CMLS, Cell. Mol. Life Sci. 56 (1999) 461–472
1420-682X/99/060461-12 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Roles of postsynaptic density-95/synapse-associated protein 90 and its interacting proteins in the organization of synapses

Y. Hata[a,b] and Y. Takai[a,c,*]

[a]Takai Biotimer Project, ERATO, Japan Science and Technology Corporation, 2-2-10 Murotani, Nishi-ku, Kobe 651-2241 (Japan)
[b]Department of Biochemistry, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510 (Japan)
[c]Department of Molecular Biology and Biochemistry, Osaka University, Graduate School of Medicine/Faculty of Medicine, Suita 565-0871 (Japan), Fax + 81 6 6879 3419, e-mail: ytakai@molbio.med.osak-u.ac.jp

Received 8 February 1999; received after revision 19 May 1999; accepted 1 June 1999

Abstract. Synapses are central stages for neurotransmission. Neurotransmitters are released from the presynaptic membrane of one neuron, and bind to the receptors accumulated at the postsynaptic membrane, followed by the activation of the other neuron. The strength of a synapse is modified depending on the history of the previous neurotransmissions. This property is called synaptic plasticity and is implicated in learning and memory. Synapses contain not only the components essential for neurotransmission but also the signalling molecules involved in synaptic plasticity. The elucidation of the molecular structures of synapses is one of the key steps to understand the mechanism of learning and memory. Recent studies have revealed postsynaptic density (PSD)-95/synapse-associated protein (SAP) 90 as a core component in the architecture of synapses. In this review, we summarize up-to-date information about PSD-95/SAP90 and its interacting proteins, and the organization of synapses orchestrated by PSD-95/SAP90.

Key words. PSD-95/SAP90; scaffolding protein; synaptic plasticity; glutamate receptor.

Introduction

Chemical synapses have two remarkable characteristics, polarity and plasticity. The presynaptic and postsynaptic structures are largely different [1]. Synapse has the machinery for neurotransmitter release at the presynaptic membrane and neurotransmitter receptors at the postsynaptic membrane. The pre- and postsynaptic membranes are separated by a synaptic cleft, and are connected with glycoproteins, which may play a role in cell-cell recognition in synaptic formation. Neurotransmitters are released from the presynaptic membrane and received at the postsynaptic membrane. The pattern of neuronal activity influences the properties of each chemical synapse and modifies the efficiency of neurotransmission. The molecular mechanism underlying synaptic plasticity is still unclear, and various molecules are proposed to play roles in this process [2, 3]. These molecules are linked to receptors and cell adhesion molecules to construct chemical synapses.

Conceptually, several steps are required for a chemical synapse to emerge from the complexity of myriad com-

* Corresponding author.

ponents (fig. 1). First, a group of specialized components are assorted into functional units, and the components of each functional unit are spatially arranged so that they cross-talk efficiently (fig. 1A). Second, individual functional units interact with each other and form homo- or heteromultiple units to amplify or modulate signals (fig. 1B). Third, the whole set of components are targeted to specific sites where they should function (fig. 1C). Molecules which assign the components of signalling networks to their proper sites through these steps are called scaffolding proteins [4]. Recent studies have revealed a protein called postsynaptic density 95 (PSD)-95/synapse-associated protein (SAP) 90 as a scaffolding protein in chemical synapses [5–14]. In other words, the study of PSD-95/SAP90 has contributed to the emergence of the entity of scaffolding proteins, and increased our knowledge of the organization not only of chemical synapses but also of other junctions in multicellular organisms.

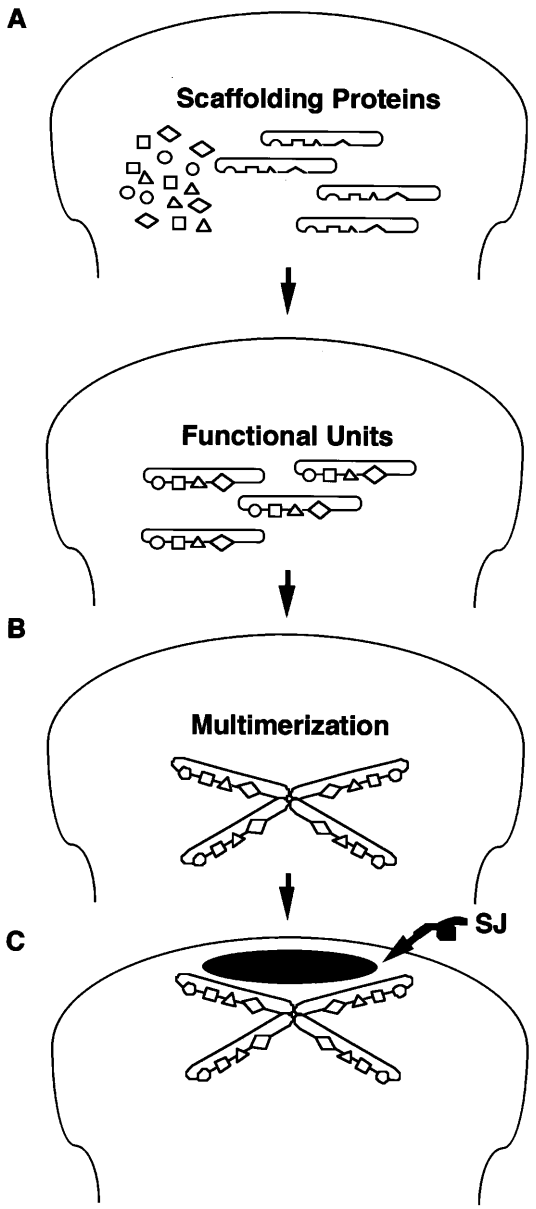

Figure 1. Scaffolding proteins in steps toward the organization of a synapse. (*A*) Various components are assembled into functional units by scaffolding proteins which have distinct binding sites for each component. (*B*) Functional units interact with each other to form a larger unit. (*C*) Functional units are localized at a synapse. SJ stands for a synaptic junction.

Identification of PSD-95/SAP90

Pioneering studies to identify the components of synapses led to the discovery of a protein of an apparent molecular weight of 90–95 kDa. Two groups named it PSD-95 and SAP90 [5, 6]. The amino acid sequence is highly homologous to the product of *Drosophila* discs-large tumor suppressor gene (*Dlg*)-*A* [15]. PSD-95/SAP90 and *Dlg-A* contain three PSD-95/*Dlg-A*/ZO-1 (PDZ) domains, one src homology (SH) 3 domain and one guanylate kinase (GK) domain. The PDZ domain was originally recognized as repeats of about 90 amino acids in PSD-95, *Dlg-A* and a tight junction protein, ZO-1, and named after these proteins [5, 6, 15–17]. The PDZ domain is a protein-interacting module [18–22]. The list of the PDZ domain-interacting proteins contains receptors, channels, cell adhesion molecules and signalling molecules, as discussed in the following sections. The SH3 domain is also a protein-interacting module [23–25], and this domain binds to the proline-rich sequence of the C-teminus of the kainate receptor subunit, KA2 [26]. The GK domain is homologous to yeast guanylate kinase [27]. The residues for the binding of GMP are conserved in the GK domain of PSD-95/SAP90, but the residues for the binding of adenosine triphosphate (ATP) are missing [5, 6]. Consistently, the GK domain of PSD-95/SAP90 has GMP-binding activity but lacks kinase activity [28]. The GK domain is recognized in several membrane-associated proteins, such as ZO-1, erythrocyte p55 and CASK/*lin-2* [16, 17, 29–31]. These proteins are proposed to play roles in the organization of the submembranous structures, and are called membrane-associated guanylate kinases (MAGUKs) [32]. PSD-95/SAP90 is a synaptic MAGUK. Four isoforms of PSD-95/SAP90 have been reported. PSD93/chapsyn-110 and SAP102 are neuronal [33–37], while SAP97/hDLG is ubiquitously expressed [38, 39]. All of these isoforms are localized at synapses. Since *Dlg-A*, a fly homologue of PSD-95/SAP90, is required for the development of the intact synaptic structure [40, 41], PSD-95/SAP90 and its iso-

Table 1. List of PSD-95/SAP90-interacting proteins.

Interacting domains of PSD-95/SAP90	Name of proteins	Description of proteins
PDZ	NR2A, B, C, D	NMDA receptor subunits
	GluR6	kainate receptor subunit
	shaker type channels	potassium channels
	APC	tumor suppressor; microtubule-associated protein
	nNOS	nitric oxide synthase
	citron	target of small GTP-binding protein, Rho
	SynGAP	GAP of small GTP-binding protein, Ras
	MAGUIN-1	homologue of fly Raf-binding protein
	CRIPT	tubulin-binding protein
	Fyn	tyrosine kinase
	neuroligin	cell adhesion molecule; ligand for neurexin
SH3	KA2	kainate receptor subunit
Guanylate kinase	GKAP/SAPAP/DAP	function unknown
	BEGAIN	function unknown
	SPAL	GAP of small GTP-binding protein, Rap1
	KA2	kainate receptor subunit
	MAP1A	microtubules-associated protein

forms are considered to be involved in the organization of synapses. Table 1 contains the list of PSD-95/SAP90-interacting proteins discussed in this review.

Interaction of PSD-95/SAP90 with receptors and channels

Two groups first reported that PSD-95/SAP90 binds to the cytoplasmic tails of *N*-methyl-D-aspartate (NMDA) receptors and Shaker type K^+ channels through the first and second PDZ domains [42–44]. Receptors and channels form clusters in the presence of PSD-95/SAP90 in transfected cells. An electrophysiological study has shown that the number of functional K^+ channels is increased in the presence of PSD-95/SAP90, which supports that the clustering of channels reflects a bona fide effect of PSD-95/SAP90 [45]. The molecular mechanism for the clustering is complex. Two molecules of receptors may be paired via two PDZ domains each of PSD-95/SAP90, although the stoichiometry of the interaction of PSD-95/SAP90 with receptors is not determined (fig. 2A). However, to form a cluster, these receptors need to be further assembled. There are several models for this process. PSD-95/SAP90 and PSD93/chapsyn-110 form homo- and heteromultimers through disulfide linkage at the N-terminus [46]. Thereby, the receptors are accumulated via head-to-head multimerization of PSD-95/SAP90 and its isoforms (fig. 2Ba). The multimerization could also be mediated by the interaction among PDZ domains (fig. 2Bb). The interactions among PDZ domains have been recognized in several PDZ domain-containing proteins, and this mechanism may be more universal [33, 47]. Alternatively, an unidentified protein may function as a linker between PSD-95/SAP90 molecules (fig. 2Bc).

The N-terminal cysteine residues of PSD-95/SAP90, which are conserved in PSD93/chapsyn-110 and SAP102, but not in SAP97/ hDLG, are palmitoylated, similarly to erythrocyte p55, and this modification regulates the interaction of PSD-95/SAP90 with K^+ channels [29, 48]. A recent study has revealed that palmitoylation is essential for synaptic targeting of PSD-95/SAP90 [49]. Phosphorylation of the C-terminal residues of K^+ channels by protein kinase A also modulates the interaction with PSD-95/SAP90 [50].

Recently, the association of PSD-95/SAP90 with kainate receptor subunits, KA2 and GluR6, has been identified [26]. PSD-95/SAP90 clusters kainate receptors and causes incomplete desensitization in COS cells expressing KA2 and GluR6, suggesting that PSD-95/SAP90 actually regulates the glutamate-mediated neurotransmission in vivo. The study using the mutant mice lacking PSD-95/SAP90 has clearly revealed that PSD-95/SAP90 is involved in NMDA receptor-mediated synaptic plasticity [51]. The assembly and localization of NMDA receptors at synapses are unaffected, and the activity of NMDA receptors does not show major changes. The isoforms of PSD-95/SAP90 may compensate the functions of PSD-95/SAP90 to some extent. However, the NMDA receptor-mediated synaptic plasticity was significantly affected. These findings suggest that the major function of PSD-95/SAP90 is to link NMDA receptors to the signalling apparatus involved in synaptic plasticity rather than to localize and regulate NMDA receptors directly. The activities of kainate receptors remain to be analyzed in mutant mice.

The localization of PSD-95/SAP90 and its isoforms is complex. For example, PSD-95/SAP90 is localized at the presynaptic membrane in cerebellum and at the postsynaptic membrane in hippocampus [52]. In the outer plexiform layer of retina, PSD-95/SAP90 and PSD93/chapsyn-110 are localized at the presynaptic membrane, whereas SAP102 is localized at the postsynaptic membrane [53]. These findings raise the question what kind of roles PSD-95/SAP90 and its isoforms play at the presynaptic membrane. Several groups report that NMDA receptors are distributed in presynaptic axon terminals [54, 55]. PSD-95/SAP90 and its isoforms may also interact with NMDA receptors at the presynaptic membrane.

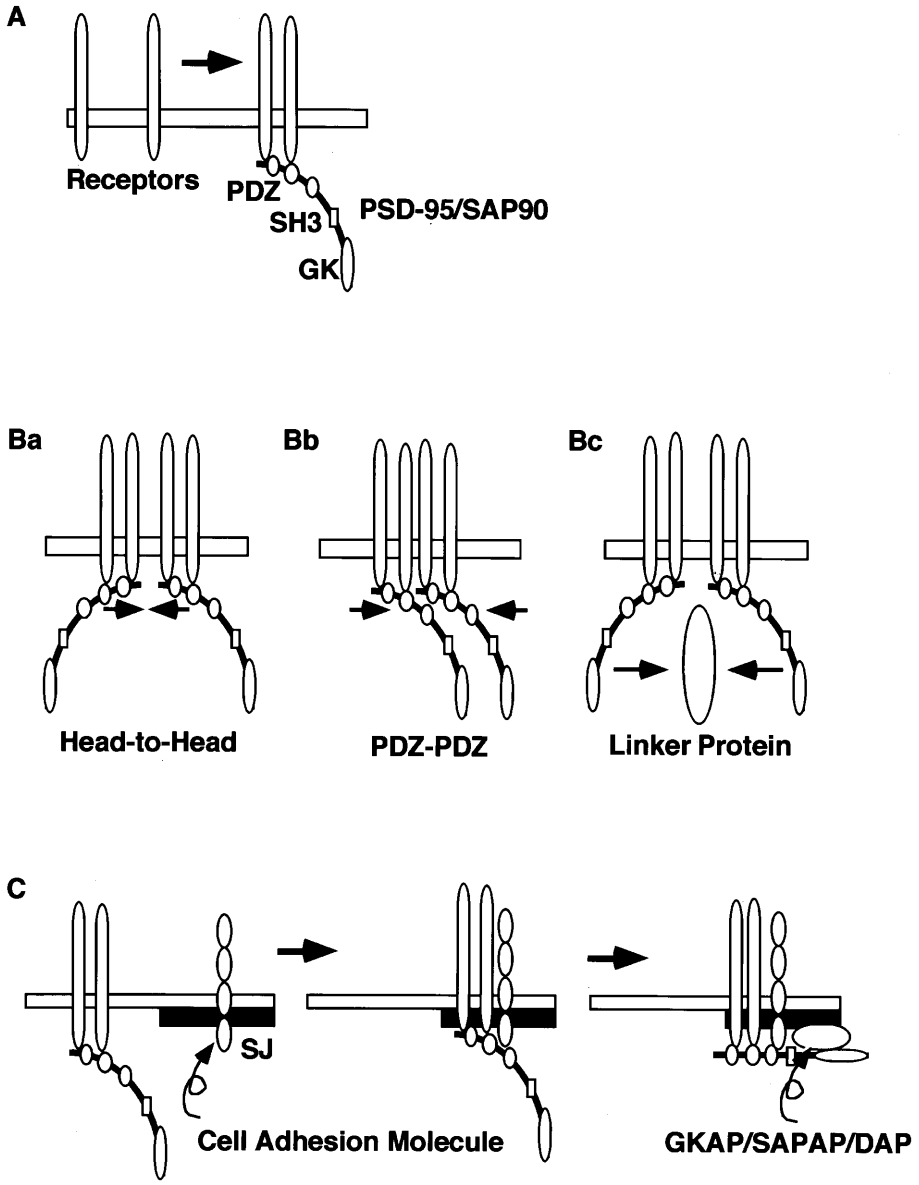

Figure 2. Conceptual model for the assembly of receptors at synaptic junctions. (*A*) Binding of receptors to PSD-95/SAP90. How many molecules of receptors bind to one molecule of PSD-95/SAP90 needs to be determined. The figure simply describes the case in which one molecule of a receptor binds to each PDZ domain. (*B*) The molecular models for the multimerization of PSD-95/SAP90. a. PSD-95/SAP90 forms a disulfide-linkage at the N-terminus. b. PDZ domains interact with each other. c. Some linker protein may mediate multimerization of PSD-95/SAP90. (*C*) Localization and fixation of the clusters of receptors at synapses. Cell adhesion molecules localize PSD-95/SAP90 at synapses, and GKAP/SAPAP/DAP may link it to the Triton X-100-insoluble structures. SJ stands for a synaptic junction.

Interaction of PSD-95/SAP90 with cell adhesion molecules

The first ligand identified for the third PDZ domain of PSD-95/SAP90 is a neuronal cell adhesion molecule, neuroligin [56]. Neuroligin is a heterophilic ligand of neurexin, which is also a neuronal cell adhesion molecule [57, 58]. The subcellular localization of neuroligin and neurexin, and the physiological significance of the adhesion between the two molecules, is still controversial, and is beyond the scope of this review. However, neuroligin is enriched in the synaptic plasma membrane (SPM) and postsynaptic density fractions, and colocalized with NMDA receptors in rat hippocampal neurons [59]. The binding of PSD-95/SAP90 to neuroligin may localize PSD-95/SAP90 at synapses (fig. 2C, middle). *Drosophila Dlg-A* seems to work in a similar manner. It binds both fasciclin II adhesion molecule and Shaker type K^+ channels at synapses [60, 61]. Among other MAGUKs, CASK/*lin-2* binds neurexin and syndecan via its PDZ domain, and interacts with Velis/rat lin-7s [31, 62–64]. Velis/rat lin-7s are also PDZ domain-containing proteins and localized at cell-cell junctions [64, 65]. They conceivably bind receptors, because its *Caenorhabditis elegans* homologue *lin-7* directly binds to EGF receptor-like *let-23* protein [66]. Taken together, MAGUKs link the clusters of receptors and channels to cell adhesion molecules to form the submembranous structures.

Interacting molecules with the GK domain of PSD-95/SAP90

Three groups independently reported the proteins interacting with the GK domain of PSD-95/SAP90 and named them guanylate kinase-associated protein (GKAP)/SAP90/PSD-95-associated protein (SAPAP)/DLG-associated protein (DAP) [67–70]. This family of proteins is composed of at least four isoforms with one minor variant. The sequences of GKAP/SAPAP/DAP do not show any significant homology to known proteins. The sequences of GKAP/SAPAP/DAP are divided into three portions. In the middle portion, there are five repeats of 14 amino acids. These repeats bind to the GK domain of PSD-95/SAP90, although it is not clear which repeats are essential for the binding. These repeats are followed by one proline-rich domain with an SH3 domain binding motif. This domain does not bind to the SH3 domain of PSD-95/SAP90, and there may be unidentified SH3 domain-containing ligands. The N-terminal portion is highly insoluble and resistant to Triton X-100 extraction, whereas the C-terminal portion is soluble. These characters suggest that GKAP/SAPAP/DAP is attached to Triton X-100-insoluble structures via the N-terminal portion and exposes the C-terminal portion to the cytosol, and that PSD-95/SAP90 interacts with GKAP/SAPAP/DAP through the hinge between the N- and C-terminal portions. PSD-95/SAP90 is Triton X-100-soluble when expressed in CHO cells, but becomes Triton X-100-insoluble when coexpressed with GKAP/SAPAP/DAP [71]. NMDA receptors also become Triton X-100-insoluble when coexpressed with PSD-95/SAP90 and GKAP/SAPAP/DAP [K., Hirao, unpublished observation], suggesting that the interaction of PSD-95/SAP90 with GKAP/SAPAP/DAP may mediate fixation of the clusters of receptors to the cytoskeleton or detergent-resistant membranes (fig. 2C, right). The truncated form of PSD-95/SAP90 containing only the first and second PDZ domains is not localized at synapses in mutant mice [51], suggesting the significance of the interaction of GKAP/SAPAP/DAP with the GK domain as well as the binding of neuroligin with the third PDZ domain for the localization of PSD-95/SAP90 at synapses.

Recent studies have revealed that there are four additional proteins that interact with the GK domain. SPA-1-like protein (SPAL) is homologous to SPA-1 protein, which may be a GTPase activating protein for small GTP-binding proteins [71, 72]. We will discuss this protein later. Brain-enriched guanylate kinase-interacting protein (BEGAIN) is neuronal, and enriched in the PSD fraction [71]. BEGAIN is recruited by GKAP/SAPAP/DAP into the Triton X-100-insoluble fraction in the presence of PSD-95/SAP90, suggesting that BEGAIN forms a complex with PSD-95/SAP90 and GKAP/SAPAP/DAP. Microtubule-associated protein 1A (MAP1A) is also a GK domain-interacting molecule [73]. This interaction is inhibited by the PDZ domains intramolecularly, and is restored when the PDZ domains are occupied by the ligands. Kainate receptor subunit KA2 binds to not only the SH3 domain but also the GK domain [26]. The activity of the receptor formed by GluR6 and KA2 is modified by the binding of KA2 to the GK domain. The receptor composed of GluR6 alone binds to the first PDZ domain, forms clusters and shows decreased desensitization in the presence of PSD-95/SAP90. KA2 itself does not form a functional receptor, but the GluR6/KA2 receptor shows decreased desensitization in the presence of PSD-95/SAP90 to a higher extent than the GluR6 receptor does. This means that the SH3 and GK domains also regulate the glutamate receptor activity. The binding to KA2 may directly change the conformation of KA2, and affect the heteromeric complex formation with GluR6. Or the binding of KA2 to the SH3 and GK domains may turn off the intramolecular interaction between the PDZ and GK domains, and enhance the effect of the PDZ domain on GluR6. Since several molecules interact with the GK domain as described above, it may be necessary to interpret the regulation by the GK domain in multiple contexts.

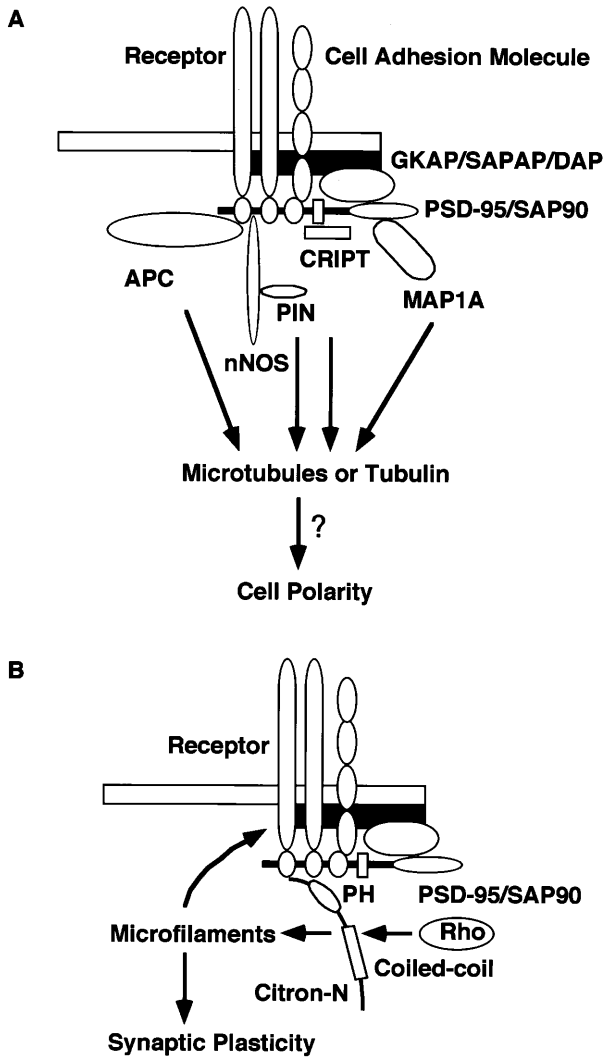

Figure 3. PSD-95/SAP90 and the cytoskeleton. (*A*) APC is a tumor suppressor and interacts with the PDZ domains of PSD-95/SAP90. MAP1A binds to the GK domain of PSD-95/SAP90. APC and MAP1A are associated with the microtubules, and CRIPT binds to tubulins. PIN is a component of dynein motors, which are associated with microtubules. (*B*) Molecules related to the regulation of microfilaments. Rho is known to regulate microfilaments. Citron-N is a target of Rho, and may be implicated in the regulation of microfilaments. Citron-N is a major component of PSD and interacts with the PDZ domains of PSD-95/SAP90. Microfilaments bind to NMDA receptors and regulate their activities.

Interaction of PSD-95/SAP90 with the cytoskeleton

The high density of the PSD is due to the accumulation of the cytoskeleton. The cytoskeleton maintains the prominent shape of neurons, is involved in the transport of membrane components and compartmentalizes the surface membranes of neurons [1]. As in other cells, there are three types of filamentous proteins in neurons. They are microfilaments, microtubules and neurofilaments. Several lines of evidence support that PSD-95/SAP90 is associated with microtubules (fig. 3A). CRIPT, which has been identified as a protein binding to the third PDZ domain of PSD-95/SAP90, interacts with tubulin [74]. Adenomatous polyposis coli gene product (APC), a ligand for the first and second PDZ domains, is also linked to microtubules, whether directly or by EB1, an APC-binding protein which is associated with microtubules [75–78]. As described in the previous section, MAP1A interacts with the GK domain of PSD93/chapsyn-110 [73]. Furthermore, neuronal nitric oxide synthase (nNOS) binds to PSD-95/SAP90 through its N-terminal PDZ domain and a protein inhibitor of nNOS (PIN) through the distinct domain [33, 79]. PIN is a component of dynein, a retrograde motor on microtubules [80]. Whether PIN and PSD-95/SAP90 bind simultaneously to nNOS is not concluded. However, all these findings support that PSD-95/SAP90 is associated with microtubules. Microtubules are dynamic polymers of tubulin and have an intrinsic polarity [81]. They transport some proteins toward the minus ends and others toward the plus ends, and are involved in protein sorting to specific membrane domains. The association of PSD-95/SAP90 with microtubules may facilitate the assembly of junctional components, which are transported on microtubules, and maintain cell polarity.

Recently, two groups have identified a protein implicated in the organization of microfilaments as a constituent of the PSD and a ligand to the PDZ domains of PSD-95/SAP90 [82, 83] (fig. 3B). This protein, citron, is one of the target proteins of a small GTP-binding protein, Rho [84]. The regulatory effect of Rho on stress fiber formation is well established [85]. Dendritic spines contain high concentrations of actin, and a sophisticated study using green fluorescent protein (GFP)-tagged actin indicates that actin shows dynamic motility in dendrites [86, 87]. Several actin-binding or -associated proteins, such as neurabin-II/spinophilin, drebrin and synaptopondin, are enriched in dendrites [88–91]. The interaction of actin and NMDA receptors has been intensively studied [92–95]. Furthermore, the β isoform of Ca^{2+}/calmodulin-dependent kinase binds to F-actin and targets the α isoform to the cytoskeleton [96]. Based on these findings, actin is considered to play roles in synaptic plasticity. How citron mediates the regulation of microfilaments by Rho is not yet clear, but PSD-95/SAP90 may be involved in actin-based synaptic plasticity via interaction with citron.

PSD-95/SAP90 and the molecules involved in NO signalling

Synaptic plasticity is assumed to be a mechanism underlying learning and memory, and mediated by various signalling molecules. Nitric oxide (NO) is also involved in synaptic plasticity [97–99]. PSD-95/SAP90 interacts with several components related to the NO synthesis (fig. 4). In neurons, NO is generated from arginine by nNOS. nNOS is a calmodulin-dependent enzyme and activated when Ca^{2+} enters through NMDA receptors [97–99]. nNOS has one PDZ domain at the N-terminus, and binds to PSD-95/SAP90 via this domain [33]. The localization of nNOS in the vicinity of NMDA receptors by PSD-95/SAP90 means that NO can quickly be produced in response to Ca^{2+} entry through NMDA receptors. nNOS has another PDZ domain-binding ligand, called CAPON [100]. CAPON inhibits the complex formation of PSD-95/SAP90 and nNOS. The mechanism by which nNOS selects its binding partner between CAPON and PSD-95/SAP90 is unknown. Because CAPON has a phosphotyrosine-binding domain [23–25], some phosphotyrosine-containing protein may increase or decrease the affinity of CAPON for nNOS, and decrease or increase the population of nNOS associated with NMDA receptors at the PSD.

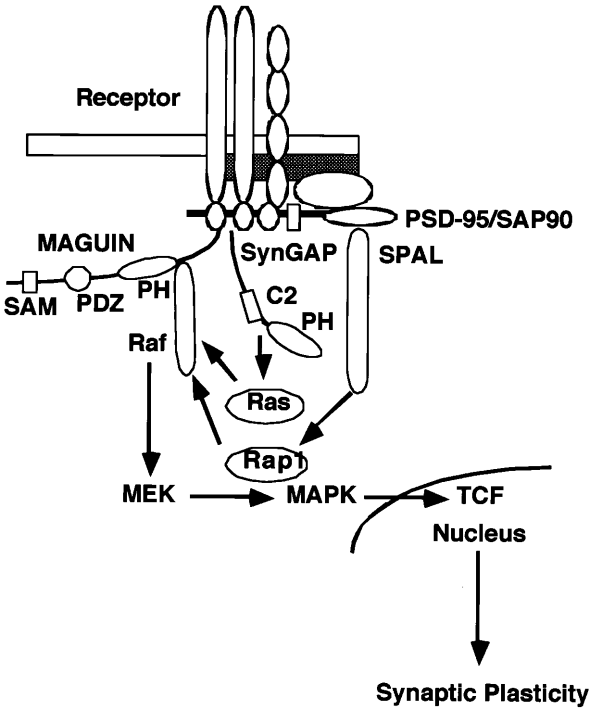

Figure 5. PSD-95/SAP90 and the MAPK cascade. SynGAP and SPAL are GTPase-activating proteins for Ras and Rap1, and bind to the PDZ and the GK domains of PSD-95/SAP90, respectively. MAGUIN-1 binds to the PDZ domains of PSD-95/SAP90 and may bind Raf kinase. The activity of Raf kinase is regulated by Ras and Rap1. Raf activates MEK, which subsequently activates MAPK. MAPK phosphorylates a transcriptional factor and induces synaptic plasticity.

PSD-95/SAP90 and mitogen-activated protein kinase cascade

Mitogen-activated protein kinase (MAPK) is involved in various biological events, and several lines of evidence have shown that MAPK is also required for synaptic plasticity. PSD-95/SAP90 binds at least three components of the MAPK cascade (fig. 5). MAPK is activated in response to LTP-inducing high frequency stimulation, and MAPK inhibitor blocks LTP in the hippocampus [101–103]. MAPK is phosphorylated and translocated into the nuclei of presynaptic cells and is necessary for long-term facilitation in *Aplysia* [104]. MAPK phosphorylates one of the ternary complex factors (TCFs), Elk-1, and induces immediate early gene transcription after the Ca^{2+} entry via NMDA receptors [105]. The activity of MAPK in neurons is regulated by a small GTP-binding protein, Ras, in a Ca^{2+}-dependent manner [106, 107]. The GTP-bound active Ras binds to and stimulates a serine/threonine kinase, c-Raf, which activates MAPK/ERK kinase (MEK), an activator of MAPK [108, 109]. The deletion of the neuron-specific Ras guanine nucleotide exchange factor, Ras-GRF, impairs memory consolidation in mice, which supports the implication of Ras in synaptic plasticity [110]. Ras-GRF has a calmodulin-binding IQ motif and is activated by Ca^{2+} influx [111]. Protein

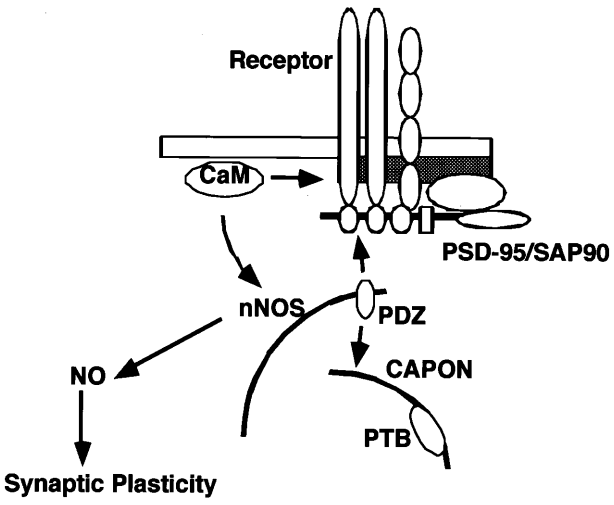

Figure 4. PSD-95/SAP90 and NO signaling. NO is synthesized by nNOS. nNOS is a calmodulin-dependent enzyme and binds to PSD-95/SAP90 via the PDZ-PDZ interaction. CAPON binds to nNOS via its PDZ domain-binding motif and competes with PSD-95/SAP90. CAPON also has a PTB domain. Calmodulin binds to the NMDA receptors.

tyrosine kinase PYK2 also confers Ca^{2+}-dependent regulation of intracellular Ca^{2+} partly through protein kinase C, and phosphorylates the adaptor protein Shc to facilitate the complex formation of Shc and Grb2 coupled with the Ras guanine nucleotide exchange factor SOS. The third regulation has been revealed by the identification of a neuronal Ras guanosine triphosphatase (GTPase)-activating protein, synGAP, which is a ligand of the PDZ domains of PSD-95/SAP90 [113, 114]. SynGAP is downregulated by Ca^{2+}/calmodulin-dependent kinase phosphorylation so that the GTP-bound active Ras is increased at high concentrations of Ca^{2+}. SynGAP also has a C_2 domain, and its activity may be directly modified by Ca^{2+}. Finally, a recent paper has reported that Ras is directly activated by NO synthesized after the activation of NMDA receptors [115]. Ras is regulated through multifold pathways and mediates NMDA receptor-dependent MAPK activation. A small GTP-binding protein, Rap1, has an effector domain with the same amino acids as that of Ras. Rap1 competes with Ras in MAPK cascade by trapping the effectors of Ras [116, 117]. In contrast, Rap1 activates B-Raf and sustains the activity of MAPK, which is initially enhanced by Ras [118–120]. The cross-talk between the signalling pathways of Ras and Rap1 may be more complicated than initially anticipated [121, 122], but in any event, the MAPK cascade is regulated by Rap1. The GK domain of PSD-95/SAP90 binds SPAL, which is homologous to Rap1 GAP [71, 72]. Actually, SPAL has Rap1 GAP activity [T. Akiyama, personal communication]. Thus, PSD-95/SAP90 binds two different GAP proteins via the PDZ and GK domains. Recently we identified a novel ligand for the PDZ domain of PSD-95/SAP90 and called it membrane-associated guanylate kinase-interacting protein (MAGUIN) [123]. MAGUIN has a characteristic molecular structure composed of a sterile α motif (SAM), a PDZ and a pleckstrin-homology (PH) domain, and is attached to the membrane through the PH domain. During our study, *Drosophila connector enhancer of ksr* (*cnk*) protein, which is required for the Ras signalling pathway in *Drosophila* eye formation, has been identified as a Raf-binding protein [124]. The molecular organization of CNK is similar to that of MAGUIN. Conceivably, MAGUIN may bind Raf and recruit it to the PSD, although the further biochemical characterization of MAGUIN is essential. The identification of MAGUIN with synGAP and SPAL suggests that PSD-95/SAP90 organizes components of the MAPK cascade. Whether PSD-95/SAP90 affects the activation of MAPK remains to be clarified.

PSD-95/SAP90 and tyrosine kinase

Tyrosine kinase is also involved in synaptic plasticity [125]. NMDA receptors are phosphorylated and regulated by tyrosine kinase, and the phosphorylation of NMDA receptors correlates with long-term potentiation [126–128]. The mutant mice of tyrosine kinase *fyn* show the impairment of learning and memory, and are rescued by the introduction of *fyn* transgene [129, 130]. PSD-95/SAP90 forms a complex with NMDA receptors and *fyn* [131]. In the case of NO synthesis and the MAPK cascade, we do not have the evidence that PSD-95/SAP90 actually improves the efficiency of signaling by binding the components of these pathways. The complex formation of *fyn* and NMDA receptors via PSD-95/SAP90 facilitates the phosphorylation of NMDA receptors by *fyn* [131].

Conclusion

The list of proteins interacting with PSD-95/SAP90 is already large and still increasing. These proteins are classified into three groups. Cell surface proteins are the first identified group, including receptors, channels and cell adhesion molecules. PSD-95/SAP90 binds receptors, channels and cell adhesion molecules to form a kind of roof structure of the postsynaptic membrane. The second group contains molecules linked to the cytoskeleton and detergent-insoluble membrane, such as MAP1A, CRIPT and presumably GKAP/SAPAP/DAP. Finally, the signaling molecules involved in synaptic plasticity are organized around PSD-95/SAP90, so that they can cross-talk efficiently. The study of mutant mice lacking PSD-95/SAP90 indicates that the major function of PSD-95/SAP90 is the linkage of the NMDA receptors to the signaling molecules of synaptic plasticity [51]. In this review, we focus on PSD-95/SAP90. However, synapses have other PDZ domain-containing proteins which are implicated in the organization of synapses. Glutamate receptor-interacting protein (GRIP) and AMPA receptor-binding protein (ABP) have seven and six PDZ domains, respectively, and both bind AMPA receptors [47, 132]. Proteins interacting with C kinase (PICK1) are also involved in the clustering of AMPA receptors [133]. Synaptic scaffolding molecule (S-SCAM) has six PDZ domains and interacts with NMDA receptors and neuroligin [134]. Channel-interacting PDZ domain protein (CIPP) is reported to interact with K^+ channel, NMDA receptors, neuroligin and neurexin [135]. PSD-95/SAP90 may cooperate with these PDZ domain-containing proteins to provide scaffolds for the components of synapses.

1 Hammond C. (1996) Cellular and Molecular Neurobiology, 1st ed., Academic Press, San Diego
2 Bear M. and Malenka R. C. (1994) Synaptic plasticity: LTP and LTD. Curr. Opin. Neurobiol. **4:** 389–399
3 Bliss T. V. and Collingridige G. L. (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature **361:** 31–39
4 Pawson T. and Scott J. D. (1997) Signaling through scaffold, anchoring and adaptor proteins. Science **278:** 2075–2080
5 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
6 Kistner U., Wenzel B. M., Veh R. W., Cases-Langhoff C., Garner A. M., Appeltauer U. et al. (1993) SAP90, a rat presynaptic protein related to the product of the *Drosophila* tumor suppressor gene dlg-A. J. Biol. Chem. **268:** 4580–4583
7 Kennedy M. B. (1993) The postsynaptic density. Curr. Opin. Neurobiol. **3:** 732–737
8 Garner C. and Kindler S. (1996) Synaptic proteins and the assembly of synaptic junctions. Trends Cell Biol. **19:** 429–433
9 Gomperts S. N. (1996) Clustering membrane proteins: it's all coming together with the PSD-95/SAP90 protein family. Cell **84:** 659–662
10 Sheng M. (1996) PDZs and receptor/channel clustering: rounding up the latest suspects. Neuron **17:** 575–578
11 Kennedy M. B. (1997) The postsynaptic density at glutamatergic synapses. Trends Neurosci. **20:** 264–268
12 Ziff E. B. (1997) Enlightening the postsynaptic density. Cell **19:** 1153–1174
13 Craven S. E. and Bredt D. S. (1998) PDZ proteins organize synaptic signaling pathways. Cell **93:** 495–498
14 Hata Y., Nakanishi H. and Takai Y. (1998) Synaptic PDZ domain-containing proteins. Neurosci. Res. **32:** 1–7
15 Woods D. F. and Bryant P. J. (1991) The discs-large tumor suppressor gene of *Drosophila* encodes a guanylate kinase homolog localized at septate junctions. Cell **66:** 451–464
16 Willott E., Balda M. S., Fanning A. S., Jameson B., Van Itallie C. and Anderson J. M. (1993) The tight junction protein ZO-1 is homologous to the *Drosophila* discs-large tumor suppressor protein of septate junctions. Proc. Natl. Acad. Sci. USA **90:** 7834–7838
17 Itoh M., Nagafuchi A., Yonemura S., Kitani-Yasuda T., Tsukita S. and Tsukita S. (1993) The 220-kD protein colocalizing with cadherins in non-epithelial cells is identical to ZO-1, a tight junction-associated protein in epithelial cells: cDNA cloning and immunoelectron microscopy. J. Cell Biol. **121:** 491–502
18 Ponting C. P. and Phillips C. (1995) DHR domains in syntrophins, neuronal NO synthases and other intracellular proteins. Trends Biochem. Sci. **20:** 102–103
19 Kennedy M. B. (1995) Origin of PDZ (DHR, GLGF) domains. Trends Biochem Sci. **20:** 350
20 Fanning A. S. and Anderson J. M. (1996) Protein-protein interactions; PDZ domains networks. Curr. Biol. **6:** 1385–1388
21 Saras J. and Heldin C-H. (1996) PDZ domains bind carboxyl-terminal sequences of target proteins. Trends Biochem. Sci. **21:** 455–458
22 Songyang Z., Fanning A. S., Fu C., Xu J., Marfatia S. M., Chishti A. H. et al. (1997) Recognitioin of unique carboxyl-terminal motifs by distinct PDZ domains. Science **275:** 73–77
23 Pawson T. (1995) Protein modules and signaling networks. Nature **373:** 573–580
24 Cohen G. B., Ren R. and Baltimore D. (1995) Modular binding domains in signal transduction proteins. Cell **80:** 237–248
25 Sudol M. (1998) From Src homology domains to other signaling modules: proposal of the 'protein recognition code'. Oncogene **17:** 1469–1474
26 Garcia E. P., Mehtal S., Blair L. A. C., Wells D. G., Shang J., Fukushima T. et al. (1998) SAP90 binds and clusters kainate receptors causing incomplete desensitization. Neuron **21:** 727–739
27 Berger A., Schiltz E. and Schulz G. E. (1989) Guanylate kinase from *Saccharomyces cerevisiae*. Isolation and characterization, crystallization and preliminary X-ray analysis, amino acid sequence and comparison with adenylate kinases. Eur. J. Biochem. **184:** 433–443
28 Kistner U., Garner C. C. and Linial M. (1995) Nucleotide binding by the synapse associated protein SAP90. FEBS Lett. **359:** 159–163
29 Ruff P., Speicher D. W. and Chishti A. H. (1991) Molecular identification of a major palmitoylated erythrocyte membrane protein containing the src homology 3 motif. Proc. Natl. Acad. Sci. USA **88:** 6595–6599
30 Hoskins R., Hajnal A. F., Harp S. A. and Kim S. K. (1996) The *C. elegans* vulval induction gene lin-2 encodes a member of the MAGUK family of cell junction proteins. Development **122:** 97–111
31 Hata Y., Butz S. and Sudhof T. C. (1996) CASK: a novel dlg/PSD95 homology with an N-terminal calmodulin-dependent protein kinase domain identified by interaction with neurexins. J. Neurosci. **16:** 2488–2494
32 Anderson J. M. (1996) Cell signalling: MAGUK magic. Curr. Biol. **6:** 382–384
33 Brenman J. E., Chao D., Gee S. H., McGee A. W., Craven S. E., Santillano D. R. 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
34 Brenman J. E., Christopherson K. S., McGee A. W. and Bredt D. S. (1996) Cloning and characterization of postsynaptic density 93, a nitric oxide synthase interacting protein. J. Neurosci. **16:** 7407–7415
35 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
36 Muller B. M., Kistner U., Kindler S., Chung W. J., Kuhlendahl S., Fenster S. D. et al. (1996) SAP102, a novel postsynaptic protein that interacts with NMDA receptor complexes in vivo. Neuron **17:** 255–265
37 Lau L-F., Mammen A., Ehlers M. D., Kindler S., Chung W. J. et al. (1996) Interaction of the N-methyl-D-aspartate receptor complex with a novel synapse-associated protein, SAP102. J. Biol. Chem. **271:** 21622–21628
38 Muller B. M., Kistner U., Veh R. W., Cases-Langhoff C., Becker B., Gundelfinger E. D. et al. (1995) Molecular characterization and spatial distribution of SAP97, a novel presynaptic protein homologous to SAP90 and the *Drosophila* discs-large tumor suppressor protein. J. Neurosci. **15:** 2354–2366
39 Lue R. A., Marfatia S. M., Branton D. and Chishti A. H. (1994) Cloning and characterization of hdlg: the human homologue of the *Drosophila* discs large tumor suppressor binds to protein 4.1. Proc. Natl. Acad. Sci. USA **91:** 9818–9822
40 Lahey T., Gorczyca M., Jia X-X. and Budnik V. (1994) The *Drosophila* tumor suppressor gene dlg is required for normal synaptic bouton structure. Neuron **13:** 823–835
41 Budnik V., Koph Y.-H., Guan B., Hartmann B., Hough C., Woods D. et al. (1996) Regulation of synapse structure and function by the *Drosophila* tumor suppressor gene dlg. Neuron **17:** 627–640
42 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
43 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

44 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
45 Horio Y., Hibino H., Inanobe A., Yamada M., Ishii M., Tada Y. et al. (1997) Clustering and enhanced activity of an inwardly rectifying potassium channel, Kir4.1 by an anchoring protein, PSD-95/SAP90. J. Biol. Chem. **272:** 12885–12888
46 Hsueh Y.-P., Kim E. and Sheng M. (1997) Disulfide-linked head-to-head multimerization in the mechanism of ion channel clustering by PSD-95. Neuron **18:** 803–814
47 Srivastava S., Osten P., Vilim F. S., Khatri L., Inman G., States B. et al. (1998) Novel anchorage of GluR2/3 to the postsynaptic density by the AMPA receptor-binding protein ABP. Neuron **21:** 581–591
48 Topinka J. R. and Bredt D. S. (1998) N-terminal palmitoylation of PSD-95 regulates association with cell membranes and interaction with K^+ channel Kv1.4. Neuron **26:** 125–134
49 Craven S. E., El-Husseini A. E. and Bredt D. S. (1999) Synaptic targeting of the postsynaptic density protein PSD-95 mediated by lipid and protein motifs. Neuron **22:** 497–509
50 Cohen N. A., Brenman J. E., Snyder S. H. and Bredt D. S. (1996) Binding of the inward rectifier K^+ channel Kir2.3 to PSD-95 is regulated by protein kinase A phosphorylation. Neuron **17:** 759–767
51 Migaud M., Charlesworth P., Dempster M., Webster L. C., Watabe A. M., Makhiston M. et al. (1998) Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. Nature **396:** 433–439
52 Hunt C. A., Schenker L. J. and Kennedy M. B. (1996) PSD-95 is associated with the postsynaptic density and not with the presynaptic membrane at forebrain synapses. J. Neurosci. **16:** 1380–1388
53 Koulen P., Fletcher E. L., Craven S. E., Bredt D. S. and Wassle H. (1998) Immunocytochemical localization of the postsynaptic density protein PSD-95 in the mammalian retina. J. Neurosci. **18:** 10136–10149
54 DeBiasi S., Minelli A., Melone M. and Conti F. (1996) Presynaptic NMDA receptors in the neocortex are both auto- and heteroreceptors. Neuroreport **7:** 2773–2776
55 Aoki C., Rhee J., Lubin M. and Dawson T. M. (1997) NMDA-R1 subunit of the cerebral cortex co-localizes with neuronal nitric oxide synthase at pre- and postsynaptic sites and in spines. Brain Res. **750:** 25–40
56 Irie M., Hata Y., Takeuchi M., Ichtchenko K., Toyoda A., Hirao K. et al. (1997) Binding of neuroligins to PSD-95. Science **277:** 1511–1515
57 Missler M. and Sudhof T. C. (1998) Neurexins: three genes and 1001 products. Trends Genet. **14:** 20–26
58 Ichtchenko K., Hata Y., Nguyen T., Ullrich B., Missler M., Moomaw C. et al. (1995) Neuroligin 1: a splice site-specific ligand for β-neurexins. Cell **81:** 435–443
59 Song J. Y., Ichtchenko K., Sudhof T. C. and Brose N. (1999) Neuroligin 1 is a postsynaptic cell-adhesion molecule of excitatory synapses. Proc. Natl. Acad. Sci. USA **96:** 1100–1105
60 Thomas U., Kim E., Kuhlendahl S., Koh Y. H., Gundelfinger E. D., Sheng M. et al. (1997) Synaptic clustering of the cell adhesion molecule fasciclin ll by dics-large and its role in the regulation of presynaptic structure. Neuron **19:** 787–799
61 Zito K., Fetter R. D., Goodman C. S. and Isacoff E. Y. (1997) Synaptic clustering of fasciclin II and Shaker: essential targeting sequences and role of Dlg. Neuron **19:** 1007–1016
62 Cohen A. R., Wood D. F., Marfatia S. M., Walther Z., Chishti A. H. and Anderson J. M. (1998) Human CASK/lin-2 binds syndecan-s and protein 4.1 and localizes to the basolateral membrane of epithelial cells. J. Cell Biol. **142:** 129–138
63 Hsueh Y.-P., Yang F.-C., Kharazia V., Naisbitt S., Cohen A. R., Weinberg R. J. et al. (1998) Direct interaction of CASK/lin-2 and syndecan heparan sulfate proteoglycan and their overlapping distribution in neuronal synapses. J. Cell Biol. **142:** 139–151
64 Butz S., Okamoto M. and Sudhof T. C. (1998) A tripartite protein complex with the potential to couple synaptic vesicle exocytosis to cell adhesion in brain. Cell **94:** 773–782
65 Irie M., Hata Y., Deguchi M., Ide N., Hirao K., Yao I. et al. (1999) Isolation and characterization of mammalian homologues of *Caenorhabditis elegans* lin-7: localization at cell-cell junctions. Oncogene **18:** 2811–2817
66 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
67 Kim E., Naisbitt S., Hsueh Y.-P., Rao A., Rothschild A., Craig A. M. et al. (1997) GKAP, a novel synaptic protein that interacts with the guanylate kinase-like domain of the PSD-95/SAP90 family of channel clustering molecules. J. Cell Biol. **136:** 669–678
68 Takeuchi M., Hata Y., Hirao K., Toyoda A., Irie M. and Takai Y. (1997) SAPAPs: a family of PSD-95/SAP90-associated proteins localized at postsynaptic density. J. Biol. Chem. **272:** 11943–11951
69 Satoh K., Yanai H., Senda T., Kohu K., Nakamura T., Okumura N. et al. (1997) DAP-1, a novel protein that interacts with the guanylate kinase-like domains of hDLG and PSD-95. Genes Cells **2:** 415–424
70 Naisbitt S., Kim E., Weinberg R. J., Rao A., Yang F.-C., Craig A. M. et al. (1997) Characterization of guanylate kinase-associated protein, a postsynaptic density protein at excitatory synapses that interacts directly with postsynaptic density-95/synapse-associated protein 90. J. Neurosci. **17:** 5687–5696
71 Deguchi M., Hata Y., Takeuchi M., Ide N., Hirao K., Yao I. et al. (1998) BEGAIN (brain-enriched guanylate kinase-associated protein), a novel neuronal PSD-95/SAP90-binding protein. J. Biol. Chem **273:** 26269–26272
72 Kurachi H., Wada Y., Tsukamoto N., Maeda M., Kubota H., Hattori M. et al. (1997) Human SPA-1 gene product selectively expressed in lymphoid tissues is a specific GTPase-activating protein for Rap1 and Rap2. Segregate expression profiles from a rap1GAP gene product. J. Biol. Chem. **272:** 28081–28088
73 Brenman J. E., Topinka J. R., Cooper E. C., McGee A. W., Rosen J., Milroy T. et al. (1998) Localization of postsynaptic density-93 to dendritic microtubules and interaction with microtubule-associated protein 1A. J. Neurosci. **18:** 8805–8813
74 Niethammer M., Valtschanoff J. G., Kapoor T. M., Allison D. W., Weinberg R. J., Craig A. M. et al. (1998) CRIPT, a novel postsynaptic protein that binds to the third PDZ domain of PSD-95/SAP90. Neuron **20:** 693–707
75 Matsumine A., Ogai A., Senda T., Okumura N., Satoh K., Baeg G.-H. et al. (1996) Binding of APC to the human homolog of the *Drosophila* discs large tumor suppressor protein. Science **272:** 1020–1023
76 Smith K. J., Levy D. B., Maupin P., Pollard T. D., Vogelstein B. and Kinzler K. W. (1994) Wild-type but not mutant APC associates with the microtubule cytoskeleton. Cancer Res. **54:** 3672–3675
77 Munemitsu S., Souza B., Muller O., Albert I., Rubinfeld B. and Polakis P. (1994) The APC gene product associates with microtubules in vivo and promotes their assembly in vitro. Cancer Res. **54:** 3676–3681
78 Berrueta L., Kraeft S. K., Tirnauer J. S., Schuyler S. C., Chen L. B., Hill D. E. et al. (1997) The adenomatous polyposis coli-binding protein EB1 is associated with cytoplasmic and spindle microtubules. Proc. Natl. Acad. Sci. USA **95:** 10596–10601
79 Jaffery S. R. and Snyder S. H. (1996) PIN: an associated protein inhibitor of neuronal nitric oxide synthase. Science **274:** 774–777

80 King S. M., Barbarese E., Dillman J. F. III, Patel-King R. S., Carson J. H. and Pfister K. K. (1996) Brain cytoplasmic and flagellar outer arm dyneins share a highly conserved Mr 8000 light chain. J. Biol. Chem. **271:** 19358–19366

81 Desai A. and Mitchison T. J. (1997) Microtubule polymerization dynamics. Annu. Rev. Cell Dev. Biol. **13:** 83–117

82 Zhang W., Vazquez L., Apperson M. and Kennedy M. B. (1999) Citron binds to postsynaptic density-95 at glutamatergic synapses on inhibitory neurons in hippocampus. J. Neurosci. **19:** 96–108

83 Furuyashiki T., Fujisawa K., Fujita A., Madaule P., Uchino S., Mishina M. et al. (1999) Citron, a Rho-target, interacts with postsynaptic density-95/synapse-associated protein 90 at glutamatergic synapses in the thalamus. J. Neurosci. **19:** 109–118

84 Madaule P., Eda M., Watanabe N., Fujisawa K., Matsuoka T., Bito H. et al. (1998) Role of citron kinase as a target of the small GTPase Rho in cytokinesis. Nature **394:** 491–494

85 Hall A. (1998) Role of GTPases and the actin cytoskeleton. Science **279:** 509–514

86 Kaech S., Fisher M., Doll T. and Matus A. (1997) Isoform specificity in the relationship of actin to dendritic spines. J. Neurosci. **17:** 9565–9572

87 Fisher M., Kaech S., Knutti D. and Matus A. (1998) Rapid actin-based plasticity in dendritic spines. Neuron **20:** 847–854

88 Allen P. B., Ouimet C. G. and Greengard P. (1997) Spinophilin, a novel protein phosphatase 1 binding protein localized to dendritic spine. Proc. Natl. Acad. Sci. USA **94:** 9956–9961

89 Satoh A., Nakainshi H., Obaishi H., Wada M., Takahashi K., Satoh K. et al. (1998) Neurabin-ll/spinophilin: an actin filament-binding protein with one PDZ domain localized at cadherin-based cell-cell adhesion sites. J. Biol. Chem. **273:** 3470–3475

90 Hayashi K., Ishikawa R., Ye L. H., He X. L., Takata K., Kohama K. et al. (1996) Modulatory role of drebrin on the cytoskeleton within dendritic spines in the rat cerebral cortex. J. Neurosci. **16:** 7161–7170

91 Mundel P., Heid H. W., Mundel T. M., Kruger M., Reiser J. and Kriz W. (1997) Synaptopodin: an actin-associated protein in telencephalic dendrites and renal podocytes. J. Cell Biol. **139:** 193–204

92 Wyszynski M., Lin J., Rao A., Nigh E., Beggs A. H., Craig A. M. et al. (1997) Competitive binding of alpha-actinin and calmodulin to the NMDA receptor. Nature **385:** 439–442

93 Allison D. W., Gelfand V. I., Spector I. and Craig A. M. (1998) Role of actin in anchoring postsynaptic receptors in cultured hippocampal neurons: differential attachment of NMDA versus AMPA receptors. J. Neurosci. **18:** 2423–2436

94 Wechsler A. and Teichberg V. I. (1998) Brain spectrin binding to the NMDA receptor is regulated by phosphorylation, calcium and calmodulin. EMBO J. **17:** 3931–3939

95 Halpain S., Hipolito A. and Saffer L. (1998) Regulation of F-actin stability in dendritic spines by glutamate receptors and calcineurin. J. Neurosci. **18:** 9835–9844

96 Shen K., Teruel M. N., Subramanian K. and Meyer T. (1998) CaMKIIbeta functions as an F-actin targeting module that localizes CaMKIIalpha/beta heterooligomers to dendritic spines. Neuron **21:** 593–606

97 Holscher C. (1997) Nitric oxide, the enigmatic neuronal messenger: its role in synaptic plasticity. Trends Neurosci. **20:** 298–303

98 Brenman J. E. and Bredt D. S. (1997) Synaptic signaling by nitric oxide. Curr. Opin. Neurobiol. **7:** 374–378

99 Huang E. P. (1997) Synaptic plasticity: a role for nitric oxide in LTP. Curr. Biol. **7:** R141–143

100 Jaffery S. R., Snowman A. M., Eliasson M. J. L., Cohen N. A. and Snyder S. H. (1998) CAPON: a protein associated with neuronal nitric oxide synthase that regulates its interactions with PSD95. Neuron **20:** 115–124

101 Bading H. and Greenberg M. E. (1991) Stimulation of protein tyrosine phosphorylation by NMDA receptor activation. Science **253:** 912–914

102 Rosen L. B., Ginty D. D., Weber M. J. and Greenberg M. E. (1994) Membrane depolarization and calcium influx stimulate MEK and MAP kinase via activation of Ras. Neuron **12:** 1207–1221

103 English J. D. and Sweatt J. D. (1996) Activation of p42 mitogen-activated protein kinase in hippocampal long term potentiation. J. Biol. Chem. **271:** 24329–24332

104 Martin K. C., Michael D., Rose J. C., Barad M., Casadio A., Zhu H. et al. (1997) MAP kinase translocates into the nucleus of the presynaptic cell and is required for long-term facilitation in *Aplysia*. Neuron **18:** 899–912

105 Xia Z., Dudek H., Miranti C. K. and Greenberg M. E. (1996) Calcium influx via the NMDA receptor induces immediate early gene transcription by a MAP kinase/ERK-dependent mechanism. J. Neurosci. **16:** 5425–5436

106 Ghosh A. and Greenberg M. E. (1995) Calcium signaling in neurons: molecular mechanisms and cellular consequences. Science **268:** 239–247

107 Finkbeiner S. and Greenberg M. E. (1996) Ca^{2+}-dependent routes to Ras: mechanisms for neuronal survival, differentiation, and plasticity. Neuron **16:** 233–236

108 Vojtek A. B. and Der C. J. (1998) Increasing complexity of the Ras signaling pathway. J. Biol. Chem. **273:** 19925–19928

109 Morrison D. K., and Cutler R.E., Jr. (1998) The complexity of Raf-1 regulation., Curr. Opin. Cell Biol. **9:** 174–179

110 Brambilla R., Gnesutta N., Minichiello L., White G., Roylance A. J., Herron C. E. et al. (1997) A role for the Ras signalling pathway in synaptic transmission and long-term memory. Nature **390:** 281–286

111 Farnsworth C. L., Freshney N. W., Rosen L. B., Ghosh A., Greenberg M. E. and Feig L. A. (1995) Calcium activation of Ras mediated by neuronal exchange factor Ras-GRF. Nature **376:** 524–527

112 Lev S., Moreno H., Martinez R., Canoll P., Peles E., Musacchino J. M. et al. (1995) Protein tyrosine kinase PYK2 involved in Ca^{2+}-induced regulation of ion channel and MAP kinase functions. Nature **376:** 737–745

113 Kim J. H., Liao D., Lau L.-F. and Huganir R. L. (1998) SynGAP: a synaptic RasGAP that associates with the PSD-95/ SAP90 protein family. Neuron **20:** 683–691

114 Chen H. J., Rojas-Soto M., Oguni A. and Kennedy M. B. (1998) A synaptic Ras-GTPase activating protein (p135 SynGAP) inhibited by CaM kinase ll. Neuron **20:** 895–904

115 Yun H. Y., Gonzalez-Zulueta M., Dawson V. L. and Dawson T. M. (1998) Nitric oxide mediates N-methyl-D-aspartate receptor-induced activation of p21ras. Proc. Natl. Acad. Sci. USA **95:** 5773–5778

116 Cook S. J., Rubinfeld B., Albert I. and McCormick F. (1993) RapV12 antagonizes Ras-dependent activation of ERK1 and ERK2 by LPA and EGF in Rat-1 fibroblasts. EMBO J. **12:** 3475–3485

117 Boussiotis V. A., Freeman G. J., Berezovskaya A., Barber D. L. and Nadler L. M. (1997) Maintenance of human T cell anergy: blocking of IL-2 gene transcription by activated Rap1. Science **278:** 124–128

118 Ohtsuka T., Shimizu K., Yamamori B., Kuroda S. and Takai Y. (1996) Activation of brain B-Raf protein kinase by Rap1B small GTP-binding protein. J. Biol. Chem. **271:** 1258–1261

119 Vossler M. R., Yao H., York R. D., Pan M. G., Rim C. S. and Stork P. J. (1997) cAMP activates MAP kinase and Elk-1 through a B-Raf- and Rap1-dependent pathway. Cell **89:** 73–82

120 York R. D., Yao H., Dillon T., Ellig C. L., Eckert S. P., McCleskey E. W. et al. (1998) Rap1 mediates sustained MAP kinase activation induced by nerve growth factor. Nature **392:** 622–626

121 Zwartkruis F. J., Wolthuis R. M., Nabben N. M., Franke B. and Bos J. L. (1998) Extracellular signal-regulated activation of rap1 fails to interfere in ras effector signalling. EMBO J. **17:** 5905–5912

122 Bos J. L. (1998) All in the family? New insights and questions regarding interconnectivity of Ras, Rap1 and Ral. EMBO J. **23:** 6776–6782

123 Yao I., Hata Y., Ide N., Hirao K., Deguchi M., Nishioka H. et al. (1999) MAGUIN: a novel neuronal membrane-associated guanylate kinase-interacting protein. J. Biol. Chem. **274:** 11889–11896
124 Therrien M., Wong A. M. and Rubin G. M. (1998) CNK, a Raf-binding multidomain protein required for Ras signaling. Cell **95:** 343–353
125 Llinas R., Moreno H., Sugimori M., Mohammadi M. and Schlessinger J. (1997) Differential pre- and postsynaptic modulation of chemical transmission in the squid giant synapse by tyrosine phosphorylation. Proc. Natl. Acad. Sci. USA **94:** 1990–1994
126 Lau L. F. and Huganir R. L. (1995) Differential tyrosine phosphorylation of N-methyl-D-aspartate receptor subunits. J. Biol. Chem. **270:** 20036–20041
127 Rosenblum K., Dudai Y. and Richter-Levin G. (1996) Long-term potentiation increases tyrosine phosphorylation of the N-methyl-D-aspartate receptor subunit 2B in rat dentate gyrus in vivo. Proc. Natl. Acad. Sci. USA **93:** 10457–10460
128 Yu X. M., Askalan R., Keil G. J. II and Salter M. W. (1997) NMDA channel regulation by channel-associated protein tyrosine kinase Src. Science **275:** 674–678
129 Grant S. G., O'Dell T. J., Karl K. A., Stein P. L., Soriano P. and Kandel E. R. (1992) Impaired long-term potentiation, spatial learning and hippocampal development in fyn mutant mice. Science **258:** 1903–1910
130 Kojima N., Wang J., Mansuy I. M., Grant S. G., Mayford M. and Kandel E. R. (1997) Rescuing impairment of long-term potentiation in fyn-deficient mice by introducing Fyn transgene. Proc. Natl. Acad. Sci. USA **94:** 4761–4765
131 Tezuka T., Umemori H., Akiyam T., Nakanishi S. and Yamamoto T. (1999) PSD-95 promotes Fyn-mediated tyrosine phosphorylation of the N-methyl-D-aspartate receptor subunit NR2A. Proc. Natl. Acad. Sci. USA **96:** 435–440
132 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. Nature **386:** 279–284
133 Xia J., Zhang X., Staudinger J. and Huganir R. L. (1999) Clustering of AMPA receptors by the synaptic PDZ domain-containing protein PICK1. Neuron **22:** 179–187
134 Hirao K., Hata Y., Ide N., Takeuchi M., Irie M., Yao I. et al. (1998) A novel multiple PDZ domain-containing molecule interacting with N-methyl-D-aspartate receptors and neuronal cell adhesion proteins. J. Biol. Chem. **273:** 21105–21110
135 Kurschner C., Mermelstein P. G., Holden W. T. and Surmeier D. J. (1998) CIPP, a novel multivalent PDZ domain protein, selectively interacts with Kir4.0 family members, NMDA receptor subunits, neurexins, and neuroligins. Mol. Cell. Neurosci. **11:** 161–172