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Classification of PDZ domains

Ilya Bezprozvanny*, Anton Maximov

Department of Physiology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75235-9040, USA

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Abstract A diverse family of PDZ domains has been identified, but the rules that govern their ligand specificity are not clear. Here we propose a novel classification of PDZ domains based on the nature of amino acids in the two critical positions in the PDZ domain fold. Using these principles, we classified PDZ domains present in the SMART database. Using yeast two-hybrid, in vitro pull-down and plasmon surface resonance assays, we demonstrated that in agreement with their position in the proposed classification the Mint1-1, hINADL-5, and PAR6 PDZ domains display similar dual ligand specificity. The proposed classification helps to organize PDZ domain containing proteins. © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: PDZ domain; Primary sequence analysis; Sequence database search; Mint1; INADL; PAR6

1. Introduction

Proper localization of signaling components in polarized cells is essential for their function. The proper targeting of signaling molecules in polarized cells is achieved and maintained via a complex network of protein–protein interactions, frequently mediated by PDZ domain containing adaptor proteins. PDZ domains bind to the last four to six carboxy-terminal amino acids in the target protein [1–4]. Determination of PDZ domain structure by crystallography and NMR methods [5–7] revealed a compact globular module formed by six β strands and two α helices, which form a carboxy-terminal peptide binding groove. Despite similarities in secondary structure and the common preference for carboxy-terminal ligands, PDZ domains display different binding specificity.

The first class of PDZ domains, identified initially by sequence analysis of *P*SD-95, *d*lg, and *Z*01 proteins, is specific for S/T-X-Φ target sequence (Φ stands for a hydrophobic residue, the most carboxy-terminal residues are indicated as PDZ domain binding motifs throughout the paper) [8]. The second class of PDZ domains, specific for Φ-X-Φ sequence, was identified by analysis of CASK PDZ domain ligand specificity [8]. The PDZ domain of nNOS is specific for a different G-E/D-X-V pattern [9,10]. Recently we demonstrated that the Mint1-1 PDZ domain is specific for a novel recognition sequence E/D-X-W-C/S [11]. These findings raise a number of important questions. How many additional classes of PDZ domains are present? Can we predict a ligand specificity of a given PDZ domain from its primary sequence?

To address these questions here we developed a novel classification of PDZ domains. The proposed classification is based on the nature of the amino acids in the two critical positions in the PDZ domain fold which were previously suggested to account for specificity between class I and class II PDZ domains [6]. Using these principles, we divided PDZ domains into 25 possible groups and applied this classification to the PDZ domains represented in the SMART database [12]. We further reasoned that the ligand specificity of PDZ domains can be predicted based on their position in the proposed classification and tested this hypothesis experimentally.

2. Materials and methods

2.1. Primary sequence analysis

Aligned sequences of 285 non-redundant (< 67% sequence identity) representative members of the PDZ domain family were downloaded from the SMART (Simple Modular Architecture Research Tool) [12,13] Website. The database of representative PDZ domains was uploaded to the NPS (Network Protein Sequence analysis) server for future analysis. In 249 out of the 285 PDZ domains *h*-G-*h* carboxylate binding loop was in alignment. Amino acids in Pos1 and Pos2 positions in these 249 PDZ domains were determined using PATTINPROT function on the NPS server. Domain structure of multi-PDZ domain proteins was determined using the SMART tool [12,13]. Assignment of PDZ domain type in these proteins was performed from amino acids in Pos1 and Pos2. Sequence alignment was performed at ClustalW Service at EBI (European Bioinformatics Institute) and shaded by BoxShade server at EMBnet (European Molecular Biology Network).

2.2. Yeast two-hybrid experiments

Liquid yeast two-hybrid experiments were performed as previously described [11]. NC4, NC4-D2334X, NR2A and NX1 baits, Mint1-1 and PSD95-1-3 preys were previously described [11,14,15]. Additional prey plasmids in pVP16-3 vector were constructed using ESTs: hINADL-5 (aa 674–782 of human INADL [16]), hMUPP1-9 (aa 1471–1574 of human MUPP1 [17]), mPAR6 (aa 147–261 of mouse PAR6 [18]). When compared to the published sequence of hINADL [16] the EST sequence contained a single base mutation resulting in substitution R744→C within the hINADL-5 sequence. This point mutation was corrected using mega-primer PCR.

2.3. In vitro binding and surface plasmon resonance

Synthetic peptides corresponding to rat N-type Ca^{2+} channel (NC = RHSYHHPDQDHWC) and rat neurexin 1a (NX1C = NKKNKDKEYYV) carboxy-termini were coupled via N-terminal amino groups to NHS activated Sepharose beads (Amersham) according to the manufacturer's instructions. Glutathione-S transferase (GST)-PDZ domains were expressed and purified as previously described [11] and incubated with peptides immobilized on Sepharose beads for 2 h at 4°C in buffer A (100 mM NaCl, 25 mM HEPES, pH 7.2). The beads were washed twice with buffer A and attached proteins were sequentially eluted with buffer B (500 mM NaCl, 25 mM HEPES, pH 7.2) and 1% sodium dodecyl sulfate (SDS). Eluted pro-

*Corresponding author. Fax: (1)-214-648 2974.
E-mail address: ilya.bezprozvanny@utsouthwestern.edu (I. Bezprozvanny).

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teins were resolved on SDS-polyacrylamide gel electrophoresis (PAGE) and visualized by Coomassie staining. For surface plasmon resonance experiments (BIAcore 2000) a biotinylated version of NX1C peptide was coupled to streptavidin coated BIAcore chip (BIAcore). GST fusion proteins were diluted in binding buffer (phosphate buffered saline (PBS), 0.1% Triton X-100) to a concentration of 30 μM (except for GST-hINAD-PDZ5 protein which was used at a concentration of 10 μM) and injected to the chip for 300 s each. Nonspecific binding was estimated from empty flow cells and subtracted from the data.

3. Results and discussion

3.1. The classification of PDZ domains

The conserved secondary structure of PDZ domains [5–7] corresponds to a compact globular module, which contains six β strands and two α helices. The two critical positions in the PDZ domain fold, the first of which immediately follows the second β strand and the second of which is in the first position in the second α helix (arrows in Fig. 1) were previously suggested to account for specificity between class I (specific for S/T-X-Φ target sequence [8]) and class II (specific for Φ-X-Φ target sequence [8]) PDZ domains [6]. Structure-guided sequence alignment of Mint1-1 (specific for E/D-X-W-C/S target sequence [11]) and nNOS (specific for G-E/D-X-V target sequence [9,10]) with the previously crystallized PSD95-3 (type I) and CASK (type II) PDZ domains (Fig. 1) supports the importance of these two positions in determination of PDZ domain ligand specificity. We reasoned that specificity of any PDZ domain may potentially be predicted by a nature of amino acids in these two critical positions, and that all PDZ domains can be classified into groups with identical or similar amino acids in these two positions, which in this paper will be referred to as 'Pos1' and 'Pos2'.

Based on this idea, we mapped PDZ domains represented in SMART database [12,13,19] to {Pos1, Pos2} space, using the procedure described in Section 2. The structure-guided sequence alignment of PDZ domains on the SMART Website is analogous to the one shown on Fig. 1 for PSD95-3, CASK, Mint1-1 and nNOS PDZ domains. The resulting distribution of 249 SMART PDZ domains in {Pos1, Pos2} space is shown on Fig. 2, with each PDZ domain represented by a single black dot. 68 out of 249 domains (27.3%) corresponded to the (G,H) combination, characteristic for the type I PDZ domains. Thus, it is not surprising that class I PDZ domains were the first to be discovered and characterized. For reference, it can be estimated from the SwissProt amino acid composition that a pair of (G,H) amino acids is expected to occur only in 0.154% of randomly selected pairs of amino acids.

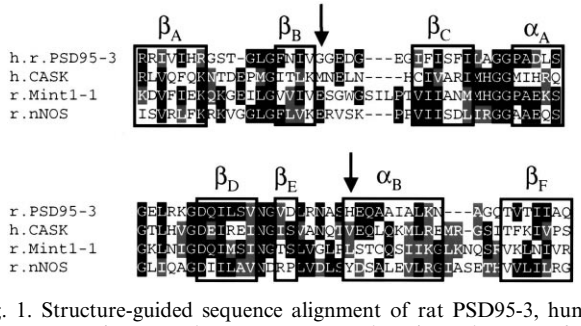

Fig. 1. Structure-guided sequence alignment of rat PSD95-3, human CASK, rat Mint1-1 and rat nNOS PDZ domains. Elements of secondary structure of PSD95-3 [5] and CASK [6] PDZ domains are as indicated. Two critical positions (Pos1,Pos2) proposed to account for ligand specificity of PDZ domains [6] are indicated by arrows.

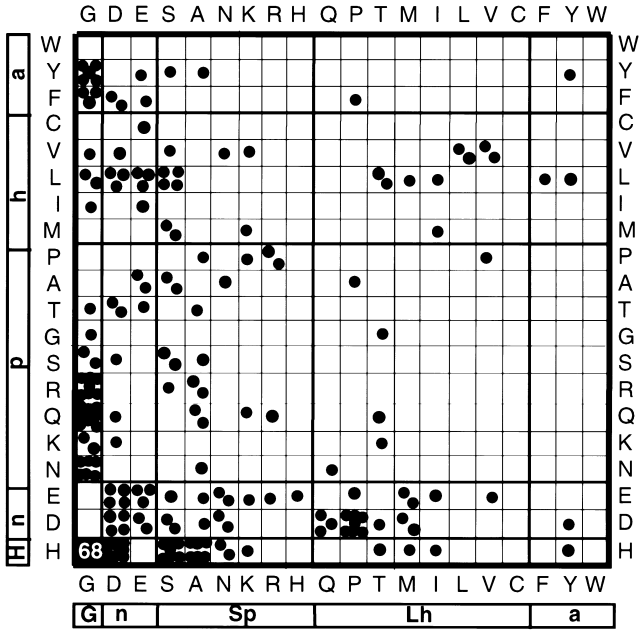

Fig. 2. Distribution of 249 representative SMART PDZ domains [12] in {Pos1, Pos2} space. Each domain is shown as single dot in the corresponding quadrant. 68 domains are found in (G,H) position. Amino acids on Pos1 and Pos2 axes are grouped into five groups as explained in the text.

To make our classification of PDZ domains more manageable, we grouped amino acids in Pos1 and Pos2 positions into five groups. As a starting point, we used both the bulkiness and the polarity of a side chain to group amino acids in Pos1 (we used the product of bulkiness and polarity scale values for each amino acid to generate the initial ranking), and the polarity of a side chain to group amino acids in Pos2. Following initial grouping, we rearranged amino acids to facilitate formation of PDZ domain clusters on Fig. 2. The matrix of PDZ domains on Fig. 2 is too sparce for an application of the formal cluster optimization algorithm, and the rearrangement of amino acids in Pos1 and Pos2 was performed manually. As a result of this procedure, the amino acids in Pos1 were divided into five groups as follows: 'G' (glycine), 'n' (negative), 'Sp' (small and polar), 'Lh' (large and hydrophobic), 'a' (aromatic). Amino acids in Pos2 were also divided into five groups as follows: 'H' (histidine), 'n' (negative), 'p' (polar), 'h' (hydrophobic), 'a' (aromatic). The distribution of SMART PDZ domains among the resulting 25 groups is shown in Table 1 (PDZ%). For reference, the calculated random frequency of the corresponding groups from the SwissProt amino acid composition is also indicated (random%). We found that PDZ domains from 23 out of 25 possible groups are represented among 249 SMART PDZ domains used in our analysis. Only (G,n) and (a,p) groups were not represented. It is not known whether these (Pos1,Pos2) combinations are prohibited or have not yet been discovered. Almost 43% of SMART PDZ domains (107 out of 249) contain G in Pos1, with (G,H) and (G,p) corresponding to the two most abundant groups (27.3 and 10.8% from all SMART PDZ domains). With the exception of the (Sp,n) combination, negative amino acids are not found in Pos2 in PDZ domains from eukaryotes, although quite abundant in bacteria.

Table 1
Classification of PDZ domains based on (Pos1,Pos2) rule

Grp	P1	P2	#	%		Ligand	Examples
				PDZ	Random		
G-H	G	H	68	27.3	0.15	-S/T-X-**Φ**	Dlg1-1,2,3; SAP97-1,2,3; IL16p-1, PAPIN-1,2; MUPP1-1,7,8,10,12,13; PTPN13-1,2; BAL1-ap1-2,4,6; hMAGI2-4; syntrophin; CIPP-4; hINADL-6,7; hTKA1-1; hKIAA0147-1,2,3,4; CortBP1; DRhoGEF2; hKIAA0380; CLIM1; rhophilin; antigen (NY-CO-38)-1; RGS12; InaD-2; GRIP1-1,2; densin-180; h.tax.c1; LNXp80-4
G-n	G	n	0	0	0.80		
G-p	G	p	27	10.8	3.52	**Ψ**-D-**Φ**	**hINADL-2,3**; InaD-4; IL16p-2; MUPP1-2,3,11; neurabin; PICK1; ZO3-1,3; CIPP-3; PAR3-2; LNXp80-3; hAPXL; TamA-3; BALI-1ap-1
G-h	G	h	4	1.6	1.78	?	MAGI2-1; KIAA0583-4 (hDLG5-2); GRIP1-6; PTPN13-5
G-a	G	a	8	3.2	0.58	?	GRIP1-4; S-periaxin; TamA-1; hKIAA0559
n-H	n	H	8	3.2	0.26	?	MAGI2-3; X11-2 (Mint-2); BAL-1ap-3; hTax.c2
n-n	n	n	13	5.2	1.37	?	CtpH; htrA
n-p	n	p	8	3.2	6.03	?	HTRA-SALTY-1; degP; ctpA; F16G10.5
n-h	n	h	8	3.2	3.05	-DXWC/EYYV	**Mint-1**; X11-1; lin10-1; **MUPP1**-5,**9**; **hINADL-5**; **PAR6**; GRIP7; mucD
n-a	n	a	4	1.6	1.00	-G-E/D-X-V	**nNOS**
p-H	Sp	H	16	6.4	0.77	**Ψ**-X-**Φ**	**hINADL-1**; TKA1-2; BAL-1ap-5; PTPN13-4; MUPP1-4; antigen (NY-CO-38)-2
Sp-n	Sp	n	12	4.8	4.00	?	InaD-1; KIAA0583-3; PAR3-3; HTRA-SALTY-2
Sp-p	Sp	p	19	7.6	17.5	E-**Φ**-**Ψ**-V	**hINADL-4**; MUPP1-6; PTPN13-3; LNXp80-2; PAR3-1; canoe
Sp-h	Sp	h	10	4.0	8.86	?	Z01-2; GRIP1-5; PALS1
Sp-a	Sp	a	2	8.0	2.89	?	Diphor1-4; cytohesin binding protein HE
Lh-H	Lh	H	3	1.2	0.87	?	Diphor1-1,2
Lh-n	Lh	n	18	7.2	4.54	?	HtrA
Lh-p	Lh	p	6	2.4	20.0	?	HDLG2; MIG-5
Lh-h	Lh	h	9	3.6	10.1	**-Φ**-X-**Φ**	p55 (**CASK**); lin2; limk; limk2; GRIP1-3
Lh-a	Lh	a	1	0.4	3.28	-EFYA	**InaD-5**
a-H	a	H	1	0.4	0.19	?	HKIAA0545
a-n	a	n	1	0.4	1.00	?	PSII D1 protease
a-p	a	p	0	0	4.36		
a-h	a	h	2	0.8	2.20	?	PSII D1 protease
a-a	a	a	1	0.4	0.72	-SGWL	**InaD-3**

The domains outside of (G,H) group with experimentally determined ligand specificity are indicated in bold: InaD-3,5 [34]; Mint1-1, hINADL-5, PAR6, MUPP1-9 (present study); nNOS [9,10]; CASK [8]; hINADL-1–4 [21].

3.2. Ligand specificity of PDZ domains in (n,h) group

In most of the literature the S/T-X-Φ carboxy-terminal sequence is considered to be the 'canonical' PDZ domain binding motif. It is not surprising, considering relative abundance of (G,H) PDZ domains (Fig. 2, Table 1). Reports of ligands for PDZ domains from other groups are less frequent and often conflicting. Thus, it is difficult to provide complete account of all reported PDZ domain interactions. In Table 1 we attempted to provide some examples of experimentally demonstrated ligands for PDZ domains. These PDZ domains are shown in bold on Table 1. In agreement with our hypothesis, the known ligands for PDZ domains outside the (G,H) group do not fit with S/T-X-Φ consensus (Table 1). Is it possible to infer ligand specificity of a given PDZ domain from its position in the proposed classification?

To test the predictive power of the proposed classification, we experimentally determined ligand specificity of representative (n,h) group members. Structure-guided sequence alignment of selected PDZ domains from the (n,h) group is shown on Fig. 3. These domains include (Fig. 3): the first PDZ domain in the X11/Mint/*lin10* family; the fifth PDZ domain in MUPP1 [17] and hINADL [16] proteins; the PDZ domain in PAR6 protein [18] (also known as human tax.c40 protein [20]); and the ninth PDZ domain in MUPP1 [17]. In our previous work [11] we used the yeast two-hybrid technique to demonstrate that the Mint1-1 PDZ domain specifically binds to N-type Ca^{2+} channel carboxy-terminal tail (NC4 bait). Using a similar approach, we demonstrated that PAR6, hINADL-5, and MUPP1-9 PDZ domains also bind to the NC4 bait (Fig. 4). Observed interactions were specific, as none of the PDZ domains tested associated with the carboxy-termini of NMDA receptor 2A (NR2A bait, a ligand of PSD95 PDZ domain) or truncated NC4-D2334X mutant (Fig. 4). That is, all four members of the (n,h) group that we tested in yeast two-hybrid assay recognize the NC4 bait. In contrast, Mint1-1 was the only PDZ domain from over 10 different PDZ domains tested in the previous work, which associated with the NC4 bait [11].

Unexpectedly, in the course of yeast two-hybrid analysis we discovered that the hINADL-5 domain, but not other tested domains, also strongly associated with neurexin 1a carboxy tail (NX1 bait) (data not shown). To rule out potential artifact of the yeast two-hybrid approach, we performed an additional analysis of the (n,h) PDZ domain ligand specificity using in vitro binding assay. In these experiments synthetic peptides corresponding to N-type Ca^{2+} channel carboxy-termini (NC) and neurexin 1a carboxy-termini (NX1C) were coupled to Sepharose beads and precipitated by PDZ domains expressed as GST fusion proteins in bacteria. In agreement with the yeast two-hybrid data, we found that Mint1-1,

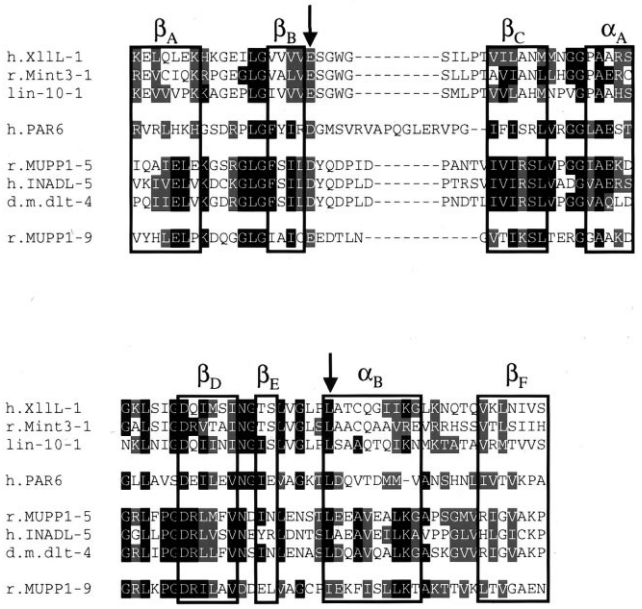

Fig. 3. Structure-guided sequence alignment of PDZ domains from (n,h) group. Human X11-1, rat Mint3-1, *lin10*-1, human PAR6, rat MUPP1-5, human INADL-5, rat MUPP1-9 SMART PDZ domains are shown as indicated. *Dlt*-4 PDZ domains are added from [23]. The elements of PDZ domain secondary structure are from Fig. 1.

PAR6, hINADL-5, and MUPP1-9 PDZ domains specifically interact with NC peptide (Fig. 5). We also found that the NXC1 peptide strongly binds to Mint1-1, PAR6, hINADL-5 PDZ domains and only weakly to the MUPP1-9 domain (Fig. 5). To further quantify these interactions, we attached NXC1 peptide to BIAcore chip and performed a series of surface plasmon resonance experiments. We found that Mint1-1, hINADL-5 and Par6 PDZ domains, but not MUPP1-9 PDZ domain associate with the NXC1 peptide (Fig. 6). Notably, MUPP1-9 is the only domain in the (n,h) group with Ile instead of Leu in Pos2 position (Fig. 3), which may relate to its distinct ligand specificity. The surface plasmon resonance assay was specific, as injection of GST protein or PSD95-1-3 PDZ domains did not result in any signal (Fig. 6). In similar experiments we demonstrated strong association of Mint1-1 PDZ domain with NC coupled BIAcore chip (data not shown), but aggregation of Mint1-1 PDZ domain on NC chip precluded collection of data with other PDZ domains.

From our experiments we concluded that Mint1-1, hINADL-5 and Par6 PDZ domains display dual ligand specificity. The dual ligand specificity may be a general feature of PDZ domains, as dual ligand specificity of the hINADL-3 PDZ domain was recently discovered [21]. Our data are in agreement with a recently reported association of Mint1-PDZ1,2 tandem construct with NC and NXC1 ligands [22]. The fourth PDZ domain in *Drosophila* protein *dlt* (*discs lost*) [23] is homologous to hINADL-5 and MUPP1-5 PDZ domains (Fig. 3). In agreement with our findings with hINADL-5 domain, Dlt-3,4 tandem construct strongly binds to the carboxy-terminal region of *Drosophila* neurexin IV homolog in GST pull-down assays [23]. Our general conclusion is that similarity in ligand specificity of PDZ domains from the (n,h) group (Figs. 4–6) supports a predictive power of the proposed PDZ domain classification.

An independent test of our classification is provided by a recent systematic analysis of hINADL PDZ domain specificity using peptide combinatorial library fused to carboxy-terminus of the capsid D protein of bacteriophage Lambda [21]. The predictive power of the proposed classification appears to be the strongest for members of the (G,H) family; for both hINADL-6 and hINADL-7 PDZ domains binding consensus

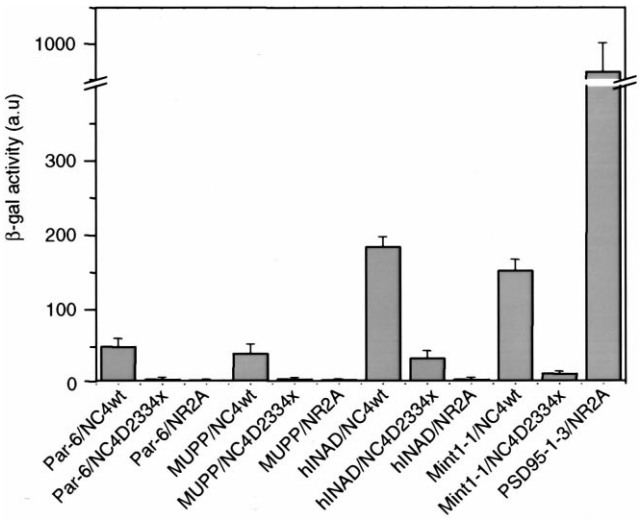

Fig. 4. Selected PDZ domains from (n,h) group bind NC4 bait in yeast two-hybrid assay. Preys encoding rat Mint1-1, human INADL-5, human MUPP1-9, and mouse PAR6 PDZ domains were tested with NC4, NC4-D2334X, and NR2 baits as indicated. Rat PSD95-1-3 prey was used as positive control for NR2 bait. β-Galactosidase activity is indicated in arbitrary units ± S.E.M. ($n \geq 3$).

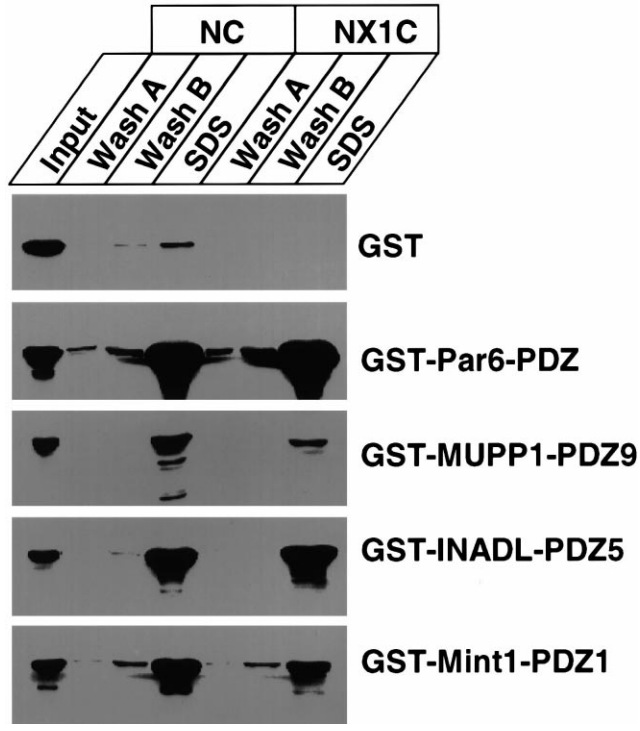

Fig. 5. Interaction of PDZ domains from (n,h) group with NC and NXC1 peptides in pull-down assay. Rat Mint1-1, human INADL-5, human MUPP1-9, and mouse PAR6 PDZ domains were expressed as GST fusion proteins and incubated with Sepharose beads coupled to NC or NXC1 peptides as indicated. Bound PDZ domains were removed from beads by SDS. GST was used as a negative control. The experiment was repeated in duplicate with similar results.

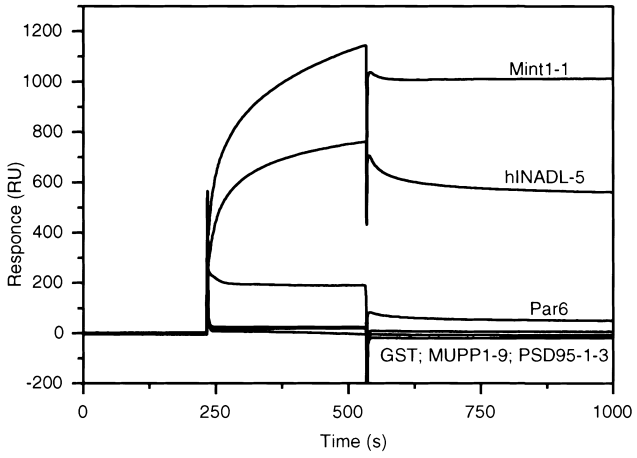

Fig. 6. Interaction of PDZ domains from (n,h) group with NXC1 peptide in surface plasmon resonance assay. NXC1 peptide was coupled to BIAcore chip. Rat Mint1-1, human MUPP1-9, mouse PAR6, rat PSD95-1-3 PDZ domains were expressed as GST fusion proteins and injected over the surface of NXC1 coupled chip in a concentration of 30 μM for 300 s. For human INADL-5 PDZ domain the concentration was 10 μM. GST (30 μM) was used as a negative control.

fits with class I PDZ domain ligand -S/T-X-Φ [21]. The classification also correctly predicted that the other five PDZ domains will have specificity distinct from the class I consensus. The Ψ-X-Φ (Ψ stands for aromatic) binding consensus of hINADL-1 PDZ domain (Sp,H) is similar to the QFV ligand of the PTPN13-4 domain [24] from the same group (Table 1). The classification also correctly predicted similar specificity of hINADL-2 and hINADL-3 PDZ domains (both from the (G,p) group) for the Ψ-D-Φ ligand, but failed to predict dual specificity of the hINADL-3 PDZ domain that also binds Φ-D/E ligand. The hINADL-4 PDZ domain (Sp,p) is specific for EΦΨV consensus, whereas the PTPN13-3 domain from the same group binds E/D-WC ligand [25]. It is not clear if (Sp,p) PDZ domains also display dual ligand specificity. In case of hINADL-5 PDZ domain (n,h) Vaccaro et al. identified S/T-W-Φ consensus, which does not match with DHWC and EYYV ligands identified for the same domain in the present report. This discrepancy highlights the importance of using multiple experimental techniques to identify PDZ domain ligands.

3.3. Diversity of multi-PDZ domain proteins

A large number of adaptor proteins contain several PDZ domains. What is a distribution of PDZ domains from different groups in multi-PDZ domain proteins? Do PDZ domains from the same or different groups tend to cluster together in the same protein? To answer these questions, we used developed classification to assign PDZ domains in multi-PDZ proteins to groups based on the (Pos1,Pos2) rule. Domain structure of multi-PDZ domain proteins found in the SMART database determined by using the SMART tool [12,13] is shown on Fig. 7 with the assignment of PDZ domains indi-

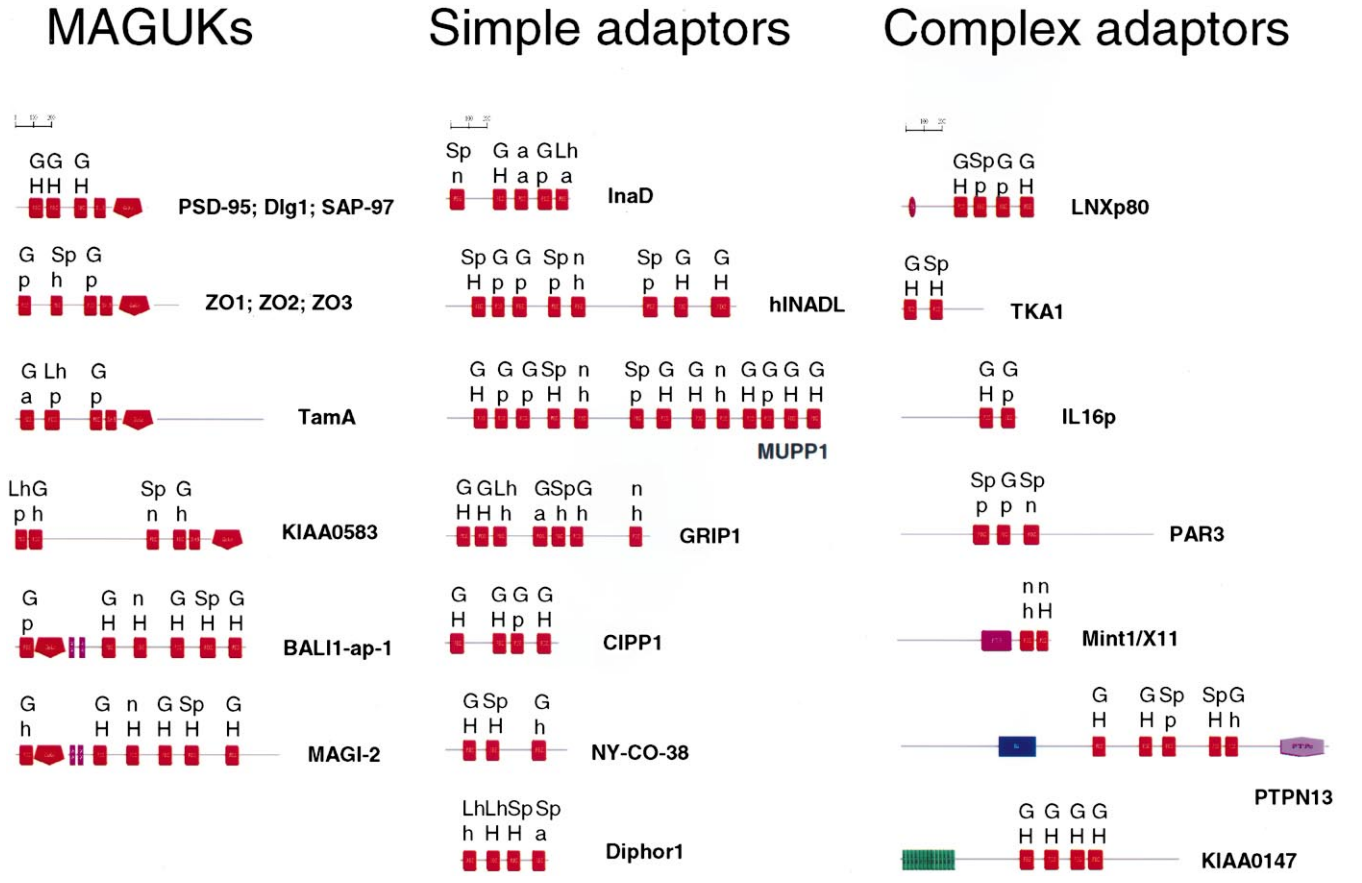

Fig. 7. Diversity of multi-PDZ domain proteins. MAGUKs (contain GUK domain). Simple adaptors (contain PDZ domains only). Complex adaptors (contain other functional and/or protein–protein interaction domains in addition to PDZ domains). The group assignment of PDZ domains is indicated as Pos1/Pos2.

cated. Based on their domain structure, we divided the multi-PDZ domain proteins into three general groups: 'membrane associated guanylate kinases' (MAGUKs), 'simple PDZ adaptors', and 'complex PDZ adaptors' (Fig. 7).

Some MAGUKs, such as PSD95, *Dlg1* and SAP97 contain only PDZ domains of the (G,H) group. Other MAGUKs, such as ZO proteins, *TamA*, KIAA0583, BALI1 associated protein 1, and MAGI2 contain PDZ domains from different groups. All simple PDZ adaptors contain PDZ domains from different groups. Probably different specificity of PDZ domains in these proteins is needed to provide flexibility required to assemble signal transduction complexes, such as for example a 'transducisome' assembled in fly photoreceptors by InaD [26]. Most of the complex PDZ adaptors, with the exception of KIAA0147, also contain PDZ domains from different groups. In addition to PDZ domains, extra signaling functions in these proteins are conferred by different types of functional and protein–protein interaction domains. In general, it appears that PDZ domains from different groups are typically combined in multi-PDZ domain proteins, with the notable exception of PSD95/*Dlg1*/SAP97 MAGUKs.

3.4. Biological significance of PDZ domain classification

Proposed classification should help to organize PDZ domains and provide some clues regarding expected ligand specificity of PDZ domains from their primary sequence. It may also point to the biological function of PDZ domain containing proteins. For example, here we predicted and experimentally demonstrated that Mint1-1 [27], hINADL-5 [16] and PAR6 [18,20] PDZ domains share similar dual ligand specificity for NC and NXC1 ligands (Figs. 4–6). The members of the X11/Mint/*lin10* family form a tripartite complex with CASK/*lin2* and Veli/*lin7* proteins which have been proposed to play a role in targeting of receptors and ion channels to specific compartments in polarized epithelia and in neurons [11,22,28–30]. In *Caenorhabditis elegans*, PAR6 protein is important for establishment of anteroposterior polarity in the single cell stage embryo, which leads to an asymmetric cell division [18]. Recent data suggest that mammalian PAR6 protein plays an important role in establishing epithelial cell polarity [31–33]. Function of INADL [16] and MUPP1 [17] adaptor proteins is not known, but based on our results we can predict that these proteins are also involved in establishing and maintenance of cell polarity in neurons and other polarized cells. Indeed, recently identified *Drosophila* homolog of INADL and MUPP *discs lost* protein (*Dlt*) is required to establish and maintain epithelial cell polarity in *Drosophila* embryo [23]. Thus, the proposed classification may not only help to organize PDZ domains and multi-PDZ domain proteins, but may also provide some clues about their function in cells.

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