Gγ13 Interacts with PDZ Domain-containing Proteins*

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The G protein γ13 subunit (Gγ13) is expressed in taste and retinal and neuronal tissues and plays a key role in taste transduction. We identified PSD95, Veli-2, and other PDZ domain-containing proteins as binding partners for Gγ13 by yeast two-hybrid and pull-down assays. In two-hybrid assays, Gγ13 interacted specifically with the third PDZ domain of PSD95, the sole PDZ domain of Veli-2, and the third PDZ domain of SAP97, a PSD95-related protein. Gγ13 did not interact with the other PDZ domains of PSD95. Coexpression of Gγ13 with its Gβ1 partner did not interfere with these two-hybrid interactions. The physical interaction of Gγ13 with PSD95 in the cellular milieu was confirmed in pull-down assays following heterologous expression in HEK293 cells. The interaction of Gγ13 with the PDZ domain of PSD95 was via the C-terminal CAAX tail of Gγ13 (where A4 indicates the aliphatic amino acid); alanine substitution of the CTAL sequence at the C terminus of Gγ13 abolished its interactions with PSD95 in two-hybrid and pull-down assays. Veli-2 and SAP97 were identified in taste tissue and in Gγ13-expressing taste cells. Immunoprecipitation of Gγ13 and PSD95 from brain and of Gγ13 and SAP97 from taste tissue indicates that Gγ13 interacts with these proteins endogenously. This is the first demonstration that PDZ domain proteins interact with heterotrimeric G proteins via the CAAX tail of Gγ subunits. The interaction of Gγ13 with PDZ domain-containing proteins may provide a means to target particular Gβγ subunits to specific subcellular locations and/or macromolecular complexes involved in signaling pathways.

A sophisticated and ordered protein network is essential to the proper functioning of cells. Precise assembly of individual components, through targeting and anchoring of proteins within designated subcellular compartments, ensures the integrity of these networks (1, 2). Specific protein-protein interactions are important for accomplishing this complex task. For example, PSD95 (postsynaptic density protein 95, also called SAP90), a member of the MAGUK (membrane-associated guanylate kinase) protein family (3), helps to assemble a complex postsynaptic protein network via its interactions with several different proteins. Some of these interactions rely on three PDZ domains (named after PSD95, Disc-large, and ZO-1) located in the N-terminal half of PSD95. PDZ domains function as protein-protein interaction modules and consist of about 90 amino acids (4–6).

PDZ domains typically bind to the extreme C terminus of a target protein in a sequence-specific manner. The PDZ domains of PSD95 recognize a canonical -X(S/T)XA motif (where X represents any amino acid and A represents an aliphatic amino acid). PDZ domains have been identified in proteins in bacteria, yeast, Drosophila, metazoans, and plants and comprise the most common protein module identified in the sequenced genome (4, 6). In addition to their affinity for C-terminal motifs, PDZ domains can also bind to internal sequences that mimic free C termini. Finally, PDZ domains can form both homo- and hetero-oligomers, enabling them to participate widely in the formation of vast and complex cellular networks (4, 6).

The PDZ domains containing Lin/MALS/Veli proteins have been implicated in protein trafficking and assembly (7–10). In mammals this group includes members that are expressed ubiquitously (e.g. Mint-3) or selectively in neurons (e.g. MALSL-1). MALSL along with CASK and Mint-1, forms a tightly associated heterotrimeric protein complex that is evolutionarily highly conserved (cf. the Lin-2-Lin-7-Lin-10 complex in Caenorhabditis elegans). Both CASK and Mint-1 also contain more than one PDZ domain, as well as other protein interaction modules, enabling the CASK-Mint-1-Veli complex to interact with an array of proteins during vesicle trafficking and protein targeting. The Lin-2-Lin-7-Lin-10 complex (the invertebrate equivalent of CASK-Mint-1-Veli) is required for the basolateral localization of LET-23, a receptor tyrosine kinase essential for vulval development in Caenorhabditis elegans (11, 12). The homologous tripartite complex in mammals, coordinated with the kinesin motor protein KIF17, has been shown to transport the N-methyl-D-aspartic acid receptors along the microtubules in neuronal dendrites (10).

Heterotrimeric G proteins, consisting of Gα and Gβγ subunits, function as signal transducers for the seven transmembrane helix G protein-coupled receptors (GPCRs). The dynamic cycle of association and dissociation of Gα and Gβγ and its role as a switch in GPCR-based signaling pathways have been well defined. Specific interactions between GPCRs and heterotrimeric G proteins are important for maintaining the specificity and fidelity of signal relay (13, 14). This is achieved in part by the membrane targeting of heterotrimeric G proteins and their specific interactions with particular receptors.

Specific lipid modification of Gα and Gγ plays an important role in targeting and anchoring these proteins to the cell membrane (15). The Gβ subunit, although not modified by lipids, is membrane-bound by virtue of its tight interaction with Gγ. The prenylation of the Gγ is dictated by the conserved CAAX motif at its C terminus (15), where A and X represent an aliphatic amino acid and any amino acid, respectively. Isoprenoids are covalently attached to the cysteine residue of the CAAX motif, whereas Gγ is still in the cytoplasm. Subsequently, the last three amino acids are proteolytically cleaved, and the free C terminus is carboxymethylated. This carboxymethylation is believed to be a membrane-associated event, and the insertion of the attached lipids into the cellular membrane completes the anchoring of Gβγ on the inner side of the cellular membrane (16, 17).

The G protein γ13 subunit (Gγ13) has a restricted and distinct pattern of expression (18, 19). Gγ13 has been shown to play a role in taste signal transduction (18). In this study, we demonstrate interactions
between Gy13 and PDZ domain-containing proteins. These interactions provide a novel means of targeting and anchoring heterotrimeric G proteins to the cellular membrane and of assigning them to macromolecular complexes of receptors and channels.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—PCR amplification was used to prepare DNA from wild type and mutant Gy13. Gy13T65A contains a single nucleotide substitution leading to a threonine to alanine substitution at amino acid 65 of Gy13. Gy13 DNA's were subcloned into the two-hybrid vector pBDU-1 (a gift of E. A. Craig and P. J. James of the University of Wisconsin, Madison (20)) to create plasmids pBD-Gy13 and pBD-Gy13T65A. Plasmid pBD-Gy13 was created by subcloning PCR-amplified GYG13 and Gy13 DNA's into the vector pBridge (Clontech). pGAD424 plasmids containing various PDZ domains from PSD95 (pGAD424-PDZ1, pGAD424-PDZ2, pGAD424-PDZ3, and pGAD424-PDZ123), and pGAD424-PDZ23, see Table 1) were the kind gifts of Dr. M. Noda and have been described previously (21).

**Table 1** Two-hybrid plasmids used

| Plasmid               | Parent   | Selection | Gene/fragment | Ref.         |
|-----------------------|----------|-----------|---------------|--------------|
| pBD-Gy13              | pBDU-1   | URA3      | Gy13          | This study   |
| pBD-Gy13T65A          | pBDU-1   | URA3      | Gy13          | This study   |
| pAD-PDZ3              | pGADT7   | LEU2      | PDZ3 of SAP97 | This study   |
| pAD-PSD95Δ38          | pACT2    | LEU2      | PSD95Δ38      | This study   |
| pAD-PSD95-PDZs        | pACT2    | LEU2      | PSD95 amino acids 39–435 | This study and MachmakerII |
| pAD-PSD95-SHUK        | pACT2    | LEU2      | PSD95 amino acids 346–724 | This study and MachmakerII |
| pAD-Veli2             | pACT2    | LEU2      | Veli-2        | This study   |
| pAD-Veli2Δ3           | pACT2    | LEU2      | Veli-2Δ3      | This study   |
| pAD-Veli2-L27         | pACT2    | LEU2      | Veli-2 amino acids 1–121 | This study and MachmakerII |
| pAD-Veli121           | pACT2    | LEU2      | Veli-2 amino acids 122–207 | This study and MachmakerII |
| pGAD424-PDZ123        | pGAD424  | LEU2      | PDZ123 of PSD95 | This study   |
| pGAD424-PDZ12         | pGAD424  | LEU2      | PDZ12 of PSD95 | 21          |
| pGAD424-PDZ23         | pGAD424  | LEU2      | PDZ23 of PSD95 | 21          |
| pGAD424-PDZ123        | pGAD424  | LEU2      | PDZ123 of PSD95 | 21          |
| pGAD424-PDZ12         | pGAD424  | LEU2      | PDZ12 of PSD95 | 21          |
| pGAD424-PDZ23         | pGAD424  | LEU2      | PDZ23 of PSD95 | 21          |
| pGAD424-PDZ123        | pGAD424  | LEU2      | PDZ123 of PSD95 | 21          |
| pGAD424-PDZ23         | pGAD424  | LEU2      | PDZ123 of PSD95 | 21          |
| pGAD424-PDZ3          | pGAD424  | LEU2      | PDZ123 of PSD95 | 21          |

**Oligonucleotides**—The following oligonucleotides were used: Gy13 5′ primer, GCCATGCTAGACCTTAGATGCTCCT; Gy13 3′ primer, GACGTCGACTCATAGGATGGTGCACTT; Gy13T65A 5′ primer, GACGTCGACTCATAGGATGGTGCACTT (the underlined C indicates the mutated residue); Gβ1 3′ primer, GGGCGCGCCATGAGTGAACGTTGAC; Gβ1 3′ primer, GGGGATCTCTTTAGTCCAAGGAGTT; Gy13 3′ primer, GGGGACCCCATTTAGGATGGATACAG; PSD95 5′ primer, CGGCTGATCTATTACAGTCTGCG; PSD95 3′ primer, GAAGATCTGTATTCTCTC; SAP97 5′ primer, CCCGGATCCATCTAGGGAGCTAGGAGG; and SAP97 3′ primer, CCCGGATCCATCTAGGGAGCTAGGAGG.

**Cell Culture**—HEK293T and STC-1 cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 100 units of penicillin/streptomycin/ml. Cultured cells were transfected with HA-tagged Gy13 and/or GW1-/myc-PSD95 using FuGENE 6 transfection reagent (Roche Applied Science).

**Antibodies**—The anti-HA monoclonal antibody was obtained from Sigma. The anti-Myc monoclonal antibody (9E10) was produced at the Hybridoma Center, Mount Sinai School of Medicine. The polyclonal antibody directed against PSD95 was obtained from Upstate Biotechnology, Inc. (Lake Placid, NY). Rabbit polyclonal antisera against Gy13 was as described previously (18). Monoclonal SAP97 antibody was from Abcam (Cambridge, MA).

**Coimmunoprecipitation and Western Blots**—HEK293 cells were transfected with Effectene (Qiagen, Valencia, CA) with wild type or mutant forms of Gy13 alone or together with PSD95. 48 h post-transfection, the cells were rinsed with phosphate-buffered saline, placed at 4 °C, and then lysed for 30 min in 200 ml of Lysis Buffer (50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 1% Triton X-100 and 1× complete protease inhibitor mixture (Roche Applied Science)). Unless otherwise indicated, all subcellular fractionation steps were performed at 4 °C. Cultured cell lysates were lysed by microcentrifugation for 15 min. Protein concentrations were determined by the Bradford assay (Bio-Rad). Equal amounts of cultured cell lysate total protein were used in immunoprecipitation assays. Lysate and brain tissues from mice were homogenized in Lysis Buffer; homogenates were centrifuged at 13,000 × g for 10 min to remove tissue debris, and the supernatants were used for immunoprecipitation pull-down assays. Antibodies to be used in pull-down assays were mixed...
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FIGURE 1. Interaction of the heterotrimeric G protein γ13 subunit (Gy13) with PSD95 and Veli-2 proteins in the yeast two-hybrid system. A, activation of the HIS3 and ADE2 reporter genes allows the growth of yeast cells on plates with synthetic medium lacking the amino acids histidine and adenine. Yeast two-hybrid vectors pAS2–1 and pACT2 were used as the negative control (−), whereas the same pair of plasmids harboring p53 and large T-antigen of SV40, respectively (MATCHmaker II, Clontech), was used as the positive control (+). B, quantitation of β-galactosidase activity in yeast cells resulting from the interaction of Gy13 with PSD95 and Veli-2 proteins. The β-galactosidase activity from the p53/T-antigen interaction (p33/T-ag), the positive control, was defined as 100. C, interaction of the Gy11γ13 with PSD95 in the two-hybrid assay allows the yeast cells to grow on plates lacking histidine and adenine. A pACT2 vector with GST-Gy13 was used as a negative control.

with protein G-Sepharose (Amersham Biosciences), incubated at room temperature for 1 h to enable conjugates to form, and then washed three times with phosphate-buffered saline to remove excess antibody. Antibody-protein G-Sepharose conjugates were mixed with cell lysates, shaken for 2 h at 4 °C, and then washed two times with RIPA buffer (20 mM Tris-HCl, pH 7.4, 137 mM NaCl, 1% glycerol, 1% Triton X-100, 0.5% deoxycholate, 0.1% SDS), two times with 0.1 M Tris-HCl containing 0.5 M LiCl, and then two times in 50 mM Tris-HCl, pH 7.5. Samples were incubated in SDS gel loading buffer for 3 min at 95 °C, then separated by SDS-PAGE, and visualized by Western blotting.

Immunocytochemistry—Immunocytochemistry using transgenic mice expressing green fluorescent protein (GFP) from the α-gustducin promoter was as described previously (18). Frozen sections (10 μm thick and fixed in 4% paraformaldehyde and cryoprotected in 20% sucrose) of lingual tissue from GFP mice were incubated in Blocking Buffer (3% bovine serum albumin, 0.3% Triton X-100, 2% goat serum, and 0.1% sodium azide in phosphate-buffered saline) for 1 h at room temperature. The sections were then incubated overnight at 4 °C with purified primary antibodies as follows: either polyclonal rabbit anti-Gy13 (18), rabbit anti-SAP97 (Affinity Bioreagent, Golden, CO), or goat anti-PSD95 (Santa Cruz Biotechnology, Santa Cruz, CA). The secondary antibodies were Cy3-conjugated goat anti-rabbit Ig for Gy13 and SAP97 and Cy3-conjugated donkey anti-goat Ig for PSD95 (Molecular Probes, Eugene, OR). Immunofluorescence images were obtained with an Olympus AX70 fluorescence microscope in the presence of Vector antifade mounting medium (Molecular Probes, Eugene, OR). Incubation of sections with primary or secondary antibodies alone produced no observable immunofluorescence (data not shown).

RESULTS

Interaction of Gy13 with PSD95 and Veli-2—The yeast two-hybrid system is a valuable tool for studying protein-protein interactions and for identifying interacting partners of known proteins (25), and it has been used to characterize the interaction between the G protein β and γ subunits (26). In a two-hybrid assay, the Gy13 subunit can interact weakly with all five known Gβ subunits and shows no distinct preference among them (27). Because the signaling specificity of the Gβγ dimer is largely dictated by Gyγ, we searched for proteins that could interact directly with Gy13. For this purpose we subcloned Gy13 into vector pGBDU-1 and used it as "bait" to screen a mouse brain cDNA library in the GAL4-based yeast two-hybrid system, where positive interactions of Gy13 with unknown proteins would activate several reporter genes (HIS3, ADE2, and lacZ) of the host yeast cells. The activation of the HIS3 or ADE2 genes was monitored by yeast cell growth on media lacking the amino acids histidine or adenine. Activation of the lacZ reporter rendered yeast colonies blue in the presence of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside in the growth media and was quantitatively measured using o-nitrophenyl β-D-galactopyranoside as a substrate (Matchmaker II; Clontech). After screening approximately 10 million independent colonies (−3 times the complexity of the library), we obtained four clones that activated the HIS3, ADE2, and lacZ reporter genes. Two of these were identical clones encoding a part of the PSD95 protein starting at amino acid 39 (pAD-PSD95Δ38). The other two clones were derivatives of the Veli-2 gene. One of these clones encoded the full-length Veli-2 protein (pAD-Veli2), although the other encoded a truncated form lacking the first three amino acids (pAD-Veli2Δ3).

Both PSD95 and Veli-2 were retested in an independent two-hybrid assay using diploid cells to confirm their interaction with Gy13 (Fig. 1A). PSD95 and Veli-2 failed to activate the reporter genes when combined with numerous unrelated genes in control two-hybrid assays (data not shown). Therefore, the interactions of Gy13 with PSD95 and Veli-2 appeared to be specific. The strength of interaction of Gy13 with PSD95 and Veli-2 was examined quantitatively using β-galactosidase activity in a yeast two-hybrid reporter assay. By this measure, PSD95 had higher affinity for Gy13 than did Veli-2 (Fig. 1B).

In these studies we relied on the ability of Gy13 to interact with other proteins in the absence of Gβ. In vivo, however, Gβγ subunits comprise a nondissociating unit. Some Gy13 domains exposed during our screening procedure might ordinarily be inaccessible within the context of the Gβγ dimer. To investigate this, we coexpressed Gy13 with Gβ1 in our two-hybrid assay. Coexpression of Gy13 with Gβ1 did not prevent PSD95 (Fig. 1C) or Veli-2 (data not shown) from activating the HIS3, ADE2, or lacZ reporter genes, suggesting that the Gy13 domain(s) responsible for interaction with PSD95 or Veli-2 is not blocked by the presence of Gβ1.

2 Z. Li and R. F. Margolskee, unpublished observations.
One PDZ Domain of PSD95 Interacts with the C Terminus of Gy13—A common feature of PSD95 and Veli-2 proteins is the presence of one or more PDZ domains. To determine whether these PDZ domains underlie the interaction of PSD95 and Veli-2 with Gy13, several deletion mutants of PSD95 and Veli-2 were generated and tested in a yeast two-hybrid assay (Fig. 2). Splitting the Veli-2 gene within the PDZ motif yielded the following two fragments: the N-terminal amino acids 1–121, containing the L27 domain (28), and the C-terminal amino acids 122–207. Neither of these fragments bound Gy13 in a two-hybrid assay (Fig. 2A), suggesting that the PDZ domain of Veli-2 must be intact for this protein to interact with Gy13. For PSD95, a deletion mutant (PDZ123, amino acids 39–435) containing all three PDZ domains, but lacking the C terminus, bound Gy13 (Fig. 2B), indicating a role for one or more of PDZ domains of PSD95 in the interaction with Gy13.

To determine whether a particular PDZ domain of PSD95 was responsible for the interaction with Gy13, individual PDZ domains of rat PSD95 were tested in a yeast two-hybrid assay (Fig. 3). Interactions with Gy13 were detected only with constructs containing the third PDZ domain of PSD95.

FIGURE 2. Identification of Veli-2 and PSD95 regions that interact with Gy13. Schematic maps of Veli-2 (A) and PSD95 (B) depict their functional domains. Deletion constructs tested are shown below the maps of the full-length proteins. The ability of deletion constructs to interact with Gy13 in a yeast two-hybrid growth assay is indicated (+ or −). Interactions with Gy13 were detected only with constructs containing the third PDZ domain of PSD95. C, a single mutation (Gy13T65A) at the C terminus of Gy13 abolishes its interaction with PSD95.

FIGURE 3. Alignment of the sequences of PDZ domains of the MAGUK protein family and Veli proteins. The secondary structure elements (five β-sheets and two α-helices) are highlighted in boxes. The phylogenetic tree of these PDZ domains is based on the neighbor-joining method of Saitou (41).
PDZ1, PDZ2, and PDZ3; Fig. 2B) (21), their combinations (PDZ123, Fig. 2B; PDZ12 and PDZ23; data not shown), and the SH3-GU kinase-like domains were subcloned and tested for their interaction with G/H9253. Plasmids containing these domains were expressed in PJ69-4a/PJ69-4a diploid cells (24) and examined for activation of the two-hybrid reporter genes HIS3 and ADE2. As shown in Fig. 2B, only the third PDZ domain of PSD95 interacted with G/H9253; the first two PDZ domains alone or in combination and the SH3-GU kinase domains failed to do so. In this regard it should be noted that the third PDZ domain of PSD95 is the most similar to the PDZ domain of Veli-2 (Fig. 3; see “Discussion”).

The PDZ domains of PSD95 and Veli recognize the canonical X(S/T)XA motif at the C terminus of proteins (where X represents any amino acid; S/T is serine or threonine; A is any aliphatic amino acid). A serine or threonine at the −3 position is critical for this interaction. Most interestingly, the C terminus of Gγ13 is CTAL, which is predicted to be a target of the PDZ domains of PSD95 and Veli-2. If this CTAL tail is indeed the target of PDZ domain binding, then mutation of the threonine at the −3 position should disrupt this interaction. As predicted, a

FIGURE 4. Heterologously expressed Gγ13 and PSD95 interact in pull-down assays. Untransfected HEK293 cells (lanes 1 and 2) or HEK293 cells were transiently transfected with wild type Gγ13-HA + vector (lanes 3 and 4), the T65A mutant Gγ13-HA + vector (lanes 5 and 6), vector + PSD95-myc (lanes 7 and 8), wild type Gγ13-HA + PSD95-myc (lanes 9 and 10), or mutant Gγ13-HA + PSD95-myc (lanes 11 and 12). HA-tagged wild type or mutant Gγ13 proteins were immunoprecipitated (IP) by an anti-HA antibody conjugated to Sepharose beads. PSD95-myc was detected in immunoblots by an anti-Myc antibody. Wild type and mutant Gγ13-HA were detected in immunoblots by an anti-HA antibody. The immunoblotted (IB) Gγ13 and PSD95 bands are at 7 and 95 kDa, respectively. A and B, immunoblots from anti-HA immunoprecipitates (IP: HA). C and D, immunoblots from cell lysates. Representative immunoblots from a single experiment that was replicated three times with equivalent results are shown. Note that PSD95 was pulled down by wild type Gγ13 but not by mutant Gγ13T65A (A, lanes 9 and 10 versus lanes 11 and 12). The right margin indicates lysate or anti-HA immunoprecipitate (IP: HA) and the antibody used in the immunoblot (IB: HA or IB: myc).

FIGURE 5. Expression of PDZ-containing proteins in taste tissue. A, immunoreactivity to PSD95 and SAP97 was detected in taste cells by indirect immunofluorescence. Taste bud containing sections of the circumvallate papillae were from a transgenic mouse (GUS-GFP) expressing GFP in the gustducin-positive taste cells (a subset of the Gγ13-expressing taste cells). B, RT-PCR amplification identified SAP97, but not PSD95, from taste tissue. In positive controls PSD95 and SAP97 were amplified from brain. In negative controls PSD95 and SAP97 were not amplified from the no DNA controls.

FIGURE 6. Expression of SAP97 and Veli-2 in taste receptor cells. A, SAP97 expression in taste receptor cells, with enhanced levels near the taste pore, was detected by indirect immunofluorescence. B, Veli-2 expression in taste receptor cells was detected by indirect immunofluorescence. Taste bud containing sections from a gustducin-GFP (GUS-GFP) mouse were as in Fig. 5.
threonine to alanine substitution mutation of Gy13 (Gy13T65A) eliminated the two-hybrid interaction of Gy13 with PSD95 (Fig. 2C) and Veli-2 (data not shown).

**Heterologously Expressed Gy13 and PSD95 Interact Physically**—The above studies indicate that the C-terminal CAAX motif of Gy13 interacts with specific PDZ domains within the setting of a yeast two-hybrid assay. To determine whether PSD95 and Gy13 could interact in a less artificial cellular setting, we used a pull-down assay with heterologously expressed tagged proteins. HA-tagged wild type or mutant Gy13, along with Myc-tagged PSD95, were coexpressed in transfected HEK293 cells; following lysis, proteins were immunoprecipitated by an anti-HA antibody and probed on Western blots (Fig. 4). Probing the blots with an anti-Myc antibody showed that HA-tagged wild type Gy13 robustly pulled down PSD95 (Fig. 4A, lanes 9 and 10). Carrying out this same experiment with mutant HA-tagged Gy13 (Gy13T65A) lacking the CAAX motif, we find that PSD95 is not pulled down at all (Fig. 4A, lanes 11 and 12). Multiple controls were done to ensure that the pull-down interaction of Gy13 and PSD95 was specific. No pull-down of PSD95 was seen with the following: (a) untransfected cell lysates (Fig. 4A, lanes 1 and 2); (b) lysates from cells transfected with wild type or mutant Gy13 and vector (Fig. 4A, lanes 3–6); (c) lysates from cells transfected with PSD95-myc and vector (Fig. 4A, lanes 7 and 8). Immunoblots of lysates confirmed reproducible expression and detection of HA-tagged Gy13 and Myc-tagged PSD95 (Fig. 4, B–D). In sum, in the cellular environment of transfected HEK293 cells there is a physical association of the C-terminal CAAX tail of Gy13 with the PDZ domain of PSD95.

**Expression of PDZ Domain-containing Proteins in Taste Cells**—Although first identified in taste receptor cells, Gy13 is also expressed in brain and retina (18, 19) two tissues in which expression of PSD95 has been well documented (29, 30). We have shown previously that in response to bitter compounds Gy13 activates phospholipase Cβ2 to elevate inositol 1,4,5-trisphosphate levels in taste receptor cells (18). Given the coexpression of PSD95 and Gy13 in brain and retina, we tested taste receptor cells for PSD95 expression. Taste bud-containing sections from transgenic mice expressing GFP from the gustducin promoter (18) were examined by indirect immunofluorescence with an anti-PSD95 antibody. PSD95 immunofluorescence was detected in taste cells (Fig. 5A, PSD95); however, amplification by RT-PCR failed to detect expression of PSD95 in taste tissue cDNA (Fig. 5B). To determine whether cross-reactivity with other PDZ domain-containing proteins explained this result, we carried out additional immunostaining and RT-PCR experiments. SAP97, like PSD95, is a MAGUK protein. Furthermore, the epitope recognized by the anti-PSD95 antibody is very similar to SAP97, and this antibody detects a 97-kDa protein (the size of SAP97) on a Western blot. When we used a more specific affinity-purified anti-SAP97 polyclonal antibody, we observed a pattern of immunostaining of mouse taste cells (Fig. 5A, SAP97) similar to that obtained with the anti-PSD95 antibody. In addition, expression of SAP97 in taste tissue cDNA was readily detected by PCR amplification (Fig. 5B).

SAP97 expression was greatest at the taste pore, where the highest levels of taste receptors are found (Fig. 6A). SAP97 is the only member of MAGUK family of proteins that is not exclusively expressed in neuronal cells. It has the highest similarity to PSD95 (Fig. 3), and perhaps it can assume the function of PSD95 in taste tissue or in non-neuronal cells. To determine whether SAP97 could indeed interact with Gy13, we cloned the third PDZ domain of SAP97 into the two-hybrid vector pGADT7, and we tested its ability to interact with pBD-Gy13 in a two-hybrid assay. As expected, the third PDZ domain of SAP97 did interact with Gy13 (data not shown). By using an antibody against Veli-2 we detected immunoreactivity in taste cells (Fig. 6B). In contrast to SAP97, taste-expressed Veli-2 immunoreactivity was more evenly placed throughout the taste bud.

**Endogenously Expressed Gy13 and PDZ Domain Proteins Interact Physically**—The observations that 1) heterologously expressed Gy13 interacts with heterologously expressed PDZ domain proteins in HEK293 cells (Fig. 4) and that 2) Gy13 is coexpressed in native tissues with PSD95 and SAP97 (Figs. 5 and 6) suggest that Gy13 may very well interact with these and other PDZ domain proteins in vivo. To determine directly if endogenously expressed Gy13 and PDZ domain proteins interact in native tissues, we carried out pull-down assays with lysates from mouse brain and taste tissue (Fig. 7). First, we confirmed the ability to detect PSD95 and Gy13 in brain extracts and SAP97 and Gy13 in taste tissue extracts on immunoblots (Fig. 7, A and B, right panels). Next, we determined that an anti-Gy13-specific antibody efficiently pulled down PSD95 from brain lysates (Fig. 7A) and SAP97 from brain lysates (Fig. 7B). To determine whether cross-reactivity with other PDZ domain-containing proteins explained this result, we carried out additional immunostaining and RT-PCR experiments. SAP97, like PSD95, is a MAGUK protein. Furthermore, the epitope recognized by the anti-PSD95 antibody is very similar to SAP97, and this antibody detects a 97-kDa protein (the size of SAP97) on a Western blot. When we used a more specific affinity-purified anti-SAP97 polyclonal antibody, we observed a pattern of immunostaining of mouse taste cells (Fig. 5A, SAP97) similar to that obtained with the anti-PSD95 antibody. In addition, expression of SAP97 in taste tissue cDNA was readily detected by PCR amplification (Fig. 5B).

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taste tissue lysates (Fig. 7B). As a control for the specificity of these interactions, we tested preimmune serum for the ability to pull down PDZ domain proteins; neither PSD95 nor SAP97 was pulled down from brain or taste tissue by the preimmune serum (Fig. 7, A and B). Thus, endogenously expressed Gy13 interacts with endogenously expressed PSD95 and SAP97 in brain and taste tissues, respectively. These results suggest that these specific interactions may have functional importance in vivo (see “Discussion”).

DISCUSSION

We have identified an interaction between the C terminus of Gy13 and three closely related PDZ domains present in PSD95, SAP97, and Veli-2 proteins. The C-terminal region of Gy13 contains the canonical binding motif for class I PDZ domains (4, 6). The third PDZ domain of PSD95 interacted with Gy13, but the other two PDZ domains of PSD95 did not, demonstrating the specificity of this interaction. One common structural feature of the three PDZ domains that interacted with Gy13 is the short sequence between the βB and βC regions; we speculate that this may be necessary to accommodate the prenyl moiety of Gy13.

Binding of Gβ to Gy13 did not interfere with the ability of Gy13 to interact with Veli-2 or PSD95 in a two-hybrid assay. Likewise, we observed interactions in the presence of Gβ between heterologously expressed Gy13 or endogenous Gy13 and PSD95 or SAP97 in pull-down assays. This is consistent with the observation that the C-terminal sequences of Gy subunits are neither required for nor part of their interaction with Gβ subunits (31). Of more than 13 Gβ subunits identified to date, only a few of them (Gγ2, Gγ5, Gγ8, Gγ12, and Gy13) have the class I type PDZ binding C-terminal sequence (-C(T/S)XX tail). The other Gy subunits are unlikely to be recognized by the conventional PDZ domain because of the lack of Thr/Ser at the critical −3 position (they have a -C(V/A)XX tail). Either these Gy subunits are not recognized by PDZ domain proteins or they may be recognized by proteins containing another type of PDZ domain.

Gy subunits are post-translationally modified by the attachment of isoprenoid lipids to the invariant cysteine residue at the −4 position at their C terminus (15). Prenylation of the Gy subunit, although not required for its dimerization with Gβ, plays an important role in the interactions of GβY with Gα subunits, receptors, and effectors (32–36). It is generally believed that prenylation of Gy is important for anchoring the GβY subunit to the membrane, presumably by direct insertion of the isoprenoid into the lipid bilayer (35, 37–39). However, whether the lipid attachment is the only signal required for membrane targeting of GβY remains unclear, because isoprenylated proteins are found in the cytoplasm as well as on cellular membranes.

The interaction of Gy13 with PDZ-containing proteins may provide a means to specifically and efficiently target certain GβY subunits to particular subcellular locations. This interaction would provide a much more efficient signal than the C-terminal prenylation required by the kinetic membrane trapping/two-signal hypothesis models for the membrane association of lipid-modified proteins (17). The higher affinity of Gy13 for PSD95 over Veli-2 would facilitate unloading of Gy13 from Veli-2-containing transport vesicles. Although the timing of prenylation relative to binding of Gy by PSD95/SAP97/Veli-2 remains unclear, we think it unlikely that prenylation of the CAAX motif of Gy13 would interfere with its binding to the PDZ domains. First, the binding of PSD95, SAP97, or Veli-2 to Gy13 could occur temporally before its prenylation. Second, the short βB to βC region of the PDZ domains of PSD95, SAP97, and Veli-2 can probably provide enough space for the lipid group of Gy.

subunits. Third, post-translational modification occurs in both the two-hybrid system and in HEK293 cells, making it likely that at least some of the Gy13 is prenylated, and Gy13 expressed in these systems interacted with PSD95, SAP97, and Veli-2.

Ultimately, the binding site of Gy13 for PDZ domain-containing proteins, its CAAX motif, would be eliminated during maturation. It is generally the case that the last three amino acids of the CAAX motif are proteolytically cleaved, followed by carboxylation of the cysteine residue. Removal of the last three amino acids has been implicated as important for the localization of some CAAX motif proteins (40). Because the cleavage and carboxylation of the CAAX motif are membrane-associated events, they would probably occur after the delivery and anchoring of Gy13 to its subcellular location by PSD95, SAP97, and Veli-2, providing an additional means to regulate the interaction of these PDZ proteins with Gy13.

Our study provides the first evidence that proteins with PDZ domains can recognize the heterotrimeric G protein through the CAAX tail of Gy. The three PDZ-containing proteins identified in this study as interacting with Gy13 are known participants in protein trafficking and macromolecular signal complex assembly. Interaction of Gy with PSD95, SAP97, or Veli-2 could efficiently and specifically target heterotrimeric G proteins to appropriate receptors and channels in neurons and taste cells. Because PSD95 is a member of the MAGUK protein family and there is broad expression of Veli proteins in both neuronal and non-neuronal cells, we propose that similar interactions may be widespread and functionally important to various signaling pathways. For example, the enhanced level of SAP97 at the taste pore could be responsible for the high local concentration of Gy13 with implications for taste receptor-mediated signaling.

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