Cell, Vol. 93, 495–498, May 15, 1998, Copyright ©1998 by Cell Press

PDZ Proteins Organize Synaptic Signaling Pathways

Minireview

Sarah E. Craven and David S. Bredt
Department of Physiology
and Program in Neuroscience
University of California at San Francisco
San Francisco, California 94143

Neurotransmission takes place at synapses, highly specialized sites of contact between neurons. Transmission across synapses occurs through a variety of mediators including amino acid neurotransmitters, peptides, electrical current, and even gaseous messengers. Processing of these signals is carried out by receptors, ion channels, and signaling molecules that are clustered at synapses. However, the molecular mechanisms that assemble these components at synapses remain largely unknown. Some insight into these mechanisms has been revealed from studies of the neuromuscular junction (NMJ), a prototypical chemical synapse where release of acetylcholine from motor neurons signals muscle contraction. During muscle development nicotinic acetylcholine receptors (nAChRs) become clustered at the muscle endplate in response to signals from the presynaptic motor neuron. A 43 kDa protein of the muscle cytoskeleton, rapsyn, has been shown by gene disruption to be required for the clustering of nAChRs and may anchor the receptors to a subsynaptic cytoskeletal complex (Apel et al., 1995). In the central nervous system, the protein gephyrin may similarly mediate synaptic clustering of glycine receptors, which are distantly related to muscle AChRs. Gephyrin directly interacts with glycine receptor subunits, and treatment of cultured neurons with antisense oligonucleotides of gephyrin disrupts the clustering of glycine receptors (Kirsch et al., 1993). Despite their similar clustering functions, rapsyn and gephyrin are neither related, nor are they members of larger families of clustering molecules that could provide insight into how other receptors and synaptic components are clustered.

Unlike the specialized rapsyn and gephyrin proteins, a large class of proteins containing PDZ domains play a general role in the localization of channels, signaling enzymes, and adhesion molecules to sites of cell–cell contact, including synapses. These functions are exemplified by the defining family of PDZ proteins (**P**SD-95, **D**lg, and **Z**O-1), known as MAGUKs for membrane-associated guanylate kinases. MAGUK proteins include PSD-95/SAP-90 and the closely related PSD-93/chapsyn-110, SAP-97/hdlg, and SAP-102, all of which are found at synapses in brain; the *Drosophila* PSD-95/SAP-90 homolog, DLG A, which localizes to septate junctions and larval NMJs; and ZO-1 and ZO-2, both of which associate with tight junctions between epithelial cells. MAGUKs share a common domain organization, with one or three N-terminal PDZ motifs, an SH3 domain, and a C-terminal region homologous to guanylate kinases. PDZ domains mediate protein–protein interactions and typically bind to short amino acid motifs at the C termini of interacting proteins that include certain ion channels and receptors. Like rapsyn and gephyrin, MAGUKs may directly cluster interacting receptors at synapses. However, there is evidence that MAGUKs play additional roles in synaptic organization by linking interacting receptors to downstream signal-transducing enzymes and regulating the structure and adhesion of synaptic junctions.

Clustering of Channels and Receptors by MAGUKs

PSD-95 was initially identified as a component of postsynaptic densities and subsequently shown to bind to the intracellular C termini of NMDA-type glutamate receptors and Shaker K^+ channels. These target proteins end with the sequence -E-S/T-D-V^* that mediates their interactions with the PDZ domains of PSD-95. All three PDZ domains of PSD-95 and homologs have similar peptide-binding preferences and interact with proteins terminating -E/Q-T/S-X-V/I^* (where X stands for any amino acid), while PDZ domains in other proteins have different binding specificities (Songyang et al., 1997). C-terminal binding specificity is consistent with the three-dimensional structures of PDZ domains, which have been determined in complex with C-terminal peptides (Doyle et al., 1996). Functionally, the interaction of PSD-95 with membrane proteins can mediate clustering. When cotransfected with NMDA receptor subunits or Kv1.4 channels, PSD-95 forms large clusters together with the interacting channels. These PSD-95/ion channel clusters are not found when the proteins are expressed alone or when the C-terminal PDZ-binding motifs are mutated (Kim et al., 1995).

While these results suggest a role for PSD-95 in receptor clustering, the first evidence for clustering in vivo came from genetic analysis in *Caenorhabditis elegans*. Here, the MAGUK protein LIN-2 and an additional PDZ protein LIN-7 are both necessary to localize the EGF receptor to cell junctions between epithelial cells (Simske et al., 1996). Further genetic evidence was provided by studies of DLG, the *Drosophila* homolog of PSD-95. DLG localizes to glutamatergic synapses at the larval NMJ where it colocalizes and interacts with clustered Shaker K^+ channels. Importantly, the clustering of Shaker K^+ channels requires the PDZ domains of DLG (Tejedor et al., 1997). Shaker channels fail to cluster in *dlg* null mutants but cluster normally in mutants with defects in the SH3 or guanylate kinase (GK) domain. Conversely, a C-terminal deletion of the Shaker channel abolishes channel clustering, although DLG remains at the NMJ (Tejedor et al., 1997). In fact, the C-terminal tail of Shaker is sufficient to localize proteins to the synapse. A chimeric protein containing the entire intracellular C terminus of Shaker linked to the extracellular and transmembrane regions of CD8 clusters at the NMJ in transgenic flies expressing this chimera in muscle. Similar to the clustering of Shaker by PSD-95 in heterologous cells, amino acids at the C terminus of the channel are necessary for synaptic clustering of the chimeric protein, as is the presence of DLG (Zito et al., 1997).

Evidence of a role for the mammalian PSD-95 family of MAGUKs in clustering K^+ channels and NMDA receptors derives mainly from experiments in cultured cells. As

discussed above, PSD-95 can cluster these proteins when coexpressed in heterologous cells. Also, mammalian MAGUKs can substitute for DLG to mediate clustering in *Drosophila*, indicative of a conserved function (Thomas et al., 1997). However, a recent study on the clustering of NMDA receptors at synapses in neuronal cultures showed that NMDA receptors initially form nonsynaptic clusters that lack PSD-95 (Rao and Craig, 1997). This indicates that NMDA receptors may initially aggregate in the absence of PSD-95, and their association with PSD-95 may function to retain preformed receptor clusters at synapses. It is also possible that other PSD-95 family members cluster NMDA receptors before they are targeted to synapses. Genetic disruptions of PSD-95 and its relatives will be necessary to resolve these questions.

Organization of Signaling Complexes

In addition to PDZ motifs, the MAGUKs contain several conserved domains indicative of a role in signal transduction. MAGUKs all have an SH3 domain, a protein–protein interaction motif found in a variety of signaling proteins. However, no binding partners have yet been identified for a MAGUK SH3 domain. The presence of a GK domain, which catalyzes the conversion of GMP to GDP using ATP, suggests a signaling function; however, critical amino acids necessary for binding ATP are not conserved in many MAGUKs and no kinase activity has been detected. A family of proteins has been identified that interact with the GK domain of the PSD-95 subset of MAGUKs, called SAPAPs or GKAPs (Naisbitt et al., 1997). These proteins are not homologous to known proteins, and their function remains unknown, although they have the potential to play a role in PSD-95 multimerization (see below). A subset of MAGUKs that includes LIN-2 and its mammalian homolog CASK also contain a CaMKII domain, but again this domain is missing amino acids critical for kinase activity.

Additional evidence that MAGUKs organize signaling cascades derives from their interactions with signaling proteins, such as neuronal nitric oxide synthase (nNOS; Brenman et al., 1996). This occurs via a PDZ–PDZ interaction in which the second PDZ domain of PSD-95 binds a single PDZ domain in nNOS. In many central neurons, nNOS activity is selectively stimulated by Ca^{2+} through NMDA receptors, while Ca^{2+} entry through other channels is not as effective. PSD-95 binding to both the NMDA receptor and nNOS may provide a scaffold to link nNOS selectively to the NMDA receptor Ca^{2+} source. Both NMDA receptor subunits and nNOS can interact with the same PSD-95 molecule, forming a ternary complex in vitro. Furthermore, internalization of a peptide that disrupts PDZ interactions into cerebellar granule cells prevents NMDA receptor–mediated stimulation of nNOS, but does not affect Ca^{2+} influx through NMDA receptors.

Many neuronal populations lack nNOS and in these cells PSD-95 may link different signaling molecules to NMDA receptors. Indeed, a recently identified ras-GTPase activating protein, p135 SynGAP (Chen et al., 1998; Kim et al., 1998), interacts with all three PDZ domains of PSD-95 and colocalizes at synapses with NMDA receptors and PSD-95. A critical source of regulation is suggested by the phosphorylation of SynGAP by CaMKII, and the inhibition of rasGAP activity in isolated postsynaptic densities by active CaMKII (Chen et al., 1998). CaMKII is an abundant PSD protein that is activated by Ca^{2+} through NMDA receptors. By analogy to nNOS, the interaction of SynGAP with PSD-95 may link its regulation to NMDA receptor gated Ca^{2+} pathways. Such a model would help explain how Ca^{2+} influx through NMDA receptors activates the MAP kinase cascade in neurons.

NMDA receptors are also required for the induction of some forms of synaptic plasticity, such as long-term potentiation (LTP) in the CA1 region of hippocampus, and C-terminal binding proteins of NMDA receptor subunits may play a central role in this process. To evaluate the functional role for the cytoplasmic domains of NMDA receptors, the C-terminal tails of the NMDA R2A, -B, or -C subunits were deleted by genetic targeting in mice, rendering these proteins unable to interact with PSD-95 or related proteins (Sprengel et al., 1998). The phenotypes of these mice closely resemble those of the corresponding gene knockouts, indicating that the C termini are critically involved in NMDA receptor function. Mice lacking the C-terminal tail of NR2A had deficits in LTP in CA1 and in spatial learning. Though receptor localization was not explored in detail, synaptic NMDA currents were normal in these mice. Therefore, the deficit in LTP is downstream of NMDA receptor activation, suggesting that the interactions of the tail of NR2A with PSD-95, GAPSYN, or other proteins are required for LTP.

Synaptic Structure and Adhesion

In addition to organizing signaling complexes, the MAGUKs may influence synaptic structure through interactions with adhesion molecules. Indeed, mutations in *dlg* alter synaptic structure at the *Drosophila* NMJ. On the postsynaptic side, the larval NMJ is surrounded by the subsynaptic reticulum, a system of convoluted membranes homologous to junctional folds of the vertebrate NMJ. In *dlg* mutants this structure is normal at early larval stages but fails to properly expand and develop as the muscle grows. On the presynaptic side, mutations in *dlg* increase the number of active zones per bouton. One clue to how DLG affects synaptic structure is that DLG clusters Fasciclin II (Fas II), an NCAM-like adhesion molecule, to the *Drosophila* NMJ in a manner similar to the Shaker K^+ channel (Thomas et al., 1997; Zito et al., 1997). Fas II functions in synaptic stabilization and growth, and without it synapses retract after larval development. Manipulating the expression level of Fas II changes the degree of synaptic growth, and down-regulation of Fas II levels by activity results in an increase in synaptic growth. Therefore, one attractive model is that activity antagonizes the interaction between DLG and Fas II, thereby reducing the level of Fas II protein clustered at the synapse and allowing for structural plasticity.

A conserved function for mammalian MAGUKs in regulating synaptic structure is suggested by the ability of PSD-95 homologs to rescue the *dlg* mutant phenotype, including the clustering of Fas II, at the NMJ. Moreover, two mammalian MAGUK family members interact with different cell adhesion molecules: PSD-95 binds the C-terminal tail of neuroligins (NL) and CASK binds the tail of neurexins (Irie et al., 1997). While the functions of these surface molecules are not fully understood, a

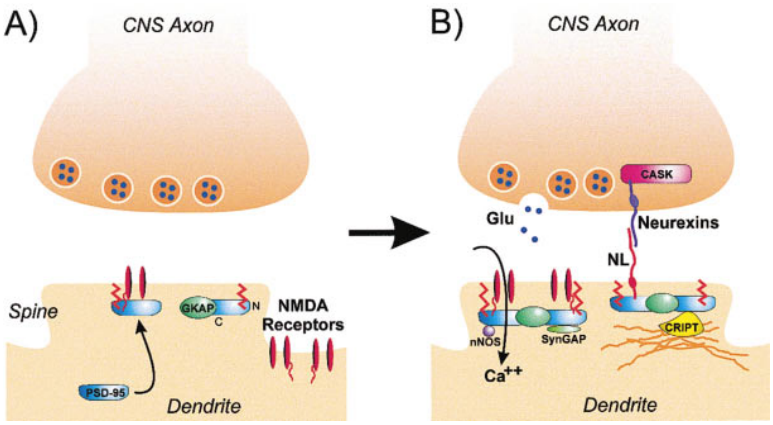

Figure 1. Postsynaptic Interactions of PSD-95

(A) N-terminal palmitoylation (jagged line) targets PSD-95 to synaptic membranes and promotes association of PSD-95 with membrane proteins including the NMDA receptor.

(B) PSD-95 then aggregates and organizes synaptic proteins through either active clustering or selective retention of interacting proteins. Through these mechanisms PSD-95 can both assemble signal transduction pathways and help determine synaptic structure.

subset of the β-neurexins interact with the neuroligins, and they can form heterotypic intercellular junctions when expressed on different cells. Therefore, PSD-95 and CASK may regulate cell adhesion and structure via these interactions.

Mechanisms of Receptor Clustering by MAGUKs

The mechanism by which PSD-95 and its homologs mediate molecular clustering is not certain. Data from *Drosophila* using the CD8/Shaker K^+ channel chimeric protein (described above) suggest that the Shaker channel is initially targeted uniformly to the membrane and then is actively clustered or selectively retained at the synapse by DLG. One model of clustering proposes that the first and second PDZ domains of PSD-95 each bind the tail of an NMDA receptor or Shaker channel subunit, while each channel, because it exists as a multimer, binds multiple molecules of PSD-95, resulting in the aggregation of the two proteins. Also, the GKAPs can bind to the GK domain of multiple PSD-95 molecules, similarly contributing to aggregation. However, this model cannot explain how the N terminus and a single PDZ domain of PSD-95 are sufficient to cluster Shaker K^+ channels or NMDA receptors in heterologous cells (Hsueh et al., 1997). Alternatively, PSD-95 might itself oligomerize and thereby aggregate interacting proteins. In support of this model, two conserved N-terminal cysteine residues in PSD-95 were found essential for both protein multimerization and K^+ channel clustering in transfected cells (Hsueh et al., 1997). In nonreducing conditions these cysteines can form disulfide bonds, allowing for the multimerization of PSD-95 molecules. However, these cysteines are also sites of palmitoylation (Topinka and Bredt, 1998). Palmitate is a fatty acid that is enzymatically added to specific proteins in a reversible fashion and that dynamically regulates protein interactions with lipid bilayers. PSD-95 in brain and in transfected COS cells is palmitoylated, and mutations in either one of the N-terminal cysteines disrupt palmitoylation and the association of PSD-95 with the membrane and with Kv1.4. Therefore, the N-terminal cysteines are important in localizing PSD-95 to the membrane so it can associate with transmembrane proteins (Figure 1). Questions therefore remain as to whether PSD-95 can multimerize in the reducing environment of cells, and whether multimerization is required for receptor clustering.

Stabilization of MAGUK-receptor clusters appears to be mediated by interactions with the cytoskeleton. In erythrocytes the MAGUK protein p55 binds protein 4.1, allowing p55 and an associated transmembrane protein, glycophorin C, to associate with the spectrin-actin lattice of the cytoskeleton. A similar interaction between neuronal MAGUKs and protein 4.1 family members at synapses could help link NMDA receptors and K^+ channel clusters to the actin-rich cytoskeleton. The NMDA R1 subunit also interacts with the actin binding protein α-actinin, providing another cytoskeletal contact point. Also, a newly identified PSD-95 binding protein, CRIPT, may mediate an association between PSD-95 and the tubulin-based cytoskeleton (Niethammer et al., 1998). CRIPT colocalizes with PSD-95 at synapses and coimmunoprecipitates with PSD-95 and tubulin from brain. When PSD-95 and CRIPT are coexpressed in heterologous cells, PSD-95 shifts from a diffuse localization to colocalize with CRIPT at the microtubule cytoskeleton. CRIPT therefore provides a possible link between PSD-95 and another intracellular structure.

While our understanding of synaptic organization has been aided by the discovery of the MAGUKs, much work remains. The most decisive evidence for MAGUKs in clustering receptors, organizing signaling cascades, and influencing synaptic structure has come from studies of the *Drosophila* NMJ, and it is not clear how this relates to synapses in the mammalian nervous system. Also, it is unknown how MAGUKs are themselves targeted to synapses, and questions remain about how they recruit interacting proteins there. The study of PSD-95 and relatives promises to enhance our understanding of synaptic structure, function, and plasticity.

Selected Reading

Apel, E.D., Roberds, S.L., Campbell, K.P., and Merlie, J.P. (1995). Neuron *15*, 115–126.

Brenman, J.E., Chao, D.S., Gee, S.H., McGee, A.W., Craven, S.E., Santillano, D.R., Huang, F., Xia, H., Peters, M.F., Froehner, S.C., and Bredt, D.S. (1996). Cell *84*, 757–767.

Chen, H.-J., Rojas-Soto, M., Oguni, A., and Kennedy, M.B. (1998). Neuron, in press.

Doyle, D.A., Lee, A., Lewis, J., Kim, E., Sheng, M., and MacKinnon, R. (1996). Cell *85*, 1067–1076.

Hsueh, Y.P., Kim, E., and Sheng, M. (1997). Neuron *18*, 803–814.

Irie, M., Hata, Y., Takeuchi, M., Ichtchenko, K., Toyoda, A., Hirao,

K., Takai, Y., Rosahl, T.W., and Sudhof, T.C. (1997). Science *277*, 1511–1515.

Kim, E., Niethammer, M., Rothschild, A., Jan, Y.N., and Sheng, M. (1995). Nature *378*, 85–88.

Kim, J.H., Liao, D., Lau, L.-F., and Huganir, R.L. (1998). Neuron *20*, 683–691.

Kirsch, J., Wolters, I., Triller, A., and Betz, H. (1993). Nature *366*, 745–748.

Naisbitt, S., Kim, E., Weinberg, R.J., Rao, A., Yang, F.C., Craig, A.M., and Sheng, M. (1997). J. Neurosci. *17*, 5687–5696.

Niethammer, M., Valtschanoff, J.G., Kapoor, T.M., Allison, D.W., Weinberg, R.J., Craig, A.M., and Sheng, M. (1998). Neuron *20*, 693–707.

Rao, A., and Craig, A.M. (1997). Neuron *19*, 801–812.

Simske, J.S., Kaech, S.M., Harp, S.A., and Kim, S.K. (1996). 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). Science *275*, 73–77.

Sprengel, R., Suchanek, B., Amico, C., Brusa, R., Burnashev, N., Rozov, A., Hvalby, O., Jensen, V., Paulsen, O., Andersen, P., et al. (1998). Cell *92*, 279–289.

Tejedor, F.J., Bokhari, A., Rogero, O., Gorczyca, M., Zhang, J., Kim, E., Sheng, M., and Budnik, V. (1997). J. Neurosci. *17*, 152–159.

Thomas, U., Kim, E., Kuhlendahl, S., Koh, Y.H., Gundelfinger, E.D., Sheng, M., Garner, C.C., and Budnik, V. (1997). Neuron *19*, 787–799.

Topinka, J.R., and Bredt, D.S. (1998). Neuron *20*, 125–134.

Zito, K., Fetter, R.D., Goodman, C.S., and Isacoff, E.Y. (1997). Neuron *19*, 1007–1016.