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BREAKTHROUGHS AND VIEWS

SAP Family Proteins

Akikazu Fujita and Yoshihisa Kurachi[1]
Department of Pharmacology II, Graduate School of Medicine, Osaka University, 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan

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Thus far, five members including Dlg, SAP97/hDlg, SAP90/PSD-95, SAP102, and PSD-93/chapsyn110 which belong to SAP family have been identified. Recent studies have revealed that these proteins play important roles in the localization and function of glutamate receptors and K^+ channels. Although most of them have been reported to be localized to the synapse, only one member, SAP97, is expressed also in the epithelial cells. In this review, we have summarized structural characters of SAP family proteins and discuss their functions in neurons and epithelial cells.

A family of synapse-associated proteins (SAPs) has recently emerged as a central player in the molecular organization of synapses. Members of SAP family include Dlg, SAP97/hDlg, SAP90/PSD-95, SAP102 and PSD-93/chapsyn110. They are also called MAGUK (for membrane-associated guanylate kinase), which are composed of multiple sites of protein–protein interactions, i.e., three PDZ (PSD-95/DLG/ZO1) domains, a src homology 3 (SH3) region, and a guanylate kinase (GK)-like sequence (Fig. 1B; 1, 2). The best characterized is the PDZ domain which binds with high affinity to the carboxyl-terminal peptide motif X-T/S-X-V/I (tSXV-motif) in a number of proteins including the NR2 subunits of the NMDA receptor, the voltage-gated and inwardly rectifying K^+ channels (1–3). This interaction appears to mediate the clustering of ion channels and receptors at specific synaptic sites.

SAPs are localized either to the pre- and/or postsynaptic sides of excitatory or inhibitory synapses. Interestingly, one family member, SAP97, is present also in epithelial cells and localized at the lateral membrane between cells. In this review, we have summarized the structural character of SAP family proteins and discuss their functions in neurons and epithelial cells.

[1] To whom correspondence should be addressed. Fax: +81-6-6879-3519. E-mail: ykurachi@pharma2.med.osaka-u.ac.jp.

SYNAPSE

Glutamate receptor/channels are actually imbedded in the postsynaptic density (PSD), an electron-dense thickening which represents a fibrous specialization of the submembrane cytoskeleton at the postsynaptic membrane. The four types of glutamate (NMDA, AMPA, kainate and metabotropic glutamate) receptors differ in their subsynaptic distribution, i.e., metabotropic glutamate receptors being located at the periphery, whereas NMDA and AMPA receptor/channels are in the central region of PSD (Fig. 1A; 4). This suggests that distinct mechanisms underlie the subsynaptic distribution of each glutamate receptor. Subunits of NMDA and kainate receptors were shown to bind to SAP family proteins (see below). AMPA (GluR2 and GluR3) and metabotropic glutamate (mGluR1 and mGluR5) receptor subunits bind to other anchoring molecules (see Fig. 1A; 44).

NMDA Receptor/Channels

SAP family proteins were reported to interact closely with the subunits of NMDA receptor/channels and to influence their locations. The postsynaptic density protein, PSD-95 (also known as the synapse-associated protein 90 kDa, or SAP90) is a cytoskeleton-associated protein of ~95-kDa abundant in the postsynaptic synaptosomal fraction (1, 5, 6). PSD-95 has been reported to interact directly with the C-terminal domains of six different NMDA receptor/channels subunits (6) (NR1-3, NR1-4, NR2A, NR2B, NR2C and NR2D; where NR1 subtypes are represented according to the nomenclature of Ref. 7) and to influence their clustered distributions. Other members of the SAP family, i.e., PSD-93, SAP102 and SAP97, were also shown to interact with the C-terminal end of NMDA receptor/

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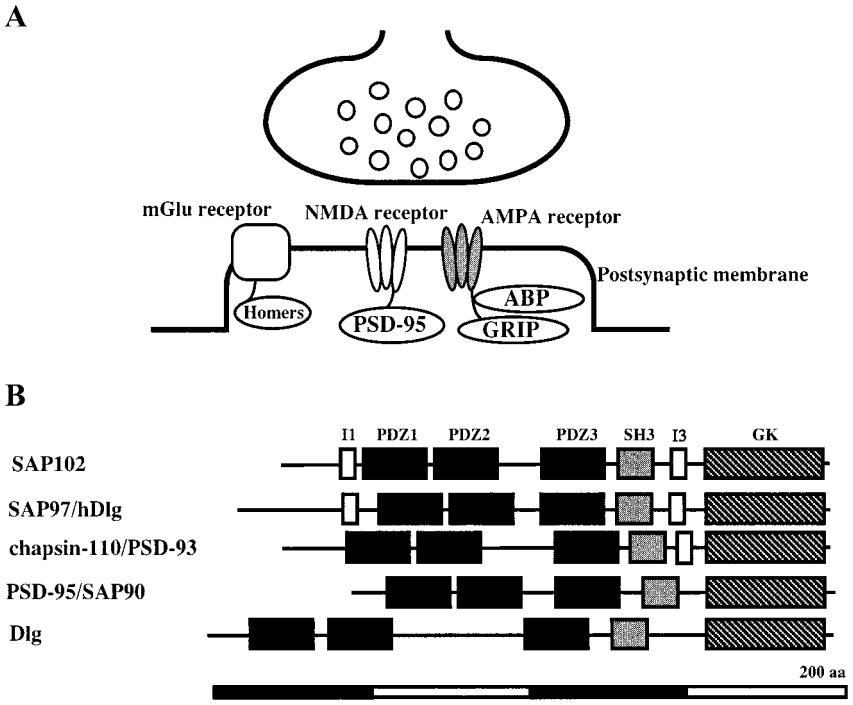

FIG. 1. Glutamate receptors in postsynaptic density (A) and the structure of SAP family proteins (B). (A) Postsynaptic NMDA receptors, AMPA receptors, and metabotropic glutamate (mGlu) receptors are shown to bind to specific PDZ domains of PSD-95, GRIP and ABP, and Homers, respectively. Subsynaptic segregation of receptors is illustrated: NMDA and AMPA receptors are located the central of the synapse, whereas mGlu receptor is located at the periphery. (B) I1: Insert 1, I3: Insert 3, GK: Guanylate kinase-like domain.

channels (6, 8–10). Each member of SAP family shows a specific tissue distribution pattern. PSD-95, PSD-93 and SAP102 are mainly distributed to PSD, while SAP97 exhibits presynaptic and axonal distributions (11). In rat brain, PSD-93 was coimmunoprecipitated with PSD-95 and showed a somatodendritic expression pattern that overlapped partly with PSD-95. These data suggest that PSD-93, but not SAP97, and PSD-95 may interact at postsynaptic sites to form a complex for the clustering of NMDA receptors (8).

These data also suggest that the specific interaction of NMDA receptor/channels with PDZ domain-containing proteins is important for determining the localization of the receptors to PSD (Fig. 1A). In order to ascertain whether this is true, the genes encoding NR2 subunits that lack their C-terminal domain were expressed in mice (12, 13). Specific phenotypes, which are similar to those observed when the gene encoding the entire subunit is deleted, were observed for both mutants. Sprengel *et al.* (13) suggested that a disturbed recruitment of the signal-transducing machinery is the main cause for the phenotypes. On the other hand, Mori *et al.* (12) visualized that the mutated NR2B subunits are not clustered and distribute to not only PSD but also in nonsynaptic regions. These data further support a role for PDZ domain-containing proteins in the maintenance of receptor clusters and the anchoring of these clusters in the subsynaptic scaffolds.

Kainate Receptor/Channels

Two subunits of kainate receptor, GluR6 and KA2, were reported to interact with PSD-95 and SAP102 (14) in SAP family. Similar to NMDA receptors, GluR6 clustering is mediated by its interaction with the PDZ domain of PSD-95. In contrast, the clustering of KA2 is caused by its interaction with the SH3 and GK domains of PSD-95 (see Protein–Protein Interaction of PDZ, SH3, and GK). These data suggest that NMDA and kainate receptor/channels may cluster at postsynaptic sites by interacting with PSD-95 and SAP102.

Scaffolding Functions of SAP

Various proteins that are involved in the intracellular signaling stimulated by glutamate-receptor activation, including nitric oxide synthase and synaptic Ras-GTPase-activating protein (SynGAP), also interact with SAP family proteins (Figs. 2A and 2B; 15, 16, 17). Additional organizer proteins, such as guanylate-kinase-associated protein (GKAP/SAPAP/DAP), which binds to the GK domain of PDZ-containing proteins, could further contribute to form a complex protein scaffold (18–20). It is possible that the PDZ domain-containing proteins are, therefore, not only involved in the maintenance of the receptor "constellation" at synapses but might also facilitate an efficient signaling by keeping key enzymes in close proximity (Figs. 2A and 2B).

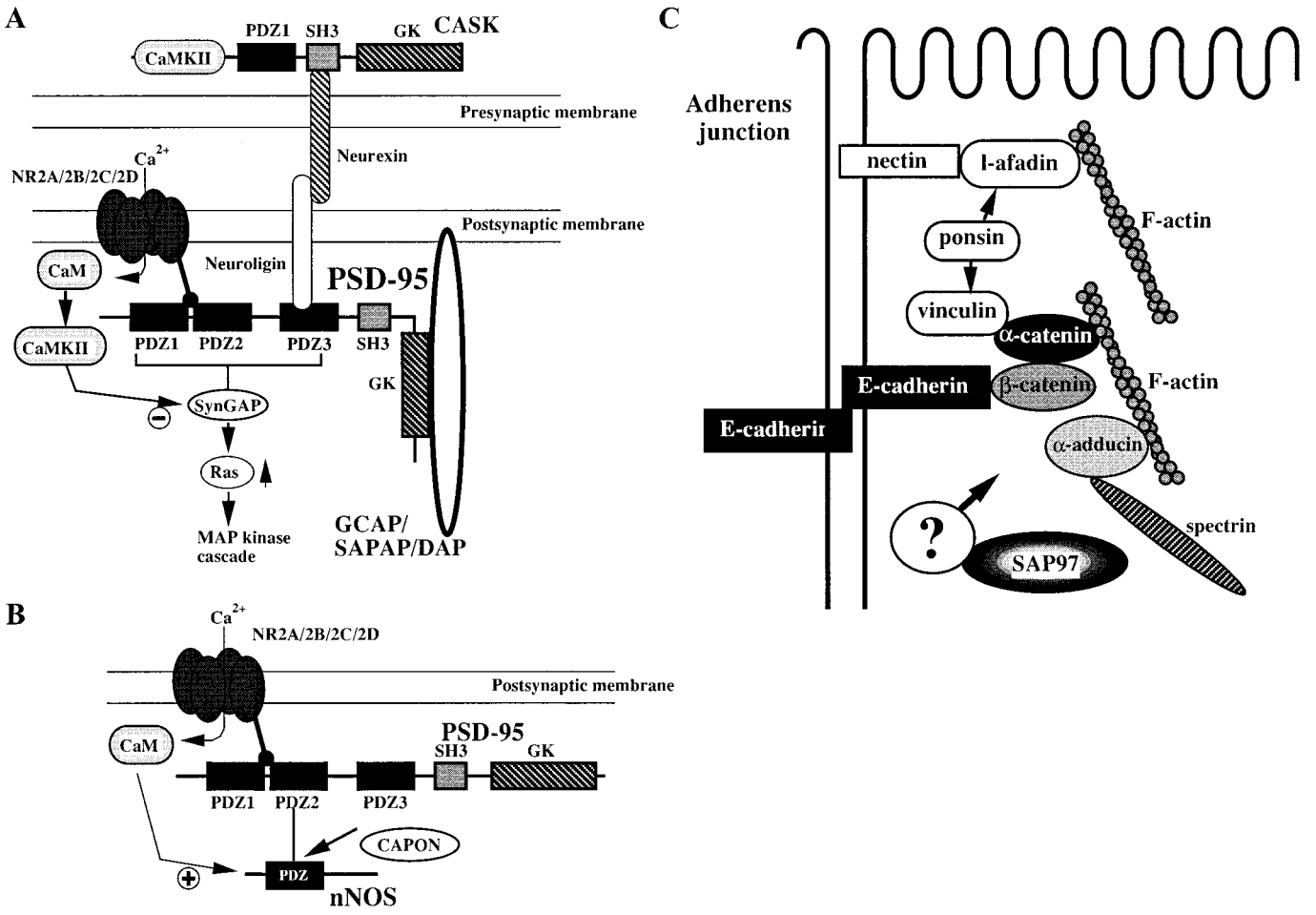

FIG. 2. Scaffolding functions of SDP-95/SAP90 (A and B) and SAP97 in epithelial cell (C). (A) Scaffolding of MAP kinase signaling. Active SynGAP at postsynaptic densities keeps the steady-state level of active Ras low near the synapse because of its rapid catalyzing hydrolysis of Ras-GTP to Ras-GDP. Activation of NMDA receptors produces an influx of Ca^{2+} that activates CaMKII at the postsynaptic density. CaMKII then phosphorylates and inactivates SynGAP, releasing the brake on the accumulation of active Ras-GTP and leading to increased activation of the MAP kinase cascade. In this manner, activation of the NMDA receptor may potentiate the action of any signal that leads to formation of Ras-GTP. Such potentiation would constitute yet another form of coincidence detection by the NMDA-type glutamate receptor. SAPAP is tightly attached to the membrane and interacts with the GK domains of PSD-95 and S-SCAM. The extracellular domain of neuroligin binds to neurexin, whose cytoplasmic domain interacts with the PDZ domain of CASK. (B) Scaffolding of nNOS signalling. NMDA receptors are coupled to nNOS through a PSD-95. These interactions are mediated by PDZ domains. In this complex, nNOS is situated close to NMDA receptor-modulated calcium influx. Binding of CAPON results in a reduction of NMDA receptor/PSD-95/nNOS complexes, leading to decreased access to NMDA receptor-gated Ca^{2+} influx and a catalytically inactive enzyme. (C) SAP97 is associated with the cortical cytoskeletons which compose the adherens junction. SAP97 was reported to be associated with, but not directly bind to, F-actin, E-cadherin, and α-, β-catenin, although it is not known whether SAP97 is associated with l-afadin, nectin, ponsin, and vinculin.

INTERACTION OF SAPs AND K^+ CHANNELS

Voltage-Gated K^+ Channels

The SAP family proteins interact also with subunits of several voltage-dependent K^+ (K_V) channels (2, 21). The voltage-dependent K^+ channels are concentrated at specific microdomains of the neural membrane, including presynaptic terminals, nodes of Ranvier, and dendrites, where they regulate local membrane excitability. Evidence has been obtained that cell-surface clustering of Shaker type-K^+ channels, such as $K_V1.4$, is mediated by SAP family of membrane- and cytoskeleton-associated proteins. This occurs through direct and specific binding of the C-terminal cytoplasmic tails of K^+ channel subunits to first and/or second PDZ domains of PSD-95 protein (Table 1).

Inwardly Rectifying and Two-Pore K^+ Channels

In addition to promoting clustering of NMDA receptors and voltage-dependent K^+ channels, PSD-95 also binds inwardly rectifying K^+ channels, including Kir2.1, Kir2.3 and Kir4.1 (Table 1). *In situ* mRNA analysis demonstrated that both Kir2.3 and PSD-95

TABLE 1

Proteins That Carry a T/SXV Motif

	E-T/SXV motif		Hydrophobic-T/SXV motif	
NMDA receptors	NR1–3[a]	-STVV		
	NR1–4[a]	-STVV		
	NR2A	-ESDV		
	NR2B	-ESDV		
	NR2C	-ESDV		
	NR2D-2	-ESDV		
Glutamate receptors	GluR6	-ETMA		
Voltage-gated K^+ channels	Kv1.4	-ETDV	Kv1.1	-LTDV
	Kv1.5	-ETDV	Kv1.2	-LTDV
			Kv1.3	-FTDV
			Kv1.6	-LTEV
			Kv3.2b	-PSIL
			Kv3.3b	-PSIL
			Kv4.1	-ISSL
			Kv4.2	-VSAL
			Kv4.3	-VSAL
Inwardly rectifying K^+ channels	IRK1/Kir2.1	-ESEI	K_{AB}-2/Kir4.1	-ISNV
	IRK2/Kir2.2	-ESEI		
	IRK3/Kir2.3	-ESRI		
	GIRK2C/Kir3.2c	-ESKV		
K^+ channels with two-pore domains			cTBAK-1 (TASK-1)	-RSSV
G-protein-coupled receptors	β1 receptor	-ETVV	5HT2A	-VSCV
			5HT2C	-ISSV
	β2 receptor	-DSLL	VIP	-VSLV
	P2Y1	-DTSL	SSTR2	-QTSI
Transportor	CFTR	-DTRL		
Others			GRK6	-PTRL
			PKCα	-QSAV

Note. Various kinds of proteins can bind to the PDZ domains of anchoring proteins via a T/SXV motif in their C-termini. Abbreviations: SSTR2; somatostatin receptor 2, CFTR; cystic fibrosis transmembrane conductance regulator, GRK6; G protein-coupled receptor kinase 6, PKCα; protein kinase Cα, see the text for other abbreviations.

[a] NR1 subtypes are represented according to the nomenclature of Ref. 7.

are specifically enriched and colocalized in granule cells of the dentate gyrus region of the hippocampus (22). PSD-95 and Kir2.3 were actually coimmunoprecipitated from hippocampal synaptic membranes (22). These data suggest that Kir2.3 and PSD-95 bound to form a protein complex in neurons of CNS.

The neuronal G-protein gated K^+ (K_G) channels are heterotetramers of Kir3.1/GIRK1 and Kir3.2/GIRK2. In substantia nigra (SN), however, K_G channels are composed of only Kir3.2 subunits. An immunological study showed that Kir3.2 is localized specifically at the postsynaptic membrane on the dendrites of dopaminergic neurons (23). Biochemical studies showed that at least some of the K_G channels in SN are composed of the splicing variants, Kir3.2a/GIRK2a and Kir3.2c/GIRK2c. Kir3.2c, but not Kir3.2a, possesses the PDZ domain-interacting motif (−ESKV) in the C-terminal domain (Table 1). The heterologously expressed K_G channels composed of Kir3.2a and Kir3.2c or Kir3.2a alone are activated by G-protein stimulation, while expression of Kir3.2c alone is not. These data suggest that the Kir3.2 splicing variants play distinct roles in the control of function and localization of some of the K_G channels in dopaminergic neurons of SN.

In addition, it was also found that a cardiac two-pore K^+ channel (cTBAK) possesses the C-terminal domain interacting with PSD-95 family proteins (Table 1; 24). Therefore, many other K^+ channels might also be under control of SAP family proteins in various organs.

PROTEIN–PROTEIN INTERACTION OF PDZ, SH3, AND GK

SAP family proteins are also called MAGUK and characterized by the existence of Src 3 homology (SH3) and guanylate kinase-like (GK) domains in the C-terminal region in addition to the presence of PDZ domains (Fig. 1B; 1, 2). PDZ domains are viewed as a module of protein-binding sites which recognizes a short consensus peptide sequence of large proteins. This is responsible for a certain type of specific protein–protein association (2). PDZ domains are composed of ~90 amino acid residues. Three repeats of PDZ domains exist in the N-terminal half of

PSD-95-related proteins (PSD-95, SAP97/hDlg, PSD-93/chapsyn110 and SAP102). The PDZ domain is also called the Dlg homologous region (DHR) or the GLGF repeat, because most of the initially identified PDZ domains contain Gly-Leu-Gly-Phe in the sequences. The C-terminal regions of the NR2 subunits of the NMDA receptor, Shaker type K^+ channels and Kir channels possess four highly conserved amino acids (-(E)S/TXV- motif) that are specifically recognized by the PDZ domains in SAP family proteins (1–3). X-ray crystallography has revealed that the third PDZ domain of PSD-95 is composed of six β sheets and two α helices. The C-terminal peptide (-TDV and -QTSV) binds to the groove between the second β sheet and the second α helix, and a carboxylate binding loop provided by GLGF (3, 25).

The ability of PDZ domains to function as independent modules for protein–protein interaction suggests that PDZ domain-containing polypeptides may be widely involved in the organization of proteins at the specialized domains of membrane (26, 27). While Shaker, Kir and NR2 proteins do not cluster when expressed alone, coexpression of them with PSD-95, SAP97, or PSD-93 results in the coclustering of channel and anchoring proteins (8, 21, 27, 28). This emphasizes the importance of SAP family proteins in directing the distribution of NMDA, voltage-dependent K^+ and Kir channels.

The possible mechanisms of membrane protein clustering by PSD-95 have been considered as follows. It was recently shown that third and fifth cysteines at the N-terminus of PSD-95 are palmitoylated, which is indispensable for its clustering and targeting (29) although it was proposed that PSD-95 formed a dimer, due to a head-to-head linkage that is mediated by disulfide bonds between the conserved N-terminal regions (30). The third PDZ domain of PSD-95 interacts with neuroligin which is a neuronal cell adhesion molecule (31). These findings suggest that PSD-95 assembles receptors and channels, and then fixes them at the specialized membrane domain through its interaction with cell adhesion molecules.

Recent studies have revealed that although it has no guanylate kinase activity (32), the GK domain of SAP family also acts as an anchoring domain to GKAP/SAPAP/DAP (18–20), MAP1A (33), BEGAIN (34), and KA2 subunit of kainate receptor/channel (14) (KA2 subunit also binds to SH3 domain of PSD-95, SAP102, as described under Kainate Receptor/Channels). GKAP/SAPAP/DAP also binds to a synaptic protein named S-SCAM (35).

EPITHELIAL CELLS

Localization of SAP in Epithelial Cells

One member of SAP family, SAP97, is present in epithelial cells of such tissues as choroidal plexus and ileum (11). It is localized along the lateral membrane at cell–cell adhesion sites (Fig. 2C). In *Drosophila*, it was shown that mutations of Dlg result in loss of apical–basolateral epithelial cell polarity and intercellular adhesion of epithelial cells within imaginal discs (36). These results suggest that SAP97/hDlg plays an important role in the determining of cell polarity and cell–cell adhesion of epithelial cells.

SAP97 was reported to be associated with the cortical cytoskeleton (F-actin, E-cadherin, and α- and β-catenin) at cell–cell contact sites in epithelial CACO-2 cells and fibroblast L-cells (37). SAP97 binds the erythrocyte cytoskeletal protein 4.1 via I3 localized between SH3 and GK domains (see Fig. 1B) and also a region between PDZ1 and PDZ2 (38, 39). Although these data led to the hypothesis that these interactions are important for the subcellular targeting of SAP97 in epithelial cells (39), it was firmly shown that the first 65 amino acid residues ($S97N_{1-65}$) in SAP97 direct the selective subcellular localization (40). The amino acid homologous region with $S97N_{1-65}$ is absent from PSD-95 and SAP102. This might be the reason why PSD-95 and SAP102 located in the cytoplasm but not at the lateral membrane, when expressed in CACO-2 cells (40). The region of $S97N_{1-65}$ is also required for the attachment of SAP97 to the cortical cytoskeleton (40). These results indicate that $S97N_{1-65}$ appears to perform a primary role in the attachment of SAP97 to the cortical cytoskeleton and in the selective localization at the lateral membrane (Fig. 2C).

SAP and Inwardly Rectifying K^+ Channel, Kir4.1

Kir4.1, a glial cell inwardly rectifying K^+ channel (41), binds to PSD-95 and SAP97, and is colocalized with SAP97 in retinal Müller cells (28) and possibly in renal tubular epithelium and in the marginal cells of cochlear stria vascularis (42, 43). This anchoring protein seems not only to cluster Kir4.1 on the cell membrane, but also to stimulate the channel current by increasing the functional channel number in the cell membrane (28).

CONCLUSION

Three main roles of the SAP family in the regulation of NMDA and kainate receptor/channels, and ion channels function have been recognized: (1) targeted distribution of receptor and channel proteins within specialized domains of the plasma membranes, (2) scaffolding of functional molecules, and (3) modulation of ion channel activity. Structural interactions between the cytoskeleton and SAP proteins may determine the highly specialized distribution of receptors and ion channel proteins within certain domains of plasma membrane. Such domain-dependent distribution and anchoring of receptors and channels are required for proper intra- and intercellular

signaling. The mechanisms underlying targeting and activity modulating of receptor and channel by SAP family proteins should be further investigated.

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