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PDZ domains – glue and guide

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Abstract

PDZ domains are small globular building blocks that are amongst the most abundant protein interaction domains in organisms. Over the past several years an avalanche of data has implicated these modules in the clustering, targeting and routing of associating proteins. An overview is given of the types of interactions displayed by PDZ domains and how this relates to the current knowledge on their spatial structure. Furthermore, the different levels on which PDZ – ligand binding can be regulated and the consequences of PDZ domain-mediated clustering for activity, routing and targeting of interacting proteins will be addressed. Finally, some cell and animal models that illustrate the impact of PDZ domain-containing proteins on (multi-) cellular processes will be discussed.

Abbreviations: APC – Adenomatous Polyposis Coli-protein; ALP – actinin-associated LIM protein; β_2AR – β_2 adrenergic receptor; CFTR – cystic fibrosis transmembrane regulator; IL-16 – interleukin-16; MAGI – membrane-associated guanylate kinase inverted; MAGUK – membrane-associated guanylate kinase; NHERF – Na^+/H^+ exchanger regulatory factor; PIP_2 – phosphatidylinositol 4,5-biphosphate; PDZ – acronym of PSD95/SAP90 DlgA ZO-1; nNOS – neuronal nitric oxide synthase; PTEN – phosphatase and tensin homologue deleted on chromosome 10.

Introduction

Cellular maintenance and responses converge on the presence, i.e. assembly / disassembly, of supramolecular complexes. The dynamic process of (re-) arranging multimeric protein complexes is critically dependent on the actions of a broad variety of protein modules. The number of different protein domains involved in interaction and signaling, e.g. SH2, SH3, PDZ, LIM, FERM, kinase and phosphatase domains, has exceeded the two hundred in the last few years and is still growing [1]. Here we will focus on the PDZ domain, a protein-protein interaction domain involved in the formation of multiprotein complexes at specific subcellular sites.

PDZ domains were first recognized approximately ten years ago as sequence repeats of ∼90 amino acid residues. Originally these modules were termed GLGF repeats (after the conserved Gly-Leu-Gly-Phe signature within the primary sequence) or Discs-large homology regions (DHR). However, presently they are named after the first three PDZ domain-containing proteins identified: the postsynaptic density protein PSD-95/SAP90, the *Drosophila* septate junction protein Discs-large, and the epithelial tight junction protein ZO-1 [2]. PDZ domains have emerged as one of the most abundant protein interaction domains in organisms as diverse as bacteria, yeast, plants, invertebrates and vertebrates [3]. Upon analysis of the human, mouse, *Drosophila melanogaster*, and *Caenorhabditis elegans* genomes, the presence of 540, 331, 171 and 117 PDZ domains in 306, 171, 107 and 84 different proteins was deduced, respectively [4].

The first indication of a clear function of the PDZ domain was explicated when the first papers

appeared describing specific interactions of the two most N-terminal PDZ domains of PSD-95/SAP90 with the extreme COOH-terminal peptide sequences of Shaker-type K^+channels [5] and NMDA receptor NR2 subunits [6,7]. Around the same time, Sato and co-workers showed an interaction of one of the PDZ domains in the human protein tyrosine phosphatase PTP1E/FAP-1 with the carboxyl-terminus of the Fas cell surface receptor [8]. This phenomenon of binding to C-terminal sequences of other proteins is now well established and is referred to as the classical or canonical PDZ binding mode. Over the past years it has become clear that in addition to this classical mode of interaction, PDZ domains are able to bind internal peptide motifs that may structurally resemble carboxyl termini [9].

PDZ domain structure

The primary amino acid sequences of PDZ domains may differ considerably (down to some 20% sequence identity) but their three-dimensional structures appear strikingly similar. The first structure that has been solved was that of the third PDZ domain of PSD-95/SAP90 [10,11]. Thereafter, numerous PDZ domain structures, occasionally in complex with their specific ligand, have been solved, including the single PDZ domains of CASK [12], syntrophin [13], and neuronal NO synthase (nNOS) [9], the first PDZ domain of the Na^+/H^+ exchanger regulatory factor (NHERF) [14], the first PDZ domain of InaD [15], the second PDZ domain of PSD-95 [16], and the second PDZ domain of both the human and mouse protein tyrosine phosphatase PTP1E/PTP-BL [17,18]. To date all described PDZ domains consist of six β-strands (βA-βF) and two α-helices (αA-αB) that are folded as a six-stranded sandwich (Figure 1). Carboxyl-terminal peptides bind as an anti-parallel β-strand in a positively charged binding groove between the βB-strand and the αB-helix. The main chain of the βB-strand forms hydrogen bonds with the main chain of the extended peptide ligand, thereby stabilizing the interaction. In addition, the groove itself ends in a hydrophobic cavity in which the last, carboxyl residue of the bound peptide can dip. Indeed, many peptide ligand sequences end with a hydrophobic amino acid residue like valine, leucine or isoleucine [19]. In addition, the conserved PDZ domain signature GLGF motif, also called the carboxylate-binding loop, forms a connecting loop between the βA- and βB-strand and, together with a highly conserved arginine residue three amino acids upstream, is critically involved in the hydrogen bond formation between the PDZ domain and the interacting carboxylated amino acid residue [10, 16]. Note that the N- and C-terminus of a PDZ domain are positioned in close proximity of each other at the opposite side of the peptide-binding cavity, an ideal setting for a globular protein interaction domain.

Carboxyl-terminal peptide recognition

PDZ target specificity is dependent on the carboxyl-terminal amino acid sequence of the interacting protein, and peptide screens revealed that for some ligands peptide residues as far back as the -8 position are influencing binding specificity [19]. The carboxylate amino acid residue (termed the 0 position) is oriented in such a way that its side chain is projected into the hydrophobic pocket of the PDZ domain. The first peptide-ligands described all show the analogy in displaying a hydrophobic amino acid at this 0 position [5, 6, 20, 21]. Variations in the depth and geometry of the PDZ binding pocket is probably discriminative in the distinct preferences of various PDZ domains for valine, leucine or isoleucine at the extreme end of the peptide ligand. In contrast, PDZ-interacting peptides ending with a negatively charged acidic amino acid residue (Asp or Glu) [22] or with the polar cysteine residue [23, 24] have been described recently. Interestingly, the third and fifth PDZ domains of hINADL, the PDZ domain of Par6, and the first PDZ domain of Mint-1 all show the phenomenon that they can interact with peptides that end on Val, Leu or Ile as well as with peptides that have Glu or Asp at the 0 position [22, 25]. Irrespective, the carboxylated amino acid at the 0 position of the peptide ligand is a major determinant in the interaction. In contrast, the amino acid residue at the -1 position is only of minor importance for PDZ binding specificity. In the crystal structure of the third PDZ domain of PSD-95/SAP90 in complex with a peptide ligand, the side chain at the -1 position is exposed from the binding surface and does not participate in the formation of hydrogen bonds [10]. Recent experiments, however, have shown that the amino acid residue at the -1 position can influence the interaction with distinct PDZ domains. For instance, amino acid substitution at the -1 position of CRIPT (-QTSV to -QTDV) affected the specificity in binding to the PDZ domains of PSD-95/SAP90, although not as critical as the 0 and

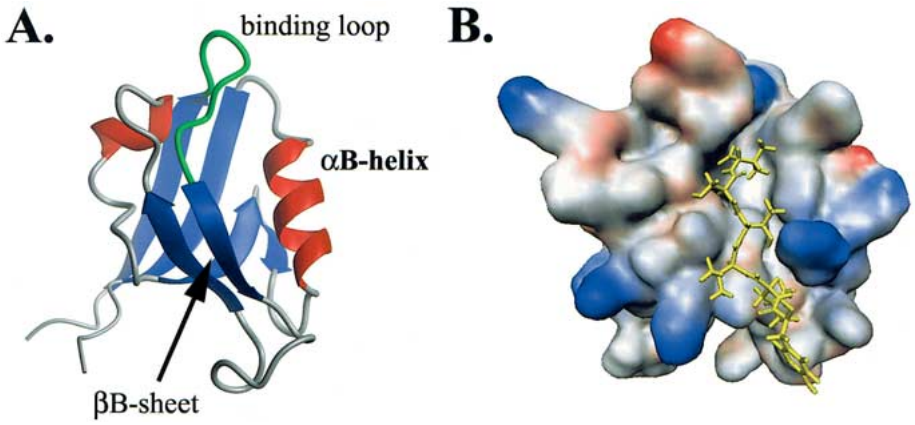

Figure 1. Three-dimensional structure of a PDZ domain with or without its peptide ligand. A: Solution structure of the second PDZ domain of mouse PTP-BL (PDB accession code: 1GM1 [18]) showing the 6 β-sheets (blue) and 2 α-helices (red) composing the modular structure characteristic for PDZ domains. The second β-sheet, second α-helix and the carboxylate binding loop (green) involved in ligand binding are indicated. B: Surface topology of the third PDZ domain of rat PSD-95 in complex with a peptide ligand ending with -KQTSV sequence. Adapted from Doyle et al. [10].

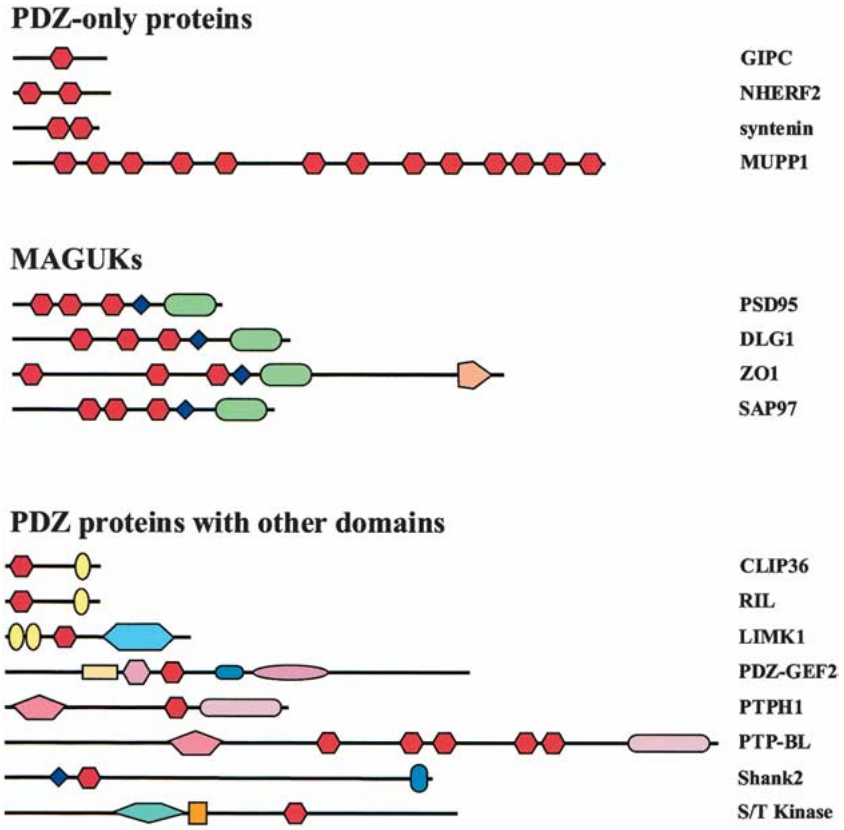

Figure 2. Schematic overview of some representative PDZ domain-containing proteins. PDZ domain-containing proteins can be divided into 3 groups: (1) PDZ-only proteins, (2) MAGUKs and (3) PDZ proteins with other domains. The protein domain build-up of various proteins from multiple species, as extracted from the SMART database, is depicted. Protein entries that were used are: GIPC, GIPC_MOUSE; NHERF2, Q920G2; syntenin, SDB1_HUMAN; MUPP1, O75970; PSD95, DLG4_RAT; DLG1, Q9BI79; ZO1, Q9BKL2; SAP97, DLG1_HUMAN; CLIP36, PDL1_HUMAN; RIL, RIL_HUMAN; LIMK1, LIK1_MOUSE; PDZ-GEF2, Q9UHV4; PTPH1, Q9NDP4; PTP-BL, Q64512; Shank2 (SH3 and multiple ankyrin repeat domains protein 2), SHK2_RAT; S/T Kinase (syntrophin-associated serine-threonine protein kinase), Q9R1L5. PDZ domains are indicated by red hexagonals. The SH3, guanylate kinase and ZU5 domains as present in MAGUKs are depicted as blue diamonds, green capsules and orange pentagonals, respectively. Additional domains include LIM (yellow ellipsoid), phosphotyrosine phosphatase (purple capsules), tyrosine/serine/threonine kinase (blue hexagonal), serine/threonine kinase (green pentagonal) and FERM (pink pentagonal) domains.

−2 positions [26]. Phage display techniques revealed that the leucine residue at position 40 in the second PDZ domain of MAGI-3 (membrane-associated guanylate kinase inverted-3) is involved in the selection of peptides containing a tryptophan residue at the −1 position [27]. Another example is the description of the arginine side chain at the −1 position of the C-terminal -DTRL sequence of the cystic fibrosis transmembrane regulator (CFTR) that forms two salt bridges with Glu^{43} of the first NHERF PDZ domain [14]. This NHERF Glu^{43} is structurally homologous to the MAGI-3 Leu^{40} suggesting that this residue in the PDZ βC-strand plays a role in determining the affinity of the PDZ/peptide interaction. A completely different function for the −1 amino acid residue is revealed in the crystal structure of the InaD N-terminal PDZ domain in complex with a peptide corresponding to the C-terminus of NorpA. Here, an intermolecular disulfide bridge is necessary for high affinity interactions [15]. Since other possible interacting C-terminal peptides also contain a cysteine at the −1 position, Kimple and coworkers proposed this 'dock-and-lock' interaction to be a relatively ubiquitous mode of coordinating signaling pathways.

Apart from the nature of the 0 position residue, the binding specificity of the PDZ domain is also crucially dependent on the amino acid residue at the −2 position. The side chain of this residue interacts with the first amino acid residue of the αB helix (αB1 position). This specific interaction is generally used for the classification of PDZ domains and their cognate ligands (discussed below). By far the largest group of C-terminal peptides that is able to associate with PDZ domains possesses a serine or threonine residue at the −2 position. Structural analysis revealed the formation of a hydrogen bond between the hydroxyl group of the −2 Thr side chain and the N-3 nitrogen of His^{372} at the αB1 position in the third PDZ domain of PSD-95/SAP90. In addition, both the carbonyl oxygen and the amide nitrogen of Thr-2 displayed hydrogen bond formation with Ile^{327} in the βB strand of this PDZ domain [10].

Another group of C-terminal peptide ligands demonstrate a hydrophobic residue at the −2 position. Again the side chain of the −2 residue exhibits hydrogen bond formation with the αB1 residue of the bound PDZ domain, whereas the main chain forms hydrogen bonds with the βB strand of the PDZ domain [12]. Yet another group of peptide ligands expose a negatively charged amino acid residue at the −2 position. For example, Tyr^{77} at the αB1 position of nNOS PDZ domain forms a hydrogen bond with the side chain carboxylate of the −2 residue. Substitution of this tyrosine residue to a histidine leads to loss of interaction to its original peptide ligand, but now, this mutated PDZ domain is able to bind C-terminal peptide harboring a serine or threonine residue at the −2 position [21]. Thus, the nature of the side chain of the amino acid residue at the −2 position is a major determinant in PDZ binding specificity. Therefore, in the near future presumably more peptide ligands containing other amino acid residues at the −2 position than described above will be uncovered as PDZ domain targets.

Although the residues at the 0 and −2 positions of the peptide ligand are the critical determinants in the association with the bound PDZ domain, several examples demonstrate that also more amino-terminal residues are involved in peptide/PDZ binding specificity. Crystallographic studies revealed that the amino acid at the −3 position in the peptide contacts the βB and βC strands of the PDZ domain. For instance, the −3 glutamine residue of CRIPT forms hydrogen bonds with amino acids at the positions βB2 (Asn^{326}) and βC4 (Ser^{339}) of the third PDZ domain of PSD-95/SAP90 [10]. Crystal structures of the NHERF PDZ domain with two different carboxyl peptide ligands, -NDSLL of the β_2 adrenergic receptor (β_2AR) and -EDSFL of the platelet-derived growth factor receptor have been determined. These studies revealed two additional hydrogen bonds between the asparagine side chain (−4 position) and Gly^{30} in the βB strand of the NHERF PDZ1 in complex with β_2AR, which contribute to the higher affinity of this interaction [28]. Regarding the −5 peptide residue, nuclear magnetic resonance (NMR) experiments demonstrated that the glutamic acid residue at this position in the human Fas receptor C-terminal tail is in contact with the extended loop following the βB strand in the second PDZ domain of the human protein tyrosine phosphatase PTP1E [17]. Already in 1997, biochemical studies demonstrated the selectivity of some PDZ domains (in mDLG, PTP-BAS, and AF-6) for the amino acid residue at the −8 position [19]. Finally, very recently, it was demonstrated that besides the C-terminal binding motif, a tripeptide sequence (SSG) at position −9 to −11 of the C-terminus of GluR-A subunit plays an essential role in the specific interaction with the second PDZ domain of SAP97 [29].

Taken together, amino acid residues at the 0 and −2 position of peptide ligands are crucial determinants in PDZ domain specificity. However, other residues in the C-terminus of the interacting protein

are also important. Still, numerous PDZ domains are able to associate with multiple peptide ligands with different C-terminal sequences, which has been shown through a large screen using PDZ domains of PICK1, GRIP and syntenin, and the C-termini of almost all AMPA receptors and glutamate receptors [30]. Thus, PDZ domains may interact with a broader range of C-terminal motifs than predicted to date, which implies a set of PDZ domain/peptide ligand interactions beyond complete predictability.

Non-C-terminal binding targets

Whilst the binding of PDZ domains to the extreme carboxyl terminal part of their associating partners is the most common mode of interaction, an increasing number of papers describe the interaction between PDZ domains and internal protein sequences. First of all, PDZ domains can associate with other PDZ domain-containing protein parts and were thought to exhibit heterodimerization capacity. For instance, the PDZ domain-containing N-terminal part of nNOS can associate with the PDZ domains of both PSD-95/SAP90 and α_1-syntrophin [31]. Crystal structures of the N-terminal part of nNOS alone and in complex with the PDZ domain of α1-syntrophin showed multiple modes of PDZ domain scaffolding [9]. The nNOS-syntrophin complex structure revealed that the domains interact in a linear head-to-tail orientation. The nNOS PDZ-containing part thus contains two distinct interaction surfaces; (1) a canonical peptide binding groove and (2) a finger-like β-hairpin extension just C-terminal to the PDZ domain. The nNOS β-finger docks as a ligand into the syntrophin PDZ binding groove leaving the nNOS PDZ domain able to interact with C-termini. The nNOS PDZ domain-containing part can also associate through the 'pseudopeptide' interaction mode with the second PDZ domain of PSD-95/SAP90 [32]. Although the β-finger is not a structural part of the nNOS PDZ domain, this kind of interaction is normally referred to as a PDZ/PDZ interaction. The same positively charged binding pocket of the PDZ domain facilitates both PDZ/PDZ and PDZ/C-terminus interactions, but important differences can be noted. For example, a single amino acid substitution in the carboxylate binding loop of the second PDZ domain of PSD-95/SAP90 ($K^{165}R$) that abolished the interaction with the PDZ domain of nNOS did not affect the binding of the C-terminus of the Shaker-type K^+ channel Kv1.4 [33]. In the literature several other PDZ/PDZ interactions have been reported [34–36], but whether these interactions also occur according to this 'pseudopeptide' interaction mode remains to be elucidated.

In addition, interactions that appear distinct from both the canonical and the PDZ/PDZ binding mode have been reported. The use of combinatorial phage libraries led to the identification of cyclic peptides as potential ligands for PDZ domains. The consensus sequence for such ligands displays residues similar to the C-terminal PDZ targets and contains in addition several cysteine residues that may form an intramolecular disulfide bond and could induce a β-turn facilitating PDZ association [37]. In a more physiological context, PDZ domains have been reported to associate with other protein modules involved in protein-protein interactions. The PDZ domain of actinin-associated LIM protein (ALP), for instance, binds to the spectrin-like motifs in α-actinin [38], and the first PDZ domain of the human protein tyrosine phosphatase PTP-BAS associates with ankyrin repeats of the transcription-factor-inhibitory protein IκBα [39]. PTP-BAS and its mouse ortholog PTP-BL also associate with their second PDZ domain to the LIM domains of the zyxin-related protein TRIP6 [40, 41]. The PTP-BL PDZ-II domain exhibits additional association with the LIM domain of RIL, a protein upregulated in Ras transformed cells [42]. Interestingly, the PDZ domain of RIL itself can also bind to its own LIM domain and to LIM domains in the above mentioned TRIP6 [41, 42]. Finally, the fourth PDZ domain of the PTP-BL also interacts with the LIM domain of RIL [42] and additionally with both LIM domains in the LIM-only protein CRIP2 (van Ham & Hendriks, unpubl.). Strikingly, these three protein modules that associate with PDZ domains (spectrin repeats, ankyrin repeats and LIM domains) all possess a three-dimensional structures that exhibit multiple β-sheets followed by sharp turns. These β-sheet/turn configurations may determine the association with PDZ domains in a way reminiscent of the pseudopeptide binding mode. Structural data for these complexes, however, remain to be attained. Finally, very recently a novel type of ligand for PDZ domains has been described; phosphatidylinositol 4,5-biphosphate (PIP_2) [43]. Some PDZ domains of syntenin, CASK, PTP-BL and Tiam-1 are able to bind PIP_2 and, furthermore, PIP_2 can compete with C-terminal peptide ligands for PDZ binding, suggesting at least partial overlap in binding sites.

On the basis of structural studies, Harris and coworkers established a set of general rules that can

explain PDZ recognition of both C-terminal and internal motifs. First, either class of ligands must exhibit the proper core linear amino acid recognition motif. Second, these amino acid residues must be followed by either a carboxyl terminus or a stabilized β-turn [44]. Structural data on the various types of ligands bound to distinct PDZ domains will be required, however, to reveal the exact modes of interaction. This may validate the general rules for PDZ ligands but, perhaps more interestingly, might also result in the disclosure of novel binding patches on the PDZ domain surface. Indeed, recently a novel hydrophobic surface patch on PDZ7 of GRIP1 was characterized as the binding area for its target sequences. The traditional carboxyl group binding pocket of this PDZ domain shows a closed conformation and the αB/βB-groove is narrow, thereby excluding peptide binding. Instead, the hydrophobic pocket between the βE-sheet and αB-helix is now serving as a peptide docking area [45].

Classification of PDZ domains

Several attempts have been undertaken to classify PDZ domains and/or their ligands. Classification based upon their cognate C-terminal peptide ligands, thus exploiting the last three or four amino acid residues of the carboxyl targets, resulted in four distinct consensus sequences: -(S/T)XΦ (class I), -(Φ/Ψ)XΦ (class II), -G(E/D)XV (class III) and -XΨ(E/D) (class IV) (where X denotes any amino acid, Φ hydrophobic residue and Ψ aromatic residue) [19,21,22]. Almost all C-terminal peptides known to associate with PDZ domains can be grouped within one of these classes, although a growing number of C-terminal 'exceptions' are being discovered [23,24]. Due to this expanding collection of peptide ligands, Bezprozvanny and Maximov devised a novel system to classify PDZ domains [25]. Their system is based on the nature of the amino acid residues at two critical positions within the binding cleft of the PDZ domain. The first (Pos1) immediately follows the βB strand and the second (Pos2) corresponds to the first position in the second α-helix (αB1). Using this {Pos1, Pos2} principle, all currently known PDZ domains could be arranged into 25 possible groups. Furthermore, it was reasoned and experimentally validated that in this way ligand specificity of certain PDZ domains could be predicted. Although in the last classification all PDZ domains can be categorized, in the literature the debate on a few omissions (e.g. PDZ domains binding to various peptide ligands) is still proceeding [46, 47].

PDZ domain-containing proteins

PDZ domain-containing proteins can be divided into three general groups (Figure 2): (1) PDZ-only proteins, (2) membrane-associated guanylate kinases (MAGUKs) and (3) PDZ proteins with other protein domains. PDZ-only proteins may contain multiple PDZ domains, even up to 13 PDZ domains as reported for MUPP1 [48]. MAGUKs are membrane-associated guanylate kinase domain-containing proteins. This large superfamily includes the PDZ founding members PSD-95/SAP90, Dlg and ZO-1, and is characterized by one to three PDZ domains, an SH3 domain, a HOOK domain and a catalytically inactive guanylate kinase-like domain. The largest and still growing group of PDZ domain-containing proteins harbors, in addition to the PDZ domain(s), one or more other protein domains. Besides protein-protein interaction domains such as WW domains, LIM domains, ankyrin repeats and leucine zippers, other protein modules (e.g. FERM, calcium/calmodulin-dependent protein kinase-like, protein tyrosine phosphatase and RGS domains) are encountered in these PDZ proteins.

PDZ domains are typically grouped into tandem arrays of pairs and triplets (Figure 2). PDZ pairing occurs for instance in PSD-95/SAP90 (PDZ1-2), in the protein tyrosine phosphatase PTP-BL (PDZ-II-III and PDZ-IV-V) and in syntenin (PDZ1-2). PDZ triplets can be found in the multi-PDZ proteins GRIP (PDZ1-3, PDZ4-6) and MUPP1 (PDZ1-3 and PDZ9-11). Why PDZ domains are grouped is not fully understood, although there is some evidence suggesting cooperation of PDZ domains enhances ligand binding. The C-terminus of the plasma membrane Ca^{2+} ATPase isoform 4b (PMCA4b), for instance, is a target for the PDZ domains of distinct MAGUKs. Using semi-quantitative yeast two-hybrid assays it was shown that combinations of PDZ domains show a two-fold increase in interaction strength to PMCA4b as compared to single PDZ domains [49]. Also, the second PDZ domain of syntenin is able to bind the carboxyl-termini of syndecan, neurexin and ephrin-B1, but for robust *in vivo* binding both syntenin PDZ domains are required [50]. The authors suggest a cooperative binding mode in which neither of the two PDZ domains is sufficient to establish binding by itself, but with PDZ2 functioning as a 'major' or 'high affinity' ligand bind-

ing domain and PDZ1 being an 'accessory' or 'low affinity' ligand binding domain the combination of the two does have this potency. Furthermore, surface plasmon resonance (SPR) measurements showed that high-affinity binding of syntenin to PIP_2-containing lipid layers also requires the presence of both PDZ domains of this protein [43]. As an interpretation of these studies, two or more compatible types of baits (proteins or lipids) could be envisioned that are in "synteny" (occurring in very close proximity) and engage multiple PDZ domains [50].

This picture of PDZ domain-cooperativity also comes from structural studies. NMR experiments provided evidence that one PDZ domain can influence the folding of the adjacent PDZ domain. PDZ4 of GRIP appeared to be correctly folded, but can spontaneously unfold, and PDZ5 of this protein is completely unstructured in solution. Neither single PDZ domain showed binding to the GluR2 C-terminal tail in solution. Strikingly, covalent binding of both PDZ domains was necessary for correct folding and stability of the PDZ domains and essential for effective GluR2 binding [51]. The occurrence of such cooperative effects between neighboring PDZ domains implies that perhaps the order in which domains occur in tandem arrays might be of importance. Indeed, domain swapping between PDZ1 and PDZ2 of PSD-95/SAP90 revealed the necessity of the correct position of the interacting domains as a prerequisite of efficient target protein cluster formation [52].

Thus, next to the tertiary structure of PDZ domains, also their quaternary structure is determining the associative capacity of these protein modules. Those features are of interest in the regulation of ligand binding, functioning in cluster formation and routing of interacting proteins to distinct subcellular environments. Current knowledge how PDZ domains are involved in these cellular processes will be presented below.

Regulation of PDZ domain/target interactions

Protein-protein interactions must be tightly regulated in order for a cell to determine direction, strength and duration of the involved signaling processes [1]. The presence of serine, threonine or tyrosine at the -2 position of many PDZ peptide ligands opens up the possibility that phosphorylation might regulate PDZ domain association. Indeed, a PKA-dependent phosphorylation of the serine residue at the -2 position in the inward rectifier K^+ channel Kir2.3 disrupts binding to PSD-95/SAP90 [53]. PKC-dependent phosphorylation of the -2 serine residue (Ser^{880}) of the AMPA receptor GluR2 subunit even regulates the interaction to multiple PDZ domain-containing synaptic proteins differentially; whereas phosphorylation of Ser^{880} drastically reduces the affinity for GRIP1, it has no effect on the association to PICK1. As a consequence, enhanced Ser^{880} phosphorylation increases internalization of surface GluR2 and ultimately recruits PICK1 to excitatory synapses [54]. In contrast, phosphorylation-dependent decrease in interaction of the PDZ domain-containing proteins is exhibited by PSD-95/SAP90 and MAGI-2 with the C-termini of stargazin and PTEN (phosphatase and tensin homologue deleted on chromosome 10), respectively [55]. Furthermore, phosphorylation of the PTEN tail is thought to act as an inhibitory switch by preventing PTEN recruitment into protein complexes. Phosphorylated PTEN appeared in a "closed" monomeric conformation, whereas, in contrast, unphosphorylated PTEN is in an 'open' conformation and strongly interacts with PDZ domain-containing proteins as MAGI-1 and -2 [56]. Thus, phosphorylation of C-terminal peptide ligands, not only affects the association to PDZ domains, but can also influence the subcellular localization of either protein and, as a consequence, down-stream signaling events.

On the other hand, ligand binding can also be regulated by modifications within or near the PDZ domain itself. For instance, a perfectly conserved CaMKII phosphorylation consensus sequence (RGNS) is present in the loop between the first and the second β-strand of the first PDZ domain in all MAGUKs. In mutant *Drosophila*, overexpression of CaMKII, and thus hyperphosphorylation of the RGNS-motif, led to an accumulation of DLG at synaptic boutons and formation of abnormal synaptic structures [57]. Phosphorylation in regions flanking the PDZ domain can also influence its interaction with associating proteins. Full-length AF6 phosphorylation is mediated by EphB3, leading to a conformational change of AF6 that results in the unmasking of the PDZ interaction pocket and thus facilitating ligand binding [58]. Another example is shown by NHERF-1, a protein containing two N-terminally located PDZ domains and a C-terminal part that harbors multiple possible phosphorylation sites. Phosphorylation at position Ser^{289} of NHERF-1 induces a conformational change that allows PDZ domain-dependent heterodimerization with its relative NHERF-2 [59]. Strik-

ingly, Cdc2-dependent phosphorylation of NHERF-1 at the positions Ser^{279} and Ser^{301} reduces this heterodimer formation and instead enables NHERF-1's PDZ domain to bind to Pin1 [60]. Thus, reversible phosphorylation of amino acid residues in or near PDZ domains may be a general mechanism to switch between at least two protein conformations, one in which the PDZ domain can be envisaged in a 'open' state, and one reflecting a 'closed' conformation.

For the second PDZ domain of PTP-BL also two different states exist of which only one can exert binding to the C-terminus of Adenomatous Polyposis Coli-protein (APC). But here the difference is caused by alternative splicing rather than post-translational modification [61]. Also the human PDZ domain-containing serine protease Omi is subject to alternative splicing. One splice variant lacks part of the PDZ domain and thus is unable to associate with its known ligand, Mxi2 [62]. Intriguingly, extensive alternative splicing results in multiple variants of RGS12 (regulator of G-protein signaling 12) that possess either no or a single PDZ domain in combination with or without a carboxyl terminus that complies to the rules of a class I PDZ ligand. Consequently, RGS12 isoforms that are capable of inter- and intramolecular binding, thereby adopting 'closed' conformations and eliminating association to other ligands, do occur [63].

Another mechanism to generate additional PDZ domain-containing isoforms is through post-translational processing by proteases. An intriguing example is set by the caspase-dependent cleavage of the cytosolic neuronal precursor of interleukin-16 (IL-16) that consists of three PDZ domains. Following cleavage the single PDZ domain-bearing, mature IL-16 is secreted, whereas the N-terminal prodomain that harbors the two remaining PDZ domains is translocated to the nucleus and functions as a nuclear scaffolding protein and affects the cell cycle [64, 65].

In summary, PDZ-ligand binding can be regulated at different levels. First of all, expression of PDZ domain-containing proteins (and their ligands) in specific cell types and their subcellular localization regulates contingence of interactions. Secondly, at the transcript level multiple splice variants may occur that lead to alternative PDZ binding properties. Thirdly, different post-translational modifications, like proteolysis and phosphorylation, can influence subcellular distribution and/or binding affinities.

PDZ domains and clustering of transmembrane proteins

As indicated above, PDZ domains were discovered as eminent binders of C-terminal peptide sequences. In the mid nineties numerous papers were published describing PDZ binding to transmembrane proteins like ion channels and receptors. Furthermore, it became apparent that PDZ domains were not just protein binding modules, but additionally facilitated clustering of transmembrane proteins. In 1995, functional and biochemical evidence was presented on PSD-95/SAP90-mediated clustering of Shaker subfamily K^+ channels [5]. Instrumental for that is the multimerization of PSD-95/SAP90 [66] which leads to aggregation of its PDZ domains and consequently modulates their clustering capacity [67]. Another PDZ domain-containing adaptor protein, GRIP, links AMPA receptor subunits to down-stream effector proteins, and plays a critical role in clustering of AMPA receptor at excitatory synapses [68]. Additionally, PDZ domains are involved in the immobilization of transmembrane proteins. By the use of Fluorescence Recovery After Photobleaching (FRAP) experiments, Burke and coworkers showed that binding of PSD-95/SAP90 to the Kv1.4 K^+ channel subunit is required for immobilization of the K^+ channel [69].

More recently it has become apparent that PDZ domain-containing proteins are also involved in the regulation of ion channel and receptor activity. Using a recombinant fragment of NHERF-1, containing the two PDZ domains through which it binds to the cytoplasmic tail of CFTR Cl^- channels, Raghuram and colleagues showed an increase in the probability of the open state for single CFTR channels in membrane patches. Low PDZ concentrations led to channel activation, whereas higher PDZ concentrations had an inhibitory effect. Furthermore, they showed the necessity of both PDZ domains for NHERF-1's concentration-dependent regulatory function [70]. Another example is given by the PSD-95/SAP90-mediated clustering of Kir5.1 K^+ channel subunits. This clustering is required for channel function, i.e. leading to Ba^{2+}-sensitive inwardly rectifying K^+ current. Furthermore, K^+ channel activity is PKA sensitive, indicating a phosphorylation-dependent interaction between PSD-95/SAP90 and Kir5.1 monomers [71]. The N-terminal two PDZ domains of PSD-95/SAP90 are involved in NMDA receptor and K^+ channel binding, respectively. Interestingly, a proper positioning of the two PDZ domains in the full length

protein is essential to induce optimal channel clustering and activity [52]. Yet another example is given by the specific binding of the third PDZ domain of IKEPP (intestinal and kidney-enriched PDZ protein) to guanylyl cyclase C, the receptor for heat-stable enterotoxins. This association is not required for targeting guanylyl cyclase C to the apical surface, but rather inhibits the receptor activation by enterotoxins [72]. Perhaps also PDZ proteins are regulating neurotransmitter transporters. Disruption of the interaction between an intracellular protein and the last eight amino acid residues of the GLAST glutamate transporter increases the glutamate affinity of GLAST and the corresponding transporter current by 40%. Although the C-terminus of GLAST bears similarity to a PDZ binding motif, no interacting PDZ domain-containing protein has yet been identified [73].

In summary, PDZ/ligand binding can be regulated but may in turn also regulate downstream processes, first at the level of anchoring and clustering of channels and receptors, and secondly at the level of activity. Perhaps the impact of PDZ domains on transmembrane proteins as described above is also applicable for intracellular protein targets. Taken together, PDZ domains should not be seen as just binding modules, but must be perceived as dynamic organizers of macromolecular complexes and thus as regulators in signaling cascades.

Protein targeting by PDZ domain-containing proteins

The multidomain structure of many PDZ domain-containing proteins reflects their involvement in protein complex formation, often at specific subcellular microenvironments (reviewed in [74, 75]). Recently it became apparent that PDZ domain-containing proteins are also involved in intracellular routing of proteins. For instance, C-terminal mutations in transmembrane TGFα decrease or even abolish the interaction with the PDZ domain-containing protein p59/GRASP55 and strongly impair cell surface expression of TGFα [76]. Also deletions of type III TGFβ receptor C-terminal residues prevent the receptor from binding to the GIPC PDZ domain and consequently abrogate receptor expression. Importantly, GIPC-mediated increase of type III TGFβ receptor surface expression is sufficient to enhance TGFβ signaling, suggesting that GIPC is involved in the TGFβ pathway [77]. SAP97 overexpression leads to intracellular accumulation of Kv1 channels and, in contrast to the former example, prevents trafficking of these channels [78]. Tyrosinase and tyrosinase-related proteins (TRPs), a family of melanosomal membrane proteins, are sorted and targeted to melanosomes by signals in their cytoplasmic domains. The carboxyl-terminal -SVV motif of the most abundant melanosomal membrane protein, gp75, is a *bona-fide* target for the PDZ domains of GIPC, but *in vivo* only newly synthesized gp75 is found associated with GIPC and primarily at the perinuclear Golgi region [79]. Cell surface expression of yet another transmembrane protein, CFTR, is regulated by dynamic interactions with at least two PDZ domain-containing proteins, CAL (CFTR associated ligand) and NHERF. CAL binds though its PDZ domain to the CFTR C-terminus, thereby retaining CFTR in the Golgi apparatus. However, the association of CFTR to NHERF appears to be stronger than that to CAL. Consequently, NHERF expression regulates the cell surface expression of CFTR by competing with CAL for the binding of CFTR [80]. These data clearly show the involvement of PDZ domain-containing proteins in the biosynthetic routing of proteins.

Besides routing, sorting of transmembrane proteins is also (partly) mediated by PDZ domain-containing proteins. Distinct subunits of the AMPA receptor are clustered in the ER and cis-Golgi area before being transported to the plasma membrane surface. In the ER, GluR1 subunits interact with SAP97, however, at the plasma membrane, SAP97 dissociates from the GluR1/GluR2 complexes [81]. In contrast, GluR2 subunits do not show interaction with SAP97, but are able to associate with the PDZ domains in GRIP1, GRIP2 and PICK1. The GluR2 C-terminus, harboring the PDZ binding motif, is required for the transport of the GluR2/3 complex [82]. The precise mechanism(s) of transport remains largely unknown but some clues are now becoming apparent. The GluR2/3 complex is bound by the multi-PDZ containing protein GRIP2 that concurrently, via its adjacent PDZ domain, binds liprin-α. Interference with the GRIP-liprin interaction disrupts the surface expression and clustering of AMPA receptors [83]. Furthermore, at the plasma membrane, the C-terminal cytoplasmic tails of AMPA receptor subunits can be phosphorylated leading to loss of binding to GRIP1 and GRIP2, but still allows binding to PICK1. Thus, phosphorylation may act as a switch from receptor retention at the membrane (bound by GRIP1/2) to receptor endocytosis (complexed to PICK1)[54]. A more complete

overview on regulated surface expression of AMPA receptors has recently been given by Barry and Ziff [84].

In addition, sorting of proteins that have been internalized from the cell surface may very well be partly dependent on PDZ domain-containing proteins. For instance, EBP50 (ERM-binding phosphoprotein-50), the human homologue of NHERF, binds through its PDZ domain the β_2AR COOH-terminus that is needed for correct sorting of endocytosed β_2ARs. Additionally, phosphorylation of the β_2AR cytoplasmic tail by GRK-5 (G-protein-coupled receptor kinase-5) inhibits recycling of the receptor, indicative for phospho-dependent PDZ/ligand interaction in the regulation of endocytotic protein sorting [85]. Strikingly, the last four -DSLL amino acid residues of the β_2AR act as a transplantable sorting signal in mediating rapid recycling. When fused to the δ opioid receptor (δOR), this sequence is sufficient to re-route endocytosed δOR into a rapid recycling pathway, whereas wild type δOR is degraded [86]. Internalization of the related β_1AR is induced upon agonist binding, and is markedly increased by co-expression with the PDZ domain-containing protein MAGI-2. Indeed, the carboxyl terminus of the β_1AR binds with high affinity to the first PDZ domains of MAGI-2. Co-expression of β_1AR and PSD-95/SAP90, on the other hand, markedly inhibits agonist-induced internalization [87]. Yet another example of PDZ domain-dependent internalization is the activin type II receptor (ActRII)/activin receptor-interaction protein 2 (ARIP2) complex. Overexpression of the PDZ domain-containing ARIP2 enhances endocytosis of ActRII, a member of the receptors for TGF-β superfamily, and reduces activin-induced transcription [88].

Thus, at least some PDZ domain-containing proteins are involved in regulated routing of transmembrane proteins to the cell surface. Once arrived at the plasma membrane, other PDZ proteins can maintain the plasma membrane expression of channels and receptors and regulate their activity. Furthermore, yet another group of PDZ domain-containing proteins is participating in endocytosis or recycling of transmembrane proteins. How the hierarchy in the consecutive ligand/PDZ domain interactions is determined or even regulated remains to be elucidated. Besides the obvious contribution of phosphorylation, the differences in interaction strength between the various complexes and the distinct subcellular concentrations of PDZ domain-containing components are amongst the important parameters in the dynamic regulation of these protein complexes.

PDZ domains and the nucleus

Most PDZ domain-containing proteins are located at or near membranous structures but other subcellular niches, like the nucleus, harbor PDZ domain-mediated interactions as well. The C-terminus of the DNA binding domain-containing protein SRY binds specifically to both PDZ domains of the nuclear protein SIP-1. This interaction positions SIP-1 as a scaffold in the nucleus and suggests a regulatory function in transcription activation [89]. The first PDZ domain of the non-receptor-type protein tyrosine phosphatase PTP-BL showed specific association with the nuclear bromodomain-containing protein BP75. In view of PTP-BL's nucleocytoplasmic shuttling behavior, this may serve in coupling cytoplasmic signaling processes to nuclear events [90]. Also many of the MAGUK proteins undergo nucleo-cytoplasmic shuttling. CASK, for example, is concentrated at neuronal synapses but through shuttling between the synapse and the nucleus it also exerts a transcription regulation function [91]. The submembranous protein ZO-2 even exhibits nuclear membranous shuttling which is cell density-dependent [92]. How nuclear-cytoplasmic shuttling is regulated is not completely understood, but obviously a balanced effect of nuclear localization signals and nuclear export signals within the PDZ-containing proteins or their travel partners is required. Intriguingly, the nuclear export of the serine/threonine kinase LIM-kinase 1 (LIMK1) is mediated by two leucine-rich nuclear export signals within the PDZ domain of this protein. Due to these NESs, LIMK1 shows a predominant cytoplasmic distribution [93]. Also proteolysis can guide the nuclear localization of PDZ domain-containing proteins. As mentioned previously, the cytoplasmic IL-16 precursor contains 3 PDZ domains and following caspase-mediated cleavage the two PDZ domains within the prodomain are redistributed to the nucleus due to an N-terminal nuclear localization signal. This then induces a G(0)/G(1) cell cycle arrest, indicative for PDZ domain functionality in the nucleus [65].

Furthermore, direct transport of PDZ domain-containing proteins into or out of the nucleus can result in subcellular relocation of bound proteins. For instance, the PDZ-binding motif in diacylglycerol kinase-ζ (DGK-ζ) is required for nuclear recruit-

ment of γ1-syntrophin. Disruption of the interaction, which may be regulated by PKC phosphorylation, affected the intracellular distribution of both proteins, resulting in DGK-ζ accumulation in the nucleus and γ1-syntrophin localization in the cytoplasm [94]. Co-expression of nNOS and the carboxyl-terminal-binding protein (CtBP), a phosphoprotein identified as a binding partner to adenovirus E1A protein, resulted in a shift of CtBP from the nucleus to the cytosol. When mutating the PDZ binding motif of CtBP this change in localization does not occur [95].

These findings illustrate that in addition to cytoplasmic assembly of multiprotein complexes, PDZ domains are also involved in complex assembly in the nucleus. Multiple PDZ domain-containing proteins are actually even shuttling between cytoplasm and nucleus thereby facilitating subcellular relocation of interacting proteins. This imposes a new dimension to PDZ domain-containing proteins as regulators of attenuation or termination of nuclear signaling pathways.

Functional impact of PDZ domain-containing proteins

It will come as no surprise that amongst the multitude of proteins that are up- or down-regulated during the tumorigenic process many PDZ-containing proteins have been identified. Some were even named after the cancers in which they are up-regulated: PCD1 (pancreatic cancer-derived protein 1) [96] and AIPC (activated in prostate cancer) [97]. The PDZ domain-containing protein RIL, on the contrary, was shown to be down-regulated in H-Ras transformed cells [98]. Also a tumor-related alteration of the PDZ domain protein p55 has been reported [99]. Here, exon skipping resulted in the deletion of parts of the p55 PDZ binding pocket in an acute megakaryoblastic chronic myeloid leukemia sample. Whether the absence of proper or presence of aberrant PDZ domain proteins or their interactions actually contribute to tumor formation is as yet unknown, but misrouting of tumor suppressor proteins may be at stake. For instance, the tumor suppressor PTEN is targeted to the plasma membrane through interaction with PDZ domain-containing proteins, and the PDZ-binding sequence in PTEN is required for efficient inhibition of cell spreading. Indeed, C-terminal PTEN mutations, similar to those found in some tumors, affect some functions of the protein but not others [100]. Also the many truncating mutations observed for the tumor suppressor APC in cancer may point to the importance of its C-terminal association to PDZ domain-containing proteins such as PTP-BL and PSD-95/SAP90 [61, 101]. The C-terminal mutations contribute to the altered subcellular distribution of APC itself and consequently its interacting proteins, as observed in polyps and carcinomas in comparison with normal tissues [102]. Of course, PTEN and APC interact with many other, non-PDZ proteins as well and therefore the precise contribution of the C-terminal truncations to the carcinogenesis process remains to be investigated.

Transgenic animal models are nowadays frequently used to gain more insight into the function(s) of proteins. Also numerous PDZ domain-containing proteins are being studied in this way and several PDZ protein knock-out mouse models have been reported over the last few years. Targeted mutation of the PSD-95/SAP90 mouse gene provided evidence that PSD-95/SAP90 plays a subtle but important role in signal transduction. Although interacting proteins, like NMDA-receptor subunits and K^+ channels, were properly targeted to postsynaptic densities in the mutant mice, NMDA-receptor-mediated synaptic plasticity was dramatically altered. In addition, synapses became inappropriately strengthened after stimulation by a wide variety of frequencies. Furthermore, the learning of PSD-95/SAP90 mutant mice was impaired, supporting bidirectional synaptic plasticity in learning and gain of memory [103]. On the other hand, targeted disruption of another MAGUK, PSD-93, did not result in any obvious structural or functional abnormalities in the mutant animals [104].

The PDZ domain-containing adaptor protein α1-syntrophin is suggested to act as a molecular linker between e.g. nNOS and the sarcolemmal dystrophin complex. Two independent transgenic mouse models underscore the importance of α1-syntrophin for proper localization of its interacting proteins nNOS and aquaporin-4 at the sarcolemma. Surprisingly, no gross differences can be observed in muscle morphology and contractile properties in both mice [105,106].

ALP (actinin-associated LIM protein) and Cypher are two cytoskeleton-associated proteins that consist of one N-terminal PDZ domain and C-terminally one or three LIM domains, respectively. Their PDZ domains bind the spectrin-like repeats of α-actinin at Z-lines in striated muscles. In mice lacking ALP protein the muscle histology appears normal; muscle sarcolemma is preserved and actin-based cytoskeleton is intact [107]. Markedly, these mice show right ven-

tricular chamber dilation and dysfunction [108]. Thus, although ALP is highly expressed in skeletal muscles and only at low levels in cardiac and other tissues, absence of expression leads to aberrant morphology in cardiac tissue only. In line with this, Cypher knock-out mice show normal muscle formation but display a severe form of congenital myopathy and die postnatally from functional failure in multiple striated muscles. Therefore, both ALP and Cypher seem not to be required for sarcomerogenesis or Z-line assembly, but rather function as a linker-strut in the maintenance of the Z-line during muscle function [109].

L- and S-periaxin, two abundant PDZ domain-containing protein isoforms, play a role in the stabilization of myelin in the peripheral nervous system. In human, loss-of-function mutations in the periaxin gene cause recessive Dejerine-Sottas neuropathy, a peripheral myelinopathy [110]. Mice lacking a functional *Prx* gene now provide an important model for studying neuropathic disorders with late onset demyelination. The animals are still able to assemble compact peripheral myelin but the resulting sheat is unstable. Older *Prx* null mice, therefore, display extensive peripheral demyelination underscoring the essential role for periaxins in stabilizing the Schwann cell/axon unit [111]. Not seldom very unexpected clues concerning protein function pop up while generating and studying transgenic animals. The seven PDZ domain-containing protein GRIP1 was thought to play a multifaceted role in assembling and localizing postsynaptic complexes through its interactions with AMPA receptor subunits, ephrin receptor tyrosine kinases, ephrin-B1 ligand, and the Ras guanine nucleotide exchange factor GRASP1. It was therefore a surprise to find that GRIP1-deficient mice suffer from a skin blistering disorder during embryogenesis, reminiscent of human dystrophic epidermolysis bullosa [112]. Undoubtedly, many more reports on PDZ protein mutant mice will appear in the near future. Taken together, such studies will help appreciating the pleiotrophic, redundant or unique contributions of this vast group of signaling and scaffolding proteins.

Concluding remarks

It has become clear that PDZ domains function at many cellular levels. Clustering and targeting of interacting proteins are the most well-known, and best-studied, effects. Most of the interactions encountered reflect the canonical C-terminal peptide-binding mode. Since amino acid residues preceding the last four positions of the peptide ligand may influence this binding and bearing in mind that protein-internal PDZ targets do exist, there is an obvious need for further structural analyses of PDZ domain – target combinations to allow full appreciation of the binding potency of the PDZ family. However, PDZ domain-containing proteins should be viewed as more than just scaffolds. Evidence is accumulating that they can also regulate the activity of associating proteins. The biggest challenge in the field of PDZ domains, however, will be to understand the spatiotemporal regulation of PDZ-mediated interactions. Dynamical studies will reveal some clues in regulated targeting of proteins by PDZ domain-containing proteins. Furthermore, studies on interaction strength and competition experiments may enlighten the hierarchy of different partner proteins in PDZ domain association. Finally, *in vivo* systems are imperative in gathering know-how on the cell biology of PDZ domain-containing proteins.

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