The postsynaptic density and dendritic raft localization of PSD-Zip70, which contains an N-myristoylation sequence and leucine-zipper motifs

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Summary

The postsynaptic site of the excitatory synapse, which is composed of the postsynaptic density (PSD) attached to the postsynaptic membrane, is a center for synaptic plasticity. To reveal the molecular organization and functional regulation of the postsynaptic site, we cloned a 70 kDa protein that is concentrated in PSDs using a monoclonal antibody against the PSD. This protein, named PSD-Zip70, is highly homologous to the human *FEZ1/LZTS1* gene product. PSD-Zip70 contains an N-myristoylation consensus sequence, a polybasic cluster in the N-terminal region and four leucine-zipper motifs in the C-terminal region. Light and electron microscopy showed that this protein was localized to the dendritic spines, especially in the PSD and the postsynaptic membrane. Fractionation of the synaptic plasma membrane demonstrated that PSD-Zip70 was localized to the PSD and the dendritic raft. In Madin-Darby canine kidney (MDCK) cells, exogenous PSD-Zip70 was targeted to the apical plasma membrane of microvilli, and its N-myristoylation was necessary for this targeting. In hippocampal neurons, N-myristoylation was also required for the membrane localization and the C-terminal region was critically involved in the synaptic targeting. These results suggest that PSD-Zip70 may be involved in the dynamic properties of the structure and function of the postsynaptic site.

Key words: Postsynaptic density, PSD, Dendritic raft, Microvilli, Hippocampus, Myristoylation, Leucine-zipper

Introduction

The postsynaptic site of the excitatory asymmetric synapse is characterized by an electron-dense structure known as the postsynaptic density (PSD), which is attached to the postsynaptic membrane. The PSD, which is linked to glutamate receptors, forms a postsynaptic protein lattice composed of numerous PSD scaffold and cytoskeletal proteins and signaling molecules (Sheng and Lee, 2000; Kennedy, 2000; Scannevin and Huganir, 2000; Xiao et al., 2000; Sheng and Sala, 2001). Following synaptic activity, the postsynaptic site contributes to the rapid and marked modulation of synaptic structure and function (Toni et al., 1999). Thus, the postsynaptic site plays a central role in synaptic plasticity. Recent studies have focused on the molecular organization and functional regulation of the postsynaptic site in association with synaptic plasticity. For these purposes, several approaches have been introduced to identify PSD scaffold proteins and their interactions. A typical example is a major PSD scaffold protein, PSD-95, which was discovered by molecular cloning on the basis of partial amino-acid sequence data (Cho et al., 1992) and by using polyclonal antibodies against PSD preparations (Kistner et al., 1993). Subsequently, the yeast two-hybrid screen was used to reveal an NMDA-receptor–PSD-95 interaction (Kornau et al., 1995) and to identify numerous PSD scaffold proteins and their interactions (Tomita et al., 2001; Sheng, 2001). We screened hybridoma cell lines producing monoclonal antibodies (mAbs) against chemically digested PSD preparations and isolated several mAbs against PSD proteins. Using one of these mAbs, we isolated PSD-Zip45, which contains an enabled/VASP homology 1 (EVH1) domain at the N-terminus and leucine-zipper motifs at the C-terminus (Sun et al., 1998; Tadokoro et al., 1999). The EVH1 domain of PSD-Zip45 interacts with the cytoplasmic domain of group 1 metabotropic glutamate receptors (mGluRs), resulting in mGluR clustering that is mediated through the self-multimerization of the extreme C-terminal leucine-zipper motif of PSD-Zip45 (Tadokoro et al., 1999). The same protein was independently isolated as Homer1c (Xiao et al., 1998) and vesl-1L (Kato et al., 1998). Recent studies further demonstrated the dynamic properties of PSD-Zip45 in the dendritic spines (Sala et al., 2001; Okabe et al., 2001). In other recent studies, two proteomic approaches were used to determine the molecular components and interactions of the NMDA-receptor-associated protein complex (Husi et al., 2000) and the core proteins of the PSD (Walikonis

et al., 2000). These approaches will greatly accelerate the discovery of novel proteins and functions of the postsynaptic sites.

Rafts, which are enriched in glycosphingolipids and cholesterol, are discrete membrane microdomains that form in the lipid bilayer (Simons and Ikonen, 1997; Resh, 1999). Specific classes of membrane proteins (e.g. GPI-linked proteins), signaling molecules (e.g. src family kinases and G protein subunits), cytoskeletal proteins (e.g. ERM proteins), scaffold proteins (e.g. GAP-43 and MARCKS), and endocytotic machinery-related proteins (e.g. caveolins) are anchored to lipid rafts and form insoluble complexes after lysis in cold detergents such as Triton X-100. Several proteins in rafts contain combinations of N-terminal myristoylated and adjacent palmitoylated sites. Some proteins are singly myristoylated and possess an adjacent or distant polybasic cluster. Accumulating evidence suggests that the most apparent roles of rafts are in membrane trafficking, signal transduction and cytoskeletal connections (Ikonen, 2001). However, rafts in neurons have not been well documented, with a few exceptions. Suzuki et al. isolated dendritic rafts from the rat forebrain and provided evidence for the localization of AMPA receptor subunits to the dendritic raft (Suzuki et al., 2001). Amyloid β peptides (Aβ), β- and γ-secretase-cleaved products of amyloid precursor protein (APP) and presenilins are potent pathogens for Alzheimer's disease. Lee et al. detected Aβ, APP and presenilin-1 in the brain raft fraction and suggested the occurrence of APP processing during membrane transport (Lee et al., 1998). Recently, parkin, a Parkinson's disease-related protein, was also localized to the brain raft fraction (Fallon et al., 2002).

Here, using one of our PSD mAbs, we identified and performed the molecular cloning of a 70 kDa protein that is concentrated in PSDs and is highly homologous to the human *FEZ1/LZTS1* gene product (Ishii et al., 1999). We found that this protein contains an N-terminal myristoylation consensus sequence and a distant polybasic cluster in the N-terminal domain in addition to four leucine-zipper motifs in the C-terminal domain. Furthermore, the expression of this protein is exclusively dominant in the neurons of some cerebral areas, including the cerebral cortex, hippocampus, olfactory bulb, striatum, and pons and is further localized to the PSD and the dendritic rafts. Experiments in cultured hippocampal neurons to test how this protein is targeted to the membrane and synapse revealed that the N-terminal myristoylation and the polybasic cluster of the N-terminal domain are required for the membrane localization and that the C-terminal domain is involved in the synaptic targeting. Because this PSD-enriched 70 kDa protein is likely to function primarily in neurons, and especially in the postsynaptic sites, we refer to this protein as PSD-Zip70 (Tachibana et al., 1999).

Materials and Methods

Antibody production

The screening of the hybridoma cells producing mAbs against the PSD preparations was described previously (Sun et al., 1998). A monoclonal antibody (mAb204H) produced by the hybridoma clone, which specifically recognized a 70 kDa protein that is concentrated in PSDs, was purified by a protein-A-Sepharose gel matrix and used for immunoscreening and western blotting. Polyclonal antibodies against PSD-Zip70 (pAbZip70) were produced as follows: the cDNA encoding the full-length (amino-acid residues 1-601) PSD-Zip70 was subcloned into pGEX6P1 (Amersham Pharmacia Biotech). GST-fused PSD-Zip70 expressed in *E. coli* BL21 was isolated using glutathione-conjugated Sepharose 4B (Amersham Pharmacia Biotech), and was used to immunize New Zealand White rabbits. The antisera were exhaustively preabsorbed with GST protein, followed by purification using a GST-PSD-Zip70-coupled Sepharose 4B gel matrix.

cDNA cloning and plasmid construction

A cDNA library was constructed from the cerebrum of 7-week-old Sprague-Dawley (SD) rats using a ZAP Express^{TM} cDNA synthesis kit (Stratagene). The cDNA library was immunoscreened with mAb204H, and two positive clones were isolated. The plasmids thus obtained were prepared by the in vivo excision method and sequenced. The wild-type PSD-Zip70 (PSD-Zip70WT) and its N-terminus (PSD-Zip70N, amino-acid residues 1-255) and C-terminus (PSD-Zip70C, amino-acid residues 246-601) were amplified by polymerase chain reaction (PCR) using Pfu polymerase (Stratagene) and subcloned into myc-tagged pcDNA3.1/Zeo(+) (modified from Invitrogen). The C-terminus of PSD-Zip70WT and PSD-Zip70N was tagged, as was the N-terminus of PSD-Zip70C. A point mutation of glycine to alanine (PSD-Zip70N/G2A), and of lysine and arginine to asparagine (R26N,K27N,K32N,K33N;PSD-Zip70Nmut) and a deletion mutant lacking amino-acid residues 21-40 (PSD-Zip70NΔ21-40) were made by site-directed mutagenesis. For the production of GST-fusion protein, the cDNAs encoding PSD-Zip70WT, GST-Zip70C, and PSD-Zip70N were subcloned into pGEX6P1 (Amersham Pharmacia Biotech) by the same method. The sequences of all the constructs were confirmed by DNA sequence analysis.

Analysis of myristoylation

Transiently transfected COS7 cells were preincubated for 30 minutes with cerulenin (2 μg/ml; SIGMA) in DMEM containing fatty-acid-free bovine serum albumin (10 mg/ml; SIGMA). Cells were then labeled with 500 μCi of [^{3}H]myristic acid (40-60 Ci/mmol; Amersham Pharmacia Biotech) for 5 hours in the same medium. [^{3}H]Myristic acid, which is supplied in ethanol solution, was concentrated under N_2 so that the final concentration of ethanol in the labeling medium was 1%.

For immunoprecipitation, [^{3}H]-labeled cells were scraped and lysed in IP buffer (20 mM Tris, pH 7.4, 1 mM EDTA, 150 mM NaCl, 1% Triton X100, 200 μM phenylmethylsulfonyl fluoride [PMSF], 10 μg/ml pepstatin, 10 μg/ml leupeptin and 10 μg/ml aprotinin) with a 26-G needle. The samples were then spun at 17,000 ***g*** for 10 min at 4°C. The supernatants were precleaned with 30 μl of protein A-Sepharose suspension (1:1 in IP buffer) and then incubated with 2 μg of polyclonal anti-myc antibody pre-coupled with protein-A-Sepharose for 3 hours at 4°C. After incubation, the Sepharose gels were pelleted, washed three times with TNE buffer and boiled with SDS-PAGE sample buffer. In brief, one half of each sample was loaded on a gel, separated by SDS-PAGE and transferred to a nitrocellulose membrane. The radioactivity was analyzed using a FUJIFIX Bioimage Analyzer (BAS5000). For the quantification of precipitated PSD-Zip70, the other half of each sample was analyzed by Western blotting as described below. The anti-myc pAb (Santa Cruz) was used as the primary antibody.

Northern blotting

Northern blotting was performed as described previously (Hayashi et al., 1999). Total RNAs were extracted from the embryonic whole brain, neonatal and adult cerebrum and cerebellum and other adult tissues of SD rats. 10 μg of total RNA per lane were separated by electrophoresis, transferred to nylon membranes and hybridized with specific probes. For the preparation of the probes, cDNA fragments

of PSD-Zip70 were labeled with [α-^{32}P]dCTP by the random priming method. The blots were subjected to autoradiography.

In situ hybridization

Fresh-frozen sections of whole brains from 7-week-old SD rats were prepared for in situ hybridization. These sections were prepared and treated as described previously (Sun et al., 1998) and hybridized with antisense or sense probes. For the preparation of the probes, PSD-Zip70 cDNAs (344-2,149 nt) were transcribed in the presence of [α-^{35}S]UTP using the Riboprobe system (Promega). The hybridized signals were visualized with a BAS-5000 phosphor imager (Fujifilm).

Subcellular fractionation, solubilization and western blotting

The subcellular and tissue distribution and developmental change of PSD-Zip70 were examined by western blotting. The subcellular fractions were prepared from the cerebrum of 7-week-old rats by a previously described method (Wu et al., 1986). In brief, the brain was homogenized and spun at 1475 ***g***. The supernatant (S1) was further spun at 17,300 ***g***, and the pellet (P2) and supernatant (S2) fractions were retained. The P2 fraction was further separated by sucrose density gradient (0.32, 1.0 and 1.4 M sucrose) centrifugation at 82,500 ***g*** for 65 minutes. The synaptosome fraction was obtained at the 1.0 M-1.4 M interface. This fraction was lysed by hypo-osmotic shock with 1 mM $NaHCO_3$, treated with 0.5% Triton X-100, and centrifuged at 15,000 ***g*** for 60 minutes. The pellet was further separated by sucrose density gradient (0.32, 1.0, 1.5, and 2.1 M sucrose) centrifugation at 201,800 ***g*** for 120 minutes. The PSD-I fraction was obtained between 1.5 M and 2.1 M sucrose. 20 μg of protein per lane were analyzed. To examine the solubilization of PSD-Zip70, the synaptosome fraction was suspended with 10 volumes of 1 mM $NaHCO_3$ and centrifuged at 17,000 ***g*** for 10 minutes at 4°C. After centrifugation, 1 mg protein of each pellet was resuspended with a 26-G needle in 500 μl of extraction buffer (10 mM Hepes, pH 7.4, 1 mM EGTA, 1 mM $MgCl_2$ 150 mM NaCl, 200 μM PMSF, 10 μg/ml pepstatin A, 10 μg/ml leupeptin and 10 μg/ml aprotinin) containing various additional reagents as indicated in Fig. 5, rotated for 30 minutes and centrifuged at 100,000 ***g*** for 30 minutes at 4°C. The pellets were resuspended in the original volume of extraction buffer. 10 μl of the pellet (ppt) and supernatant (sup) fractions were analyzed by western blotting. Samples were loaded on gels, separated by SDS-PAGE and transferred to nitrocellulose membranes. mAb204H or pAbZip70 were used as the primary antibodies and visualized using peroxidase-conjugated secondary antibodies followed by ECL (Amersham Pharmacia). For the preabsorption of antibodies, mAb204H and pAbZip70 diluted for western blotting were preabsorbed with an excess amount of purified GST-PSD-Zip70.

Cells

Hippocampal neurons were prepared from rat embryonic brains at embryonic day 18 (E18). The hippocampus was dispersed with 0.25% trypsin in HBSS solution, and the cell suspension was plated onto 0.5 mg/ml poly-L-lysine-coated glass coverslips at a density of 10,000-15,000 cells/cm^2. Neurons were cultured in a Neurobasal medium (Life Technologies) containing 2% B27 supplement (Life Technologies) and 0.5 mM L-glutamine. Half of the medium was changed per week. COS7 and MDCK cells were cultured in DMEM supplemented with 10% FCS and transfected with expression vectors using Lipofectamine 2000 (Life Technologies). The cells were fixed at 16 hours (for immunocytochemistry) or 36 hours (for biochemical analysis) after transfection.

Transfection using microinjection

Using a micromanipulator (Narishige), plasmid DNAs (5-200 ng/μl) were microinjected through a glass capillary into the nuclei of hippocampal neurons. After 24 hours, the neurons were fixed for immunocytochemistry as described below.

Immunocytochemistry

After 6 or 30 days in culture, hippocampal neurons were fixed in 4% paraformaldehyde and 4% sucrose in PBS for 1 hour at 4°C. In some cases, the neurons were further treated with methanol at –20°C for 10 minutes. Fixed neurons were permeabilized with the blocking solution containing Triton X-100 (10% normal goat serum, 0.2% BSA, and 0.1% Triton X-100 in PBS) for 1 hour at 37°C and incubated with primary antibodies in the blocking solution for 2 hours at 37°C. Anti-MAP2 (clone HM-2, SIGMA) and anti-Tau-1 (Boehringer Mannheim) mAbs were used for dendritic and axonal markers, respectively. Presynaptic and postsynaptic sites were labeled with anti-synaptotagmin mAb produced by our laboratory (mAbSV96) and anti-PSD-95 mAb (clone 6G6-1C9, Affinity Bioreagents), respectively. F-actin was labeled with Alexa Fluor™ 568-phalloidin. For PSD-Zip70 staining, pAbZip70 was used. After incubation with the primary antibody, the neurons were labeled with Alexa Fluor™ 488 and/or 546 secondary antibodies (2 μg/ml, Molecular probes) in the blocking solution. After washing with PBS, the coverslips were mounted onto glass slides using a Prolong Antifade Kit (Molecular Probe). Fluorescence images were acquired using a cooled CCD camera (Roper Scientific) mounted on an Olympus IX70 microscope with Metamorph imaging software (Universal Imaging Corporation).

Transfected MDCK cells were fixed in 4% paraformaldehyde in PBS for 10 minutes at 37°C. For in vivo extraction, cells were washed with PBS and treated with the extraction buffer (0.2% Triton X-100, 20 mM PIPES, pH 6.8, 100 mM NaCl, 300 mM sucrose, 1 mM $MgCl_2$, 1 mM EGTA, 200 μM PMSF, 10 μg/ml pepstatin, 10 μg/ml leupeptin and 10 μg/ml aprotinin) for 1 minute at 4°C. After extraction, cells were washed with the extraction buffer without Triton X-100 and then fixed. The anti-myc mAb and pAb (Santa Cruz) and an anti-ezrin mAb (CHEMICON) were used as the primary antibodies. To stain the mitochondria, MitoTracker (Molecular Probes) was added to the growth medium and the cells were further cultured for 30 minutes. After labeling, the cells were fixed and double stained as described above.

Immunohistochemistry

To prepare the sections, rat brains (7-week-old) were fixed by perfusion with 4% paraformaldehyde in 0.1 M phosphate buffer and further fixed in the same buffer for 4 hours at 4°C. 50 μm sections were prepared using a vibratome. These sections were pre-treated with the blocking solution as described above for 1 hour at room temperature and incubated with pAbZip70 or control rabbit IgG (0.5 μg/ml) in the blocking solution for 36 hours at 4°C. A peroxidase-conjugated anti-rabbit IgG antibody was used as the secondary antibody, and the staining was visualized using an ABC kit (Vector).

Immunoelectron microscopy

Adult SD rats were perfused with a fixative containing 4% paraformaldehyde, 0.01 M sodium periodate and 0.1 M lysine-HCl in 0.1 M phosphate buffer. Small pieces of forebrain tissues including the cerebral cortex and hippocampus were dissected out and kept in the same fixative for 4 hours at 4°C. After washing with 0.1 M phosphate buffer, specimens were infused with a mixture of 20% poly(vinylpyrrolidone) and processed for ultracryotomy on an Ultracuts microtome (Reichert) equipped with FCS (Reichert) following the method of Tokuyasu (30). Briefly, sections of frozen specimens were collected with drops of 2.3 M sucrose, placed on a grid and thawed at room temperature. After blocking with 10% normal goat serum, they were incubated in 0.1 M Tris-buffered saline

(TBS) containing pAbZip70 for 24-48 hrs at 4°C, then washed with TBS and incubated with goat anti-rabbit IgG antibodies conjugated with 5 or 10 nm gold particles (Amersham) for 2 hours at room temperature. Thereafter, the sections were rinsed and embedded with a mixture of 1% poly(vinyl alcohol) containing 0.1% uranyl acetate and observed under an electron microscope (Hitachi H-7100) after drying.

Sucrose floatation gradient separation of a crude synaptosomal fraction and isolation of dendritic rafts

All procedures were carried out at 4°C. 1 mg protein of the synaptosome fraction obtained from the rat cerebrum was suspended with a 26-G needle in 1 ml of TNE (20 mM Tris, pH 7.4, 150 mM NaCl, 1 mM EDTA, 200 μM PMSF, 10 μg/ml pepstatin A, 10 μg/ml leupeptin and 10 μg/ml aprotinin) containing 1% Triton X-100 and rotated for 30 minutes. The sample was adjusted to 40% sucrose using 80% sucrose in TNE, placed in the bottom of a centrifuge tube and overlaid with 3 ml of 30% sucrose, 3 ml of 25% sucrose and 5 ml of 5% sucrose in TNE. The sample was then centrifuged at 201,000 ***g*** for 14 hours. After centrifugation, the upper 3 ml of the gradient solution was discarded and 10 fractions were collected from the top. The pellet fraction (ppt) was resuspended in 50 μl of TNE, and 500 μl of each collected fraction was precipitated with tricarboxylic acid (TCA), resuspended in 50 μl of TNE and separated by SDS-PAGE followed by western blotting as described above. The methods for preparing the synaptic plasma membrane (SPM), dendritic raft and PSD fractions have been described previously (Suzuki et al., 2001). 5 μg of each sample per lane were analyzed by western blotting. Anti-PSD-95 mAb, pAbZip70, anti-synaptophysin mAb (PROGEN), anti-fyn pAb (Upstate biotechnology), anti-α-internexin pAb (CHEMICON) and anti-NR1 pAb (Upstate biotechnology) were used as the primary antibodies. The methods for electron microscopy and the lipid assay for the SPM, dendritic rafts and PSD were described previously (Suzuki et al., 2001). The PSD preparation purified by this method was referred to 'PSD-II'. For electron microscopy, the dendritic raft preparation was fixed with 2% glutaraldehyde in HEPES/KOH buffer (5 mM, pH 7.4), spun briefly, further fixed with 1% osmium tetroxide, dehydrated through a graded ethanol series and embedded in Epon. An ultrathin section was cut and stained with uranyl acetate and lead citrate. For negative staining, dendritic raft suspension was dropped on a Formvar-coated grid and stained with 4% uranyl acetate. Specimens were examined with an electron microscope (JE-M-1200 EX; JEOL). For the lipid assay, lipids were extracted from the SPM, dendritic raft and PSD-II preparations and separated by thin layer chromatographies (TLC). The separated lipids were re-extracted and quantified using an enzymatic colorimetric assay kit for cholesterol and sphingomyelin.

Results and Discussion

Cloning of a 70 kDa protein that is concentrated in the PSD fraction and recognized by mAb204H

Western blotting of the cerebral subcellular fractions showed that mAb204H specifically recognized a closely related 70 kDa doublet that is concentrated in the PSD-I fraction. A small amount of these 70 kDa proteins was detected in the S2 fraction, which contains the light membrane and the cytosol (Fig. 1A). mAb204H preabsorbed with GST-Zip70WT (the control) did not detect any proteins in the PSD-I fraction. In our subcellular preparations, a major PSD scaffold protein, PSD-95, was also densely localized to the PSD-I fraction (Fig. 1A), suggesting that the 70 kDa protein recognized by mAb204H is a component of the PSD. Because mAb204H was not useful for immunocytochemistry, we generated a rabbit polyclonal antibody against GST-fused recombinant PSD-Zip70 (pAbZip70), which also specifically recognized PSD-Zip70 in the PSD-I fraction (Fig. 1A). pAbZip70 that was preabsorbed with GST-Zip70WT was also unable to detect any proteins in the PSD-I fraction. Thus, we used pAbZip70 for all the following experiments, except for the molecular cloning.

A rat cerebrum cDNA library (3×10^5 clones) was screened with mAb204H, and two positive clones were isolated. These clones had identical sequences in the open reading frame (GenBank Accession Number AB075607), which encoded a 601 amino-acid protein with the calculated M_r of 67,562 (Fig. 1B). An N-terminal myristoylation consensus sequence (MGxxxS/T) and a polybasic cluster (RKxxxxKK) were found in the N-terminus, and four leucine-zipper motifs were present in the C-terminal region* (Fig. 1B). A database search revealed that the predicted amino-acid sequence of PSD-Zip70 was 89% identical to that of the human *FEZ1/LZTS1* gene product (Ishii et al., 1999), which contains multiple leucine-zipper motifs; therefore, PSD-Zip70 is a rat homologue of the human *FEZ1/LZTS1* gene product. Recent allelotyping studies have shown that the allelic loss of chromosome region 8p21-22 is closely associated with various human tumors (Jenkins et al., 1998). The *FEZ1/LZTS1* gene was identified by Ishii et al. in a genetic analysis of tumor suppressor genes at 8p22; they found this gene to be altered in many tumors including esophageal, prostate, and breast cancers (Ishii et al., 1999). The PSD-Zip70 protein recognized by mAb204H and pAbZip70 was a closely related 70 kDa doublet in the rat cerebral subcellular fractions (Fig. 1A). When an expression plasmid carrying wild-type PSD-Zip70 was transfected into COS7 cells, the expressed protein also appeared as a 70 kDa doublet (data not shown). As described above, this protein contains a consensus sequence for N-terminal myristoylation. Indeed, the N terminus of PSD-Zip70 (PSD-Zip70N) was myristoylated when it was expressed in COS7 cells (Fig. 1C), but its mutant (PSD-Zip70N/G2A) was not, indicating that the N-terminal myristoylation of PSD-Zip70 in vivo is specific. In this case, myristoylated PSD-Zip70WT and non-myristoylated PSD-Zip70N/G2A both appeared as the doublet (Fig. 1C). Although PSD-Zip70N also contains a candidate sequence for palmitoylation (^{15}SKH**C**RA^{20}), it was not palmitoylated in COS7 cells (data not shown). These results suggest that the doublet of PSD-Zip70 may be due to post-translational modifications other than acylation. Consistent with our results, Ishii et al. demonstrated the serine phosphorylation of the *FEZ1/LZTS1* gene product by cAMP-dependent protein kinase, which resulted in differently migrating protein bands on SDS-PAGE (Ishii et al., 2001).

Unique expression of PSD-Zip70 in the brain

Northern blotting revealed that the approximately 5.0 kb PSD-Zip70 mRNA was already expressed in whole embryonic brains at 15 days (E15) and that its expression gradually increased in the cerebrum thereafter. By contrast, the PSD-Zip70 mRNA in the cerebellum increased abruptly between 1 and 3 weeks after birth and then disappeared (Fig. 2A). Western blotting using pAbZip70 showed only a trace amount

*The nucleotide sequence reported in this paper has been submitted to the GenBank, Accession Number AB075607.

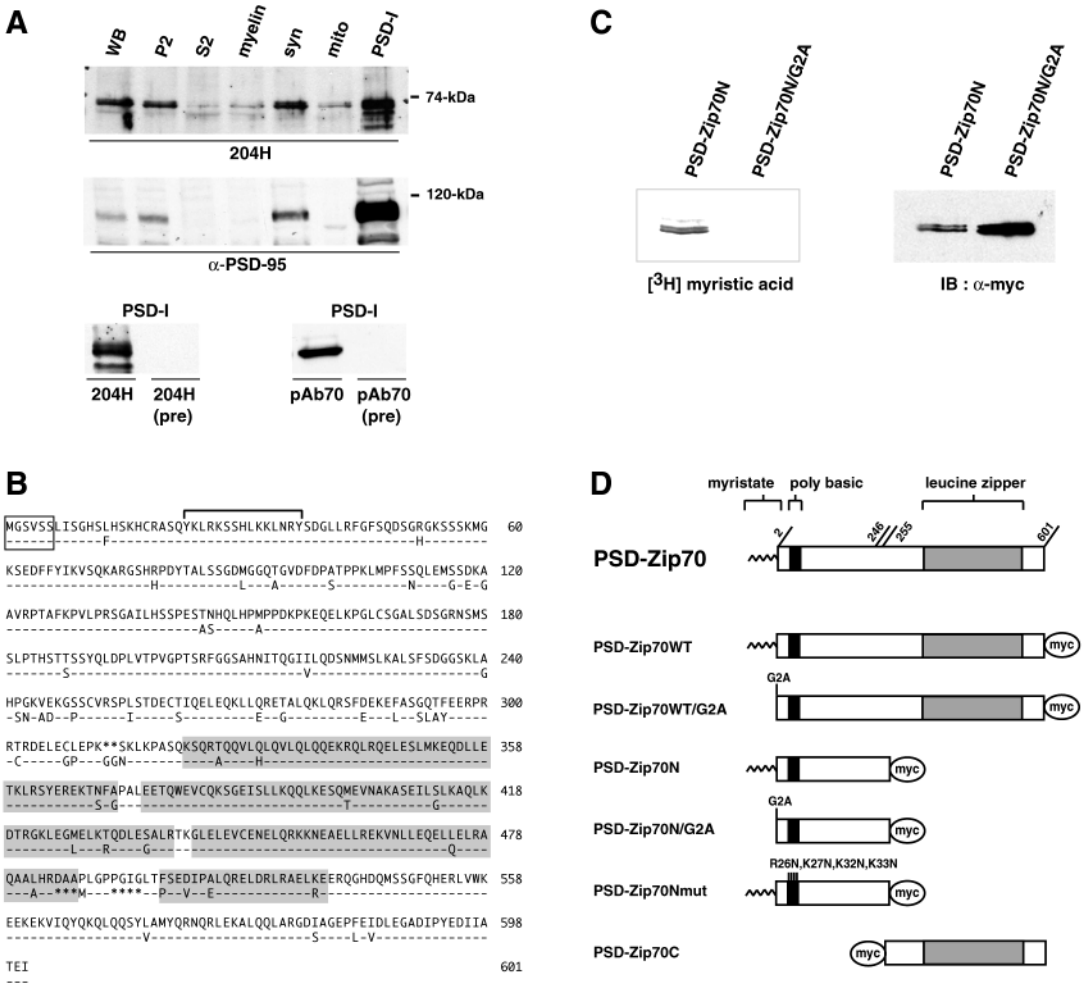

Fig. 1. Subcellular distribution, predicted amino-acid sequence and N-terminal myristoylation of PSD-Zip70. (A) The subcellular distribution of PSD-Zip70 in the rat cerebrum using mAb204H (204H, upper panel). Each fraction was prepared as described in Materials and Methods. WB, Syn and mito indicate the whole brain lysate, synaptosome and mitochondria fraction, respectively. pAbZip70 recognized a 70 kDa protein in the PSD-I fraction (pAb70, lower right panel). Preabsorption (pre) means that mAb204H and pAbZip70 were preabsorbed with an excess amount of GST-PSD-Zip70 (lower two panels). PSD-95 is shown as a control of the subcellular fractionation (middle panel). (B) The predicted amino-acid sequences of rat PSD-Zip70 (upper letters) and the human *FEZ1/LZTS1* gene product (lower letters). The N-terminal myristoylation consensus sequence is boxed. The four putative leucine-zipper motifs of PSD-Zip70 and the *FEZ1/LZTS1* gene product are marked in gray. The thin line and hatch marks in the *FEZ1/LZTS1* sequence indicate identical amino acids within PSD-Zip70 and the polybasic region, respectively. (C) N-terminal myristoylation of PSD-Zip70 in COS7 cells. Left and right panels show the autoradiographs of incorporated [^{3}H] myristic acid and western blotting, using anti-myc pAb, respectively. PSD-Zip70N/G2A is mutated at the myristoylation site. (D) Design of the PSD-Zip70 variants used for transfection.

of PSD-Zip70 protein in embryonic brains at E15-18, whereas the protein expression in the cerebrum increased between 1 and 3 weeks after birth and reached a plateau at 7-12 weeks (Fig. 2B). The protein in the cerebellum was present at lower levels than in the cerebrum during all developmental stages. Thus, PSD-Zip70 showed distinctly different expression patterns at the mRNA and protein levels during brain development. This discrepant expression of PSD-Zip70 in the brain may be due to extremely different turnover rates of the PSD-Zip70 mRNA and protein or to the critical regulation at the translational level during development. Further studies are required to elucidate the precise mechanism governing these discrepant expression patterns.

We next examined the expression of PSD-Zip70 at the mRNA and protein levels in adult rat tissues. Northern and western blot analyses revealed the presence of PSD-Zip70 mRNA and protein only in the cerebrum and not in the other tissues examined (Fig. 2C,D). A long exposure of northern and western blots, however, showed trace amounts of the PSD-Zip70 mRNA and protein in the cerebellum, lung, kidney and stomach (data not shown).

In situ hybridization of adult rat brain showed a unique distribution of the PSD-Zip70 mRNA, in which intense signals were found in the cerebral cortex, hippocampus, olfactory bulb, striatum and pons (Fig. 3A). In the hippocampus, the transcripts were intensely labeled in the CA2 region compared with the CA1 and CA3 regions. Faint signals were detected in other cerebral regions, such as the thalamus and brain stem. However, no signals were observed in the cerebellum. In control sections using a sense probe, no signals were detected

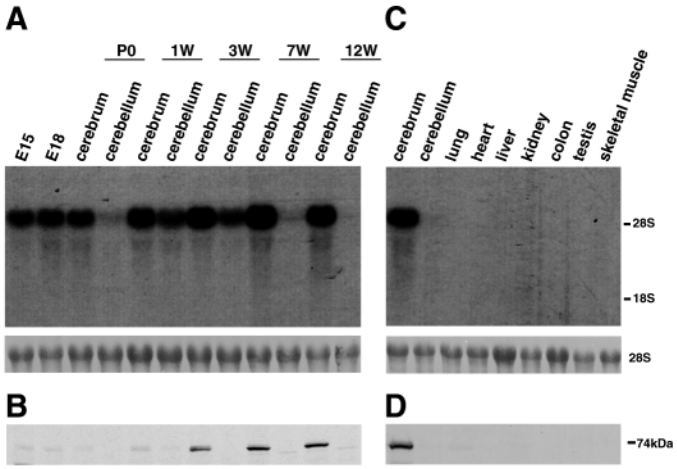

Fig. 2. Expression of PSD-Zip70 mRNA and protein during brain development and in tissue distribution. (A,C) Northern blotting using specific ^{32}P-labeled probes for PSD-Zip70 mRNA. 20 μg of total RNAs per each lane were analyzed. The two lower panels indicate methylene blue staining of 28S ribosomal RNAs, which was used to quantify the amounts of total RNA loaded. The positions of the 28S and 18S ribosomal RNAs are indicated at the right. (B,D) Western blotting using pAbZip70. 20 μg of the indicated protein samples per lane were analyzed.

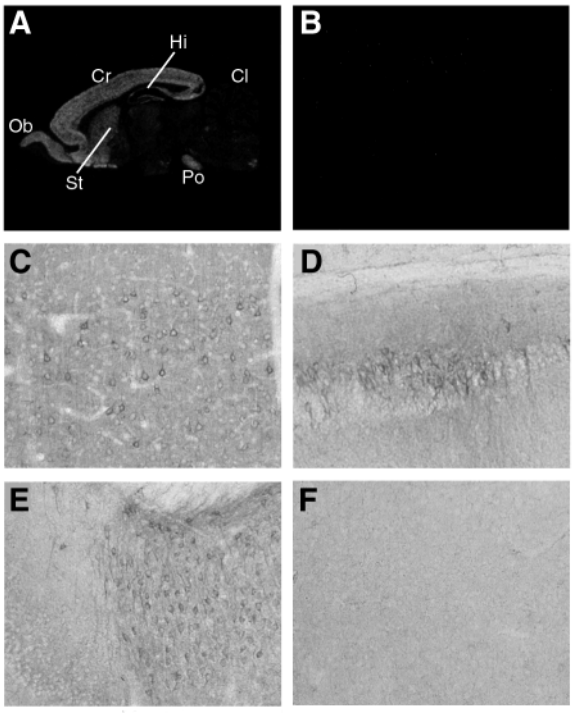

Fig. 3. Distribution of PSD-Zip70 in the brain. In situ hybridization (A,B) and immunohistochemistry (C-F) were performed to determine the distribution of PSD-Zip70 mRNA and protein in the brain. Sagittal sections from the adult rat brain were hybridized with specific ^{35}S-labeled cRNA antisense (A) or sense (B) probes for PSD-Zip70. Ob, olfactory bulb; Cr, cerebral cortex; Hi, hippocampus; St, striatum; Po, pons; Cl, cerebellum. Immunohistochemistry of sagittal sections from the adult rat brain was performed using pAbZip70. The cerebral cortex (C), hippocampal CA2 region (D) and subiculum (E) are shown. The cerebral cortex in a control section using normal rabbit IgG is shown in F.

(Fig. 3B). Immunostaining for pAbZip70 showed intense labeling in the cerebral cortex, hippocampal CA2 region, subiculum and other cerebral regions. In the cerebral cortex and hippocampal CA2 region, immunolabeling was localized to the soma and dendritic processes of the principal neurons but not to glial cells (Fig. 3C-E). These immunoreactivities were specific for PSD-Zip70, because no immunolabeling was detected using a control IgG as the primary antibody (Fig. 3F). These results indicate that PSD-Zip70 is specifically expressed in the neurons of some cerebral areas.

Postsynaptic localization of PSD-Zip70

Immunocytochemistry of cultured hippocampal neurons after 6 days in vitro (DIV) revealed patchy immunofluorescence for PSD-Zip70 in the dendrites and the cell bodies (Fig. 4A1,B1). Double immunofluorescence for PSD-Zip70 and MAP2, a dendritic marker, completely overlapped in the dendrites and the cell bodies (Fig. 4A1-A3). However, PSD-Zip70 and tau protein, an axonal marker, showed distinctly different distributions (Fig. 4B1-B3). These results indicated a preferential localization of PSD-Zip70 to the dendrite. In cultured mature neurons (~30 DIV), numerous PSD-Zip70-positive neurons were seen among some populations of morphologically excitatory neurons but not in all excitatory neurons. This finding may reflect the dominant expression of PSD-Zip70 in the hippocampal CA2 region, as shown in Fig. 3A,D. In addition to the patchy fluorescence in the dendrites, intense immunofluorescent clusters of PSD-Zip70 were distributed along the dendrites (Fig. 4C1,D1 and E1). To further characterize the localization of PSD-Zip70, double staining was performed using pAbZip70 and antibodies against pre- and postsynaptic markers or phalloidin for F-actin. Clusters of PSD-Zip70 were colocalized with PSD-95 clusters (Fig. 4C1-C3 and C1′-C3′) and with many F-actin clusters (Fig. 4D1-D3 and D1′-D3′). High-resolution images showed that the areas of PSD-Zip70 cluster within the dendritic spines were much wider than those of PSD-95 clusters (Fig. 4C1′-C3′) and that the PSD-Zip70 clusters also partially overlapped with synaptotagmin clusters (Fig. 4E1-E3 and E1′-E3′), suggesting the synaptic localization of PSD-Zip70.

To further define the synaptic localization of PSD-Zip70, we performed postembedding immunoelectron microscopy. Immunogold labeling for PSD-Zip70 was predominantly observed in axospinous and axodendritic asymmetric synapses of pyramidal neurons in the hippocampal CA2 region and cerebral cortex. Most of the immunogold label appeared over the perisynaptic regions of the PSD and the postsynaptic membrane in excitatory asymmetric synapses of pyramidal neurons in the hippocampus and cerebral cortex (Fig. 4F-I), indicating the postsynaptic localization of PSD-Zip70.

Solubility of PSD-Zip70 from the synaptic membrane fraction

We examined the solubility of PSD-Zip70 (Fig. 5). Non-ionic detergents (Triton or Lubrol WX), CHAPS or high salt alone did not significantly solubilize the PSD-Zip70 from the synaptosome membrane. A combination of non-ionic detergent or CHAPS and high salt could solubilize 10-30% of the total PSD-Zip70, indicating that high salt is partially effective for

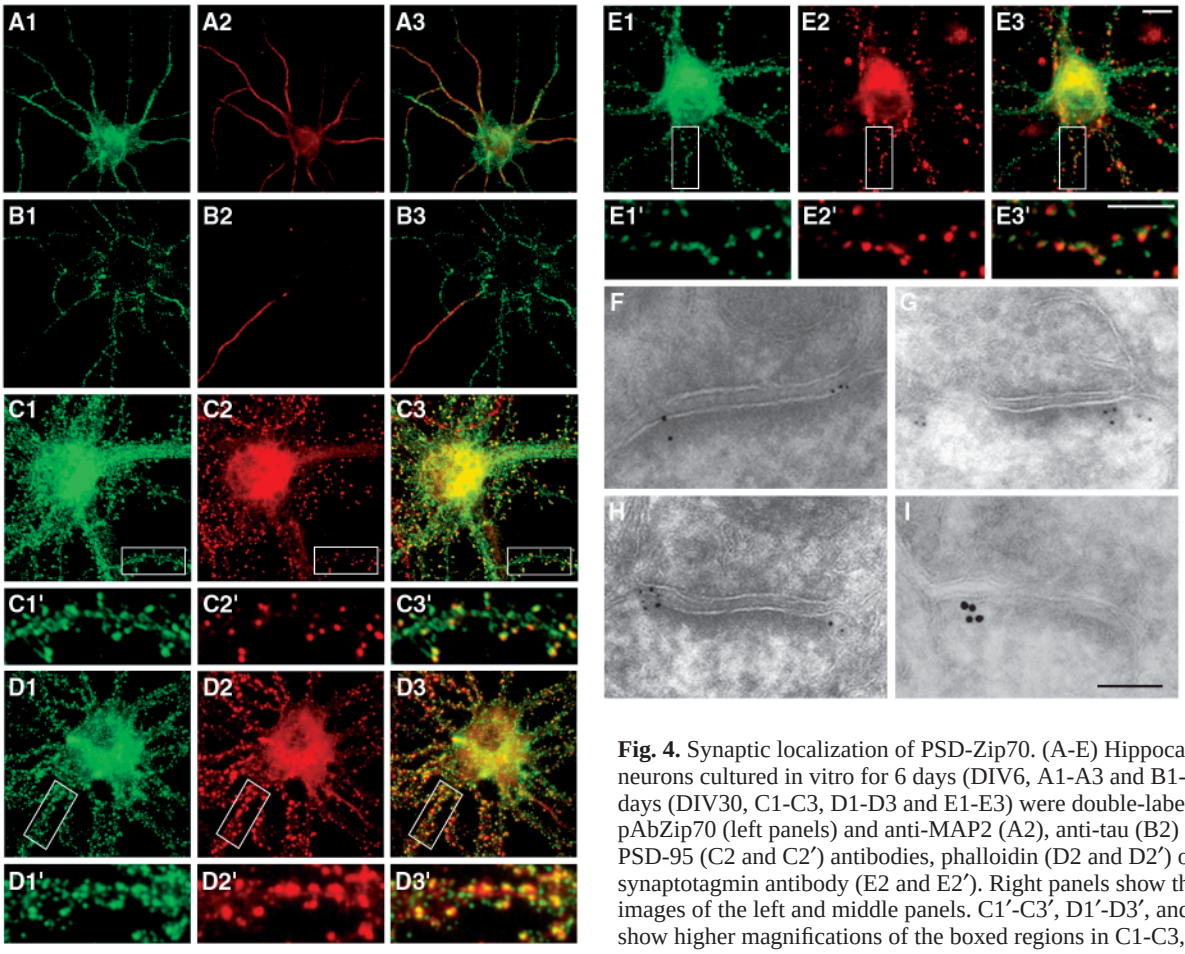

Fig. 4. Synaptic localization of PSD-Zip70. (A-E) Hippocampal neurons cultured in vitro for 6 days (DIV6, A1-A3 and B1-B3) or 30 days (DIV30, C1-C3, D1-D3 and E1-E3) were double-labeled using pAbZip70 (left panels) and anti-MAP2 (A2), anti-tau (B2) and anti-PSD-95 (C2 and C2′) antibodies, phalloidin (D2 and D2′) or anti-synaptotagmin antibody (E2 and E2′). Right panels show the merged images of the left and middle panels. C1′-C3′, D1′-D3′, and E1′-E3′ show higher magnifications of the boxed regions in C1-C3, D1-D3 and E1-E3, respectively. Bar, 10 μm. (F-I) Immunoelectron microscopy of the adult rat brain was performed using pAbZip70. The cerebral cortex (F-H, 5 nm gold particles) and the CA2 region of hippocampus (I, 10 nm gold particles) are shown. Bar, 100 nm.

the solubilization of this protein from the detergent-resistant synaptosome membrane. A high concentration of Tris-HCl, which can partly solubilize the membrane cytoskeleton, including spectrin (Tanaka et al., 1991), had no effect on the solubility of PSD-Zip70. Alkaline conditions, which solubilize the peripheral membrane proteins, did not solubilize PSD-Zip70. Deoxycholate (1%, pH 9.0), which was previously used to extract the PSD proteins (Husi et al., 2000), and urea could release nearly a half of the total PSD-Zip70. Consistent with the perisynaptic localization of PSD-Zip70 in the PSD and the postsynaptic membrane, N-lauroyl sarcosinate (NLS) completely solubilized the protein. These results suggest that most of the PSD-Zip70 tightly associates with the detergent-insoluble compartments of the postsynaptic membrane, such as the PSD, rafts, and detergent-insoluble cytoskeleton via lipid-lipid and/or lipid-protein interactions.

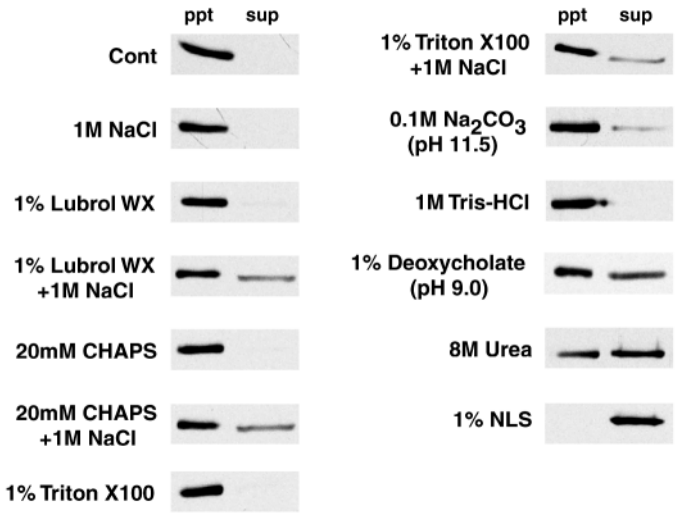

Fig. 5. Solubilization of PSD-Zip70 from the synaptosome membrane fraction. The synaptosome membrane fraction was treated with extraction buffer with or without (cont) various detergents and salts as indicated on the left side of the panels. After centrifugation, the pellet (ppt) and the supernatant (sup) fractions were analyzed by western blotting using pAbZip70. NLS indicates N-lauroyl sarcosinate.

Localization of PSD-Zip70 to the PSD and the dendritic rafts

To investigate whether PSD-Zip70 was associated with the membrane rafts, we performed a sucrose floatation gradient separation of the Triton-X-100-treated synaptosome fraction modified from a previously reported method (Röper et al.,

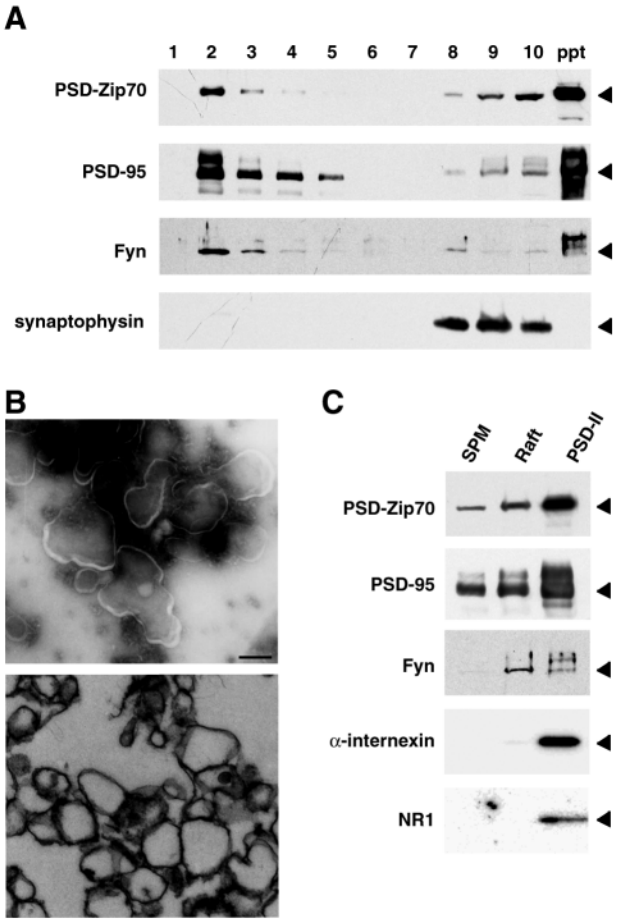

Fig. 6. The PSD and raft localization of PSD-Zip70 in the synaptosome fraction. (A) Sucrose floatation gradient separation of the 1% Triton-X-100-treated synaptosome fraction was performed at 4°C. Gradient fractions were analyzed by western blotting using pAbZip70, anti-PSD-95, anti-Fyn, and anti-synaptophysin antibodies. Fraction 1 is from the top of gradient, and ppt indicates the pellet fraction. Fractions 2-4 are the raft fraction, and fractions 7-10 mainly contain Triton-soluble proteins. (B) Electron microscope observation of the dendritic raft fraction prepared from the rat forebrain. Photographs show negative staining (upper panel) and thin-section (lower panel) of the dendritic raft fraction. Bar, 200 nm. (C) Localization of PSD-Zip70 in the PSD-II and raft preparations. The synaptic plasma membrane (SPM), dendritic raft (raft) and PSD-II fractions were prepared using the method of Suzuki et al. (Suzuki et al., 2001). These preparations were analyzed by western blotting using pAbZip70, anti-PSD-95, anti-Fyn, anti-α-internexin and anti-NR1 antibodies.

Table 1. Lipid contents in the subcellular fractions

	μg/mg protein	
Fraction	Cho	SM
SPM	56.3±12.2	18.7±1.8
Raft	645.7±3.5	91.0±11.1
PSD-II	14.5±0.5	13.1±1.0

Contents of cholesterol (Cho) and sphingomyelin (SM) were measured in the synaptic plasma membrane (SPM), the dendritic raft (Raft), and the PSD-II fractions (n=3).

2000). Fyn and PSD-95 are reported to be raft-associated proteins (Wolven et al., 1997; Perez and Bredt, 1998). As shown in Fig. 6A, PSD-Zip70 and PSD-95 were recovered in both the detergent-insoluble low-density fraction (the raft fraction, fractions 2-4) and the pellet fraction, which probably included the PSD. Fyn was mainly found in the raft fraction. By contrast, synaptophysin was detected in the detergent-soluble fraction (fractions 7-10). To confirm the neuronal distribution of PSD-Zip70, we prepared the dendritic rafts by the method of Suzuki et al. (Suzuki et al., 2001). The dendritic raft fraction thus obtained was homogenous in its morphology according to both negative staining and thin-sectioned electron micrographs (Fig. 6B). Both types of imaging showed membrane sacs of 200-1000 nm in diameter, to which fuzzy structures that probably consist of proteins were attached. One of the criteria for the raft structure is a highly ordered concentration of cholesterol and sphingomyelin in the raft membrane (Simons and Ikonen, 1997). In agreement with this criterion, the levels of cholesterol and sphingomyelin in the dendritic raft fraction were much higher than those in the SPM and PSD fractions (Table 1). As shown in Fig. 6C, PSD-Zip70 and PSD-95 were found in both the PSD-II and the dendritic raft fractions, and Fyn was mainly in the dendritic raft fraction. Consistent with our previous report (Suzuki et al., 2001), the NR1 subunit of the NMDA receptors and α-internexin were only detected in the PSD fraction. Thus, two different fractionations indicated that PSD-Zip70 was localized to both the PSD and the dendritic rafts.

Targeting of PSD-Zip70 into the microvilli and the plasma membrane of non-neuronal cells

As shown in Fig. 6, PSD-Zip70 was localized to the membrane raft microdomain in the brain. We further examined if PSD-Zip70 is also targeted to the microvilli-like structure of the plasma membrane, which is a membrane lipid raft-rich structure in MDCK cells (Röper et al., 2000), and determined the critical domains of PSD-Zip70 for its localization.

The PSD-Zip70 protein was not detected in MDCK cells by immunocytochemistry or western blotting (data not shown). However, when myc-tagged PSD-Zip70WT or PSD-Zip70N was expressed in MDCK cells cultured under serum-supplemented conditions, the immunolabeling with the anti-myc antibody was highly concentrated in structures at the apical plasma membrane and also showed a submembranous distribution (Fig. 7A1,B1). Double labeling for PSD-Zip70WT or PSD-Zip70N (with the anti-myc antibody) and F-actin (with phalloidin; Fig. 7A1-A3 and B1-B3) or ezrin, a marker for microvilli-like structures (with an anti-ezrin antibody; Fig. 7E1-E3 and E1′-E3′), revealed speckled immunolabeling for PSD-Zip70WT or PSD-Zip70N overlapping with the positive staining for F-actin or ezrin in the apical structures. After in vivo extraction with Triton X-100, PSD-Zip70N was retained in the microvilli, but its membrane localization was abolished (data not shown). It has been demonstrated that such detergent-insoluble microvilli belong to a subclass of membrane rafts (Röper et al., 2000). Ishii et al. demonstrated the colocalization of cytoplasmic microtubules and the exogenous *FEZ1/LZTS1* gene product in HEK 293 cells and some cancer cells and reported the *FEZ1/LZTS1*-dependent inhibition of cell growth mediated

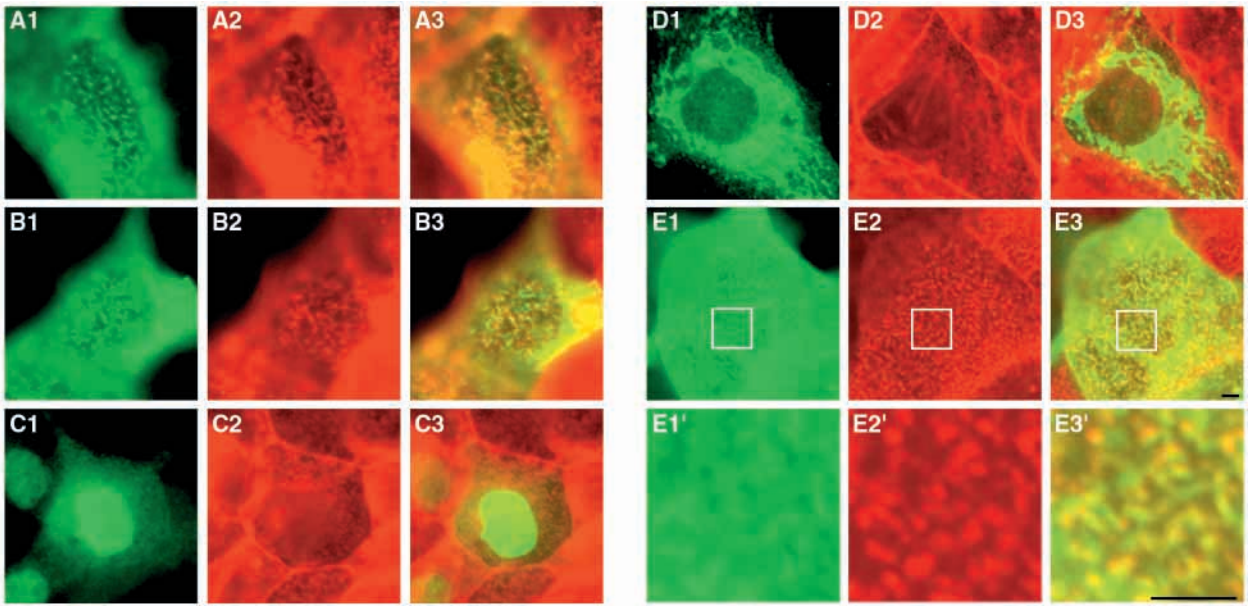

Fig. 7. Localization of myc-tagged PSD-Zip70 and its deletion mutants in MDCK cells. Madin-Darby canine kidney (MDCK) cells were transfected with myc-tagged PSD-Zip70 and its deletion and point mutant expression vectors. Expressed PSD-Zip70WT (A1-A3), PSD-Zip70N (B1-B3, E1-E3, and E1′-E3′), PSD-Zip70N/G2A (C1-C3) and its mutation in the polybasic region, PSD-Zip70Nmut (D1-D3) were visualized with an anti-myc antibody (all left panels) and double-labeled with phalloidin (A2, B2, C2 and D2) and the anti-ezrin antibody (E2 and E2′). E1′-E3′ show higher magnifications of the boxed regions in E1-E3. Right panels show the merged images of the left and middle panels. Bar, 5 μm.

through a suppression of microtubule assembly and interaction with $p34^{cdc2}$ (Ishii et al., 2001). However, we found that myc-tagged PSD-Zip70WT and PSD-Zip70N in MDCK cells were predominantly targeted to the microvilli and the plasma membrane but not to the cytoplasmic microtubules. One possibility for this discrepancy may be due to a different cell line used; however, the exact reason is not yet known.

Many proteins have covalently attached fatty acids (myristate and palmitate) at their N-terminus (Resh, 1999). This is seen in src family tyrosine kinases, G protein subunits, endothelial nitric oxide synthase and others. Several proteins are singly myristoylated and possess an adjacent or distant polybasic cluster. Other proteins contain two or more covalently linked palmitates at their N-terminus. In all cases, multiple N-terminal acylation sites or an acylation site juxtaposed to a polybasic cluster are required for the membrane anchorage of these modified proteins. The presence of these modified proteins and additional protein-protein interactions are further required to form the raft structure. Because PSD-Zip70 is myristoylated on its N-terminus (Fig. 1D) and also contains the distant polybasic cluster (Fig. 1B), we examined whether PSD-Zip70's localization to the microvilli may be mediated through N-terminal myristoylation and/or the polybasic cluster. PSD-Zip70N/G2A exclusively accumulated in the nucleus and the perinuclear region (Fig. 7C1-C3). By contrast, PSD-Zip70C was diffusely distributed throughout the cytoplasm but was not localized to the microvilli and the plasma membrane. Deletion of amino-acid residues 21-40 containing the polybasic cluster (PSD-Zip70N/Δ21-40) resulted in the loss of the protein's localization to the microvilli and plasma membrane; instead, this mutant protein translocated to the mitochondria (data not shown). A mutation of the polybasic cluster (PSD-Zip70N/mut) resulted in a protein that showed the same distribution as PSD-Zip70N/Δ21-40 (Fig. 7D1-D3). Staining the mitochondria with MitoTracker clearly showed that the polybasic cluster mutant was localized to the mitochondria (data not shown). These results indicate that both N-terminal myristoylation and the polybasic cluster are required for the targeting of PSD-Zip70 to the microvilli and the plasma membrane in epithelial cells.

Synaptic targeting of PSD-Zip70

We also analyzed the synaptic targeting of PSD-Zip70 in cultured hippocampal neurons. The synaptic localization of the exogenous proteins was monitored by observing the colocalization with PSD-95. When myc-tagged PSD-Zip70WT was expressed, it was localized to the dendrites and the dendritic spines. The fluorescence intensity of the PSD-Zip70WT in the dendritic spines was much higher than that in the dendrites (Fig. 8A1-A3 and A1′-A3′). PSD-Zip70C was also localized to the dendrites and dendritic spines, but the spine localization of PSD-Zip70C was slightly lower than that of PSD-Zip70WT (Fig. 8C1-8C3 and 8C1′-8C3′). By contrast, PSD-Zip70N was ubiquitously distributed from the dendrites to the dendritic spines. The fluorescence intensities of PSD-Zip70N in the dendritic spines and the dendrites were identical, suggesting the submembranous localization of PSD-Zip70N (Fig. 8B1-B3 and B1′-B3′). The non-myristoylated form of the full-length protein, PSD-Zip70WT/G2A, was also localized to postsynaptic sites similar to PSD-Zip70C (Fig. 8D1-D3 and D1′-D3′). By contrast, PSD-Zip70N/G2A lost the submembranous localization but accumulated in the nuclei and the perinuclear regions (Fig. 8E1-E3). These results indicate that, consistent with the results using MDCK cells, the N-terminal myristoylation of PSD-Zip70 is required for its

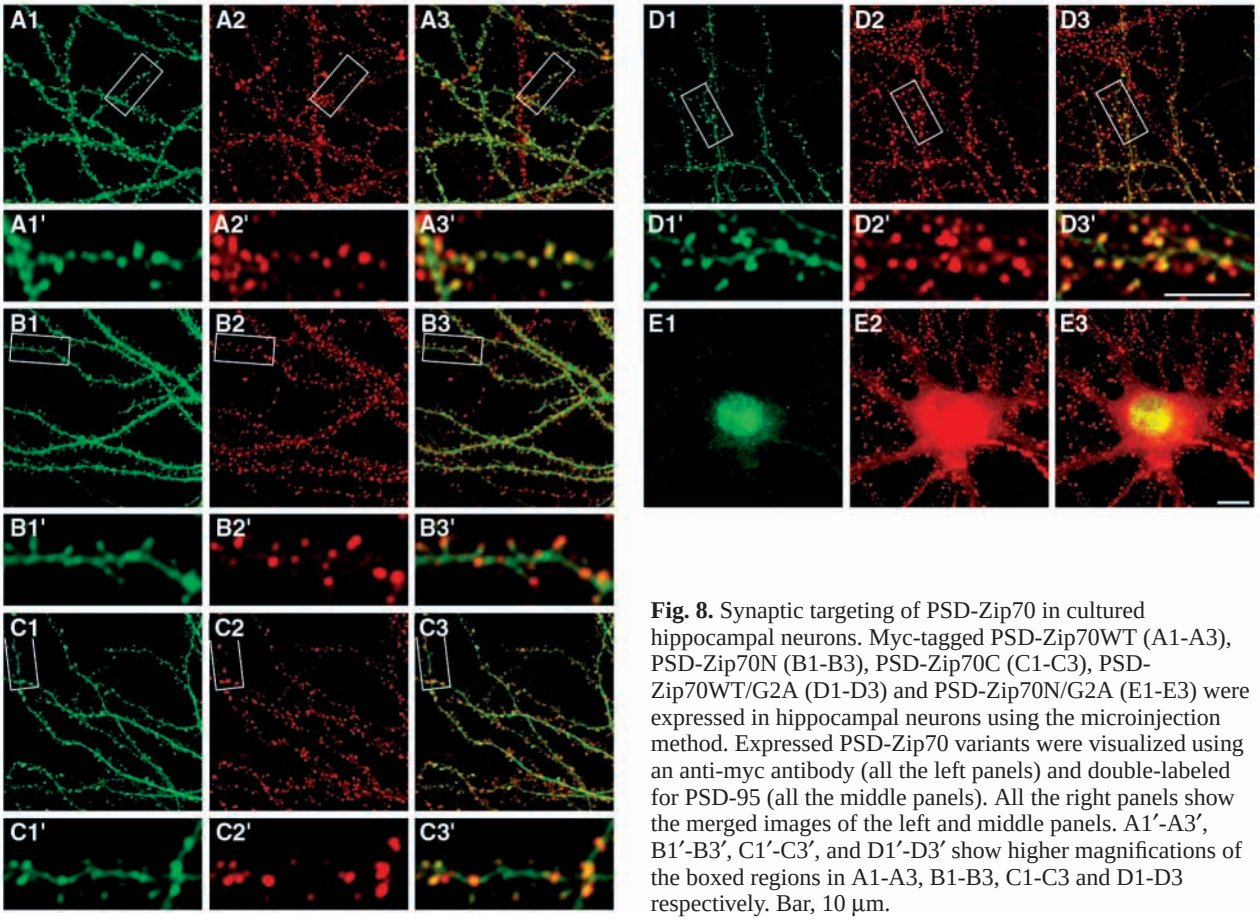

Fig. 8. Synaptic targeting of PSD-Zip70 in cultured hippocampal neurons. Myc-tagged PSD-Zip70WT (A1-A3), PSD-Zip70N (B1-B3), PSD-Zip70C (C1-C3), PSD-Zip70WT/G2A (D1-D3) and PSD-Zip70N/G2A (E1-E3) were expressed in hippocampal neurons using the microinjection method. Expressed PSD-Zip70 variants were visualized using an anti-myc antibody (all the left panels) and double-labeled for PSD-95 (all the middle panels). All the right panels show the merged images of the left and middle panels. A1′-A3′, B1′-B3′, C1′-C3′, and D1′-D3′ show higher magnifications of the boxed regions in A1-A3, B1-B3, C1-C3 and D1-D3 respectively. Bar, 10 μm.

plasma membrane localization in hippocampal neurons and the C-terminal domain is critically involved in synaptic targeting. We have identified a 200 kDa protein that is also concentrated in PSDs as a candidate binding partner for the C-terminal domain of PSD-Zip70, which mainly contains leucine-zipper motifs (data not shown). Therefore, these motifs may be critically involved in the synaptic targeting of PSD-Zip70 via this protein. We are currently investigating the characterization of this 200 kDa protein and its role in the PSD.

Here, we have demonstrated that the PSD-Zip70 protein is exclusively and highly expressed in the neurons of restricted cerebral areas and is mainly localized to the PSD and the dendritic rafts. These results suggest that the main function of PSD-Zip70 is likely to be carried out at its postsynaptic site. The N-terminal myristoylation of PSD-Zip70 is required for its membrane localization, and the C-terminal domain, which contains leucine-zipper motifs, is critically involved in its targeting to the synapse, which is mediated through protein-protein interaction. It has been well documented that the leucine-zipper motif is important for the dimerization of several transcription factors, such as c-fos and c-jun. Furthermore, this motif is also present in a variety of cytoplasmic and transmembrane proteins, including cytoskeletal proteins (Lupas et al., 1996). Leucine-zipper-motif-containing proteins in the postsynaptic region of the neuromuscular junction have been reported and include rapsin, dystrophin and dystrobrevin. Rapsin, a neuromuscular 43 kDa myristoylated protein, is involved in acetylcholine receptor (AchR) clustering. The other two neuromuscular proteins, dystrophin and dystrobrevin, bind to each other to form heterodimers via their leucine-zipper motifs (Sadoulet-Puccio et al., 1997). A few leucine-zipper proteins in the central nervous system have also been reported. One of them is PSD-Zip45, which is involved in the clustering of mGluRs mediated through self-multimerization via the extreme C-terminal leucine-zipper of PSD-Zip45 (Tadokoro et al., 1999). Together, previous reports on the neuromuscular junction and our previous and present studies suggest that the leucine-zipper motif might be a critical motif for protein-protein interactions contributing to the postsynaptic cytoarchitecture and function.

The currently accepted concept is that both the PSD and the rafts are cytoarchitectural centers for receptor-linked signal transduction, membrane trafficking and cytoskeletal connections (Sheng and Lee, 2000; Kennedy, 2000; Scannevin and Huganir, 2000; Xiao et al., 2000; Sheng and Sala, 2001; Simons and Ikonen, 1997; Resh, 1999; Ikonen, 2001). However, the relationship between the rafts and the PSD in neurons is largely unknown, with a few exceptions. Recently, several studies have indicated the importance of acylation for postsynaptic functions. El-Husseini Ael-D et al. reported that the palmitoylated PSD protein, PSD-95, regulates synaptic plasticity through the activity-dependent regulation of palmitate cycling (El-Husseini Ael-D et al., 2002). They suggest that in synapses, protein acylation is not only involved in simple membrane anchoring but also in the regulation of several cell processes, including endocytosis, that are reported

to be raft functions. One of us previously demonstrated the localization of AMPA receptors to the dendritic rafts (Suzuki et al., 2001). It is well documented that AMPA receptors are recruited to postsynaptic sites in a neuron-stimulation-dependent fashion (Carroll et al., 1999; Shi et al., 1999; Hayashi et al., 2000). Furthermore, EphrinB ligands recruit GRIP family members, which are AMPA-receptor-binding proteins, into raft membrane microdomains (Brückner et al., 1999). As demonstrated here (Fig. 6) and in previously reported studies (Wolven et al., 1997; Perez and Bredt, 1998), PSD-95 and Fyn are also localized to both the PSD and the dendritic rafts. Thus, some important postsynaptic molecules are present in both structures.

As shown in Fig. 4, PSD-Zip70 was localized to the edge of the PSD and/or the perisynaptic region. The significance of this localization is not clear. However, several studies report that some neurotransmitter receptors, such as the α7 nicotinic acetylcholine receptor subunits (α7 nAChRs) and type I mGluRs, also localize to the perisynaptic region of the postsynapse (Fabian-Fine et al., 2001). Specifically, α7 nAChRs are localized to membrane lipid rafts, and rafts are necessary for their maintenance in ciliary neurons (Bruses et al., 2001). These reports and our data suggest that a close relationship exists between the dendritic rafts and postsynaptic structures, including the PSD. Taken together, the findings support the idea that the dendritic rafts may be active centers of recruitment for some proteins to postsynaptic sites, especially to the PSD, in addition to the generally accepted roles of rafts. The unique expression and localization of PSD-Zip70 indicate that it may be involved in the dynamic properties of postsynaptic structure and function.

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