDVPV). **G.** The neuronal nitric oxide synthase (nNOS) PDZ domain complexed with VVKVDSV. **A, D** and **E.** The hydrogen bonding in PDZ domains (blue ribbon representation) and peptide (red sticks) complexes is shown (*green dashed lines*). Water molecules are in yellow. **C**, **F** and **I**. Surface topology of PDZ domain bound to their peptides. The figure was made using program PyMOL (DeLano). **B**, **D** and **H**. Two-dimensional representation of the interaction of PDZ domains (orange) and their peptides (purple) was made using program LIGPLOT (Wallace *et al.*, 1995) hydrogen bonds as *dashed lines* and hydrophobic interactions as *arcs* with *radial spokes*. Water molecules were not included in this presentation.

Densin PDZ domain to C-terminus of MAGUIN-1 (membrane-associated guanylate kinase-interacting protein 1) protein what is essential for assembly of PSD-95, MAGUIN-1, Densin ternary complex at the postsynaptic membrane of hippocampal neurons (Ohtakara *et al.*, 2002). The *Drosophila* tumor suppressor Scribble is a PDZ-containing protein belonging to the LAP family and required for maintaining epithelial cell polarity. At the larval neuromuscular junction, Scribble colocalizes and indirectly interacts with another tumor suppressor and PDZ protein, Dlg. Scribble was identified as an essential regulator of synaptic architecture, plasticity and physiology (Roche *et al.*, 2002).

Shank family. The scaffold proteins, Shank1, Shank2, and Shank3 (SH3 and multiple ankyrin repeat domains proteins) are members of the Shank family. These complex proteins (each about 2000 aa) possess several types of binding modules such as (from the N to C-terminus) multiple ankyrin repeats, an SH3 domain, a PDZ domain, a long proline-rich region and a sterile alpha motif (SAM) domain. All Shank proteins are highly concentrated in postsynaptic density of brain excitatory synapses (Boeckers *et al.*, 1999; Lim *et al.*, 1999; Naisbitt *et al.*, 1999) where they play an important role in assembly of signaling complexes between membrane and cytoplasmic proteins. The Shank PDZ domain was shown to bind the C-terminus of GKAP (guanylate kinase-associated protein) protein, that is also abundant in PSD of brain synapses (Boeckers *et al.*, 1999; Naisbitt *et al.*, 1999; Tu *et al.*, 1999; Yao *et al.*, 1999). Additionally, the C-termini of mGluRs and of SSTR2 (somatostatin receptor type 2) were reported to interact directly with the Shank PDZ domain in yeast two-hybrid (Tu *et al.*, 1999).

PDZ-containing proteins not classified to families

Besides PDZ-containing proteins classified to the families and subfamilies, there are many proteins possessing PDZ modules in a unique arrangement with other signaling domains and which cannot be simply grouped. This situation demonstrates and confirms a gigantic potential and spreading of PDZ domains among many types of existing proteins. Several interesting examples of such proteins are briefly described below.

The protein tyrosine phosphatase, PTP-BL, localized to the submembranous region of epithelial cells is characterized by having the N-terminal FERM (4.1, ezrin, radixin, moesin) domain, five PDZ domains and the C-terminal catalytic phosphatase domain. Its PDZ domains are involved in interactions with several partners; in particular, PDZ2 and PDZ4 interact with two LIM domain containing proteins, RIL (reversion-induced LIM protein) and TRIP-6 (thyroid receptor interacting protein 6) (Cuppen *et al.*, 2000), which are found in actin-rich structures of the cell. In addition, PDZ1 can interact with BP75 (bromodomain-containing protein) (Cuppen *et al.*, 1999), PDZ2 with the tumor supressor protein APC (adenomatous polyposis coli) (Erdmann *et al.*, 2000) and PDZ3 with the Rho effector kinase PRK2 (protein kinase C-related kinase 2) (Gross *et al.*, 2001). Another interesting PDZ-containing protein, Delphilin, is the first reported protein that contains a single PDZ domain in combination with two forming homology (FH) domains. This unique protein has been reported to interacts with the GluRδ2 C-terminus *via* its PDZ domain (Miyagi *et al.*, 2002). PDZ-RhoGEF and LARG (leukemia-associated Rho guanine-nucleotide exchange factor) proteins, essential for activation of biochemical pathways specific to Rho-like GTPases also possess a single N-terminal PDZ domain in their multidomain architecture. Recent studies have shown the interaction between PDZ domain of PDZ-RhoGEF and LARG and the C-terminus of B-plexins, suggesting B-plexin-mediated activation of Rho signaling (Swiercz *et al.*, 2002). Another signaling pathway, extremely important for vertebrate and non-vertebrate embryogenesis, called the canonical

Wnt signal transduction cascade, also employs the PDZ-containing protein, Dishevelled, with a conserved arrangement of three domains: DAX (domain present in Dishevelled and axin), PDZ and DEP (Dishevelled, Egl-10, and pleckstrin). The PDZ domain of Dishevelled is necessary for its ability to induce nuclear accumulation of β-catenin (Kishida *et al.*, 1999).

UNCONVENTIONAL FUNCTIONS OF PDZ-CONTAINING PROTEINS IN A LIVING CELL

Emphasizing a primary and critical role of PDZ proteins in the organization of large signaling complexes at the plasma membrane, several additional functions of PDZ-containing proteins were reported.

Protein targeting

Lin2, Lin7 and Lin10 are *C. elegans* proteins required for the normal basolateral localization of LET-23 receptor in vulval epithelial cells (Simske *et al.*, 1996; Kim, 1997; Bredt, 1998; Whitfield *et al.*, 1999). On the other hand, mammalian homologues of these proteins (mLin-2/CASK/PALS, mLin-7/VELI/MALS and mLin-10/MINT/X11) are mainly localized to neuronal cells where are responsible for trafficking of the NMDA receptors to cell membrane. According to the proposed mechanism of this targeting, Lin-2/CASK, Lin-7/VELI and Lin-10/MINT have ability to form a ternary complex (Borg *et al.*, 1998; Butz *et al.*, 1998; Kaech *et al.*, 1998). In case of *C. elegans* homologue in epithelial cells, both Lin-7 and Lin-10 bind to the Lin-2 protein (MAGUK protein) through the non-PDZ-mediated fashion and the PDZ domain of Lin-7 binds directly to the C-terminus of the LET-23 (lethal) receptor. In mammalian neurons, PDZ domain of VELI protein from the CASK/VELI/MINT complex, binds to the C-terminus of NMDA receptor subunit NR2B. Targeting of NMDA receptor to plasma membrane along microtubules is dependent on the PDZ domain of MINT protein

Table 2. PDZ containing proteins and their interaction partners.

In case of proteins containing additional domains, only interactions involving PDZ domains are included. Digits in the brackets indicate the number of PDZ domains.

	C-TERMINUS OF MEMBRANE PROTEINS			C-TERMINUS OF CYTOPLASMIC PROTEINS	C-TERMINUS OF CYTOSKELETAL PROTEINS
	RECEPTORS	ION CHANNELS	ADHESION PROTEINS		
Single-PDZ domain	PICK-1 (1) – AMPA PICK-1 (1) – mGluR PICK-1 (1) – ERBB2	PICK-1 (1) - ASIC		PICK-1 (1) - PKC	
Multi-PDZ domain proteins	GRIP (7) – AMPA NHERF (2) – NHE3 CIPP (4) – NMDA NHERF (2) - β_2 AR	NHERF (2) - CFTR INAD (5) – TRP NHERF (2) - SOC CIPP (4) – ASIC CIPP (4) – Kir 4.1	MUPP1 (13) – NG2 MUPP1 (13) – claudins MUPP1 (13) – JAM CIPP (4) – neurexin CIPP (4) – neuroligin	INAD (5) – PLC INAD (5) – PKC NHERF–2 (2) – TAZ NHERF (2) - PLCβ1,2,3 GRIP (7) – GRASP1	
Dlg MAGUKs	PSD95 (3) – NMDA PSD95 (3) – ErbB4	PSD-95 (3) – Shaker K+ Dlg-1 (3) - Shaker K+ PSD-95 (3) – Kv1 K+	PSD-95 (3) – neuroligin Dlg (3) – fasciclin II		
ZO-1 MAGUKs			ZO-1,2,3 (3) - claudins		
p55 MAGUKs			p55 (1) – glycophorin C		
Lin-2 MAGUKs			PALS1 (1) – Crumbs CASK (1) – neurexin CASK (1) – JAM hCASK (1) - syndecan		
PDZ-LIM family					CLP-36 (1) - α-actinin Cypher-1,-2 (1) - β-actinin Enigma (1) - β- tropomyosin
Shank family	Shank (1) – mGluR Shank (1) – SSTR2			Shank (1) - GKAP	
LAP family	Erbin (1) – ERBB2			Erbin (1) - δ catenin Erbin (1) – ARVCF Densin (1) – MAGUIN-1	

(in CASK/VELI/MINT complex), which was shown to bind to the kinesin superfamily motor protein KIF17 (kinesin family member 17) (Jo *et al.*, 1999; Setou *et al.*, 2000).

Regulation of gene expression

TAZ (transcriptional co-activator with PDZ-binding motif) and YAP (Yes-associated protein) function as co-activators of transcription factors and their activity is regulated by interactions with 14-3-3 and PDZ containing proteins. The transcriptional co-activation function of both proteins is critically dependent on the C-terminal residues, which consitute a PDZ binding motif. PDZ-domain proteins involved in binding of C-terminal motifs of TAZ and YAP are E3KARP (NHE3 kinase A regulatory protein or NHERF-2) and NHERF (Na^+/H^+ exchanger regulatory factor or NHERF-1), both containing two tandem PDZ domains and ERM binding region. TAZ specifically binds to the PDZ1 domain of E3KARP, whereas YAP interacts with the PDZ2 domain of both NHERF and E3KARP. Removal of the last four C-terminal amino acids from TAZ eliminates the E3KARP interaction. Therefore, NHERF and E3KARP, which also bind channels and receptors to cytoskeleton, positively regulate transcriptional activation of TAZ and YAP proteins and link membrane and cytoskeleton proteins to nuclear transcription (Kanai *et al.*, 2000).

Regulation of receptors activity

Interactions between cystic fibrosis transmembrane conductance regulator (CFTR) and two different PDZ-domain proteins: NHERF-1 (Raghuram *et al.*, 2001) and CAP70 (Wang *et al.*, 2000) are a clear example of involvement of PDZ-containing proteins in regulation of ion channels activity. The functional CFTR channel is a dimer containing two PDZ-binding motifs. It has been demonstrated that NHERF-1 binds to the cytoplasmic tails of CFTR Cl^- channels through either of its two PDZ domains. This interaction cross-links the C-termini within pre-existing dimeric channel complexes and causes a conformational change in the channels that affects Cl^- gating (Raghuram *et al.*, 2001).

Another studies have found that dimeric binding of multi-PDZ protein CAP-70 (hydrophilic CFTR-binding protein) to the C-termini of CFTR channels is sufficient to potentiate the chloride current (Wang *et al.*, 2000). They proposed a model of CAP-70-mediated potentiation. According to this model, in the absence of CAP-70 protein, CFTR Cl^- channels exist either as monomers or as transient dimers. Bivalent binding mediated by CAP-70 PDZ domains increases the binding affinity and improves the contact geometry between the two interacting CFTR molecules.

Selection of substrates

Bacterial tail-specific proteases are periplasmic enzymes, which cleave proteins with non-polar C-termini. It was shown that substrate specificity of these proteases is provided by the presence of PDZ domain independent of the catalytic domain. Two possible mechanisms of substrate recognition were proposed. One assumes that the PDZ domain initially recognizes a cognate substrate by binding to its non-polar C-terminus and this event recruits a substrate to the catalytic site. In this case PDZ domain helps to increase the enzyme affinity towards the substrate and creates enzyme-substrate complex. An alternative mechanism assumes that binding of the C-terminus of the substrate to the PDZ domain causes a conformational change that, in turn, activates proteolytic domain (Beebe *et al.*, 2000).

Members of another family of bacterial proteases, HtrA, also combine a proteolytic domain with at least one C-terminal PDZ domain (Pallen & Wren, 1997). Finally, tricorn proteases forming proteasome-like capsids in *Archea* also contain PDZ domains which in cooperation with a β-propeller fold play a

role in substrate selection (Pallen *et al.*, 2001).

EFFECTS OF PDZ DOMAIN MALFUNCTIONS

Malfunction of many PDZ domain-containing proteins is implicated in a variety of pathophysiological phenomena, including cancer. Analysis of p55 MAGUK protein mRNA from patients with acute megacaryoblastic CML revealed a 69 base pair deletion in the PDZ domain. This observation is the first abnormality of a PDZ domain linked to a human disease. Mutations in a gene encoding harmonin cause Usher syndrome type 1C, an autosomal recessive disorder characterized by congenital sensorineural deafness, vestibular dysfunction and blindness (Bitner-Glindzicz *et al.*, 2000; Montell, 2000; Verpy *et al.*, 2000). PDZ1 and PDZ2 domains of harmonin interact with two complementary binding surfaces of the Cadherin 23 (CDH23) cytoplasmic domain. Interaction of PDZ1 with CDH23 is perturbed by the insertion of 35 amino acids within CDH23 (Siemens *et al.*, 2002). Mutations in Periaxin gene cause Dejerine-Sottas neuropathy, a severe demyelinating form of peripheral neuropathy (Boerkoel *et al.*, 2001; Sherman *et al.*, 2001).

In flies, mutations in the gene encoding INAD, a protein composed solely of PDZ domains, disrupt the photoinduction cascade resulting in the light-dependent retinal degeneration (Shieh & Zhu, 1996). Mutations in PDZ domain-containing protein result in subcellular mislocalization of the LET-23 protein and the lack of vulval differentiation. The LAP proteins are recently described family of scaffold proteins that are involved in the formation of membrane complexes and the maintenance of epithelial and neuronal cell shape and polarity (Bryant & Huwe, 2000). For example, in *Drosophila* mutation of the Scribble LAP protein (16 leucine rich repeats and four PDZ domains) results in loss of epithelial cell polarity and morphology as well as uncontrolled, tumor-like growth (Bilder & Perrimon, 2000). Moreover, disruption of *Scribble* gene (*Scrb1*) causes severe neural tube defects (termed craniorachischisis) in the *circletail* mouse. In this disorder, almost the entire brain and spinal cord are affected, owing to a failure to initiate neural tube closure. It was found, that the *Scrb1* gene mutated in *circletail* (Crc) contains a single base insertion that creates a frame shift and leads to a premature termination of the Scrb1 protein. Scrb1 may control the subcellular localization of the Vangl2 protein alternatively Scrb1 and Vangl2 may form a part of a protein complex, perhaps through a direct interaction of the C-terminal PDZ-binding motif of Vangl2 with the PDZ domains of Scrb1 (Murdoch *et al.*, 2003).

Syntenin was originally discovered as a protein containing a tandem of PDZ domains and interacting with transmembrane proteoglycans called syndecans (Grootjans *et al.*, 1997). Syntenin was subsequently shown to bind class B ephrins, proTGF-α, neurofascin, schwannomin (also known as merlin), IL5 receptor α (ILR5α) and various glutamate receptor subtypes. Very recently, it was discovered that syntenin is overexpressed and promotes cell migration in metastatic human breast and gastric cancer cell lines (Koo *et al.*, 2002). Expression analysis shows that level of syntenin correlated well with invasive and metastatic potential in these cell lines. Furthermore, syntenin-trasfected cells migrated more actively, and showed numerous cell surface extensions, suggesting that syntenin is active upstream of pathways affecting actin cytoskeleton (Koo *et al.*, 2002). There is some experimental evidence that PDZ domains constitute for good drug targets. Fas (APO-1/CD95), a member of the tumor necrosis factor receptor superfamily and a cell surface receptor, which induces apoptosis, interacts with the PDZ domain of the Fas-associated phosphatase-1 (FAP-1). Direct cytoplasmic micro-

injection of a tripeptide (Ac-SLV) corresponding to the C-terminal fragment of Fas, resulted in apoptosis in a colon cancer cell line that expresses both Fas and FAP-1 (Yanagisawa *et al.*, 1997). It is therefore possible that other PDZ-mediated pathways may be equally sensitive to selective inhibitors.

PDZ domains are involved in tumorigenesis, cell migration and metastasis. Among highly expressed proteins in the human primary prostate tumors is AIPC (activated in prostate cancer), a protein containing six PDZ domains (Chaib *et al.*, 2001). It is possible, that disrupting the pathways mediated by these domains might inhibit early promotion of prostate tumorigenesis. In colon, breast, liver, lung, pancreas, stomach, and prostate tumors, a protein containing PDZ and LIM domains, denoted PCD1, was significantly overexpressed, in contrast to normal tissues (Kang *et al.*, 2000). It has been suggested that it participates in cytoskeletal reorganization in cancer, and that it could be a target for drug design.

CONCLUDING REMARKS

PDZ domains are ubiquitous element of cytoplasmic proteins in organisms from bacteria to mammals. Due to a common multiple copy occurrence within a single protein they mediate formation of extensive protein–protein networks. Diversity and size of such protein complexes is further enhanced by combination of PDZ domains with other protein interaction modules (SH3, PTB, LIM, WW, and ankiryn repeats). Among major cellular targets of PDZ domains are proteins associated directly with the plasma membrane like ion channels, receptors and cytoskeleton proteins The structural basis of their specificity to bind four to six C-terminal residues of these proteins appears relatively simple and suggests redundancy of recognized target sequences. However, since PDZ domains can also bind other PDZ domains in a head-to-tail fashion, recognize internal structural motifs in their target proteins or bind phosphatidylinositol derivatives, it is likely that diversity of their cellular interactions is much broader. Significant problems can be expected in deciphering cellular function and regulation of PDZ containing proteins since currently the technology to study *in vivo* transient multidomain protein complexes is not developed.

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