MAGUIN, a Novel Neuronal Membrane-associated Guanylate Kinase-interacting Protein*

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Postsynaptic density (PSD)-95/Synapse-associated protein (SAP) 90 and synaptic scaffolding molecule (S-SCAM) are neuronal membrane-associated guanylate kinases. Because PSD-95/SAP90 and S-SCAM function as synaptic scaffolding proteins, identification of ligands for these proteins is important to elucidate the structure of synaptic junctions. Here, we report a novel protein interacting with the PDZ domains of PSD-95/SAP90 and S-SCAM and named it MAGUIN-1 (membrane-associated guanylate kinase-interacting protein-1). MAGUIN-1 has one sterile α motif, one PDZ, and one plekstrin homology domain. MAGUIN-1 is localized at the plasma membrane via the plekstrin homology domain and the C-terminal region and interacts with PSD-95/SAP90 and S-SCAM via a C-terminal PDZ domain-binding motif. MAGUIN-1 has a short isoform, MAGUIN-2, which lacks a PDZ domain-binding motif. MAGUINs are expressed in neurons and localized in the cell body and neurites and are communoprecipitated with PSD-95/SAP90 and S-SCAM from rat crude synaptosomes. MAGUIN-1 may play an important role with PSD-95/SAP90 and S-SCAM to assemble the components of synaptic junctions.

Synaptic junctions are interneuronal cell-cell junctions differentiated for neurotransmission. Neuronal transmitters are released from the synaptic vesicles into the synaptic cleft and bind to the receptors accumulated at the postsynapse, opening the ion channels and generating the second messengers in-leased from the synaptic vesicles into the synaptic cleft and released into the synaptic cleft. Among six PDZ domains, PDZ1 and -5 bind to the C termini of glutamate receptor-interacting protein have not been so far reported. Synaptic scaffolding molecule (S-SCAM) was originally identified as a SAPAP-interacting protein (24). We have first reported that S-SCAM has one GK, two WW, and five PDZ domains (24). The GK domain of S-SCAM is shorter than that of PSD-95/SAP90. The WW domain is a protein-interacting module binding a proline-rich sequence (25). The recent version of simple modular architecture research tool recognizes an additional PDZ domain at the N terminus of S-SCAM (26). We number these PDZ domains consecutively from 0 to 5 (PDZ0, -1, -2, -3, -4, and -5) to keep consistency with the first report. Among six PDZ domains, PDZ1 and -5 bind to the C termini of neuroligin and NMDA receptors, respectively (24). Because S-SCAM has more PDZ domains than PSD-95/SAP90, it may integrate more components of synaptic junctions. Based on this assumption, we have performed a yeast two-hybrid screening using the PDZ domains of S-SCAM to obtain a novel neuronal molecule. Eventually, this molecule binds not only to S-SCAM but also to PSD-95/SAP90. We have named this protein MAGUIN-1 (membrane-associated guanylate kinase-interacting protein-1).

MATERIALS AND METHODS

Yeast Two-hybrid Screening and cDNA Cloning—Rat brain yeast two-hybrid library was constructed using pVP16 vector and screened (27). Rat brain cDNA libraries were screened with the -1-3’PDgcdCP-labeled random-primer probes (27).

Construction of Expression Vectors—Various expression vectors were constructed by conventional molecular biology techniques and polymerase chain reaction method using pBTM116, pBTP116-2, pCMV Myc, pCMV Myc2, pClneo Myc, pGex5X-3 (Amersham Pharmacia Biotech), pGex4T-1 (Amersham Pharmacia Biotech), and pGexRG. pBTP116-2, pCMV Myc, and pClneo Myc were constructed from pBTM116, pCMV5, and pClneo, respectively (24). pCMV Myc2 was constructed by ligating attcattgagatctcgggtaccacgcgtatcgatatcgcggccg/ctagcggccgcgatatcgatacgc-

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‡The abbreviations used are: PSD, postsynaptic density; SAP, synapse-associated protein; PDZ, PSD-95/Dlg-A/ZO-1; GK, guanylate kinase; NMDA, N-methyl-D-aspartate; SAPAP, SAP90/PSD-95-associated protein; S-SCAM, synaptic scaffolding molecule; GST, glutathione S-transferase; SAM, sterile α motif; PH, plekstrin homology; SPM, synaptic plasma membrane; MAPK, mitogen-activated protein kinase; CHO, Chinese hamster ovary.

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** The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM and EBI Data Bank with accession number(s) AF102853 and AF102854.


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two-hybrid screening is underlined.

beads were washed four times with 50 mM NaOH/Hepes, pH 8.0, containing 100 mM NaCl and 1% (w/v) Triton X-100, proteins on enized in 16 ml of 50 mM Hepes/NaOH, pH 8.0, containing 100 mM NaCl and 1% (w/v) Triton X-100 and centrifuged at 100,000 g for 30 min to collect the supernatant and the pellet. The pellet was homogenized in 220 mlo f2 0m M Hepes/NaOH, pH 7.4, by sonication. 80 mlo of the homogenate was kept for the analysis, and the remaining samples were centrifuged at 100,000 × g for 30 min to separate the supernatant and the pellet.

RESULTS

Identification of MAGUINs—We performed the yeast two-hybrid screening using baits containing the PDZ domains of S-SCAM (pBTM116 S-SCAM-13, -14, and -16). We obtained 34 positive independent clones from 5 × 10⁶ clones of a rat brain cDNA library. The sequences of 22 clones were novel, and we performed Northern blot analysis for these clones. The messages of two clones (pPrey 4233 and pPrey 4514) were detected only in the brain. During the study, the presumptive full-length coding sequences of pPrey 4233 through the conventional hybridization screening using a rat brain cDNA library and polymerase chain reaction using rat brain cDNAs as templates. Two isoforms were detected and named MAGUIN-1 and -2. MAGUIN-1 had 1032 amino acids and was composed of one novel, and we performed Northern blot analysis for these clones. The messages of two clones (pPrey 4233 and pPrey 4514) were detected only in the brain. During the study, the presumptive full-length coding sequences of pPrey 4233 through the conventional hybridization screening using a rat brain cDNA library and polymerase chain reaction using rat brain cDNAs as templates. Two isoforms were detected and named MAGUIN-1 and -2. MAGUIN-1 had 1032 amino acids and was composed of one gene and two PDZ domains. MAGUIN-2f contain the amino acid residues 568–896 and 1–896 of MAGUIN-1, respectively. pGex5X-3 MAGUIN-16 and pCMV Myc PSD-95-1, -5, and -6 contain the amino acid residues 1–724, 1–407, and 432–724 of PSD-95/SAP90, respectively.
His-Val at the C terminus, which corresponded to the PDZ domain-binding motif. pPrey 4233 contained the C-terminal 319 residues of MAGUIN-1 (amino acids 714–1032, underlined in Fig. 1A). MAGUIN-2 contained the N-terminal 895 amino acids of MAGUIN-1 and is terminated with Ser as the last (896th) residue, lacking the PDZ domain-binding motif (Fig. 1B). The SAM domain of MAGUINs is about 30% homologous to those of *Caenorhabditis elegans* R01H10.8 and yeast *byr2* and about 15% homologous to that of yeast *STE50* (28–31). The PDZ domain of MAGUINs is about 40% homologous to that of *C. elegans* R01H10.8 (Fig. 2B). The homology with the PDZ domain of PSD-95/SAP90 or CASK is about 15%, and among others the residues of the first α helix are rather well conserved (Fig. 2B) (32, 33). The PH domain of MAGUINs is about 20% homologous to those of *C. elegans* R01H10.8 (Fig. 2C). Although the complete coding sequence of KIAA0403 is not reported, it may be a human isoform of MAGUINs. Because *C. elegans* R01H10.8 has a similar molecular structure, it may be a *C. elegans* homologue of MAGUINs.

**Interaction of MAGUIN-1 with S-SCAM and PSD-95/SAP90**—To confirm the interaction of MAGUIN-1 with S-SCAM, the extract of COS cells expressing S-SCAM was incubated with either GST-MAGUIN-12 containing the C terminus of MAGUIN-1 or GST-MAGUIN-16 containing the C terminus of MAGUIN-2. The C terminus of MAGUIN-1 interacted with S-SCAM, whereas the C terminus of MAGUIN-2 did not (Fig. 3A). MAGUIN-1 was coimmunoprecipitated with S-SCAM from the rat crude synaptosomal fraction (Fig. 3B and C). To determine the interacting region of S-SCAM with MAGUIN-1, various Myc-tagged constructs of S-SCAM were incubated with GST-MAGUIN-12 containing the C terminus of MAGUIN-1 (Fig. 4A). Myc-S-SCAM-1, -4, -11, and -10 interacted, whereas Myc-S-SCAM-2, -3, and -9 did not (Fig. 4B), suggesting that the fourth and fifth PDZ domains were involved in the interaction. From the reverse yeast two-hybrid screening using pBTM116 MAGUIN-9 as a bait, the PDZ domains of PSD-95/SAP90, PSD93/chapsyn110, and SAP97 were obtained (36–38), suggesting that MAGUIN-1 interacted with not only S-SCAM but also PSD-95/SAP90 and its isoforms. MAGUIN-1 was coimmunoprecipitated with PSD-95/SAP90 from the rat crude synaptosomal fraction (Fig. 5A). The interaction of MAGUIN-1 with the PDZ domains of PSD-95/SAP90 was confirmed using Myc-tagged constructs of PSD-95/SAP90 and the GST construct of MAGUIN-1 (Fig. 5B).

**Tissue and Subcellular Distribution of MAGUINs**—Northern blot analysis revealed 4.4- and 5.4-kilobase pair messages...
only in brain (Fig. 6). The two messages with different sizes may reflect differential polyadenylation. No message was detected in heart, spleen, lung, liver, kidney, skeletal muscles, or testis. In the subcellular fractionation of rat brain, MAGUINs were detected mainly in the synaptic plasma membrane (SPM) and PSD fractions (Fig. 7A). Two bands with different molecular sizes may represent protein degradation, post-translational modifications, or alternative splicing isoforms. In rat hippocampal neurons, MAGUINs were distributed in the cell body and the neurites and colocalized with NMDAR1 (Fig. 7B).

Interaction of MAGUINs with Membrane—The PH domain is known to interact with the phospholipid membrane (reviewed in Refs. 39 and 40). To test whether MAGUINs associate with the plasma membrane through the PH domain, various Myc-tagged constructs of MAGUINs were transfected in CHO cells (Fig. 8A). Full-length MAGUIN-1 and -2 and the construct containing the PH domain with the C-terminal stretch were localized at the plasma membrane (Fig. 8B, If, 2f, and m). The construct containing the SAM and PDZ domains was distributed in the cytosol (Fig. 8B, n). The construct containing the C-terminal region of MAGUIN-1 was localized at the plasma membrane, although it lacked the PH domain (Fig. 8B, c). The similar results were obtained in the subcellular fractionation of CHO cells transfected with these constructs. Full-length MAGUIN-1 was recovered in the membrane fraction (Fig. 9A). The N-terminal construct containing the SAM and PDZ domains was distributed more in the cytosol than in the membrane fraction (Fig. 9B). The PH domain with the C-terminal region of MAGUIN-1 was localized at the plasma membrane, although it lacked the PH domain (Fig. 8C). The C-terminal construct was recovered mainly in the membrane fraction with a smaller amount in the cytosol (Fig. 9C). Because the construct containing only the PH domain was not expressed, we could not determine whether the PH domain was directly involved in the membrane association of MAGUIN-1. However, these findings suggest that MAGUIN-1 associates with the plasma membrane through the region containing the PH domain and the C-terminal stretch of the PH domain. The N-terminal, PH domain, and C-terminal constructs were Triton X-100-soluble, whereas the full-length construct of MAGUIN-1 was Triton X-100-insoluble (Fig. 9), suggesting that the whole structure of MAGUIN-1 is necessary for the interaction with the Triton X-100-insoluble structures.
Recruitment of PSD-95/SAP90 and S-SCAM into Triton X-100-insoluble fraction by MAGUIN-1—In the last set of experiments, we tested whether MAGUIN-1 affected the subcellular localization of PSD-95/SAP90 and S-SCAM in the transfected cells. PSD-95/SAP90 and S-SCAM were distributed in the Triton X-100-soluble fraction in CHO cells (Fig. 10, A and B). MAGUIN-1 and -2 were distributed in the Triton X-100-insoluble fraction (Fig. 9A and data not shown). PSD-95/SAP90 and S-SCAM were recruited into the Triton X-100-insoluble fraction, when coexpressed with MAGUIN-1 (Fig. 10, C and D). In contrast, PSD-95/SAP90 and S-SCAM remained in the Triton X-100-soluble fraction, when coexpressed with MAGUIN-2 (data not shown).

DISCUSSION

In this paper, we have identified a novel ligand for S-SCAM and named it MAGUIN-1. We have also reported its short isoform, MAGUIN-2. MAGUIN-1 binds to PSD-95/SAP90 as well as to S-SCAM. MAGUINs have a unique combination of protein modules including SAM, PDZ, and PH domains. SAM domain is proposed to mediate protein binding or DNA binding (reviewed in Refs. 28 and 29). The subcellular localization of MAGUINs precludes a DNA binding role, but some protein may interact with MAGUINs via the SAM domain. The PDZ domain is a well known protein module that binds to the C terminus of other proteins (reviewed in Refs. 2–6). The study using the peptide library revealed the existence of two classes of the PDZ domains (41). Class I PDZ domains, such as those of PSD-95/SAP90, select the peptides containing Glu-(Ser/Thr)-Xaa-(Val/Ile) (Xaa is any amino acid) at the C terminus, whereas class II PDZ domains, such as that of CASK, select the peptides with hydrophobic or aromatic side chains at the C-terminal three residues (41). The PDZ domains are also reported to interact with other PDZ domains (36, 42). The PDZ domain is composed of two α helices and six β sheets, and the second α helix and second β sheet provide a carboxyl-binding loop (32, 33). The residues of this loop of MAGUINs are diverged from those of PSD-95/SAP90 or CASK, suggesting that the ligand for the PDZ domain of MAGUINs has the C-terminal residues different from either group 1 or group 2 consensus motif (41). No interacting protein was obtained from the yeast two-hybrid screening using the PDZ domain of MAGUINs as a bait (data not shown), and whether the PDZ domain of MAGUINs functions as a protein-interacting module needs to be investigated. The PH domain binds to inositol phosphates and phosphoinositides and regulates the membrane association of many signaling proteins (reviewed in Refs. 39 and 40). We could not determine whether the PH domain was required for the membrane association, but we observed that MAGUIN-1 binds to the plasma membrane through the region containing the PH domain and the C-terminal region. This finding suggests that the PH domain can support the membrane attachment, as well as the C-terminal region. The C-terminal stretch of the PH domain of β-adrenergic receptor kinase is reported to bind the βγ subunits of heterotrimeric GTP-binding proteins (Gβγ) (43). The C-terminal stretch of the PH domain of MAGUINs is diverged from that of β-adrenergic receptor kinase, and the interaction of MAGUIN-1 with Gβγ is not detected (data not shown). However, because the C-terminal region of MAGUIN-1 also mediates the membrane association, this region also has a binding activity for lipid or some membrane protein.

MAGUIN-1 is a common ligand for PSD-95/SAP90 and S-SCAM, which are both neuronal multiple PDZ domain-containing proteins. PSD-95/SAP90 and S-SCAM bind NMDA receptors, K⁺ channels, and neuroligin through distinct PDZ domains and assemble these molecules at synaptic junctions. PSD-95/SAP90 further interacts with neuronal nitric-oxide synthase, synGAP, and CRIP T (16–18, 36). synGAP regulates the activity of a small GTP-binding protein, Ras (16, 17). Dur-
ing this study, a Drosophila gene that genetically interacts with kinase suppressor of ras (ksr) has been reported and named connector enhancer of ksr (cnk) (44). cnk functions in the Ras/mitogen-activated protein kinase (MAPK) pathway, and the product of cnk physically binds Raf kinase. CNK has a molecular structure similar to that of MAGUNs. Therefore, MAGUN-1 may also bind Raf kinase and links it to PSD-95/SAP90 and S-SCAM. The yeast two-hybrid screening using the
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GK domain of PSD-95/SAP90 revealed SPA-1-like protein besides SAPAP and BEGAIN (22). SPA-1 is a GAP protein for Rap1 (45), and Rap1 plays roles in the MAPK pathway (46). We have not confirmed the interaction of SPA-1-like protein with PSD-95/SAP90 using other methods and have not tested whether it has a GAP activity. However, these findings suggest a model that the components implicated in the Ras/MAPK pathway are assembled through the complex of PSD-95/SAP90 and MAGUIN-1. This model is interesting, because the Ras/MAPK pathway is suggested to be implicated in the synaptic plasticity (reviewed in Ref. 47). We are now testing this model.

MAGUIN-1 is Triton X-100-insoluble and recruits PSD-95/SAP90 and S-SCAM into the Triton X-100-insoluble fraction. SAPAP has a similar activity for PSD-95/SAP90 and S-SCAM (22). These findings suggest that PSD-95/SAP90 and S-SCAM are connected to the Triton X-100-insoluble structures via the PDZ domain by MAGUIN-1 and via the GK domain by SAPAP. MAGUIN-2 does not bind to PSD-95/SAP90 or S-SCAM. MAGUIN-2 may compete with MAGUIN-1 for the same ligands, such as Raf kinase, and switch off these ligands from the network around PSD-95/SAP90 and S-SCAM.

K. Hirao, unpublished observation.
C. elegans R01H10.8 also has a structure similar to that of MAGUIns and may be a homologue of MAGUIns (28). C. elegans has a putative S-SCAM homologue, K01A.6. Analysis of the mutants of R01H10.8 and K01A.6 may enlighten the physiological function of MAGUIns and the significance of the interaction of MAGUIN-1 with S-SCAM.

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