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Organization of Signaling Complexes by PDZ-Domain Scaffold Proteins

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ABSTRACT

Transduction of biological signals from receptors at the plasma membrane to their targets in cytoplasm and nucleus relies on specific protein−protein interactions. A common strategy used by cells is to organize proteins in the same signaling cascade into large molecular weight, multiprotein complexes. PDZ domain proteins have been shown to play important roles in assembling various signaling complexes. Here, we first present biophysical basis of the advantages of organizing proteins in a signaling cascade into a clustered multiprotein complex. We then discuss the structure, ligand binding, and function of PDZ domains in organizing synaptic signaling complexes.

Introduction

A biological signal transduction process is in many ways analogous to signal transmission in an integrated electronic circuit, which generally involves the following steps: signal initiation, reception, amplification, readout, and signal termination. Rather than relying on various electronic components, a biological signaling pathway is composed of various protein molecules that are capable of binding to each other in a specific and often reversible manner. Biological signals normally arise from the external milieu of a cell in the form of hormones or protein ligands or extracelluar matrix. These signaling molecules (often in very low concentrations) exert their effects by binding to receptors displayed on the surface of the cells. These signals are then amplified by enzymes in the signaling cascade before reaching the final cues. Finally, a signal transduction process is completed with the termination of the signal so that the cell can reset itself for next round of signaling events. Tremendous research efforts over the past two decades have culminated in establishing following principles: (1) various protein molecules in a signaling cascade are often connected by modular protein−protein interaction domains; (2) signaling proteins are usually associated with each other to form very large molecular weight complexes that are loosely termed as signaling complexes, signalsomes, or transducisomes; and (3) rather than freely diffusing in cytoplasm, signaling complexes are often attached to cell membranes via transmembrane receptors and/or other membrane-associated proteins.

In this Account, we first present the biophysical advantages of assembling signaling components into large molecular weight complexes. We then use PDZ domain proteins as examples to illustrate how cells use such multimodular proteins as scaffolds in organizing synaptic signaling complexes.

Biophysical Advantages of Signaling Complexes

Once various components in a signaling pathway are organized into large molecular weight, multiprotein clusters in cells, the chemical reactions occurring inside the clusters differ considerably from the reactions taking place in freely diffusible environments. The general advantages of cellular signaling complexes are as follows:

(i) Enhancing Signaling Efficiency. In an assembled signaling complex, protein molecules in the same pathway are juxtaposed. A reaction product from an upstream signaling protein can be directly channeled to downstream protein as a reactant without equilibrating with the bulk solution (Figure 1). This protein−protein interaction-mediated channeling mechanism is a well recognized phenomenon in many metabolic pathways including amino acid and nucleic acid syntheses, lipid metabolism and tricarboxylic acid cycle.[1] The channeling of reaction intermediates in a signaling reaction cascade can provide kinetic benefits by increasing the effective concentration of the substrate and protecting labile intermediates from unproductive reactions. The end result of such spatially coupled reactions is enhanced signaling speed, which is often critical for cells to function properly.

(ii) Ensuring Signaling Specificity. A common and difficult task faced by almost every cell is the conversion of each of extracellular signals into intracellular responses that should retain the proper specificity of signaling. Rather than acting as a watery bag filled with protein soup, a living cell contains many compartments with distinct biological functions. For example, neurons contain specialized cellular compartments such as the cell soma, dendrites, and axons. Signaling complexes comprising receptors and downstream machineries are often localized to specific membrane regions of different cellular compartments. Since many receptors often share a common pool of downstream signaling proteins, organization of proteins in a specific signaling pathway into a spatially enclosed complex should insulate pathways from cross-

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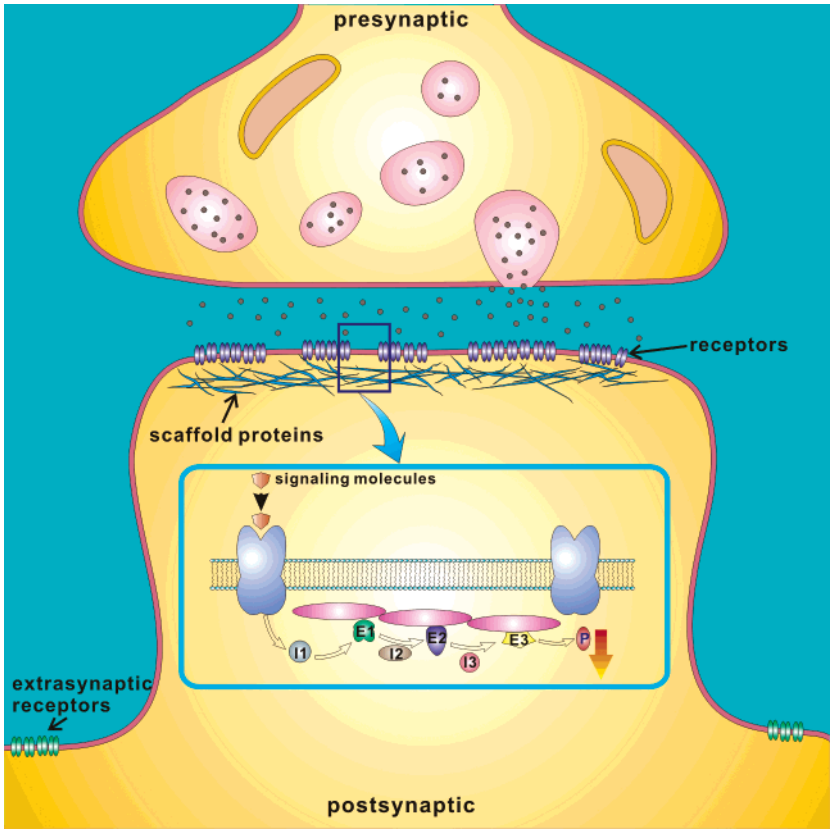

FIGURE 1. Schematic diagram showing receptor-mediated signal transduction in a synapse. In this diagram, signaling molecules (black dots) are released from the presynaptic neuron and bind to receptors on the postsynaptic membrane. The receptors and the downstream signaling proteins are clustered in high concentration at the postsynaptic membrane specialization by scaffold proteins. The insert shows a magnified view of the receptor signaling complex organization by the scaffold proteins. In this drawing, we show the substrate (intermediate products I1, I2, and I3) channeling from the receptor to the enzymes (E1, E2, and E3) in the reaction cascade. Due to the membrane localization of the complex, the final product ("P") displays a concentration gradient from the membrane to the deeper side of cytoplasm (block arrow). The diagram also illustrates that the receptors located outside the synapse will not be activated by the neurotransmitters released from presynaptic neurons.

talking with unwanted signaling pathways and allow the system to ignore sporadic signals generated in certain regions of a cell. For example, protein kinases such as protein kinase C are used by many receptors. If substrates in different signaling pathways are not properly segregated away from these common kinases, the initial signal received by a particular receptor would, in principle, leak to all the substrates. In addition to selectively activating downstream proteins in the cytoplasm, targeting the signaling complexes to specific regions of the cell membrane allow the system to response selectively to extracellular signals at discrete locations. For example, localization of a signaling complex (e.g., a glutamate receptor complex) to the postsynaptic membrane would allow receptors only to be activated by ligands released from the presynaptic terminus. Signals originated from other locations (e.g., extrasynaptic signals) would not activate the signaling pathway localized at synapses (Figure 1).

A spatially anchored enzyme is expected produce a concentration gradient of its catalytic product at the specific region of a cell (Figure 1). Mounting experimental evidence has demonstrated that it is the concentration gradient, rather than the absolute concentration, that is important for proper function of many biological signals.[2,3] For example, concentration gradients of morphogens determine the patterning of cells during development.[3]

(iii) Increasing Signaling Sensitivity by Oligomerization of Signaling Complexes. Numerous experiments have indicated that the assembled signaling complex can further multimerize into very large molecular weight clusters.[4,5] Theoretical calculations have predicted that clustered receptor systems have greatly enhanced sensitivity (by several orders of magnitude) to activating signals when compared with unclustered receptors. Equally important, a clustered receptor system has a much wider dynamic range (i.e., receptors can detect relative concentration changes of ligands over a much wider range) than an unclustered system can.[6]

Large molecular weight clusters of signaling complexes also impose special requirements on cells. Any defects in assembling a particular signaling component could seriously compromise the function of a signaling cascade. For example, phototransduction signaling proteins (protein kinase C, ion channel TRP, phospholipase C, and calmodulin) in *Drosophila* are assembled by a multi-PDZ scaffold protein, InaD, in the rhabdomeres of photoreceptor cells. Mutation of *InaD* leads to random distributions of protein kinase C, TRP, and phospholipase C, and

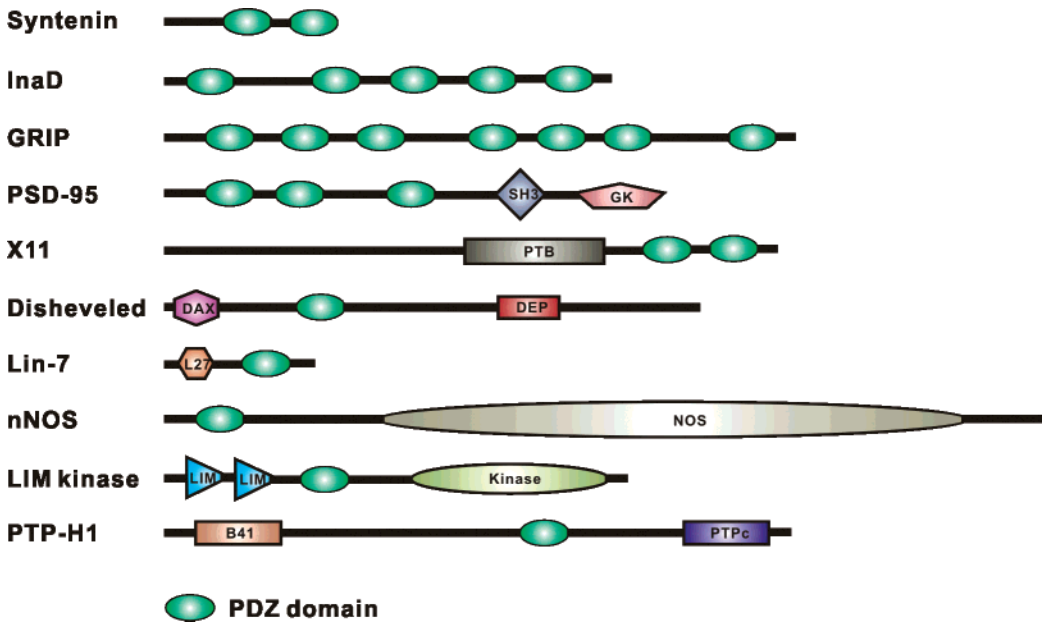

FIGURE 2. Modular architecture of some PDZ-domain proteins. The detected protein domains (as defined by SMART[57]) are indicated by their names. The abbreviated domain names are as follows: SH3, Src-homology 3 domain; GK, guanylate-kinase-like domain; L27, Lin-2 and Lin-7 domain; PTB, protein tyrosine-binding domain; LIM, zinc-binding domain present in Lin-11, Isl-1, and Mec-3; DAX, dishevelled and axin-homology domain; DEP, dishevelled, Egl-10, and pleckstrin-homology domain; NOS, nitric oxide synthase; B41, Band 4.1 homology domain; PTPc, protein tyrosine phosphatase catalytic domain.

the mutant flies are completely blind.[7] In addition, macromolecular signaling complexes are usually too bulky to diffuse freely to specific regions of cells. Instead, these complexes usually require molecular motors for active transport along microtubules or actin tracks (see ref. 8 for an example). In the rest of this Account, we use PDZ domain scaffold proteins as examples to illustrate how cellular signal transduction complexes are organized.

Discovery of PDZ Domains

PDZ domains were originally identified as ~90 amino acid residue repeats of unknown function in several structurally related proteins, including the postsynaptic density-95 (PSD-95), the *Drosophilia* tumor suppressor protein disks-large-1 (DLG), the tight junction protein zonula occludentes 1 (ZO-1), and the human erythrocyte membrane cytoskeletal protein p55.[9−11] At the time of the discovery, the domain was named the "GLGF repeat" after a signature Gly-Leu-Gly-Phe sequence in the domain.[9] It was also referred to as DHR for the DLG homology repeat.[12] The unified domain name is derived from the three founding members of PDZ-domain proteins: *P*SD-95, *D*LG, and *Z*O-1.[13] Today, we know that PDZ domains are among the most abundant protein domains in multicellular eukaryotic genomes. Analysis of the genome sequences of *Caenorhabditis elegans*, *Drosophila*, and human estimates the presence of 89, 128, and 320 PDZ domain containing proteins, respectively. The number of PDZ domains is significantly larger in each genome, as many PDZ proteins contain multiple PDZ domains (Figure 2). PDZ domains can coexist with a wide variety of modular signaling domains. A large number of PDZ proteins are pure scaffold proteins, as they do not contain intrinsic enzymatic activities, and some PDZ proteins contain recognizable catalytic domains (Figure 2).

Discovery of the binding of the very carboxyl terminal tails of Shaker-type K^+ channels[14] and NMDA receptor NR2 subunits[15,16] to the PSD-95 PDZ domains demonstrated the function of PDZ domains as protein−protein interaction modules. These pioneering studies also established the most common function of PDZ domains as protein interaction modules that recognize a short stretch of amino acid residues (<10 amino acids) at the carboxyl termini of target proteins.[17−19] Recent studies have demonstrated other modes of PDZ domain-mediated protein interactions. For example, PDZ domains can interact with internal peptide sequences that adopt a β-hairpin structure.[20−22] Interaction of the seventh PDZ domain of glutamate receptor interacting protein (GRIP) with its target, GRASP1, is mediated via an entirely different mechanism.[23] A study by Zimmermann et al. raised the provocative idea that PDZ domains can function as lipid-binding modules.[24]

PDZ Domain Structure and Ligand Binding

The structures of a number of PDZ domains alone, or in complex with their respective ligands, have been solved. A canonical PDZ domain contains six β-strands (βA to βF) and two α-helices (αA and αB). The six β-strands form a partially opened barrel and the opening sides of the barrel are each capped with an α-helix (Figure 3A). As a signature feature of modular signaling domains, the amino- and carboxyl-termini of PDZ domains are spatially close to each other. Carboxyl peptide ligands bind to an extended groove formed by the βB strand and the αB helix of PDZ domains by augmenting the βB strand in an antiparallel

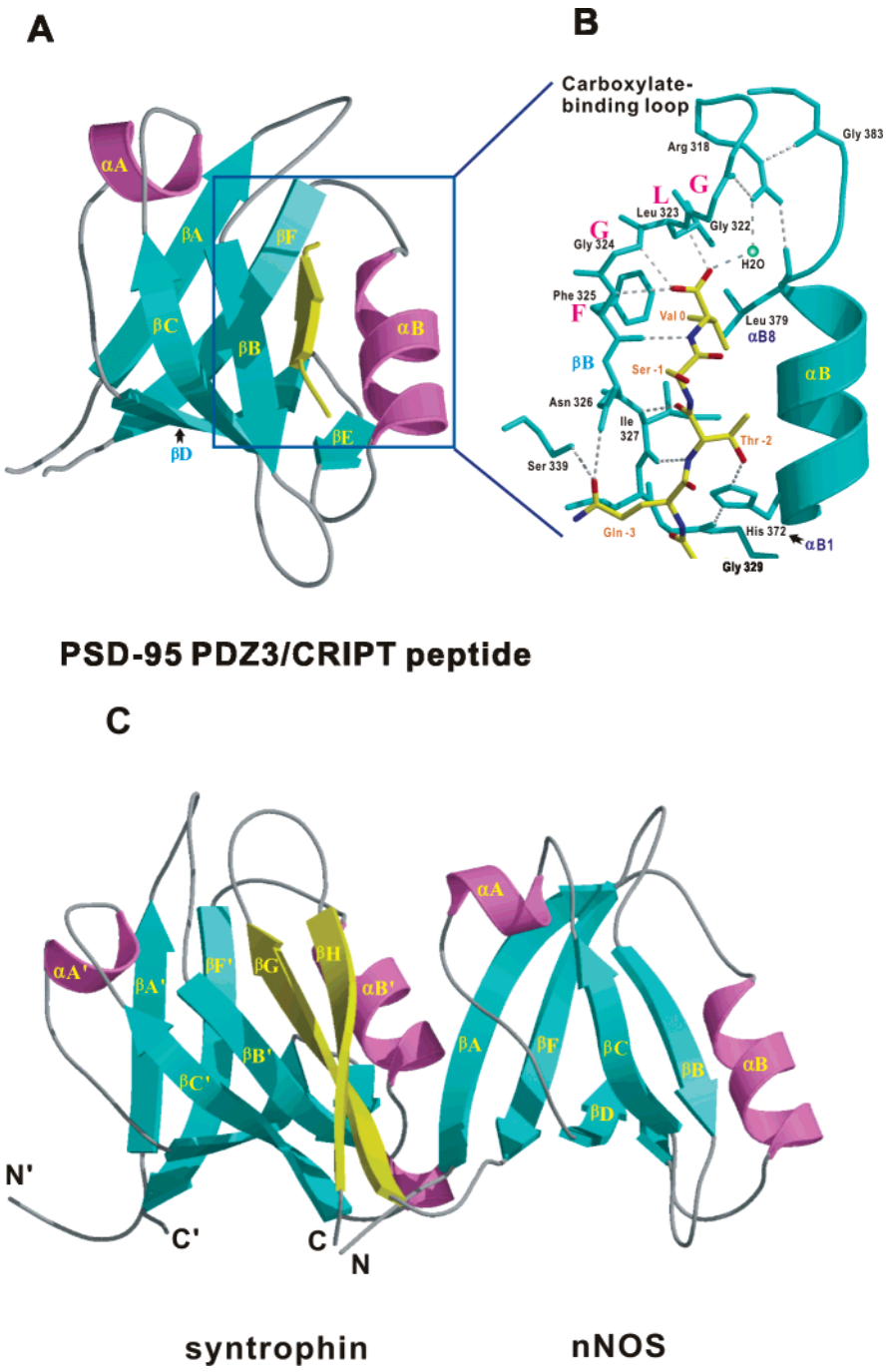

FIGURE 3. The structure and ligand binding of PDZ domains. (A) Ribbon diagram drawing showing the crystal structure of the third PDZ domain of PSD-95 in complex with a target peptide (in yellow).[25] (B) Detailed view of the interaction between the peptide ligand (in yellow stick model) and the αB/βB groove of the PSD-95 PDZ3. The oxygen, nitrogen, and carbon atoms of the peptide are shown in red, blue, and yellow, respectively. A bridging water molecule is drawn using a green sphere. The hydrogen bonds are indicated using gray dashed lines. (C) The 3D structure of the syntrophin/nNOS PDZ dimer showing the binding of a PDZ domain to an internal peptide sequence.[20]

fashion (Figure 3A). Binding of a peptide ligand induces little conformational changes to a PDZ domain.[25]

Extensive structural and peptide library screening studies in the past few years have established general principles of ligand-binding specificities for PDZ domains.[25,27−29]

Carboxylate Binding. The carboxylate of peptide ligands binds to the so-called "carboxylate-binding loop" located at one end of the αB/βB groove (Figure 3B). The carboxylate-binding loop of PDZ domains contains the well-conserved sequence motif K/R-X-X-X-G-Ψ-G-Ψ (the GLGF-motif in originally identified PDZ domains, Ψ is a hydrophobic residue). The second Gly is absolutely conserved; the first Gly can be replaced by small hydrophilic residues in some PDZ domains. The carboxylate of a peptide ligand forms hydrogen bonds with the main chain amides of the last three residues in the GLGF motif. The negatively charged carboxylate is further stabilized by the Arg or Lys at the beginning of the loop. Backbone dynamic studies of PSD-95 PDZ domains showed that the αB/βB groove, as well as the carboxylate-binding loop, has

limited flexibility in the absence of peptide ligands, suggesting that PDZ domains have preformed ligand-binding grooves.[30]

Recognition of Side Chains of Peptide Ligands. Due to the formation of antiparallel β-sheet with the βB-strand of the PDZ domain, the side chains of the residues at the 0 and -2 positions point directly to the base of the αB/βB groove, and the side chains at the -1 and -3 positions face the surface of the protein and are solvent accessible (Figure 3B). The hydrophobic side chain of the 0 position residue sites in a pocket formed by several highly conserved hydrophobic residues from the carboxylate-binding loop (both hydrophobic residues) and the αB helix (αB5 and αB8). The side chain at the -2 position is in direct contact with the side chain of the first residue in the αB helix (αB1), and this contact is almost the sole determinant of the specificity for the -2 residue. For example, the αB1 residue in the class I PDZ domains is a His, and this class of PDZ domains specifically recognize a Ser/Thr residue in the -2 position of the peptide ligands. This specificity originates from a strong hydrogen bond formed between the N-3 nitrogen of the His and the hydroxyl group of Ser/Thr from the peptide. In the nNOS PDZ domain, the αB1 residue is a Tyr, and the domain recognizes an Asp or Glu in the peptide ligands.[21,31] Mutation of the αB1 Tyr to a His changes the -2 residue in the corresponding peptide into a Ser or Thr.[31] The interactions of the side chains at the -1 and -3 positions with the PDZ domains are generally unique to individual PDZ domains. These structural features predict that the amino acids at the 0 and -2 positions play dominant roles in binding to PDZ domains, whereas residues at the -1 and -3 positions and possibly further upstream residues are expected to play supplementary roles in fine-tuning the binding specificity as well as the affinity to PDZ domains. A number of peptide library screening studies have confirmed the general peptide ligand selection principles of PDZ domains deduced from structural studies.[27,31,32]

Several biochemical studies indicated that amino acid residues upstream of the -3 position of the carboxyl peptides can also play roles in binding to PDZ domains.[32,33] Recent structural studies have shown that regions outside the αB/βB groove of the PDZ domains can directly participate in binding to these upstream residues in the carboxyl peptides.[29,34,35] Specifically, some PDZ domains contain an extended βB/βC loop with a defined structure, and the βB/βC loop can serve as an anchoring site for residues as far as the -7 position in the peptide ligands.[29,30,34,35]

Recognition of Internal Peptide Sequence. Other than binding to carboxyl peptides, some PDZ domains can also interact with internal peptide fragments in target proteins.[36] The best example of this type of interaction is the heterodimer formation between PDZ domains of nNOS and PSD-95 or syntrophin.[20−22,37] The nNOS PDZ domain contains a ~30-residue extension at its C-terminus, and this extension forms a short (3 residues in each strand), two-stranded β-sheet. This mini β-sheet is anchored to the canonical PDZ domain by a pair of salt-bridges formed between Arg121 in the extension and Asp62 in the PDZ domain. Disruption of this salt bridge leads to the melting of the β-sheet structure, and the mutant nNOS PDZ loses its binding capacity to PSD-95 PDZ.[22] Upon binding to the syntrophin/PSD-95 PDZ, the mini β-sheet grows into a rigid β-hairpin (referred as the β-finger) with 10 residues in each strand.[20,22] The first β-strand of the finger inserts into the αB/βB-groove of the syntrophin/PSD-95 PDZ domain (Figure 3C). A "-E-T-T-F-" motif in the β-finger mimics the canonical C-terminal peptide in its sequence-specific interactions with the peptide-binding groove of syntrophin/PSD-95 PDZ (which can also bind to conventional C-terminal peptides), and a sharp turn immediately following the "-E-T-T-F-" motif replaces the carboxylate of conventional C-terminal peptides. Since a predefined conformation and a specific amino acid sequence are both required for an internal peptide sequence to bind to a PDZ domain, one would expect that such an interaction mode would be relatively rare. It is interesting to note that the binding of syntrophin/PSD-95 PDZ to nNOS PDZ does not interfere with nNOS PDZ domain's own ligand binding (Figure 3C), and such head-to-tail PDZ domain binding has a potential advantage of assembling PDZ domain proteins into very large molecular weight oligomers.

There are more than 400 different PDZ domains identified in the human genome. Many of the PDZ domains share remarkably similar peptide ligand-binding properties in in vitro assays. At first glance, it seems that such overlap in bindings by various PDZ domains would create problems in cellular function. However, in addition to the specificities conferred by their amino acid sequences, the distinct cellular localizations of both PDZ domains and their respective ligands provide a crucial spatial regulation of their binding specificities (i.e., many PDZ domains and their potential binding ligands may never come close to each other in living cells). The enrichment of many PDZ domain proteins into large molecular weight clusters further creates ligand binding properties distinct to those of the same set of PDZ domains assayed under test tube conditions.

Function of PDZ Domain Proteins in Synaptic Signaling

A striking feature of PDZ proteins is that most of them are localized to specific regions of cell membrane, although these proteins usually do not contain transmembrane domains. The multimodular nature of PDZ proteins and the PDZ domain's ability to bind to cytoplasmic tails of membrane-spanning proteins (e.g., ion-channels, receptors, and adhesion molecules) enable PDZ domain proteins to assemble large protein complexes in specific microdomains of cell membrane. The ability of many PDZ domains to form higher order homomeric or heteromeric oligomers brings another layer of complexity to PDZ-based protein complexes. Multimodular PDZ proteins have been shown to play major roles in establishing/maintaining cell polarities and scaffolding signal transductions (reviewed

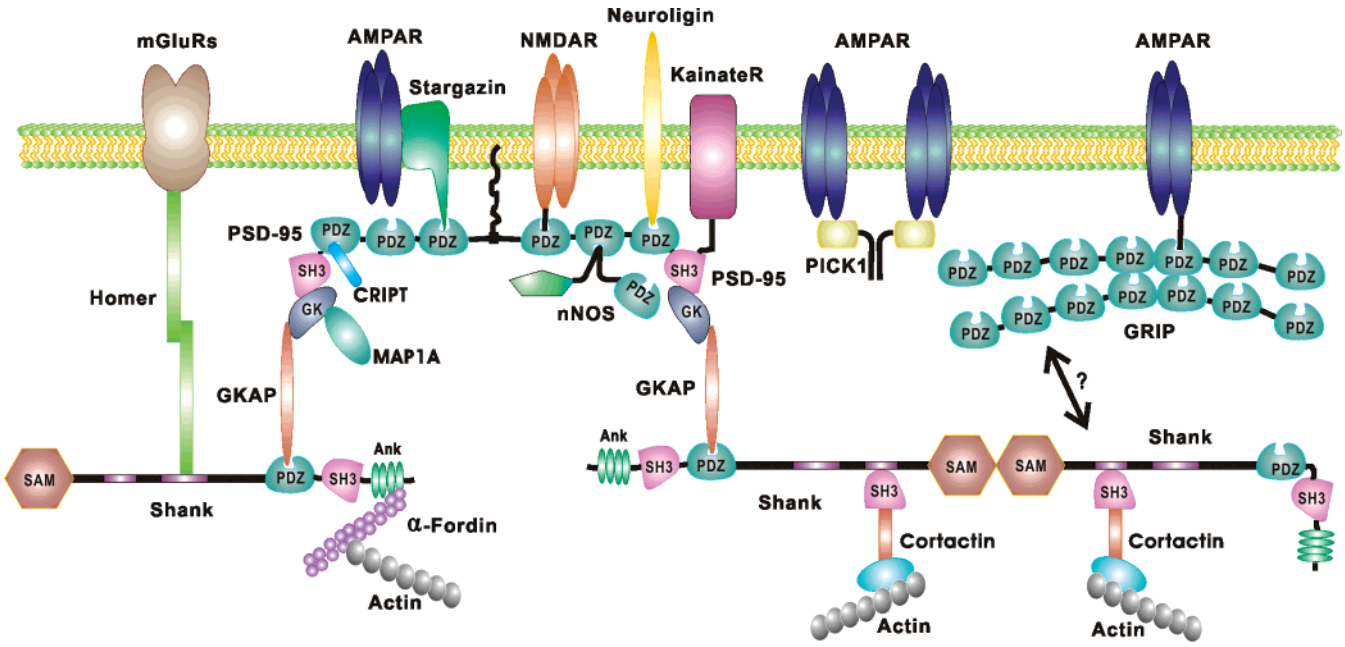

FIGURE 4. Glutamate receptor signaling complex organization in PSD by PDZ proteins. Clustering of NMDA receptors (NMDAR) is mediated by direct binding of the cytoplasmic tail of its NR2 subunits to the first two PDZ domains of PSD-95. The PDZ domains of PSD-95 can also interact with the C-terminus of the cell-adhesion protein neuroligin and AMPA receptor (AMPAR)-targeting protein stargazin. Kainate receptors (KainateR) interact with the SH3 domain of PSD-95. PSD-95 is attached to the postsynaptic membrane via the N-terminal palmitate group (wiggly line). The AMPA receptors also bind to two additional PDZ proteins GRIP/ABP and PICK1 via PDZ domain-mediated protein interactions. These glutamate receptors are further organized by Shank situated at the deeper side of the synapse. The GK domain of PSD-95 binds to GKAP, and the C-terminal tail of GKAP directly binds to the PDZ domain of Shank. Shank also couples the metabolic glutamate receptors (mGluRs) via a bridging protein Homer. Shank may also directly interact with the GRIP/AMPAR complex. Like many other scaffold proteins, Shank can multimerize via its SAM domain. Finally, Shank is directly linked to the cytoskeleton via two actin-binding proteins cortactin and α-fordin.

in refs 17−19 and 38). Here, we use glutamate receptor signaling in the central nervous system as an example to illustrate the ability of PDZ proteins to organize postsynaptic synaptic density structures as well as to assemble synaptic signaling complexes.

Neurons are extremely asymmetric cells with distinct processes including axons and dendrites. Communications between neurons occur at intercellular junctions referred to as synapses, where axons of signal-originating neurons meet with dendrites of signal-receiving cells. In excitatory synapses of the central nervous system, the postsynaptic membrane is specialized in responding rapidly to glutamate released from the presynaptic terminus. Three classes of ionotropic glutamate receptors have been characterized to response to the released glutamates. These include the following: AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole) receptors, which are responsible for fast excitatory synaptic transmission; NMDA (*N*-methyl-D-aspartate) receptors, which are critical mediators of synaptic plasticity; and kainate receptors, which also contribute to the synaptic activity. These glutamate receptors and the corresponding downstream signaling proteins are concentrated at a membrane specialization called the postsynaptic density (PSD), which is a structured, protein rich thickening (∼50 nm) easily visible under electron microscope.[39] Recent experimental evidence strongly suggests that PDZ domain proteins play important roles in both maintaining the architecture of PSD and organizing glutamate receptor signaling complexes within PSD.[17−19]

Clustering of NMDA receptors on the plasma membrane and organization of the receptor signaling complexes beneath the membrane are mediated by PSD-95, a prototype membrane associated guanylate kinase family PDZ domain. PSD-95 contains three PDZ domains, an SH3 domain and a guanylate kinase like domain (Figure 2). PSD-95 contains two Cys residues (Cys3 and Cys5) in its N-terminus, and the pair of Cys residues can be reversibly palmitoylated. Palmitoylation of N-terminal Cys residues of PSD-95 is essential for multerization and clustering of PSD-95 at PSD.[40,41] One can envision that PSD-95 forms a two-dimensional lattice directly beneath the postsynaptic membrane (Figure 4). This lattice provides an extremely large docking capacity for transmembrane receptors as well as their downstream signaling proteins. For example, the first two PDZ domains of PSD-95 can bind to the NR2 subunits of NMDA receptors as well as nNOS.[36,42] The third PDZ domain of PSD-95 can interact with cell adhesion molecule neuroligin and cytoskeletal protein CRIPT.[33,43] The SH3-GK domain of PSD-95 can interact with the kainite receptor as well as other proteins including microtubule associated protein MAP1A, guanylate kinase associated protein GKAP.[44,45] One would expect that the PSD-95-organized NMDA signaling complexes clustered at high concentrations in PSD would provide a molecular basis for high efficiency and specific-

ity of the receptor-mediated signaling, as we discussed at the beginning of this article.

Other than clustering, organizing and firmly anchoring the NMDA receptor complexes in PSD, PSD-95 has recently been shown to directly regulate synaptic AMPA receptor concentration and activity. PSD-95-mediated synaptic AMPA receptor concentration regulation is mediated by a bridging transmembrane protein called stargazin.[46] The cytoplasmic tail of stargazin binds to the first two PDZ domains of PSD-95, and the main body of stargazin can interact with the AMPA receptor.[46,47] The formation of the PSD95/stargazin/AMPA receptor ternary complex, together with activity regulated palmitoylation of PSD-95, directly controls the synaptic AMPA receptor number and activity.[41,47] It has been shown that overexpression of PSD-95 in hippocampal neurons enhances synaptic clustering and activity of AMPA receptors and drives synaptic maturation by increasing both the number and the size of spines.[48]

In addition to the indirect association with PSD-95, the AMPA receptors can directly interact with a number of PDZ domain proteins. For example, the cytoplasmic tail of the GluR2/3 subunits of the receptors can selectively bind to multi-PDZ domain proteins GRIP and ABP.[49,50] Both GRIP and ABP can multimerize via their PDZ domains, and the scaffold protein can form a large weblike structure in PSD. The receptor tail binds to the fourth and fifth PDZ domain of the protein, and the rest of the PDZ domains of the scaffold protein can serve as docking sites for AMPA receptor downstream signaling and regulatory proteins.[51] The GluR2/3 subunit of the AMPA receptors can also interact with single PDZ domain protein PICK1, and interaction between PICK1 and GluR2 is required for protein kinase C-mediated long-term depression in cerebellum[52,53] (Figure 4). Our current view is that the interactions of the AMPA receptors with PICK1 and stargazin/PSD-95 regulate synaptic targeting and trafficking of AMPA receptors, and the interaction of the receptor with GRIP and ABP is likely to be important for the retention of the receptors at PSD.

The association of glutamate receptor complexes with cytoskeleton at synapses is crucial for the architecture of PSD. PDZ domain proteins play important roles in mediating such receptor-cytoskeleton interactions. A PDZ protein called Shank seems to be particularly important in interfacing with the receptor complexes at the postsynaptic membrane with the cytoskeleton at the cytoplasmic face of dendritic spines[54,55] (Figure 4). Subcellular localization studies demonstrated that Shank is concentrated in deeper parts of the PSD as compared with PSD-95 and GKAP,[55] consistent with the model shown in Figure 4. Shank contains an ankyrin repeat, an SH3 domain, a PDZ domain, a proline-rich domain, and a SAM (sterile α motif) domain from its N- to C-terminal sequence. The PDZ domain of Shank binds to the C-terminal tail of GKAP. Through GKAP, Shank physically links with the PSD-95/glutamic receptor complex. The proline-rich domain of Shank in return binds to cortactin, and cortactin serves as a bridge to couple the Shank complex with the actin filament in dendritic shafts (Figure 4). The proline-rich region of Shank has also been shown to associate with the metabolic glutamate receptors via a bridging protein Homer.[54] In addition, Shank can associate with cytoskeleton via its ankyrin repeat by directly binding to α-fordin. Various isoforms of Shank can form homo- and hetero-oligomers via its SAM domain.[55] We may regard Shank as a "scaffold of scaffolds" that is able to organize all families of glutamate receptor complexes as well as maintaining PSD archetectures (Figure 4). The master scaffolding property of Shank provides a molecular basis for the signaling crosstalk between various glutamate receptors. Overexpression of Shank in neurons leads to enlarged spines with increased concentrations of a number of its association partners, including Homer, GKAP, PSD-95, NMDA receptors, and glutamate receptors.[56]

Conclusions and Perspectives

Increasing experimental data have shown that PDZ domain proteins play central roles in assembling receptors and associated signaling complexes as well as establishing and maintaining specialized structures at specific membrane regions of polarized cells such as neurons and epithelial cells. The scaffolding capacity of many PDZ domain proteins originates from their multimodular nature, their propensities to multimerize, and their direct binding to short carboxyl terminal sequences of target proteins. Biochemical and structural studies have provided mechanistic insights into the molecular basis of the interactions between PDZ domains and their target proteins. Many questions remain to be answered. For example, more and more data have indicated that PDZ protein-organized multiprotein assemblies are dynamic complexes. However, we have very limited knowledge of how such complexes are regulated both at spatial and temporal levels. Another interesting area for future study is to develop small molecular weight compounds that are capable of specifically disrupting certain PDZ domain mediated interactions. Given that PDZ domains can specifically recognize ~5 amino acid peptide ligands, the design and development of such PDZ-ligand intervening compounds is conceptually feasible. The use of such compounds may lead to new directions in combating human diseases in which PDZ domain-mediated protein interactions have been implicated.

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