Polarized Targeting of Peripheral Membrane Proteins in Neurons*

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Differential targeting of neuronal proteins to axons and dendrites is essential for directional information flow within the brain, however, little is known about this protein-sorting process. Here, we investigate polarized targeting of lipid-anchored peripheral membrane proteins, postsynaptic density-95 (PSD-95) and growth-associated protein-43 (GAP-43). Whereas the N-terminal palmitoylated motif of PSD-95 is necessary but not sufficient for sorting to dendrites, the palmitoylation motif of GAP-43 is sufficient for axonal targeting and can redirect a PSD-95 chimera to axons. Systematic mutagenesis of the GAP-43 and PSD-95 palmitoylation motifs indicates that the spacing of the palmitoylated cysteines and the presence of nearby basic amino acids determine polarized targeting by these two motifs. Similarly, the axonal protein paralemmin contains a C-terminal palmitoylated domain, which resembles that of GAP-43 and also mediates axonal targeting. These axonally targeted palmitoylation motifs also mediate targeting to detergent-insoluble glycolipid-enriched complexes in heterologous cells, suggesting a possible role for specialized lipid domains in axonal sorting of peripheral membrane proteins.

Proper neuronal function requires selective protein targeting to specialized cellular and plasma membrane domains including the nerve terminal, node of Ranvier, axon hillock, and postsynaptic density. An early step in this targeting decision tree involves a polarized sorting of proteins to either dendritic (postsynaptic) or axonal (presynaptic) domains. However, the mechanisms by which neurons target specific proteins to dendrites versus axons are poorly understood. Better characterized is protein sorting to apical versus basolateral plasma membranes in polarized epithelial cells, which share certain features with axonal versus dendritic targeting in neurons (1, 2). That is, short cytosolic C-terminal protein-sorting motifs are one route for both dendritic and basolateral targeting (2), whereas specialized lipid rafts can mediate both axonal and apical sorting of certain transmembrane and glycosylphosphatidylinositol-anchored membrane proteins (3).

The concept of specialized lipid rafts mediating polarized protein targeting emerged from observations that apical and basolateral cell membranes have different lipid compositions. Apical membranes are enriched in sphingolipids that aggregate with cholesterol to form packed raft-like domains within the fluid membrane bilayer. These rafts are insoluble in non-ionic detergents and, hence, are termed detergent-insoluble glycolipid-enriched complexes (DIGs). These complexes form in the trans-Golgi network and incorporate certain transmembrane, GPI-anchored, and axially acylated proteins, which are then targeted to the apical plasma membrane (4, 5). The inhibition of DIG formation by sphingolipid or cholesterol depletion disrupts this apical/axonal sorting pathway (3, 6, 7). However, the polarized targeting of cytosolic proteins via DIGs has not been explored.

Postsynaptic density-95 (PSD-95) is a peripheral membrane protein that localizes exclusively to the PSD in hippocampal neurons and is believed to mediate the targeting and assembly of other synaptic proteins, including neurotransmitter receptors and signaling enzymes (8–11). The N terminus of PSD-95 is posttranslationally modified with palmitate (12), a 16-carbon-saturated fatty acid linked via thiioester bonds to specific cysteine residues (13–15). Dual palmitoylation of PSD-95 is necessary for appropriate postsynaptic localization (16, 17). However, not all dually acylated proteins are found at postsynaptic membranes; GAP-43 is a dually palmitoylated protein that occurs predominantly at axonal membranes (18). Both PSD-95 and GAP-43 accumulate in the secretory pathway in a palmitoylation-dependent manner (17, 19), but it is unclear how they sort to separate vesicles destined for dendritic versus axonal membranes.

To elucidate mechanisms for axonal versus dendritic sorting of peripheral membrane proteins, we analyzed the polarized targeting of PSD-95 and GAP-43 in hippocampal neurons. We find that the palmitoylation motif of PSD-95 is necessary but not sufficient for dendritic targeting, whereas the palmitoylation motif of GAP-43 is sufficient for axonal targeting. Systematic mutagenesis of these two palmitoylation motifs reveals that axonal targeting by the GAP-43 motif requires two adjacent cysteines as well as nearby basic residues, features that are conserved in other palmitoylated axonal proteins. Palmitoylation motifs that mediate axonal targeting also localize to DIGs in heterologous cells, indicating that lipid rafts probably mediate axonal targeting of certain cytosolic proteins.

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1 The abbreviations used are: DIG, detergent-insoluble glycolipid-enriched complexes; PSD, postsynaptic density; GAP, growth-associated protein; GFP, green fluorescent protein; HBS, 150 mM NaCl, 20 mM Hepes, pH 7.4; MAP, microtubule-associated protein; A/D ratio, ratio of axonal versus dendritic expression; ANOVA, analysis of variance; TEE, 50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 1 mM EGTA; PAGE, polyacrylamide gel electrophoresis; SAP, synapse-associated protein; par, paralemmin.
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EXPERIMENTAL PROCEDURES

**cDNA Cloning and Mutagenesis**—GW1 PSD-95, PSD-95(C3,5S), and PSD-95(1–26) fused to GFP were described previously (16, 17). The mutations of the palmitoylation motif of PSD-95, the addition of the GAP-43 N-terminal palmitoylation motif to PSD-95, and the mutations within the GAP-43 palmitoylation motifs of the 43-PSD-95 chimera were constructed with oligos encoding the appropriate wild-type or mutated motif and restriction sites that were annealed and subcloned into GW1 PSD-95 GFP at a HindIII site upstream of the starter methionine and a silent KpnI site at amino acid 13 of PSD-95. The addition of the C-terminal prenyl-palmitoylation motif of parallemrin was added to the extreme C terminus of PSD-95(C3,5S) GFP with primers encoding the appropriate wild-type or mutated motif and restriction sites, which were used to amplify the C-terminal GFP. Dr. David Sretavan (University of California, San Francisco) kindly provided wild-type GAP-43. Parallemrin was obtained by reverse transcriptase-polymerase chain reaction from mouse brain RNA and subcloned into pEGFP (CLONTECH) at the BglII and HindIII sites.

**Primary Neuronal Culture and Transfection**—Neuronal cultures were prepared from the hippocampi of E18/E19 rats. Hippocampi were dissociated by enzyme digestion with papain followed by brief mechanical trituration. Cells were plated on poly-D-lysine (Sigma)-treated glass coverslips (12 mm diam) and maintained in neurobasal medium (Life Technologies, Inc.) supplemented with B27, penicillin, streptomycin, and l-glutamine as described in Brewer et al. (39). Hippocampal cultures were transfected by lipid-mediated gene transfer just before plating as described previously (20). 2 μg of DNA and 10 μl of 1,2-dioleoyl-sn-glycero-3-trimethylammonium-propane (Roche Molecular Biochemicals) were mixed in 25 μl of HBs and added to the cells (1 million cells/0.25 ml) with immediate and gentle mixing. Cells were incubated for 37°C and then washed with buffer containing l-glutamine and glass coverslips (Fisher) in 24-well plates (Falcon). To visualize transfected cells, coverslips were removed from the wells and mounted live onto slides (Frost Plus slides, Fisher) with Fluoromount-G (Southern Biotechnology Associates, Inc.). Transfection efficiency was never >0.01%, and on average, 15–30 transfected cells were obtained for each independent transfection.

**Immunofluorescence**—Coverslips were removed from culture wells and fixed in 4°C paraformaldehyde for 15–20 min. The cells were washed with Tris-buffered saline containing 0.1% Triton X-100 Tris-buffered saline and blocked in Triton X-100 Tris-buffered saline with 3% normal goat serum for 1 h at room temperature. Primary antibodies against MAP-2 (monoclonal) (Pharmingen) or Thy-1 (MRC OX-7, Serotec) to stain dendrites or axons, respectively, were added to blocking solution for 1 h at room temperature followed by donkey anti-mouse or donkey anti-rabbit antibodies conjugated to Cy3 or 7-amino-4-methylcoumorin-3-acetic acid fluorophores (diluted 1:200 in blocking solution) for 30 min. The cells were washed with ice-cold phosphate-buffered saline and resuspended in 0.4 ml of lysis buffer containing 25 mM Tris-HCl, pH 7.6, 5 mM EDTA, 150 mM NaCl, 0.5% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml each of aprotinin and leupeptin. After extracting for 10 min at 4°C, the homogenate was adjusted to 40% sucrose and placed at the bottom of an ultracentrifuge tube. A linear sucrose gradient (5–30%) was overlaid on top, and samples were centrifuged for 18 h at 200,000 × g in a SWTI 50 rotor (46,245 rpm) at 4°C. Sequential fractions across the gradient were collected, separated by SDS-PAGE, and immunoblotted using antibodies to caveolin-1 (Transduction Laboratories, Lexington, KY) and GFP (1:150 dilution, CLONTECH).

**RESULTS**

**Palmitoylation Is Required for Axon Exclusion of PSD-95**—PSD-95 and SAP-97 are highly homologous membrane-associated guanylate kinase proteins, however, PSD-95 is restricted to postsynaptic sites in forebrain neurons, whereas SAP-97 occurs both pre- and postsynaptically (11). To test whether this polarized sorting can be reproduced in cell transfections, we expressed GFP fusions of PSD-95 or SAP-97 in hippocampal neurons (Table 1). As shown in Fig. 1, we find that PSD-95 is restricted to postsynaptic clusters in the dendrites, whereas SAP-97 occurs both in dendrites and axon. As PSD-95 is palmitoylated and SAP-97 is not, we asked whether differential lipophilic modifications of these proteins account for their differential sorting to axons versus dendrites. Indeed, we found that mutating the palmitoylated cysteines of PSD-95 to serine (PSD-95(C3,5S)) disrupts axon exclusion and causes the mutant to enter the axon. Conversely, appending the palmitoylated N terminus of PSD-95 to SAP-97 yields a chimera that is excluded from the axon (Fig. 1).

Axons were unambiguously identified both by their morphology and by the absence of MAP-2 immunoreactivity (Fig. 1). To quantitate the extent of axonal targeting, the average fluorescent pixel intensity of GFP fusions in the axon versus the dendrites were determined (A/D ratio) (see Materials and Methods). Experiments were normalized with the A/D ratio of GFP alone. This analysis revealed that wild-type PSD-95 protein and 95–95– SAP-97 are excluded from the axon, whereas SAP-97 and PSD-95(C3,5S) are present in the axon to a similar extent as is GFP alone (Fig. 2C).

**The Palmitoylation Motif of GAP-43 Targets PSD-95 to the Axon**—Whereas palmitoylation of PSD-95 is required for axon exclusion, not all palmitoylated neuronal proteins are
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We next asked whether the adjacent cysteine/basic amino acid motif might play a general role in the axonal sorting of palmitoylated proteins. Paralemmin is a neuronal prenyl-palmitoyl-anchored protein that is found at axonal membranes (22). The C-terminal palmitoylation motif of paralemmin contains adjacent prenylated/palmitoylated cysteines and nearby basic amino acids (43-PSD-95(CC)) no longer targets PSD-95 to the axon, but rather the protein localizes solely to the postsynaptic membrane similar to wild-type PSD-95 (Fig. 3). These results suggest that the basic amino acids are critically involved but do not entirely explain the targeting differences between these motifs.

Finally, we combined alternations in spacing between the cysteines and mutations in the basic amino acids. A PSD-95 palmitoylation motif with juxtaposed cysteines and the T8R mutation (PSD-95(CC-T8R)) redistributes the mutant to the axon with almost half the efficiency of 43-PSD-95 (Fig. 3). Conversely, mutating the basic amino acids of the 43-PSD-95 palmitoylation motif to isoleucines (43-PSD-95(R(6,7))I) does not alter protein palmitoylation but reduces axonal targeting of 43-PSD-95 by approximately half (Fig. 3). These results suggest that the basic amino acids are critically involved but do not entirely explain the targeting differences between these motifs.

The Palmitoylation Motif of Paralemmin Resembles That of GAP-43 and Mediates Axonal Targeting—We next asked whether the adjacent cysteine/basic amino acid motif might play a general role in the axonal sorting of palmitoylated proteins. Paralemmin is a neuronal prenyl-palmitoyl-anchored protein that is found at axonal membranes (22). The C-terminal palmitoylation motif of paralemmin contains adjacent prenylated/palmitoylated cysteines and nearby basic amino acids (43-PSD-95(CC)) no longer targets PSD-95 to the axon, but rather the protein localizes solely to the postsynaptic membrane similar to wild-type PSD-95 (Fig. 3). These results suggest that these two features account entirely for differential targeting by these domains.

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| Construct | Palmitoylation Motif |
|-----------|----------------------|
| PSD-95    | MDCLCIVYTVKXVY-      |
| PSD-95(C3,5S) | MDLSGLIYTVKXVY-  |
| PSD-95(CC) | MLCCIVYTVKX-         |
| PSD-95(TR) | MLCCIVTVKXVY        |
| PSD-95(CTXR) | MLCCIVTRKX-         |
| 43-PSD-95 | MLCCIVTVKXVY         |
| 43-PSD-95(R6,7I) | MLCCIMTVKXRV-     |
| 43-PSD-95(CXC-R6,7I) | MLCCIMTVKXRV-    |
| Par       | GFP                  |
| GFP-par   | -DEKKRRKRKCGSIN     |
| (C3,5S)-par-palmitoyl | -DEKKRRKRKCGSIN |
| (C3,5S)-par-B | -DEKKRRKRKCGSIN |

Strikingly, a PSD-95 mutant containing adjacent cysteines (PSD-95(CC)) is not exclusively dendritic; the protein also localizes to the axon though not to the degree of 43-PSD-95 (Fig. 3). On the other hand, the addition of a single amino acid between the contiguous cysteines of the 43-PSD-95 palmitoylation motif (43-PSD-95(CC)) does not affect axonal targeting of 43-PSD-95, and the A/D ratio is unchanged (Fig. 3). These mutated constructs are all efficiently palmitoylated (Fig. 5), so changes in protein targeting are not attributed to alterations in protein palmitoylation. These results suggest that the spacing of the cysteines is important but is not the only feature of these motifs that determines targeting.

Additionally, the GAP-43 motif contains two basic amino acids that is one residue away from the palmitoylated cysteines, whereas the PSD-95 motif does not have basic residues near the cysteines. Remarkably, adding a single basic amino acid to PSD-95 two amino acids away from the cysteines (PSD-95(TSR)) redistributes the mutant to the axon with almost half the efficiency of 43-PSD-95 (Fig. 3). Conversely, mutating the basic amino acids of the 43-PSD-95 palmitoylation motif to isoleucines (43-PSD-95(R6,R7I)) does not alter protein palmitoylation but reduces axonal targeting of 43-PSD-95 by approximately half (Fig. 3). These results suggest that the basic amino acids are critically involved but do not entirely explain the targeting differences between these motifs.

Finally, we combined alternations in spacing between the cysteines and mutations in the basic amino acids. A PSD-95 palmitoylation motif with juxtaposed cysteines and the T8R mutation (PSD-95(CC-T8R)) targets PSD-95 to the axon similar to 43-PSD-95 (Fig. 3). In addition, a GAP-43 palmitoylation motif both with cysteines separated and with mutations in the basic (43-PSD-95(CXC-R6,R7I)) no longer targets 43-PSD-95 to the axon, but rather the protein localizes solely to the postsynaptic membrane similar to wild-type PSD-95 (Fig. 3). These results suggest that these two features account entirely for differential targeting by these domains.

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We next determined whether axonal targeting by the paralemmin motif requires similar features as the GAP-43 motif.
Adjusting the spacing of the cysteines interferes with prenylation and subsequent palmitoylation (data not shown), so we were unable to assess the importance of cysteine residue spacing in protein trafficking. However, mutating the palmitoylated cysteines to serines (PSD-95(C3,5S-par-palmitoyl)) retains prenylation but blocks palmitoylation (Fig. 5) and disrupts axon targeting (Fig. 4). In addition, mutations in the basic amino acids maintain lipidation but reduce axonal targeting, consistent with a requirement for nearby basic amino acids in axonal targeting (Fig. 4).
Palmitoylation Motifs That Mediate Axonal Targeting Are Incorporated into DIGs—Dually acylated proteins can be incorporated into DIGs, and these complexes have been implicated in targeting to axonal membranes (4, 7, 23). We, therefore, asked whether the palmitoylation motifs of GAP-43 and PSD-95 might differentially associate with DIGs. Resident proteins of DIGs float in sucrose gradients and are found in light membrane fractions together with caveolin (24). As previously published, only a small amount of PSD-95 associates with DIGs, and this is independent of palmitoylation (Fig. 6) (25, 26). In contrast, the palmitoylation motif of GAP-43 efficiently targets GFP preferentially to the axon (arrows in Fig. 43(1–14)), and this is independent of palmitoylation (Fig. 6). Furthermore, when the palmitoylation motif of PSD-95 is replaced with that of GAP-43 or palammin, these chimeras also associate with DIGs, whereas the isolated palmitoylation motif of GAP-43 targets GFP preferentially to the axon (arrows in Fig. 43(1–14)), and this is independent of palmitoylation (Fig. 6). Thus, there is a strong correlation between the ability of palmitoylation motifs to target proteins to DIGs and to axonal membranes.

DISCUSSION

This analysis of polarized sorting of peripheral membrane proteins demonstrates that palmitoylation motifs can mediate either dendritic/postsynaptic or axonal targeting. Previous work shows that dual palmitoylation is necessary for targeting PSD-95 to postsynaptic membranes (16, 17). We now find that palmitoylation is also necessary to exclude PSD-95 from axons, although the isolated palmitoylation motif is not sufficient for axonal exclusion. In contrast, the dually palmitoylated motifs of the axonal proteins GAP-43 and palammin are sufficient to mediate protein targeting to axonal membranes and, therefore, are the first identified axonal targeting motifs for peripheral membrane proteins.

Differential sorting of the PSD-95 and GAP-43 palmitoylation motifs depend on two features, the spacing of the cysteine residues and the presence of nearby basic amino acids. The PSD-95 and GAP-43 palmitoylation motifs also differ in their capacity to associate with DIGs; PSD-95 is only faintly incorporated into DIGs, whereas the isolated palmitoylation motif of PSD-95 is not.
FIG. 3. Sequence requirements for axonal targeting by the GAP-43 palmitoylation motif: Adjacent cysteines and nearby basic amino acids. The N-terminal 12 amino acids of the PSD-95 and GAP-43 palmitoylation motifs are shown with the lipid-modified cysteines in red and the basic residues underlined. Modification of the PSD-95 palmitoylation motif to include two adjacent cysteines results in some protein expression in the axon (arrow in PSD-95(CC), bottom middle). The combination of two adjacent cysteines and the addition of a nearby basic residue T8R result in efficient axonal targeting (arrow in PSD-95(CC-T8R), left). Conversely, mutating the GAP-43 motif to separate the cysteines and remove the nearby basic amino acids disrupts axonal targeting (43-PSD-95(CXC-R6I,R7I), top middle). However, separating the cysteines alone does not alter localization to the axon (arrow in 43-PSD-95(CXC), top left). In all transfections, the axon was identified by its morphology and the lack of MAP-2 immunoreactivity. The MAP-2 labeling for the 43-PS-95(CXC) transfection is shown as a typical example (MAP-2, bottom left). A graph shows mean A/D ratios for the different constructs (bottom). PSD-95(CC) and PSD-95(CC-T8R) are statistically different from wild-type PSD-95 (***, p < 0.001), and 43-PSD-95(R6L,R7I) and 43-PSD-95(CXC-R6L,R7I) are statistically different from 43-PSD-95 (**, p < 0.01; ***, p < 0.001; scale bar = 10 μm).
FIG. 4. Axonal targeting by the paralemmin C-terminal lipidated motif. The C-terminal 13 amino acids of the prenylation-palmitoylation motif of paralemmin is shown with the adjacent cysteines in red and basic residues underlined. Full-length paralemmin GFP is enriched in the axon (arrows in top panels). The C-terminal palmitoylation motif is sufficient to target GFP to the axon (arrow in GFP-par), which is identified by the morphology and absence of MAP-2 immunoreactivity (MAP-2, top right). This motif also targets PSD-95(C3,5Spar-B) to axons (arrows in PSD-95(C3,5S-par)), and this is partially dependent on basic amino acids within the motif PSD-95(C3,5S-par-B) and completely dependent upon palmitoylation (PSD-95(C3,5S-par-palmitoyl)). Mean A/D ratios for the various constructs are shown (bottom). Par, GFP-par, and PSD-95(C3,5S-par) are statistically different from PSD-95(C3,5S) and GFP. ***, p < 0.001; scale bar = 10 μm.
are not as polarized as their isolated palmitoylation motifs, nor when axonally targeted palmitoylation motifs from GAP-43 and paralemmin are added to PSD-95, the resulting chimeras are not as polarized as their isolated palmitoylation motifs, nor are they as well associated with DIGs. These results suggest that additional dendritic targeting or axonal exclusion signals reside within PSD-95. Consistent with this finding, the palmitoylation motif of PSD-95 is insufficient for dendritic targeting. Yet because unpalmitoylated PSD-95(C3,5S) is distributed similar to GFP, the expression of the dendritic targeting signal within the body of PSD-95 probably requires an association with membranes via palmitoylation. The identity of the additional region(s) of PSD-95 involved in dendritic targeting/axonal exclusion remains to be uncovered.

In contrast to the absolute polarization of dendritically targeted PSD-95, axonally targeted constructs, such as GAP-43 and paralemmin, are also expressed in dendrites. These proteins are considered axonal because of their enhanced density in the axon compared with diffusely expressed GFP. Previous studies have also found that exogenous expression of axonal proteins often yields some protein in dendrites (30). This dendritic expression may occur for a number of reasons, including inadequate axonal retention or missorting due to saturation of targeting mechanisms. Alternatively, dendritic localization may be explained by the presence of dendritic targeting signals and/or the absence of dendritic exclusion signals, as axonal proteins can also occur in dendritic/postsynaptic localizations (22). Indeed, we found that PSD-95 constructs containing GAP-43 or paralemmin palmitoylation motifs concentrate in the axon but also occur at the PSD.

PSD-95 and related membrane-associated guanylate kinases display complex expression patterns that depend on the specific protein isoform and neuronal cell type. In hippocampal neurons, PSD-95 and PSD-93 are excluded from axons, whereas SAP-97 and SAP-102 occur both in axons and dendrites (17). As PSD-95 and PSD-93 are palmitoylated and SAP-97, and SAP-102 are not (16), this differential targeting in vivo may reflect the lipid-dependent mechanisms as described here.

Unlike its dendritic localization in hippocampal and other forebrain neurons, PSD-95 occurs prominently in axons of cerebellar basket cells (27). Interestingly, basket cells are unusual in that their axons are devoid of microtubules (28). Axons of hippocampal and most other neurons contain plus end distal microtubules, and dendrites contain both plus and minus end-directed microtubules (29). Therefore, dendritic localization of PSD-95 may reflect a selective association with minus end-directed microtubule motors that cooperate with palmitoylation to target PSD-95 into dendrites and to exclude PSD-95 from axons. Thus, the presence of PSD-95 in basket cell axons is potentially explained by the loss of microtubule-dependent axon exclusion.

A recent study (30) has determined the axonal exclusion signal for a metabotropic glutamate receptor. This work shows that dendritic/axonal targeting of these transmembrane proteins relies on signals within their cytoplasmic C termini. By contrast, our analysis of palmitoylation-dependent sorting of peripheral membrane proteins implicates a role for DIGs. These complexes are rich in glycosphingolipids and cholesterol, and their formation in the secretory pathway is thought to serve as a sorting platform to direct proteins to the apical membrane of epithelial cells (4). Polarized sorting of proteins to axonal membranes has been compared with this apical sorting (23). Indeed, an incorporation into sphingolipid-cholesterol rafts appears to mediate axonal targeting of the GPI-anchored protein, Thy-1 (3, 7).

We find that dual palmitoylation motifs that associate with these rafts can also target proteins to axonal membranes. In contrast, the dual palmitoylation motif of PSD-95 is not incorporated into DIGs, and rather than mediating axonal targeting, it plays a role in dendritic targeting/axonal exclusion. To

**Fig. 5. Palmitoylation of PSD-95 GFP fusion constructs.** COS cells were transiently transfected with various PSD-95 constructs described in Figs. 1–4. Cells were lysed in radioimmune precipitation buffer, and the solubilized material was immunoprecipitated with an antibody to GFP. Immunoprecipitates were loaded onto duplicate gels that were analyzed for [3H]palmitate by fluorography (upper gel) or were immunoblotted for GFP (lower gel).

**Fig. 6. Palmitoylation motifs that target to axonal membranes also associate with DIGs.** A, the isolated palmitoylation motif of PSD-95 fused to GFP (PSD-95(1-26)) is found in the heavy fractions (lanes 8–10) of a sucrose gradient similar to cytosolic GFP and, thus, is not significantly associated with DIGs. In contrast, the palmitoylation motif of GAP-43 (GAP-43(1-14)) or paralemmin (GFP-par) is sufficient to incorporate GFP into lipid rafts, and these proteins are found in the lighter membrane fractions. B, PSD-95 is only modestly associated with lipid rafts similar to the non-DIG-associated transmembrane protein Tac. In contrast, replacing the palmitoylation of PSD-95 with that of GAP-43 (43-PSD-95) or paralemmin (PSD-95(C3,5S-par)) localizes a portion of these chimeric proteins to lighter membrane fractions containing α-caveolin, a DIG-resident protein.

GAP-43 is sufficient for association with these complexes. These data suggest that the incorporation of peripheral membrane proteins into lipid rafts may mediate axonal trafficking.

Whether dendritic sorting of PSD-95 results from active dendritic targeting or axonal exclusion is unclear. Interestingly, when axonally targeted palmitoylation motifs from GAP-43 and paralemmin are added to PSD-95, the resulting chimeras are not as polarized as their isolated palmitoylation motifs, nor
mediate palmitoylation of neuronal proteins will help to clarify
solic or associated with membranes other than the
Golgi network followed by the incorporation into lipid rafts for
recognized by a palmitoyl-transferase enzyme in the
sequence specificity for palmitoylation identified here suggests
that this is indeed an enzymatic process. For instance, palmi-
tcosity associated with DIGs because different palmitoyl-transferase en-
ses recognize their distinct palmitoylation motifs. Although
association for protein trafficking to the axonal membrane.
Indeed, previous studies have found that GAP-43 is enriched in
DIGs from brain homogenates (31) and is transported to axons
on vesicles derived from the secretory pathway (32). On the
other hand, signals within the body of PSD-95 working to-
gether with palmitoylation may mediate its association with
dendritic targeting vesicles and its exclusion from rafts. Near
its C terminus, PSD-95 possesses a tyrosine-based protein-
Freeze: 5–6
unpublished results.
uncover a more direct relationship between DIG association
and axonal targeting, we attempted to disrupt DIG formation
using lovastatin and methyl-β-cyclodextrin to deplete choles-
terol. However, this treatment did not affect axonal targeting of
endogenous Thy-1, suggesting that cholesterol depletion was
not successful in our cultures.
Both GAP-43 and PSD-95 are found in the secretory pathway
(17, 19) where lipid rafts are first formed. And it is here that
these two proteins may be segregated to separate secretory
vesicles for transport to dendritic versus axonal membranes.
The palmitoylation motif of GAP-43 can mediate lipid raft
association for protein trafficking to the axonal membrane.
Its C terminus, PSD-95 possesses a tyrosine-based proteincar-bon
trans-Golgi network followed by the incorporation into lipid rafts for
axonal trafficking. In contrast, the palmitoylation motif of
PSD-95 may be recognized by a separate enzyme that is cyto-
solic or associated with membranes other than the trans-Golgi
network, and thus PSD-95 is not incorporated into DIGs. Iden-
tification of the putative palmitoyl-transferase enzyme(s) that
mediate palmitoylation of neuronal proteins will help to clarify
these issues. Furthermore, elucidating how protein palmitoy-

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