Mechanism and role of PDZ domains in signaling complex assembly

Baruch Z. Harris and Wendell A. Lim*

Department of Cellular and Molecular Pharmacology, University of California San Francisco, 513 Parnassus Avenue, San Francisco, CA 94143-0450, USA

*Author for correspondence (e-mail: wlim@itsa.ucsf.edu)

Journal of Cell Science 114, 3219-3231 (2001) © The Company of Biologists Ltd

Summary

PDZ domains are protein-protein recognition modules that play a central role in organizing diverse cell signaling assemblies. These domains specifically recognize short C-terminal peptide motifs, but can also recognize internal sequences that structurally mimic a terminus. PDZ domains can therefore be used in combination to bind an array of target proteins or to oligomerize into branched networks. Several PDZ-domain-containing proteins play an important role in the transport, localization and assembly of supramolecular signaling complexes. Examples of such PDZ-mediated assemblies exist in *Drosophila* photoreceptor cells and at mammalian synapses. The predominance of PDZ domains in metazoans indicates that this highly specialized scaffolding module probably evolved in response to the increased signaling needs of multicellular organisms.

Key words: PDZ, Signaling, Scaffolding

Introduction

An emerging paradigm in eukaryotic signal transduction is that modular protein-protein recognition domains are used to wire the complex biochemical circuits that control cellular responses to external stimuli. These recognition domains play a central role in assembling multiprotein signaling complexes, thereby coordinating and guiding the flow of regulatory information.

Here, we focus on the PDZ domain, one of the most common modular protein-interaction domains. The primary function of these domains is to recognize specific ~5-residue motifs that occur at the C-terminus of target proteins or structurally related internal motifs. We examine the structure of PDZ domains, the mechanism of PDZ-domain-mediated recognition and how PDZ-domain-containing proteins function as key elements that organize diverse signaling pathways.

The discovery of PDZ domains as protein-interaction modules

PDZ domains were first identified as regions of sequence homology found in diverse signaling proteins (Cho et al., 1992; Woods and Bryant, 1993; Kim et al., 1995). The name PDZ derives from the first three proteins in which these domains were identified: PSD-95 (a 95 kDa protein involved in signaling at the post-synaptic density), DLG (the *Drosophila melanogaster* Discs Large protein) and ZO-1 (the zonula occludens 1 protein involved in maintenance of epithelial polarity). These domains have also been referred to as DHR (Discs large homology repeat) domains or GLGF repeats (after the highly conserved four-residue GLGF sequence within the domain).

PDZ domains function as protein-protein interaction modules. The first identified (and by far the most common) function of PDZ domains is the recognition of specific C-terminal motifs found in partner proteins, most often in the cytoplasmic tails of transmembrane receptors and channels (Kornau et al., 1995; Niethammer et al., 1996). More recently, examples of PDZ-domain-mediated recognition of non C-terminal motifs have also been discovered (Xu et al., 1998; Hillier et al., 1999; Christopherson et al., 1999; Fouassier et al., 2000).

Organismal distribution of PDZ domains

Clearly identifiable PDZ domains are primarily concentrated in metazoans. PDZ-domain-containing proteins are numerous in all three currently sequenced metazoan genomes (*Caenorhabditis elegans*, *D. melanogaster* and *Homo sapiens*), representing 0.2-0.5% of open reading frames (Schultz et al., 2000; Schultz et al., 1998b). Domains similar to PDZ domains have also been identified in yeast, bacteria and plants (Pallen and Ponting, 1997; Ponting, 1997). However, the putative PDZ domains found in both the *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* genomes exhibit extremely low sequence similarity to the metazoan consensus PDZ domain amino acid sequence (E values > 0.1), calling into question whether these are truly PDZ domains. Moreover, there is no evidence that these putative domains function in protein-protein recognition. The bacterial and plant PDZ domains, by contrast, show considerably higher homology to those found in metazoan proteins. The structure of one such bacterial PDZ domain has recently been solved, and it reveals that this domain has a fold related to but distinctly different from canonical PDZ domains (Liao et al., 2000; see below). Thus, many of these bacterial ‘PDZ-like’ domains are likely to be significantly different from canonical metazoan PDZ domains.

Since canonical PDZ domains are rare in non-metazoans, PDZ domains might have co-evolved with multicellularity. In this sense they are similar to SH2 domains, which appear only

in metazoans, but differ from SH3 domains, which are well established in yeast as well as metazoans (Zagulski et al., 1998; The *C. elegans* Sequencing Consortium, 1998; International Human Genome Sequencing Consortium, 2001; Venter et al., 2001). The fact that clear PDZ domains are absent in yeast, but numerous PDZ-like domains are present in bacteria and plants, has led to the hypothesis that the domains may have entered these other genomes by horizontal genetic transfer (Pallen and Ponting, 1997). This hypothesis is also consistent with the finding that PDZ domains are absent in Archaea.

The structure of PDZ domains and their mechanism of recognition

Overall PDZ fold and peptide-binding site

The structures of eight PDZ domains (and two 'PDZ-like' domains) have been determined by crystallography and/or NMR (Berman et al., 2000; Daniels et al., 1998; Doyle et al., 1996; Hillier et al., 1999; Karthikeyan et al., 2001a; Karthikeyan et al., 2001b; Kozlov et al., 2000; Liao et al., 2000; Morais Cabral et al., 1996; Mühlhahn et al., 1998; Schultz et al., 1998a; Tochio et al., 2000; Tochio et al., 1999; Webster et al., 2001). Like many other protein-protein interaction domains, PDZ domains are relatively small (≥90 residues), fold into a compact globular fold and have N- and C-termini that are close to one another in the folded structure. Thus the domains are highly modular and could easily have been integrated into existing proteins without significant structural disruption through the course of evolution. The PDZ fold consists of six β-strands (βA-βF) and two α-helices (αA and αB; Fig. 1a).

The structures of several PDZ-peptide complexes have also been determined **(**Fig. 1b,c; Doyle et al., 1996; Daniels et al., 1998; Tochio et al., 1999). Peptide ligands bind in an extended groove between strand βB and helix αB by a mechanism referred to as β-strand addition (Harrison, 1996). Specifically, the peptide serves as an extra β-strand that is added onto the edge of a pre-existing β-sheet within the PDZ domain. The peptide ligand backbone participates in the extensive hydrogen-bonding pattern normally observed between main-chain carbonyl and amide groups in a β-sheet structure. The structure of the PDZ domain does not change upon ligand binding; the free and peptide-bound structures of the third PDZ domain of PSD-95 show an α carbon root-mean-squared deviation (RMSD) of only 0.9 Å (Doyle et al., 1996).

The affinity of PDZ-peptide interactions

Confusion exists concerning the binding affinities of PDZ domains for their peptide ligands. Estimates for dissociation constants (K_d), measured using a variety of techniques, have ranged from low nanomolar to high micromolar. Much of this confusion, however, may result from the methods used. Most of the high-affinity measurements (Kd ~ nM) were obtained by solid-phase methods such as ELISA or surface plasmon resonance. The caveats of these methods and their tendency to overestimate the magnitude of molecular interactions are well known (Myszka, 1999a; Myszka, 1999b). To date, two studies have measured the affinities of PDZ domains for their ligands by solution methods, giving figures for the affinities of the α1-syntrophin PDZ domain and PSD-95 PDZ domains 2 and 3 in the low micromolar range (1-10 μM) (Harris et al., 2001; Niethammer et al., 1998). Thus, this is probably the true affinity range of most PDZ domain interactions, which would place them in the same range as SH2 and SH3 interactions. These moderate affinities are likely to be more suitable for regulatory functions (Nguyen et al., 1998).

Specificity in C-terminal peptide binding

PDZ domains recognize specific C-terminal sequence motifs that are usually about five residues in length, although in some rare cases specificity of recognition extends beyond these terminal five residues (Niethammer et al., 1998). The nomenclature for residues within the PDZ-binding motif is as follows: the C-terminal residue is referred to as the P_0 residue; subsequent residues towards the N-terminus are termed P_{-1}, P_{-2}, P_{-3}, etc.

Extensive peptide library screens pioneered by Songyang et al. have revealed the specificities of distinct PDZ domains

Table 1. Examples of PDZ ligands

PDZ Domain	Consensus binding sequence*					Ligand protein	Reference
	P_{-3}	P_{-2}	P_{-1}	P_0			
Class I		S/T	X‡	Φ§	-COOH		
Syntrophin	E	S	L	V	-COOH	Voltage-gated Na^+ channel	Schultz et al., 1998a
PSD-95 #1, 2	E	T	D	V	-COOH	Shaker-type K^+ channel	Kim et al., 1995
Class II		Φ	X	Φ	-COOH		
hCASK	E	Y	Y	V	-COOH	Neurexin	Songyang et al., 1997
Erythrocyte p55	E	Y	F	I	-COOH	Glycophorin C	Marfatia et al., 2000
Class III		X	X	C	-COOH		
Mint-1	D	H	W	C	-COOH	N-type Ca^{2+} channel	Maximov et al., 1999
SITAC		Y	X	C	-COOH	L6 antigen	Borrell-Pages et al., 2000
Other							
nNOS	G	D	X	V	-COOH	MelR	Stricker et al., 1997
MAGI PDZ #2		S/T	W	V	-COOH	(phage display)	Fuh et al., 2000
Engineered from AF6		K/R	Y	V	-COOH	Synthesized peptide	Schneider et al., 1999

*By convention, P_0 is the C-terminal residue, P_1 is one residue N-terminal to it, etc.
‡X denotes any amino acid (no specificity defined at this position for this class).
§Φ denotes a hydrophobic amino acid, usually V, I or L.

(Songyang et al., 1997; Schultz et al., 1998a). Together, these studies suggest that the P_0 and P_{-2} residues are most critical for recognition. These studies also show that PDZ domains can be divided into at least three main classes on the basis of their pReferences for residues at these two sites: class I PDZ domains recognize the motif S/T-X-Φ-COOH (where Φ is a hydrophobic amino acid and X is any amino acid); class II PDZ domains recognize the motif Φ-X-Φ-COOH; and class III PDZ domains recognize the motif X-X-C-COOH. There are a few other PDZ domains that do not fall into any of these specific classes (Table 1).

The structural basis of specific recognition

Why do PDZ domains recognize these motifs only if they are at the C-terminus of the ligand? Structures of PDZ-peptide complexes reveal that at the end of the peptide-binding groove is a loop termed the 'carboxylate-binding loop'. This loop contains the well-conserved sequence motif R/K-X-X-X-G-L-G-F. In the complex, the ligand terminal carboxylate is coordinated by a network of hydrogen bonds to main-chain amide groups in this loop, as well as an ordered water molecule that is coordinated by the side chain of a conserved arginine/lysine residue at the beginning of the loop (Fig. 1b,c). The coordination of the terminal carboxylate, as well as the extensive β-strand-β-strand interactions between the peptide and strand βB, firmly positions the peptide in the binding groove.

Given the manner in which the peptide ligand is positioned, the side chains of the P_0 and P_{-2} ligand residues point directly into the base of the peptide-binding groove. This mode of docking explains the importance of these residues in specificity. The P_0 residue points into a large hydrophobic pocket. In the case of the third PDZ domain from PSD-95, which prefers valine at site P_0, the pocket is formed by Phe325, Leu379 and other hydrophobic residues (Doyle et al., 1996). A slightly larger hydrophobic pocket in the first PDZ domain from NHERF/EBP50 has been hypothesized to result in the preference for leucine at the P_0 position (Karthikeyan et al., 2001a). The P_{-2} side chain points into a separate pocket, which in class I PDZ domains contains a histidine residue (His-372 in PDZ 3 of PSD-95). The N-3 nitrogen of this residue forms a specific hydrogen bond with the hydroxylated side chains of either a serine or threonine residue, thus giving rise to the distinct P_{-2} residue preference of class I domains. In class II domains, which recognize a hydrophobic residue at position P_{-2}, the histidine residue is replaced by leucine or methionine (Daniels et al., 1998). In the nNOS PDZ domain, which recognizes aspartic acid at the P_{-2} position, the corresponding residue is tyrosine (Stricker et al., 1997).

PDZ-complex structures also reveal that residues other than

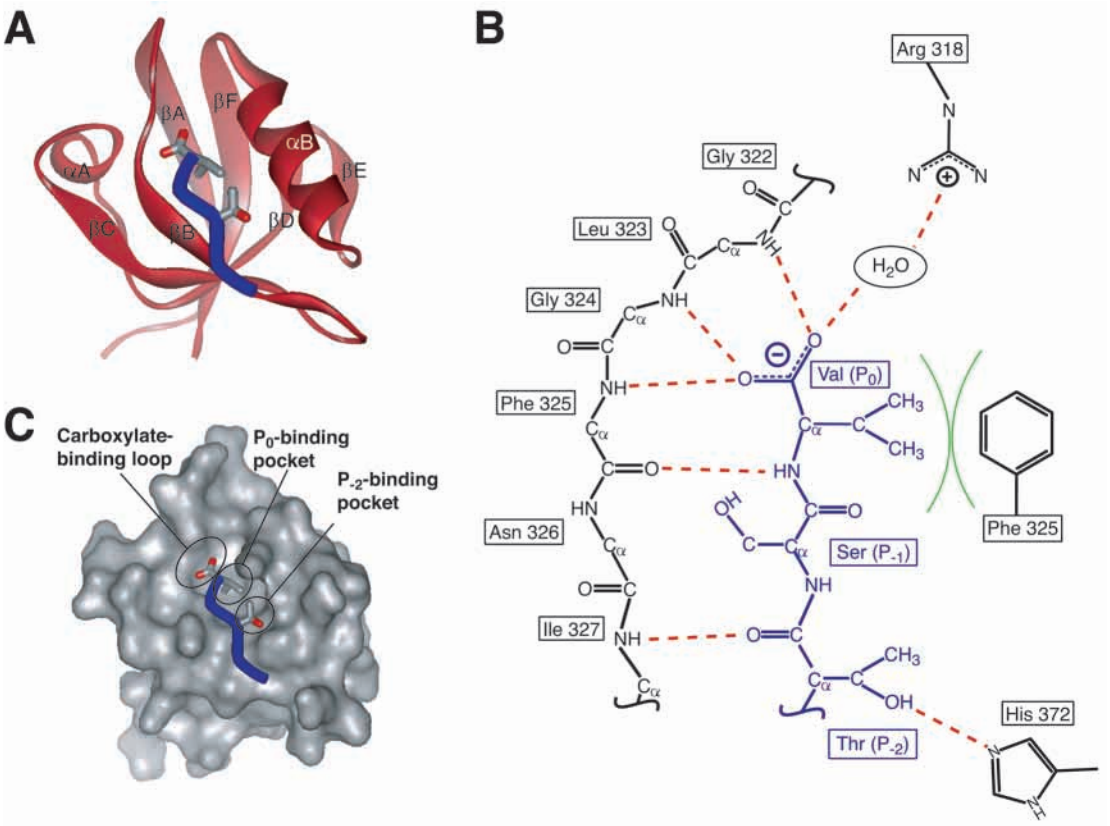

Fig. 1. Structure of the PDZ domain and mechanism of peptide recognition. (A) Ribbon diagram of PSD-95 PDZ domain 3 (residues 306-394, shown in red) with a bound peptide (NH_2-KQTSV-COOH, shown in blue). Names of β-strands and α-helices are indicated. The side chains of the peptide P_0 residue (valine) and P_{-2} residue (threonine) are shown in stick form, as is the terminal carboxylate. (B) Diagram of the peptide-binding pocket. Residues in the PDZ-domain-binding pocket are shown in black; the peptide is shown in blue. Hydrogen bonds are drawn as red dotted lines, and hydrophobic packing is indicated by green arcs. (C) Solvent-accessible surface representation of the structure shown in (A) (probe radius=1.4 Å). The peptide is drawn as in A, and key binding pockets are indicated by circles (Doyle et al., 1996).

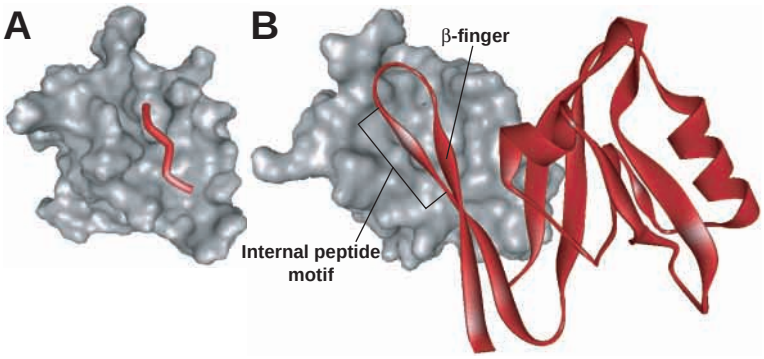

Fig. 2. The same PDZ domain can recognize two structurally distinct ligands. The α1-syntrophin PDZ domain is shown as a gray solvent-accessible surface. (A) The ligand is canonical peptide NH_2-VKESLV-COOH, shown as a red tube (Schultz et al., 1998). (B) The ligand is the nNOS PDZ domain with its distinctive β-finger motif (indicated), shown as a red ribbon diagram. The internal peptide motif that mimics a C-terminal peptide is indicated (Hillier et al., 1999).

the P_{-2} and P_0 residue can also participate in specific interactions with PDZ domain residues that are adjacent to the peptide-binding groove (Doyle et al., 1996; Tochio et al., 1999; Karthikeyan et al., 2001b). These interactions tend to be unique to individual PDZ domains and are likely to fine tune specificity within each domain class.

Thus, the specificity of PDZ domains is primarily determined by the chemical nature of the P_0- and P_{-2}-binding pockets. Distinct sequence variations in the residues that line these pockets can change the size and shape preference for particular hydrophobic P_0 residues. Variations in the P_{-2} pocket can yield distinct preferences for hydroxylated, charged or hydrophobic amino acids.

This model of recognition is supported by the relative ease with which PDZ domain specificity can be altered. Stricker et al. changed the specificity of the nNOS PDZ domain from -D-X-V-COOH to T-X-V-COOH by mutating Tyr77 and Asp78 in the P_{-2}-binding pocket to histidine and glutamate residues, respectively (Stricker et al., 1997). In addition, Schneider et al. used phage-display methods to select for mutations in the AF-6 PDZ domain that bind to a variety of mutant target peptides (Schneider et al., 1999). In most cases, the resulting amino acid substitutions are in or directly adjacent to strand βB and helix αB, the secondary-structure elements lining the peptide-binding pocket. Interestingly, in no case were more than three residue substitutions necessary to change the binding specificity of the AF-6 PDZ domain. These experiments suggest that specificity of PDZ domains is easily altered without significantly compromising the overall domain structure.

Although the fundamental basis for PDZ specificity is well understood, many questions remain. How specific are individual PDZ domains? Even if there are at least 5-10 general specificity classes, do the 394 different PDZ domains found in humans each recognize a unique set of sequences? These questions may be answered as large-scale proteomics efforts begin to elucidate the specificities of large sets of interaction domains.

The recognition of internal motifs

A growing number of examples show that some PDZ domains can also recognize internal peptide motifs. This second mode of interaction at first seems at odds with the strict requirement for a C-terminal recognition motif. However, structural and mechanistic studies of internal motif complexes reveal that the two modes of interaction are related. Internal motifs can be recognized if they are presented within a specific tertiary structure context that conformationally mimics a chain terminus.

The best-characterized example of an internal-motif-mediated PDZ interaction is the heterodimer involving the PDZ domain of nNOS and the PDZ domain of either syntrophin or PSD95 (PDZ #2; Fig. 2). Both syntrophin and PSD95 PDZ domains recognize C-terminal motifs, but they can also recognize the nNOS PDZ domain in a manner that does not depend on the C-terminal sequence of either partner protein. A 30-residue extension on the nNOS PDZ domain, however, is required for interaction (Christopherson et al., 1999). In this structure, the two domains interact in an unusual linear head-to-tail arrangement. The 30-residue extension to the nNOS PDZ domain adopts an extended β-hairpin fold, the 'β-finger'. This nNOS β-finger docks in the syntrophin peptide-binding groove, mimicking a peptide ligand through its P_0 (Phe) and P_{-2} (Thr) pocket interactions. However, in the β-finger, the normally required C-terminus is replaced with a sharp β-turn.

Gee et al. have obtained further evidence that PDZ domains can bind such ligands. They obtained internal sequence motifs that bind to the syntrophin PDZ domain by phage display; the ligands maintain the same consensus recognition motif as all other syntrophin PDZ domain ligands (Gee et al., 1998). However, binding occurs only if the C-terminal end of the peptide is structurally constrained by an intramolecular disulfide bond, which suggests that a 'loop' structure similar to the nNOS β-hairpin turn is necessary.

These studies reveal that internal motif recognition is highly analogous to C-terminal motif recognition. Thus, all PDZ domains might recognize internal motifs, if the latter are presented within the correct structural context. However, internal motif recognition is likely to be more rare, given that the structural requirements for PDZ binding of internal sequence motifs are so stringent.

Several other examples of putative internal motif recognition involve PDZ-PDZ homo- or hetero-dimerization. These interactions might mediate oligomerization of PDZ-containing proteins. Marfatia et al. showed that the PDZ-containing protein hDlg forms oligomers in solution (Marfatia et al., 2000). PDZ domains from the mammalian proteins PAR-3 and PAR-6 appear to bind each other in vitro (Lin et al., 2000). Several recent papers have also recently shown that the first two PDZ domains of NHERF/EBP50 can dimerize or form higher-order oligomers in a manner that may be dependent on phosphorylation (Fouassier et al., 2000; Lau and Hall, 2001); it should be noted, however, that others have been unable to replicate these findings (Reczek and Bretscher, 2001). Finally,

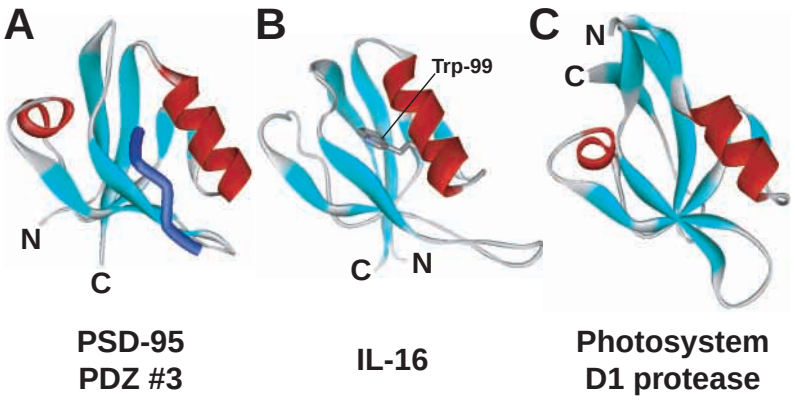

Fig. 3. Structures of related 'PDZ-like' domains. Proteins are shown in ribbon form; β-strands are shown in turquoise, and α-helices are shown in red. (A) A canonical metazoan PDZ domain exemplified by the third PDZ domain of PSD-95 (residues 306-394), shown with a bound peptide (dark blue) as in Fig. 1 (Doyle et al., 1996). (B) Interleukin 16 (IL-16), residues 25-119. Trp-99, shown in stick form, occludes the normal PDZ peptide-binding site (Mühlhahn et al., 1998). (C) The photosystem D1 protease (residues 157-249) from *Scenedesmus obliquus*. The structure is circularly permuted relative to the structures in (A) and (B), but the fold is essentially the same (Liao et al., 2000). Note that, in all structures, the N- and C-termini of the domains are close together.

Xu et al. showed that PDZ domains 3 and 4 from the *Drosophila* protein INAD can bind to each other and form homo-oligomers (Xu et al., 1998). More examples of internal PDZ interactions may exist, but these will be very difficult to identify on the basis of sequence alone, given that a key determinant of binding is a structural feature that can be highly degenerate at the sequence level. It remains to be seen whether these other examples share structural similarities with the nNOS β-finger.

General model for PDZ recognition

The studies described above reveal a unified mechanism for PDZ recognition of diverse ligand types. PDZ domains are extremely selective for specific C-terminal peptide motifs. Internal motif recognition is not an exception to the rules of PDZ recognition but a different way to satisfy the same energetic requirements. Recent mutagenic studies support this idea: alteration of residues in the nNOS β-finger that bind at the P_0 and P_{-2} sites can eliminate binding, just as analogous changes made in C-terminal peptide ligands do. In addition, mutation of nNOS residues that disrupt the β-finger conformation also destroys binding (Harris et al., 2001; Tochio et al., 2000).

One hypothesis that emerges from this analysis is that direct chemical recognition of the terminal carboxylate group is energetically less critical than spatial recognition of the chain terminus. Indeed, PDZ-peptide complexes differ from other carboxylate-recognition proteins in that they lack any direct salt-bridges between the protein and the ligand carboxylate. The indirect nature of terminus recognition might serve two purposes. First it might prevent PDZ-mediated interactions from having too high an affinity for their regulatory purpose. Second, it might prevent nonspecific recognition of free carboxylates or carboxylated amino acids, which could compete with peptide ligands in the cytoplasm.

The focus of PDZ domains on recognition of termini or terminus-like structures might provide a distinct specificity advantage over other protein-recognition modules. Recognition of internal motifs can occur at any location within a given polypeptide sequence. C-terminal recognition, however, can occur at only one location. Given a mean protein length of ~500 residues in the metazoans, it is 500 times less likely that, within the proteome, a given peptide motif will be found at the C-terminus than as an internal sequence (on a purely random basis). In short, a module that constrains its ligands to be at the C-terminus requires far less chemical discrimination power to select its ligands within a complex cellular environment, largely because there are so few termini in comparison with possible internal motifs. In this sense, PDZ domains appear to be highly specialized for specific, non-

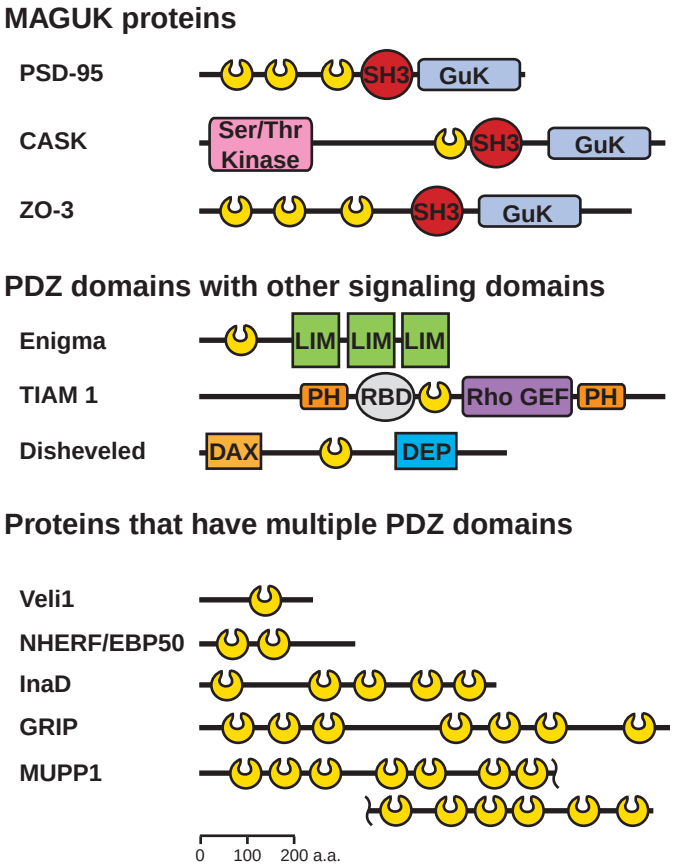

Fig. 4. Examples of higher-order organization of PDZ domains found in signaling proteins. Proteins are indicated in black lines scaled to the length of the primary sequence of the protein; PDZ domains are shown in yellow. Other domains are indicated as abbreviations (from SMART; Schultz et al., 2000) as follows: SH3, Src-homology 3 domain; GuK, guanylate-kinase-like domain; LIM, zinc-binding domain present in Lin-11, Isl-1, Mec-3; PH, pleckstrin-homology domain; RBD, Raf-like Ras-binding domain; RhoGEF, Rho-like GTP-exchange factor; DAX, Dishevelled- and axin-homology domain; DEP, Dishevelled-, Egl-10- and pleckstrin-homology domain.

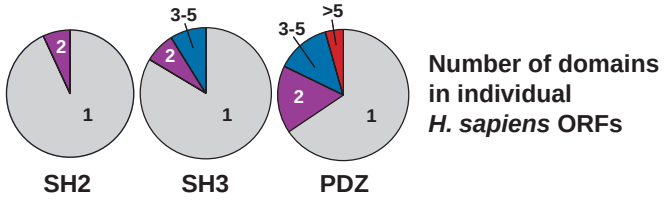

Fig. 5. PDZ domains co-occur more frequently than comparable signaling domains in the human genome. Distributions of multiple signaling domains in a single ORF as predicted by SMART (Schultz et al., 2000) are shown for SH2, SH3 and PDZ domains. ORFs are separated according to those that contain 1 (gray), 2 (purple), 3-5 (blue) and >5 (red) of a single type of domain in the same polypeptide. PDZ domains are much more likely to occur in multiple copies.

invasive recognition (i.e. without interfering with the overall structure) of target proteins.

Related, 'PDZ-like' domains

Two recent structures have called attention to the fact that the PDZ domain fold can be used in other contexts (Fig. 3). As stated above, PDZ-like domains have been identified in bacteria and plants. Although these are clearly related in sequence to metazoan PDZ domains, their homology is more distant, and in most cases it has yet to be determined whether they share a common structure and function.

Recently, however, Liao et al. determined the first crystal structure of a bacterial PDZ-like domain, from the photosystem II D1 protease in *Scenedesmus obliquus* (Liao et al., 2000). The central domain of the protease resembles a PDZ domain at the primary and tertiary structural level (RMSD < 2.0 Å, compared with several metazoan PDZ domains). Nonetheless, there is one striking structural difference: the fold contains a circular permutation. Specifically, the strand that would be the βA strand in a metazoan PDZ domain (at the N-terminus) is at the C-terminus in the primary sequence. In the folded structure, however, this strand occupies the same location and orientation as its corresponding strand in canonical PDZ domains.

It is not known whether this bacterial PDZ-like domain binds C-terminal peptides, although the complete protease is known to recognize substrates based on C-terminal tags. The new structural data suggest that this PDZ-like domain should bind C-terminal peptides as metazoan PDZ domains do. Beebe et al. have found that the putative PDZ-like domain from the related Tsp protease recognizes C-terminal peptides with an apparent affinity of 1.9 μM (Beebe et al., 2000). Thus, this class of PDZ-like domains is likely to share a similar function and overall type of fold but have a distinct topology.

On the basis of sequence analysis (B.Z.H. and W.A.L., unpublished), a large number of the sequences of other bacterial PDZ domains are more consistent with the circularly permuted fold, which suggests that a large family of proteins have this related fold. However, it is still formally possible that a subset of bacterial PDZ domains share the precise structure of metazoan PDZ domains.

Mühlhahn et al. also recently showed that the cytokine interleukin 16 (IL-16; also known as lymphoctye chemoattractant factor) has the same fold as metazoan PDZ domains (Mühlhahn et al., 1998). This secreted cell signaling molecule can activate $CD4^+$ T cells and suppress HIV replication. The central domain adopts a fold strikingly similar to PDZ domains; the overall structure is maintained, as are conserved residues such as the GLGF loop and the buried lysine residue. The carboxylate-binding loop, however, is smaller than in canonical PDZ domains, and the peptide-binding pocket is partially occluded by a tryptophan side chain. Not surprisingly, IL-16 does not bind C-terminal peptides (Mühlhahn et al., 1998) and probably represents an example of divergent evolution, because it differs from a typical PDZ domain sequence by only a few residues.

Higher-order organization of PDZ domain-containing proteins

The wealth of current whole genome information affords us the unprecedented opportunity to examine, at a sequence level, how PDZ domains are utilized in whole proteins. Several distinct patterns are observed (Fig. 4).

The multiplicity of PDZ domains

One of the most striking aspects of PDZ domains is the frequency with which multiple domains occur within the same polypeptide. Of the human PDZ-domain-containing proteins, 18% have three or more PDZ domains within the same polypeptide, and one protein, MUPP1, contains a remarkable 13 PDZ domains. The multiplicity of PDZ domains within a single chain is much higher than for other modular signaling domains (Fig. 5), except for those that are obligate repeats (such as WD40 domains, which only fold as an ensemble of repeats).

MAGUK proteins

PDZ domains are also often found in particular multidomain arrangements. One particularly common class is the so-called MAGUKs (membrane-associated guanylate kinases). These proteins contain between one and three PDZ domains, an SH3 domain and a guanylate kinase homology (GuK) domain (Gomperts, 1996). Although the GuK domain is similar to the yeast enzyme guanylate kinase, it lacks key catalytic residues and guanylate kinase activity. However, both the SH3 and GuK domains of MAGUKs have been implicated in other protein-protein interactions. 24 MAGUK proteins are found in the human genome; several of these have been proposed to organize multiprotein signaling complexes by virtue of their multiple interaction domains.

PDZ domains organize multiprotein signaling complexes

PDZ-domain-containing proteins appear to function in vivo by organizing multiprotein complexes that function in signaling, as well as establishment and maintenance of cell polarity. For a cell to communicate effectively with its neighbors, relevant receptors and downstream effectors must be localized and organized at the proper regions of the cell. This type of organization is particularly critical for the highly specialized cells found in multicellular organisms.

The best-understood examples of these PDZ-organized signaling complexes occur in neurons or epithelial cells. Both cell types function in cell-cell communication and have highly

polar structures that are critical for this function. Neurons, for example, have topologically distinct presynaptic (axonal) and postsynaptic (dendritic) structures. Proper neuronal communication requires maintainance of this polarity. Analogously, epithelial cells have distinct basolateral and apical sides, which share certain topological similarities with the dendritic and axonal domains of neurons, respectively (Bredt, 1998; Dotti and Simons, 1990). A general problem facing both of these cell types is how to establish and maintain this polarity and how to target and assemble the proper signaling proteins at these sites.

PDZ-domain-containing proteins appear to play key roles in organizing polar sites of cell-cell communication. PDZ proteins organize receptors and their downstream effectors. They also play a crucial role in transporting and targeting appropriate proteins to sites of cellular signaling. Both of these functions capitalize on the ability of PDZ-domain-containing proteins to crosslink many different polypeptides by binding to small C-terminal tags. Below, we discuss four specific examples of biological processes involving PDZ-containing proteins: organization of the *Drosophila* phototransduction pathway by INAD, organization of the postsynaptic density by PSD95, regulation of membrane protein activity and trafficking by NHERF/EBP50, and receptor localization by the LIN-2–LIN-7–LIN-10 complex. This discussion is by no means exhaustive and we refer readers to several more detailed reviews of these subjects (Sheng, 2001; Shenolikar and Weinman, 2001; Tsao et al., 2001; Kennedy, 2000; Tsunoda and Zuker, 1999; Craven and Bredt, 1998; Lefkowitz, 1998; Ranganathan and Ross, 1997). Moreover, we do not discuss the important role of PDZ domain proteins in several other processes, such as the maintenance of epithelial cell polarity and morphology, which have been recently reviewed elsewhere (Bredt, 1998; Fanning and Anderson, 1998).

The PDZ protein INAD scaffolds the *Drosophila* phototransduction pathway

One of the best-studied PDZ-containing proteins is the *Drosophila* protein INAD, which is composed almost entirely of five PDZ domains. INAD appears to serve as a scaffold for the G-protein-coupled phototransduction cascade in the fly eye (Fig. 6).

Drosophila photoreceptor cells are specialized for the sensitive detection of photons (for review see Tsunoda and Zuker, 1999; Fanning and Anderson, 1999; Ranganathan and Ross, 1997). Activation of rhodopsin, the receptor protein, by a photon triggers a G-protein-coupled cascade involving $G\alpha_q$, $G\beta_e$, $G\gamma_e$ and phospholipase Cβ (PLC-β). The second-messenger products of PLC-β, diacylglycerol and Ins(1,4,5)P_3, in turn open the transient receptor potential (TRP) Ca^{2+} channel, causing depolarization of the cell. Subsequent deactivation of this response is mediated by a Ca^{2+}-dependent adaptation process involving eye-specific protein kinase C (eye-PKC), calmodulin, arrestin and calmodulin-dependent kinase. Both activation and deactivation are fast (a millisecond timescale), robust and strongly amplified – a single photon reliably results in the opening of a large number of channels. This unitary, highly amplified response is referred to as a 'quantum bump'.

InaD was first identified as a mutation that disrupts the phototransduction cascade and causes light-dependent retinal degeneration (Shieh and Zhu, 1996). Moreover, *InaD* mutants show mislocalization of several proteins in the signaling cascade (Chevesich et al., 1997; Tsunoda et al., 1997; Tsunoda et al., 2001). INAD, therefore, appears to be required for the assembly, function and maintenance of the phototransduction signaling pathway, despite the fact that it is composed solely of PDZ domains and appears to have no direct catalytic function.

Why is INAD so indispensable for this signaling cascade? Extensive studies reveal that the five PDZ domains in INAD interact with individual proteins in the signal transduction pathway, including PLC-β, PKC and TRP (Fig. 6; Tsunoda et al., 2001; Li and Montell, 2000; Bähner et al., 2000; Huber et al., 1998; Xu et al., 1998; van Huizen et al., 1998; Adamski et al., 1998; Chevesich et al., 1997; Tsunoda et al., 1997; Huber et al., 1996; Shieh and Zhu, 1996). In addition, the third and fourth PDZ domains participate in oligomerization – probably through PDZ-PDZ interactions (Xu et al., 1998). There is some debate as to whether certain members of the

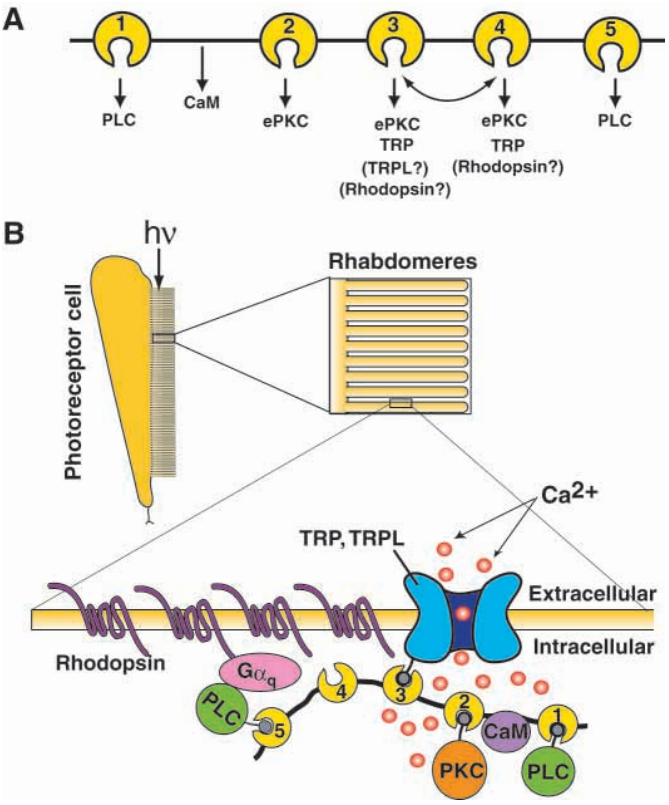

Fig. 6. INAD coordinates the *Drosophila melanogaster* phototransduction cascade. (A) Domain structure of INAD; arrows indicate putative protein-protein interactions. (B) Diagram of the *Drosophila* phototransduction cascade. The full photoreceptor cell is at top left. At top right, a section of the cell is expanded to show the stacking of rhabdomeres. Below, a schematic representation of the signaling pathway coordinated by INAD. INAD is indicated by five yellow PDZ domains connected by a black line. Abbreviations for proteins that form the phototransduction signaling cascade are as follows: TRP/TRPL, transient-receptor-potential-type Ca^{2+} channels; $G\alpha_q$, the Gα subunit of the heterotrimeric G protein involved in the *Drosophila* phototransduction pathway; PLC, phospholipase Cβ; PKC, eye-specific protein kinase C; CaM, calmodulin.

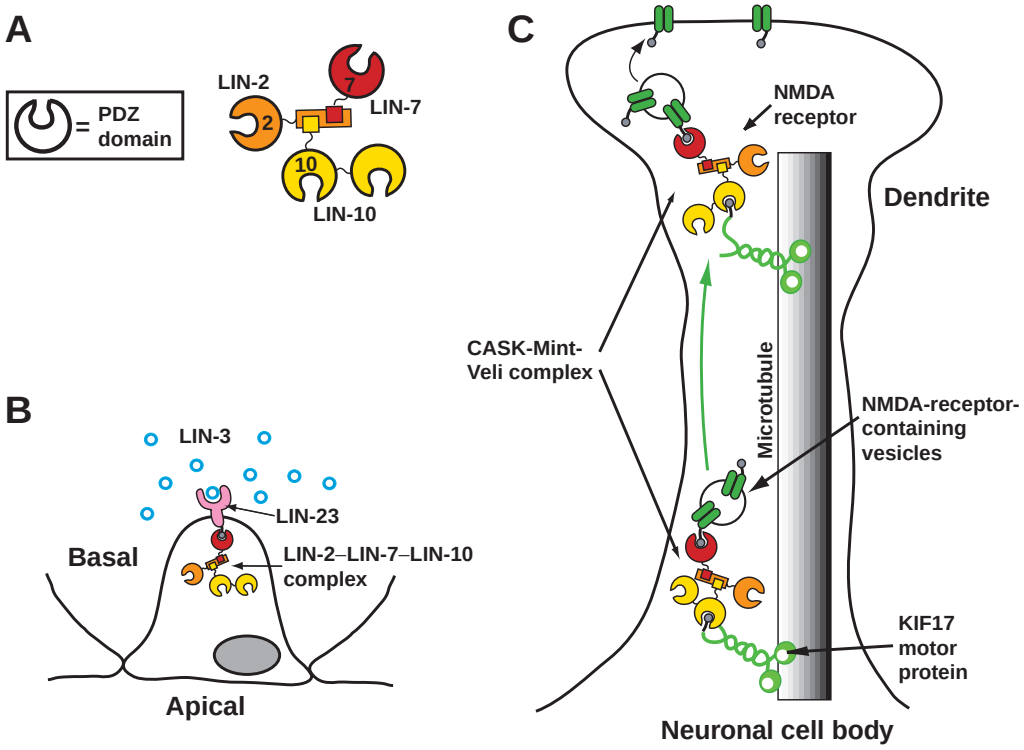

Fig. 7. LIN-2, LIN-7 and LIN-10 form a conserved PDZ-mediated transport complex in polarized cells. (A) Diagram of the three proteins, showing their interactions with each other, which leave all three PDZ domains free to bind ligands. (B) Diagram of *C. elegans* vulval precursor epithelial cells. LIN-2, LIN-7 and LIN-10 form a tripartite complex that is essential for targeting of the tyrosine kinase receptor LET-23; proper localization of LET-23 is required for it to detect its ligand, LIN-3, which is present only on the basolateral side (Kaech et al., 1998; Simske et al., 1996). (C) Schematic diagram of the mammalian neuron, showing transport along microtubules of vesicle containing NMDA receptors. The mammalian homolog of LIN-7 (MALS) binds to the C-terminal tail of NMDA receptor subunit 2B, whereas the LIN-10 homolog (MINT1) binds the kinesin superfamily motor protein KIF17. CASK, the LIN-2 homolog, links MALS and MINT1 together. The entire complex, including NMDA-receptor-containing vesicles, is proposed to move along microtubules to the neuronal dendrite (Setou et al., 2000). This complex thus forms a transport system that targets proteins to their appropriate subcellular locations.

pathway, most notably rhodopsin, directly bind INAD; studies by Chevesich et al. (Chevesich et al., 1997) and Xu et al. (Xu et al., 1998) indicate that it does, whereas studies by Tsunoda et al. suggest that it does not (Tsunoda et al., 1997). Li and Montell suggest that binding of rhodpsin and certain other pathway components may be transient or more highly regulated, possibly explaining some of the above inconsistencies (Li and Montell, 2000). Nonetheless, there is general agreement that INAD binds multiple proteins in this pathway, forming what has been dubbed a ‘transducisome’ complex that may increase signaling efficiency. The ability of INAD to multimerize via PDZ oligomerization interactions may also help organize these cascades into large supramolecular complexes, possibly explaining the quantum bump phenomenon, in which at least a few hundred Ca^{2+} channels are activated in response to a single photon within 20 milliseconds.

As in the case of other PDZ-scaffolding proteins, much remains to be learned about how INAD functions. For example, what is the architecture of INAD-mediated ‘tranducisome’ complexes? Do they form a single type of complex that has a unique, fixed composition and structure, much like a ribosome? Alternatively, do they have heterogenous and dynamic compositions? Recent studies by Scott and Zucker suggest that the answer lies somewhere in the middle (Scott and Zuker, 1998): INAD-scaffolded complexes appear to have variable compositions, but each individual complex appears to have a relatively fixed architecture. The evidence for this model comes from studies in which stimulation of a given rhodopsin molecule yields a highly reproducible quantum bump amplitude. However, stimulation of different individual rhodopsin molecules within a large population produces a much higher degree of variability. These results argue that INAD-scaffolded complexes are fixed, each acting as a defined elementary unit of response. However, the responses produced by individual INAD-scaffolded complexes vary in size.

Much also remains to be learned about the role of INAD in signal processing. For example, one hypothesis has been that cascades such as that used in phototransduction might allow for high levels of amplification at each step in the cascade. A scaffold like INAD might therefore promote amplification. Recent data, however, argue that INAD may actually limit amplification at some steps in the pathway. Scott and Zucker show that varying the expression levels of $G\alpha_q$ and PLCβ does not change the size of the quantum bump (Scott and Zuker, 1998), which is inconsistent with a model in which the availability of these intermediate pathway members determines the magnitude of signal amplification. Amplification appears to occur only downstream of PLCβ, probably at the level of channel activation. Tethering of the upstream protein components by INAD appears not to facilitate amplification but instead play a role in increasing signaling efficiency and tuning response properties such as adaptation.

PDZ scaffolds organize the postsynaptic density

Many PDZ-containing proteins have been implicated in organizing the signaling machinery at synapses and neuromuscular junctions. At the dendritic side of the neuronal synapse, there is a dense complex of proteins termed the postsynaptic density (PSD), which contains many of the

signaling components necessary for responses to incoming signals. Several multi-PDZ-domain proteins are found in the PSD and are implicated as scaffolds in the assembly and maintenance of this structure.

The postsynaptic MAGUK protein PSD95, which has three PDZ domains as well as an SH3 and GuK domain, is one of the best-studied of the putative PSD scaffolds (Kim et al., 1996; Kornau et al., 1995; Müller et al., 1996; Niethammer et al., 1996; Kistner et al., 1993). The PDZ domains of PSD95 can bind to a number of proteins found in the PSD, some of which we describe below. The first and second PDZ domains can specifically bind to the C-terminus of the NR2B subunit of the *N*-methyl-D-aspartic acid (NMDA) glutamate receptor, which suggests a role in receptor clustering at the synapse (Kornau et al., 1995; Niethammer et al., 1996). The second PDZ domain can also participate in a heterodimeric PDZ-PDZ interaction with the PDZ domain from nNOS (Brenman et al., 1996). The localization of nNOS (which is a Ca^{2+}/calmodulin-activated enzyme) to the NMDA receptor complex is thought to allow efficient synthesis of the second messenger nitric oxide in response to local Ca^{2+} influxes resulting from NMDA channel opening. In addition, the GuK domain of PSD95 also participates in interactions with other PSD components, such as the protein GKAP (Naisbitt et al., 1999). PSD95 has been implicated in synapse formation and receptor clustering (El-Husseini et al., 2000). However, its precise biological role is still unclear. PSD95-knockout mice do not have catastrophic neurological or developmental defects, perhaps because the multiple MAGUK proteins present in neurons have overlapping functions (Migaud et al., 1998).

Other PDZ-containing proteins also contribute to organization of the PSD. For example, the seven-PDZ-containing protein GRIP is thought to act as a scaffold that can both bind to α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) receptors and oligomerize (Dong et al., 1997; Srivastava et al., 1998; Wyszynski et al., 1999). The PDZ-containing protein SHANK is also a component of the PSD, and this protein appears directly or indirectly to crosslink the scaffold proteins PSD-95 and GRIP, perhaps acting as a 'scaffold of scaffolds' (Sheng and Kim, 2000; Kennedy, 2000; Naisbitt et al., 1999; Tu et al., 1999).

It has been difficult to determine the precise role of these PDZ scaffolding proteins in organizing the PSD. Does the PSD have a precise arrangement and composition? Is the PSD highly heterogeneous or dynamic in nature – perhaps involving multiple redundant interactions? This view is supported by studies showing that elimination of some PDZ-containing scaffolds from the synapse does not lead to a dramatic and predictable loss-of-function phenotype, but rather to a specific decrease in efficiency of synaptic receptor clustering (El-Husseini et al., 2000) or a change in synaptic plasticity (Migaud et al., 1998).

Multiple functions of PDZ domains in regulating membrane protein activity and trafficking.

The protein NHERF/EBP50 (Na$^+$/H$^+$ exchanger regulatory factor, or ezrin-radixin-moesin binding phosphoprotein of 50 kDa), which contains two PDZ domains, is an excellent example of a PDZ protein that uses a variety of mechanisms to control the function of several cell surface proteins.

NHERF/EBP50 is hypothesized to control activity of the Na$^+$/H$^+$ antiporter NHE3 through two related mechanisms, both of which require PDZ domain function. First, NHERF/EBP50 is proposed to maintain the antiporter in an inactive basal state by linking NHE3 to protein kinase A (PKA). PKA is thought to downregulate the antiporter (Weinman et al., 2000; Lamprecht et al., 1998). The PDZ domains of NHERF/EBP50 bind to the C-terminal tail of NHE3, whereas the ERM-binding domain in NHERF/EBP50 is proposed to associate with PKA indirectly through the protein ezrin. Second, NHERF/EBP50 is also required for activation of NHE3 upon stimulation of the β2 adrenergic receptor, a G-protein-coupled receptor. The C-terminal tail of the β2 adrenergic receptor can bind to the PDZ domains from NHERF/EBP50. However, this interaction seems to occur only upon receptor activation, perhaps mediated by a conformational change or change in phosphorylation state of the receptor. In a simple model, this agonist-induced interaction could competitively disrupt the inhibitory NHERF/EBP50-NHE3 interaction, resulting in antiporter activation (Hall et al., 1998a; Hall et al., 1998b).

NHERF/EBP50 can also directly regulate the activity of the β2 adrenergic receptor by controlling its recycling and subcellular localization. Interactions between the β2 adrenergic receptor and the NHERF/EBP50 PDZ domains are essential for recycling following agonist-induced endocytosis. This interaction is probably blocked by phosphorylation of the β2 adrenergic receptor C-terminal tail (Cao et al., 1999). The authors propose a model in which the PDZ domain interactions in the endosome direct recycling of the β2 adrenergic receptor to the plasma membrane following internalization; inhibition of the PDZ interaction by phosphorylation instead directs the receptor to the lysosome for degradation. This model may help explain the phenomenon known as receptor desensitization, in which cells containing the β2 adrenergic receptor show a lessened response to agonist following initial stimulation.

Perhaps the most distinct functional aspect of NHERF/EBP50 is the way in which binding of its two PDZ domains appears to modulate directly the activity of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) chloride channel. Two recent studies have shown that NHERF/EBP50, as well as CAP70, another multi-PDZ protein, can directly increase conductance through the CFTR channel through binding of the PDZ domains to the channel C-terminal tail (Wang et al., 2000; Raghuram et al., 2001; Bezoprozvanny and Maximov, 2001). These authors suggest a model in which binding and/or tethering of two separate channel subunits by the tandem PDZ domains causes an activating conformational change. This model is supported by the following observations: first, the tandem PDZ domains of NHERF/EBP50 or CAP70 are required for activation; second, no other regulatory co-factor appears to be required; and third, overexpression of the PDZ proteins above a certain level eventually leads to a decrease in channel activity, a behavior expected given the ratio of PDZ protein to channel subunit shifts from 1:2 (active) to 2:2 (inactive). It can be argued that, in this situation, the PDZ-domain containing proteins are not acting as simple scaffolds (Bezprozvanny and Maximov, 2001).

Many of the mechanistic details of how NHERF/EBP50 functions in these different processes are still unknown. Moreover, it is still unclear whether these different processes constitute independent function of the protein or if they are

inter-related. Nonetheless, these studies illustrate the multitude of ways in which PDZ domains can be utilized in regulation.

A PDZ-mediated localization machine: LIN-2, LIN-7 and LIN-10

The *C. elegans* proteins LIN-2, LIN-7 and LIN-10, as well as their mammalian homologs, form a conserved heterotrimeric complex that is essential for delivery of certain receptors to proper subcellular locations in both neurons and epithelia (Fig. 7). Each of these three proteins contains PDZ domains. These PDZ domains do not function in formation of the heterotrimeric complex, but instead bind to other specific partner proteins that either function as cargo or participate in the localization process (Borg et al., 1998; Butz et al., 1998; Kaech et al., 1998).

In *C. elegans*, LIN-2, LIN-7 and LIN-10 are all required to localize the EGFR-like tyrosine kinase receptor LET-23 to the basolateral side of vulval precursor epithelial cells; loss of LIN-2, LIN-7 or LIN-10 results in a loss of LET-23-dependent signaling and a defect in vulval induction (Kaech et al., 1998; Simske et al., 1996). It is hypothesized that LIN-2, LIN-7 and LIN-10 are required simply for receptor localization, since overexpression of the receptor can overcome the *lin-2* or *lin-7* mutant phenotypes (Simske et al., 1996); presumably overexpression of LET-23 results in a sufficient amount of receptor localized to the basolateral surface. Interaction of LET-23 with a PDZ domain in LIN-7 is essential for targeting; deletion of the last six residues of LET-23 results in mislocalization of the receptor. Moreover, wild-type localization is observed even when the native PDZ-peptide pair from LET-23 and LIN-7 are simultaneously replaced with a heterologous interaction pair (Kaech et al., 1998). These proteins appear to function in neurons as well as epithelia, since *lin-10* mutants also have a defect in targeting of the glutamate receptor GLR-1 to neuronal synapses (Rongo et al., 1998).

Mammalian homologs of LIN-2, LIN-7 and LIN-10 also exist (CASK/PALS, VELI/MALS and MINT/X11α, respectively); they are localized throughout the nervous system in the presynaptic (axonal) and postsynaptic (dendritic) subcellular compartments, and also form a trimeric complex (Misawa et al., 2001; Setou et al., 2000; Butz et al., 1998). In mammalian dendrites, these proteins appear to be involved in NMDA receptor trafficking. VELI/MALS binds to the C-terminal tail of the NMDA receptor subunit NR2B through its PDZ domain (Setou et al., 2000; Jo et al., 1999). MINT1/X11α, in turn, binds to the kinesin superfamily motor protein KIF17 (Setou et al., 2000). The entire complex is proposed to transport NMDA-receptor-containing vesicles along microtubules to the dendrite. More recently, these mammalian homologs of LIN-2 and LIN-7 have also been shown to be important in epithelial cells as well as neurons, possibly playing a role in endosomal sorting (Straight et al., 2001; Straight et al., 2000).

The available evidence suggests that the LIN-2–LIN-7–LIN-10 complex forms a conserved transport system that targets proteins to their appropriate subcellular location. The exact mechanism of receptor transport in *C. elegans* vulval precursor cells is not known, but it is tempting to speculate that it closely parallels that discovered in mammalian neurons. If true, the overall conservation of the transport mechanism would lend credence to the notion that neurons and epithelial cells are topologically similar (Dotti and Simons, 1990). Elucidating the precise role of this heterotrimeric complex in mammals has been difficult, in part because multiple, possibly redundant homologs of each protein exist (Misawa et al., 2001). Remaining questions concerning this complex include the following. Why do cells make use of a trimeric complex instead of a single scaffold to mediate transport? What mediates assembly of the heterotrimeric complex? Most importantly, how is the assembly and targeting process regulated?

Conclusions

How does the unique structure and function of PDZ domains allow them to scaffold and organize signaling complexes? The structure and recognition mechanisms of PDZ domains are highly specialized for their diverse roles in transport, localization and assembly of multi-protein machines.

PDZ domains bind small C-terminal tags in a ‘non-invasive’ way. This allows them to bind practically any target protein, especially membrane proteins such as ion channels, which have very small free C-termini. They bind their ligands with modest affinities (Kd ~1 μM), which makes them appropriate for reversible and regulatable interactions. Recognition based on termini yields added specificity when compared with recognition of internal motifs, because there are far fewer protein termini in a cell than internal sequence motifs. Moreover, since PDZ domains can recognize select internal sequences, certain sets of PDZ domains can hetero-oligomerize in a head-to-tail fashion. PDZ-domain scaffolding proteins can therefore be used not only to bind an array of target proteins but also to crosslink one another into branched assemblies. All of these factors make PDZ-containing scaffolds well suited for assembly of signaling molecules into large supramolecular signaling complexes. Such PDZ-mediated assemblies appear to exist in *Drosophila* photoreceptor cells and mammalian neurons. Additionally, the predominance of PDZ domains in metazoans indicates that this highly specialized scaffolding module may have evolved in response to the increased signaling needs of multicellular organisms.

Although much is known about PDZ domains, several fundamental questions remain. For example, how specific are the 394 individual PDZ domains in the human genome? This is especially important in multivalent scaffolds that bind many different proteins. In addition, what is the nature of PDZ-mediated assemblies – do they have a specific composition or are they heterogeneous? Are they constitutive or dynamically regulated? Studies over the next few years should shed light on these and other critical issues.

We thank D. Bredt, P. Chien, A. McGee, R. Nicoll, E. Schnell and M. von Zastrow, as well as members of the Lim lab, for helpful discussions and critical reading of the manuscript.

References

Adamski, F., Zhu, M., Bahiraei, F. and Shieh, B. (1998). Interaction of eye protein kinase C and INAD in *Drosophila*. *J. Biol. Chem.* **273**, 11713-17719.

Bähner, M., Sander, P., Paulsen, R. and Huber, A. (2000). The visual G protein of fly photoreceptors interacts with the PDZ domain assembled

INAD signaling complex via direct binding of activated Gα(q) to phospholipase Cβ. *J. Biol. Chem.* **275**, 2901-2904.

Beebe, K. D., Shin, J., Peng, J., Chaudhury, C., Khera, J. and Pei, D. (2000). Substrate recognition through a PDZ domain in tail-specific protease. *Biochemistry* **39**, 3149-3155.

Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalov, I. N. and Bourne, P. E. (2000). The Protein Data Bank. *Nucl. Acids Res.* **28**, 235-242.

Bezprozvanny, I. and Maximov, A. (2001). PDZ Domains: more than just a glue. *Proc. Natl. Acad. Sci. USA* **98**, 787-789.

Borrell-Pages, M., Fernandez-Larrea, J., Borroto, A., Rojo, F., Baselga, J. and Arribas, J. (2000). The carboxy-terminal cysteine of the tetraspanin L6 antigen is required for its interaction with SITAC, a novel PDZ protein. *Mol. Biol. Cell* **12**, 4217-4225.

Borg, J. P., Straight, S. W., Kaech, S. M., de Taddéo-Borg, M., Kroon, D. E., Karnak, D., Turner, R. S., Kim, S. K. and Margolis, B. (1998). Identification of an evolutionarily conserved heterotrimeric protein complex involved in protein targeting. *J. Biol. Chem.* **273**, 31633-31636.

Bredt, D. S. (1998). Sorting out genes that regulate epithelial and neuronal polarity. *Cell* **94**, 691-694.

Brenman, J. E., Chao, D. S., Gee, S. H., McGee, A. W., Craven, S. E., Santillano, D. R., Wu, Z., Huang, F., Xia, H., Peters, M. F. et al. (1996). Interaction of nitric oxide synthase with the postsynaptic density protein PSD-95 and α1-syntrophin mediated by PDZ domains. *Cell* **84**, 757-767.

Butz, S., Okamoto, M. and Südhof, T. C. (1998). A tripartite protein complex with the potential to couple synaptic vesicle exocytosis to cell adhesion in brain. *Cell* **94**, 773-782.

***C. elegans* Sequencing Consortium.** (1998). Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* **282**, 2012-2018.

Cao, T. T., Deacon, H. W., Reczek, D., Bretscher, A. and von Zastrow, M. (1999). A kinase-regulated PDZ-domain interaction controls endocytic sorting of the β2-adrenergic receptor. *Nature* **401**, 286-290.

Chevesich, J., Kreuz, A. J. and Montell, C. (1997). Requirement for the PDZ domain protein, INAD, for localization of the TRP store-operated channel to a signaling complex. *Neuron* **18**, 95-105.

Cho, K. O., Hunt, C. A. and Kennedy, M. B. (1992). The rat brain postsynaptic density fraction contains a homolog of the *Drosophila* discs-large tumor suppressor protein. *Neuron* **9**, 929-942.

Christopherson, K. S., Hillier, B. J., Lim, W. A. and Bredt, D. S. (1999). PSD-95 assembles a ternary complex with the N-methyl-D-aspartic acid receptor and a bivalent neuronal NO synthase PDZ domain. *J. Biol. Chem.* **274**, 27467-27473.

Craven, S. E. and Bredt, D. S. (1998). PDZ proteins organize synaptic signaling pathways. *Cell* **93**, 495-498.

Daniels, D. L., Cohen, A. R., Anderson, J. M. and Brünger, A. T. (1998). Crystal structure of the hCASK PDZ domain reveals the structural basis of class II PDZ domain target recognition. *Nat. Struct. Biol.* **5**, 317-325.

Dong, H., O'Brien, R. J., Fung, E. T., Lanahan, A. A., Worley, P. F. and Huganir, R. L. (1997). GRIP: a synaptic PDZ domain-containing protein that interacts with AMPA receptors (see comments). *Nature* **386**, 279-284.

Dotti, C. G. and Simons, K. (1990). Polarized sorting of viral glycoproteins to the axon and dendrites of hippocampal neurons in culture. *Cell* **62**, 63-72.

Doyle, D. A., Lee, A., Lewis, J., Kim, E., Sheng, M. and MacKinnon, R. (1996). Crystal structures of a complexed and peptide-free membrane protein-binding domain: molecular basis of peptide recognition by PDZ. *Cell* **85**, 1067-1076.

El-Husseini, A. E., Schnell, E., Chetkovich, D. M., Nicoll, R. A. and Bredt, D. S. (2000). PSD-95 involvement in maturation of excitatory synapses. *Science* **290**, 1364-1368.

Fanning, A. S. and Anderson, J. M. (1998). PDZ domains and the formation of protein networks at the plasma membrane. *Curr. Top. Microbiol. Immunol.* **228**, 209-233.

Fanning, A. S. and Anderson, J. M. (1999). Protein modules as organizers of membrane structure. *Curr. Opin. Cell Biol.* **11**, 432-439.

Fouassier, L., Yun, C. C., Fitz, J. G. and Doctor, R. B. (2000). Evidence for ezrin-radixin-moesin-binding phosphoprotein 50 (EBP50) self-association through PDZ-PDZ interactions. *J. Biol. Chem.* **275**, 25039-25045.

Fuh, G., Pisabarro, M. T., Li, Y., Quan, C., Lasky, L. A. and Sidhu, S. S. (2000). Analysis of PDZ domain-ligand interactions using carboxyl-terminal phage display. *J. Biol. Chem.* **275**, 21486-21491.

Gee, S. H., Sekely, S. A., Lombardo, C., Kurakin, A., Froehner, S. C. and Kay, B. K. (1998). Cyclic peptides as non-carboxyl-terminal ligands of syntrophin PDZ domains. *J. Biol. Chem.* **273**, 21980-21987.

Gomperts, S. N. (1996). Clustering membrane proteins: it's all coming together with the PSD-95/SAP90 protein family. *Cell* **84**, 659-662.

Hall, R. A., Ostedgaard, L. S., Premont, R. T., Blitzer, J. T., Rahman, N., Welsh, M. J. and Lefkowitz, R. J. (1998a). A C-terminal motif found in the β2-adrenergic receptor, P2Y1 receptor and cystic fibrosis transmembrane conductance regulator determines binding to the Na^+/H^+ exchanger regulatory factor family of PDZ proteins. *Proc. Natl. Acad. Sci. USA* **95**, 8496-8501.

Hall, R. A., Premont, R. T., Chow, C. W., Blitzer, J. T., Pitcher, J. A., Claing, A., Stoffel, R. H., Barak, L. S., Shenolikar, S., Weinman, E. J., Grinstein, S. and Lefkowitz, R. J. (1998b). The β2-adrenergic receptor interacts with the Na^+/H^+-exchanger regulatory factor to control Na^+/H^+ exchange. *Nature* **392**, 626-630.

Harris, B. Z., Hillier, B. J. and Lim, W. A. (2001). Energetic determinants of internal motif recognition by PDZ domains. *Biochemistry* **40**, 5921-5930.

Harrison, S. C. (1996). Peptide-surface association: the case of PDZ and PTB domains. *Cell* **86**, 341-343.

Hillier, B. J., Christopherson, K. S., Prehoda, K. E., Bredt, D. S. and Lim, W. A. (1999). Unexpected modes of PDZ domain scaffolding revealed by structure of nNOS-syntrophin complex. *Science* **284**, 812-815.

Huber, A., Sander, P., Gobert, A., Bähner, M., Hermann, R. and Paulsen, R. (1996). The transient receptor potential protein (Trp), a putative store-operated Ca^{2+} channel essential for phosphoinositide-mediated photoreception, forms a signaling complex with NorpA, InaC and InaD. *EMBO J.* **15**, 7036-7045.

Huber, A., Sander, P., Bähner, M. and Paulsen, R. (1998). The TRP Ca^{2+} channel assembled in a signaling complex by the PDZ domain protein INAD is phosphorylated through the interaction with protein kinase C (ePKC). *FEBS Lett.* **425**, 317-322.

International Human Genome Sequencing Consortium (2001). Initial Sequencing and Analysis of the human genome. *Nature* **409**, 860-921.

Jo, K., Derin, R., Li, M. and Bredt, D. S. (1999). Characterization of MALS/Velis-1, -2, and -3: a family of mammalian LIN-7 homologs enriched at brain synapses in association with the postsynaptic density-95/NMDA receptor postsynaptic complex. *J. Neurosci.* **19**, 4189-4199.

Kaech, S. M., Whitfield, C. W. and Kim, S. K. (1998). The LIN-2/LIN-7/LIN-10 complex mediates basolateral membrane localization of the *C. elegans* EGF receptor LET-23 in vulval epithelial cells. *Cell* **94**, 761-771.

Karthikeyan, S., Leung, T., Birrane, G., Webster, G. and Ladias, J. A. (2001a). Crystal structure of the PDZ1 domain of human Na^+/H^+ exchanger regulatory factor provides insights into the mechanism of carboxyl-terminal leucine recognition by class I PDZ domains. *J. Mol. Biol.* **308**, 963-973.

Karthikeyan, S., Leung, T. and Ladias, J. (2001b) Structural basis of the Na^+/H^+ exchanger regulatory factor PDZ1 interaction with the carboxyl-terminal region of the cystic fibrosis transmembrane conductance regulator. *J. Biol. Chem.* **276**, 19683-19686.

Kennedy, M. B. (2000). Signal-processing machines at the postsynaptic density. *Science* **290**, 750-754.

Kim, E., Niethammer, M., Rothschild, A., Jan, Y. N. and Sheng, M. (1995). Clustering of Shaker-type K^+ channels by interaction with a family of membrane-associated guanylate kinases. *Nature* **378**, 85-88.

Kim, E., Cho, K. O., Rothschild, A. and Sheng, M. (1996). Heteromultimerization and NMDA receptor-clustering activity of Chapsyn-110, a member of the PSD-95 family of proteins. *Neuron* **17**, 103-113.

Kistner, U., Wenzel, B. M., Veh, R. W., Cases-Langhoff, C., Garner, A. M., Appeltauer, U., Voss, B., Gundelfinger, E. D. and Garner, C. C. (1993). SAP90, a rat presynaptic protein related to the product of the *Drosophila* tumor suppressor gene *dlg-A*. *J. Biol. Chem.* **268**, 4580-4583.

Kornau, H. C., Schenker, L. T., Kennedy, M. B. and Seeburg, P. H. (1995). Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. *Science* **269**, 1737-1740.

Kozlov, G., Gehring, K. and Ekiel, I. (2000). Solution structure of the PDZ2 domain from human phosphatase hPTP1E and its interactions with C-terminal peptides from the Fas receptor. *Biochemistry* **39**, 2572-2580.

Lamprecht, G., Weinman, E. J. and Yun, C. H. (1998). The role of NHERF and E3KARP in the cAMP-mediated inhibition of NHE3. *J. Biol. Chem.* **273**, 29972-29978.

Lau, A. G. and Hall, R. A. (2001). Oligomerization of nherf-1 and nherf-2 pdz domains: differential regulation by association with receptor carboxyl-termini and by phosphorylation. *Biochemistry* **40**, 8572-8580.

Lefkowitz, R. J. (1998). G protein-coupled receptors III. New roles for

receptor kinases and β-arrestins in receptor signaling and desensitization. *J. Biol. Chem.* **273**, 18677-18680.

Li, H. S. and Montell, C. (2000). TRP and the PDZ Protein, INAD, form the core complex required for retention of the signalplex in *Drosophila* photoreceptor cells. *J. Cell Biol.* **150**, 1411-1421.

Liao, D. I., Qian, J., Chisholm, D. A., Jordan, D. B. and Diner, B. A. (2000). Crystal structures of the photosystem II D1 C-terminal processing protease. *Nat. Struct. Biol.* **7**, 749-753.

Lin, D., Edwards, A. A., Fawcett, J. P., Mbamalu, G., Scott, J. D., Pawson, T. (2000) A mammalian PAR-3-PAR-6 complex implicated in Cdc42/Rac1 and aPKC signalling and cell polarity. *Nat. Cell Biol.* **2**, 540-547.

Marfatia, S. M., Byron, O., Campbell, G., Liu, S. C. and Chishti, A. H. (2000). Human homologue of the *Drosophila* discs large tumor suppressor protein forms an oligomer in solution. Identification of the self-association site. *J. Biol. Chem.* **275**, 13759-13770.

Maximov, A., Sudhof, T. C. and Bezprozvanny, I. (1999). *J. Biol. Chem.* Association of neuronal calcium channels with modular adaptor proteins. *J. Biol. Chem.* **274**, 24453-24456.

Migaud, M., Charlesworth, P., Dempster, M., Webster, L. C., Watabe, A. M., Makhinson, M., He, Y., Ramsay, M. F., Morris, R. G., Morrison, J. H. et al. (1998). Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. *Nature* **396**, 433-439.

Misawa, H., Kawasaki, Y., Mellor, J., Sweeney, N., Jo, K., Nicoll, R. A. and Bredt, D. S. (2001). Contrasting localizations of MALS/LIN-7 PDZ proteins in brain and molecular compensation in knockout mice. *J. Biol. Chem.* **276**, 9264-9272.

Morais Cabral, J. H., Petrosa, C., Sutcliffe, M. J., Raza, S., Byron, O., Poy, F., Marfatia, S. M., Chishti, A. H. and Liddington, R. C. (1996). Crystal structure of a PDZ domain. *Nature* **382**, 649-652.

Mühlhahn, P., Zweckstetter, M., Georgescu, J., Ciosto, C., Renner, C., Lanzendörfer, M., Lang, K., Ambrosius, D., Baier, M., Kurth, R. et al. (1998). Structure of interleukin 16 resembles a PDZ domain with an occluded peptide binding site. *Nat. Struct. Biol.* **5**, 682-686.

Müller, B. M., Kistner, U., Kindler, S., Chung, W. J., Kuhlendahl, S., Fenster, S. D., Lau, L. F., Veh, R. W., Huganir, R. L., Gundelfinger, E. D. et al. (1996). SAP102, a novel postsynaptic protein that interacts with NMDA receptor complexes in vivo. *Neuron* **17**, 255-265.

Myszka, D. G. (1999a). Improving biosensor analysis. *J. Mol. Recog.* **12**, 279-284.

Myszka, D. G. (1999b). Survey of the 1998 optical biosensor literature. *J. Mol. Recog.* **12**, 390-408.

Naisbitt, S., Kim, E., Tu, J. C., Xiao, B., Sala, C., Valtschanoff, J., Weinberg, R. J., Worley, P. F. and Sheng, M. (1999). Shank, a novel family of postsynaptic density proteins that binds to the NMDA receptor/PSD-95/GKAP complex and cortactin. *Neuron* **23**, 569-582.

Nguyen, J. T., Turck, C. W., Cohen, F. E., Zuckerman, R. N. and Lim, W. A. (1998) Exploiting the basis of proline recognition by SH3 and WW domains: design of N-substituted inhibitors. *Science* **282**, 2088-2092.

Niethammer, M., Kim, E. and Sheng, M. (1996). Interaction between the C terminus of NMDA receptor subunits and multiple members of the PSD-95 family of membrane-associated guanylate kinases. *J. Neurosci.* **16**, 2157-2163.

Niethammer, M., Valtschanoff, J. G., Kapoor, T. M., Allison, D. W., Weinberg, T. M., Craig, A. M. and Sheng, M. (1998). CRIPT, a novel postsynaptic protein that binds to the third PDZ domain of PSD-95/SAP90. *Neuron* **20**, 693-707.

Pallen, M. J. and Ponting, C. P. (1997). PDZ domains in bacterial proteins (letter). *Mol. Microbiol.* **26**, 411-413.

Passafaro, M., Sala, C., Niethammer, M. and Sheng, M. (1999). Microtubule binding by CRIPT and its potential role in the synaptic clustering of PSD-95. *Nat. Neurosci.* **2**, 1063-1069.

Pippig, S., Andexinger, S. and Lohse, M. J. (1995). Sequestration and recycling of β2-adrenergic receptors permit receptor resensitization. *Mol. Pharmacol.* **47**, 666-676.

Ponting, C. P. (1997). Evidence for PDZ domains in bacteria, yeast, and plants. *Protein Sci.* **6**, 464-468.

Raghuram, V., Mak, D. D. and Fosket, J. K. (2001). Regulation of cystic fibrosis transmembrane conductance regulator single-channel gating by bivalent PDZ-domain-mediated interaction. *Proc. Natl. Acad. Sci. USA* **98**, 1300-1305.

Ranganathan, R. and Ross, E. M. (1997). PDZ domain proteins: scaffolds for signaling complexes. *Curr. Biol.* **7**, R770-773.

Reczek, D. and Bretscher, A. (2001). Identification of EPI64, a TBC/rabGAP domain-containing microvillar protein that binds to the first PDZ domain of EBP50 and E3KARP. *J. Cell Biol.* **153**, 191-205.

Rongo, C., Whitfield, C. W., Rodal, A., Kim, S. K. and Kaplan, J. M. (1998). LIN-10 is a shared component of the polarized protein localization pathways in neurons and epithelia. *Cell* **94**, 751-759.

Schneider, S., Buchert, M., Georgiev, O., Catimel, B., Halford, M., Stacker, S. A., Baechi, T., Moelling, K. and Hovens, C. M. (1999). Mutagenesis and selection of PDZ domains that bind new protein targets (see comments). *Nat. Biotechnol.* **17**, 170-175.

Schultz, J., Hoffmüller, U., Krause, G., Ashurst, J., Macias, M. J., Schmieder, P., Schneider-Mergener, J. and Oschkinat, H. (1998a). Specific interactions between the syntrophin PDZ domain and voltage-gated sodium channels. *Nat. Struct. Biol.* **5**, 19-24.

Schultz, J., Milpetz, F., Bork, P. and Ponting, C. P. (1998b). SMART, a simple modular architecture research tool: identification of signaling domains. *Proc. Natl. Acad. Sci. USA* **95**, 5857-5864.

Schultz, J., Copley, R. R., Doerks, T., Ponting, C. P. and Bork, P. (2000). SMART: a web-based tool for the study of genetically mobile domains. *Nucl. Acids Res.* **28**, 231-234.

Scott, K. and Zuker, C. S. (1998). Assembly of the Drosophila phototransduction cascade into a signalling complex shapes elementary responses. *Nature* **395**, 805-808

Setou, M., Nakagawa, T., Seog, D. H. and Hirokawa, N. (2000). Kinesin superfamily motor protein KIF17 and mLin-10 in NMDA receptor-containing vesicle transport. *Science* **288**, 1796-1802.

Sheng, M. (2001). Molecular organization of the postsynaptic specialization. *Proc. Natl. Acad. Sci. USA* **98**, 7058-7061.

Sheng, M. and Kim, E. (2000). The Shank family of scaffold proteins. *J. Cell Sci.* **113**, 1851-1856.

Shenolikar, S. and Weinman, E. J. (2001) NHERF: targeting and trafficking membrane proteins. *Am. J. Physiol. Renal Physiol.* **280**, F389-F395.

Shieh, B. H. and Zhu, M. Y. (1996). Regulation of the TRP Ca^{2+} channel by INAD in *Drosophila* photoreceptors. *Neuron* **16**, 991-998.

Simske, J. S., Kaech, S. M., Harp, S. A. and Kim, S. K. (1996). LET-23 receptor localization by the cell junction protein LIN-7 during *C. elegans* vulval induction. *Cell* **85**, 195-204.

Songyang, Z., Fanning, A. S., Fu, C., Xu, J., Marfatia, S. M., Chishti, A. H., Crompton, A., Chan, A. C., Anderson, J. M. and Cantley, L. C. (1997). Recognition of unique carboxyl-terminal motifs by distinct PDZ domains. *Science* **275**, 73-77.

Srivastava, S., Osten, P., Vilim, F. S., Khatri, L., Inman, G., States, B., Daly, C., DeSouza, S., Abagyan, R., Valtschanoff, J. G. et al. (1998). Novel anchorage of GluR2/3 to the postsynaptic density by the AMPA receptor-binding protein ABP. *Neuron* **21**, 581-591.

Straight, S., Chen, L., Karnak, D. and Margolis, B. (2001). Interaction with mLin-7 alters the targeting of endocytosed transmembrane proteins in mammalian epithelial cells. *Mol. Biol. Cell* **12**, 1329-1340.

Straight, S. W., Karnak, D., Borg, J. P., Kamberov, E., Dare, H., Margolis, B. and Wade, J. B. (2000). mLin-7 is localized to the basolateral surface of renal epithelia via its NH_2 terminus. *Am. J. Physiol. Renal Physiol.* **278**, F464-475.

Stricker, N. L., Christopherson, K. S., Yi, B. A., Schatz, P. J., Raab, R. W., Dawes, G., Bassett, D. E., Jr, Bredt, D. S. and Li, M. (1997). PDZ domain of neuronal nitric oxide synthase recognizes novel C-terminal peptide sequences. *Nat. Biotechnol.* **15**, 336-342.

Tochio, H., Mok, Y. K., Zhang, Q., Kan, H. M., Bredt, D. S. and Zhang, M. (2000). Formation of nNOS/PSD-95 PDZ dimer requires a preformed β-finger structure from the nNOS PDZ domain. *J. Mol. Biol.* **6**, 359-370.

Tochio, H., Zhang, Q., Mandal, P., Li, M. and Zhang, M. (1999). Solution structure of the extended neuronal nitric oxide synthase PDZ domain complexed with an associated peptide. *Nat. Struct. Biol.* **6**, 417-421.

Tsao, P., Cao, T. and von Zastrow, M. (2001) Role of endocytosis in mediating downregulation of G-protein-coupled receptors. *Trends Pharmacol. Sci.* **22**, 91-96.

Tsunoda, S. and Zuker, C. S. (1999). The organization of INAD-signaling complexes by a multivalent PDZ domain protein in *Drosophila* photoreceptor cells ensures sensitivity and speed of signaling. *Cell Calcium* **26**, 165-171.

Tsunoda, S., Sierralta, J., Sun, Y., Bodner, R., Suzuki, E., Becker, A., Socolich, M. and Zuker, C. S. (1997). A multivalent PDZ-domain protein assembles signalling complexes in a G-protein-coupled cascade. *Nature* **388**, 243-249.

Tsunoda, S., Sun, Y., Suzuki, E. and Zuker, C. (2001). Independent

anchoring and assembly mechanisms of INAD signaling complexes in *Drosophila* photoreceptors. *J. Neurosci.* **21**, 150-158.

Tu, J. C., Xiao, B., Naisbitt, S., Yuan, J. P., Petralia, R. S., Brakeman, P., Doan, A., Aakalu, V. K., Lanahan, A. A., Sheng, M. et al. (1999). Coupling of mGluR/Homer and PSD-95 complexes by the Shank family of postsynaptic density proteins. *Neuron* **23**, 583-592.

van Huizen, R., Miller, K., Chen, D. M., Li, Y., Lai, Z. C., Raab, R., Stark, W., Shortridge, R. D. and Li, M. (1998). Two distantly positioned PDZ domains mediate multivalent INAD-phospholipase C interactions essential for G protein-coupled signaling. *EMBO J.* **17**, 2285-2297.

Venter, J. C., Adams, M. D., Myers, E. W., Li, P. W., Mural, R. J., Sutton, G. G., Smith, H. O., Yandell, M., Evans, C. A., Holt, R. A. et al. (2001). The sequence of the human genome. *Science* **291**, 1304-1351.

Wang, S., Yue, H., Derin, R. B., Guggino, W. B. and Li, M. (2000). Accessory protein facilitated CFTR-CFTR interaction, a molecular mechanism to potentiate the chloride channel activity. *Cell* **103**, 169-179.

Webster, G., Leung, T., Larthikeyan, S., Birrane, G. and Ladias, J. (2001). Crystallographic characterization of the PDZ1 domain of the human Na^+/H^+ exchanger regulatory factor. *Acta Crystallogr.* **57**, 714-716.

Weinman, E. J., Steplock, D., Donowitz, M. and Shenolikar, S. (2000). NHERF associations with sodium-hydrogen exchanger isoform 3 (NHE3) and ezrin are essential for cAMP-mediated phosphorylation and inhibition of NHE3. *Biochemistry* **39**, 6123-6129.

Woods, D. F. and Bryant, P. J. (1993). Zo-1, DlgA and PSD-95/SAP90: homologous proteins in tight, septate and synaptic cell junctions. *Mech. Dev.* **44**, 889.

Wyszynski, M., Valtschanoff, J. G., Naisbitt, S., Dunah, A. W., Kim, E., Standaert, D. G., Weinberg, R. and Sheng, M. (1999). Association of AMPA receptors with a subset of glutamate receptor-interacting protein in vivo. *J. Neurosci.* **19**, 6528-6537.

Xu, X. Z., Choudhury, A., Li, X. and Montell, C. (1998). Coordination of an array of signaling proteins through homo- and heteromeric interactions between PDZ domains and target proteins. *J. Cell Biol.* **142**, 545-555.

Zagulski, M., Herbert, C. J. and Rytka, J. (1998). Sequencing and functional analysis of the yeast genome. *Acta Biochim. Pol.* **45**, 627-643.