PDZ Protein Regulation of GPCR Trafficking and Signaling Pathways

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MOL Manuscript # 98509

Running Title: GPCR regulation by PDZ proteins

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Body words: 6636  
Figures: 2  
Tables: 2  
References: 228  
Abstracts words: 187  
Introduction words: 595

Abbreviations:

GPCR, G protein-coupled receptor; PDZ, PSD-95, Disc large, Zona occludens-1; PSD, post-synaptic density; PSD-95/93, post-synaptic density protein of 95/93 kilodaltons; SAP97/102, synapse-associated protein of 97/102 kilodaltons; DLG5, discs, large homolog 5; CARD, caspase activation and recruitment domain; CARMA3, CARD and MAGUK domain-containing protein 3; MPP3, membrane protein, palmitoylated 3; CASK, calcium/calmodulin-dependent serine protein kinase; MAGI-1/2/3, membrane-associated guanylate kinase protein 1/2/3; NHERF1/2, Na+/H+ exchanger regulatory factor 1/2; PDZK1/2, PDZ domain-containing kidney protein 1/2; GIPC, GAIP interacting protein, C terminus; CAL, CFTR-associated ligand; PDZ-GEF1/2, PDZ domain-containing guanine nucleotide exchange factor; RGS3/12, regulator of g protein signaling; RH-RhoGEF, RGS-homology domain containing Rho guanine nucleotide exchange factor; LARG, leukemia-associated RhoGEF; PDZ-RhoGEF, PDZ domain-containing RhoGEF; SH3, SRC Homology 3 domain; Shank1/2/3, SH3 and multiple ankyrin repeat domains 1/2/3; Par3/6, Partitioning defective protein 3/6; MUPP1, multiple PDZ protein 1; nNOS, neuronal nitric oxide synthase; PICK1, protein interacting with PRKCA 1; SNX27, sorting nexin 27; β1/2AR, β1/2 adrenergic receptors; 5-HT1-7R, serotonin 1-7 receptor; D1-3R, dopamine 1-3 receptor; CRFR1/2, corticotropin-releasing factor receptor 1/2; A1/2R, adenosine 1/2 receptor; VPAC1, vasoactive intestinal peptide receptors 1; mGlur, metabotropic glutamate receptor; α12AR, α1/2 adrenergic receptor; TP, thromboxane A2 receptor; hκ-OR, human κ opioid receptor; SSTR, somatostatin receptor; PTH1R, parathyroid 1 receptor; CCR5, chemokine (C-C motif) receptor 5; PAFR, platelet-activating factor receptor; P2Y1/12R, purinergic P2Y receptors; hLHR, human prostacyclin receptor; CL1, α-Latrotoxin receptor CIRL/latrophilin 1; μ/δOR, μ/δ opioid receptor; GPR10, prolactin-releasing peptide receptor; GHRHR, growth hormone-releasing hormone receptor; LPA1/2R, lysophosphatidic acid 1/2 receptor; hLHR, human luteinizing hormone receptor; BAI1,
brain; MT₁, melatonin 1 receptor; M₁-4/mAChR1-4, muscarinic acetylcholine receptor 1-4 receptor; CB₁, cannabinoid receptor 1; ET₁R, endothelin 1 receptor; FSHR, follicle-stimulating hormone receptor; PAR1, protease-activated receptor 1; AT₁R, angiotensin II receptor 1; OR2AG1, olfactory receptor 2AG1; GABA₆, gamma-aminobutyric acid 6 receptor; B₂R, bradykinin 2 receptor; GRPR, gastrin-releasing peptide receptor; GRHR, gonadotropin-releasing hormone receptor; C₅aR, chemo-attractant C₅a receptor; PAC1R, pituitary adenylate cyclase-activating polypeptide 1 receptor; S₁PR2; sphingosine-1-phosphate receptor 2; GPR132, G2 accumulation protein/g protein-coupled receptor 132; H₁R, histamine 1 receptor; MAS1, proto-oncogene mas; Mam2, pheromone p-factor receptor; ERK, extracellular signal-related kinase; IP₃, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; PLC, phospholipase C; PKA, protein kinase A; PKC, protein kinase C; Akt, protein kinase B; cAMP, cyclic adenosine monophosphate; CREB, cAMP response element-binding protein; cFos; FAK, focal adhesion kinase; Fzd, frizzled; GPR37, G protein-coupled receptor 37 (endothelin receptor type B-like).
Abstract

G protein-coupled receptors (GPCRs) contribute to the regulation of every aspect of human physiology and are the therapeutic targets for the treatment of numerous diseases. As a consequence, understanding the myriad of mechanisms controlling GPCR signaling and trafficking is essential for the development of new pharmacological strategies for the treatment of human pathologies. Of the many GPCR-interacting proteins (GIPs), PDZ domain-containing proteins appear most abundant and have similarly been implicated in disease mechanisms. PDZ proteins play an important role at regulating receptor and channel protein localization of synapses and tight junctions and function to scaffold intracellular signaling protein complexes. In the current study, we review the known functional interactions between PDZ domain-containing proteins and GPCRs, and provide insight into the potential mechanisms of action. These PDZ domain-containing proteins include the membrane-associated guanylate-like kinases (MAGUKs) (PSD-95, SAP97, PSD-93, SAP102, DLG5, CARMA3, MPP3, CASK, MAGI-1, MAGI-2, MAGI-3), NHERF proteins (NHERF1, NHERF2, PDZK1, PDZK2), Golgi-associated PDZ proteins (GIPC and CAL), PDZ-GEFs (PDZ-GEF1 and PDZ-GEF2), RGS-Homology-RhoGEFs (PDZ-RhoGEF and LARG), RGS3 and RGS12, spinophilin and neurabin-1, Shank proteins (Shank1, Shank2, Shank3), Par3 and Par6, MUPP1, Tamalin, nNOS, syntrophins, PICK1, syntenin-1 and SNX27.
Introduction

In the central nervous system, G protein-coupled receptors (GPCRs) and ion channels are targeted at the membrane of dendritic post-synaptic terminals in and around a region termed the post-synaptic density (PSD) (Feng and Zhang, 2009; Neubig and Siderovski, 2002; Magalhaes et al., 2012). Each post-synaptic density is specifically organized such that dozens to hundreds of receptors are targeted to this specialized membrane domain via the interaction of scaffolding proteins with the receptors. These scaffold proteins containing multiple protein-protein interaction domains that allow them to interact with a multitude structural and signaling proteins holding them in close proximity with one another (Feng and Zhang, 2009). Of these scaffolding proteins, it is believed that PSD-95, Disc large, Zona occludens-1 (PDZ) domain-containing proteins are the most abundant, and often provide direct contact with both GPCRs and ion channels at the post-synaptic density (Cheng et al., 2006; Feng and Zhang, 2009). PDZ proteins are not only important for targeting GPCRs to synapses, but they an important role in regulating tight junctions and signaling protein complexes. In the current review, we will overview the growing understanding of the role PDZ domain-containing proteins in the regulation of GPCR subcellular localization, endocytosis, trafficking and signal transduction.

PDZ Domains

PDZ domains are approximately 80-90 amino acid residues in size and represent the most common protein-protein interaction domain (Doyle et al., 1996; Feng and Zhang, 2009; Magalhaes et al., 2012). Although there are hundreds of unique PDZ domain sequences, they all contain a conserved glycine-leucine-glycine-phenylalanine (GLGF) sequence that provides the domain’s folded, globular, cup-like structure that is capable of recognizing short, finger-like peptides (Harris and Lim, 2001). Because of this structure, PDZ domains appear best suited for
binding the distal regions of receptor carboxyl terminal tails, labelled the PDZ-binding motif (Kornau et al., 1995; Niethammer et al., 1996; Harris and Lim, 2001; Magalhaes et al., 2012). Interestingly, additional studies have identified internal PDZ ligands that, like a carboxyl terminal tail, project outwardly from the protein (Xu et al., 1998; Hillier et al., 1999; Christopherson et al., 1999; Fouassier et al., 2000; Harris and Lim, 2001; Paasche et al., 2005; Trejo, 2005). In this case, the internal PDZ-binding motif is manifest as a sharply folded, finger-like projection.

PDZ-Binding Motifs

Although seemingly imperfect and likely biased against internal PDZ ligands (reviewed by Trejo, 2005), a simple classification system has evolved to identify potential PDZ-binding motifs and helps to predict potential PDZ domain-containing protein interactions (Songyang et al., 1997; Bezprozvanny and Maximov, 2001; Sheng and Sala, 2001; Vaccaro and Dente, 2002). Although there is some deliberation over how many classes of PDZ-binding motifs there are, it is most commonly limited to three classes (Sheng and Sala, 2001; Tonikian et al., 2008; Magalhaes et al., 2012). Class I PDZ-binding motifs are the most described class within the literature and are classified by their final 3 amino acid sequence of S/T-x-ϕ, where x indicates any amino acid and ϕ indicates any hydrophobic amino acid (Songyang et al., 1997; Bezprozvanny and Maximov, 2001; Sheng and Sala, 2001; Vaccaro and Dente, 2002). However, valine, isoleucine, or leucine appear to be most common of the hydrophobic amino acids that contribute to the formation of a Class I PDZ binding motif (Songyang et al., 1997; Bezprozvanny and Maximov, 2001; Sheng and Sala, 2001; Vaccaro and Dente, 2002). Class II and III PDZ-binding motifs are not as well characterized and show slightly more ambiguous sequences: with class II having its final 3 amino acids as ϕ-x-ϕ, and class III having Ψ-x-Ψ, where Ψ represents any acidic amino acid residue (Sheng and Sala, 2001).
GPCR-interacting PSD-95 Family PDZ Domain-Containing MAGUK Proteins

**PSD-95 (DLG4):** Post-Synaptic Density protein of 95 kDa (PSD-95) contains three PDZ domains, an SH3 domain, and a GK domain (Fig. 1) and is prototypically localized within the post-synaptic density (Sampedro et al., 1981; Cho et al., 1992). PSD-95 has been demonstrated to modulate both AMPA and NMDA receptor function, as well as a number of GPCRs. In regards to AMPA and NMDA receptors, it appears PSD-95 is important for enhancing and/or maintaining these receptors at the synaptic membrane, thereby potentiating receptor activation, channel opening, receptor-mediated currents and receptor trafficking (Elias et al., 2006; Elias and Nicoll, 2007). PSD-95 is able to indirectly bind and regulate AMPA receptors via a shared association with transmembrane AMPA receptor regulating proteins, such as stargazin (Chen et al., 2000). The β1-adrenergic receptor (β1AR) is the first GPCR to be reported as a PSD-95 interacting GPCR and PSD-95 is responsible for antagonizing β1AR endocytosis in response to agonist activation, thereby stabilizing the receptor at the cell surface (Hu et al., 2000) (Table 1). Despite the potentiation of β1AR membrane expression, this interaction appears to have no functional consequence on Gαs-coupled signaling, as measured by cAMP accumulation (Hu et al., 2000). In contrast, PSD-95 interactions with the serotonin 2A receptor (5-HT2AR) facilitate Gαq-coupled signaling by the receptor (Xia et al., 2003) (Table 2). PSD-95 has similarly been shown to antagonize the agonist-induced endocytosis of 5-HT2AR (Xia et al., 2003). G protein-coupled receptor kinase 5 phosphorylation also disrupts PSD-95 interactions with the β1AR which is consistent with a PSD-95/β-arrestin competition model (Hu et al., 2002). Moreover, the recruitment of β-arrestin2 to the 5-HT2AR corresponds with the dissociation of PSD-95, suggesting competitive binding for 5-HT2AR with mechanistic implications for the regulation of endocytosis of PSD-95 associated GPCRs (Schmid and Bohn, 2010). Notably, PSD-95 is documented to have an opposing role in 5-HT2AR trafficking, where PSD-95 overexpression is suggested to suppress cell surface receptor expression and promote receptor endocytosis (Gavarini et al., 2006). This
decrease in receptor expression at the cell surface is correlated with enhanced desensitization of
5-HT\textsubscript{2C}R-mediated Ca\textsuperscript{2+} accumulation (Gavarini et al., 2006). In PSD-95 null mice, 5-HT\textsubscript{2C}R-mediated cfos induction is impaired (Abbas et al., 2009). Despite significant sequence homology, PSD-95 appears to have opposing roles in regulating their trafficking and signaling pathways of the 5-HT\textsubscript{2A}R and 5-HT\textsubscript{2C}R (Xia et al., 2003; Gavarini et al., 2006). PSD-95 was recently suggested to form a complex with GPR30, AKAP5 and the PKA RII\textsubscript{β} regulatory subunit thereby promoting GPR30 membrane localization and facilitating the constitutive inhibition of cAMP (Akama et al., 2013; Broselid et al., 2014). PSD-95 has also been reported to positively regulate dopamine 1 receptor (D\textsubscript{1}R) endocytosis and to inhibit D\textsubscript{1}R-mediated cAMP formation (Zhang et al., 2007). A more recent study suggests that PSD-95 contributes to D\textsubscript{1}R recycling and resensitization without influencing D\textsubscript{1}R-mediated G\textsubscript{α}s activation (Sun et al., 2009). However, the methods and cellular contexts utilized to arrive at these conclusions in these various studies are not directly comparable. Nevertheless, this highlights the importance of considering the specific GPCR in question when determining the regulatory role of a PDZ domain-containing protein, as well as the endogenous trafficking and signaling machineries available within each specific cellular context.

\textbf{SAP97 (DLG1):} Although synapse-associated protein of 97 kDa (SAP97) shares ~60% sequence homology with PSD-95 (including three PDZ domains, an SH3 domain, a GK domain, and an additional L27 domain on the amino terminal), less is known about the role of SAP97 in regulating GPCR activity (Fig. 1). Nevertheless, SAP97 has been demonstrated to promote \(\beta\textsubscript{1}AR\) phosphorylation via cyclic AMP-dependent protein kinase (PKA), despite having no effect on \(\beta\textsubscript{1}AR\)-stimulated adenyl cyclase activation and cAMP accumulation (Gardner et al., 2007). Additionally, SAP97 promotes recycling of the \(\beta\textsubscript{1}AR\) by a mechanism that involves the formation of a complex between \(\beta\textsubscript{1}AR\), AKAP79 and PKA (Gardner et al., 2007; Nooh et al., 2013; Nooh et al., 2014). In contrast, SAP97 promotes membrane stabilization of the corticotropin-releasing factor receptor 1 (CRFR1) by suppressing CRFR1 endocytosis (Dunn et al., 2013). Although
SAP97 does not contribute to the regulation of CRFR1-mediated cAMP accumulation via G\(\alpha_s\), endogenous SAP97 is essential for CRF-mediated extracellular signal regulated kinase (ERK1/2) phosphorylation via the ERK1/2 signaling pathway (Dunn et al., 2013). In contrast, similar to what is observed for PSD-95-mediated enhancement of 5-HT\(_2A\)R-stimulated inositol phosphate formation, the loss of endogenous SAP97 expression results in a reduction in 5-HT\(_2A\)R-activated inositol accumulation via G\(\alpha_q\) (Xia et al., 2003, Dunn et al., 2014). However, SAP97 also suppresses 5-HT\(_2A\)R endocytosis and facilitates 5-HT-mediated ERK1/2 phosphorylation. The role of endogenous SAP97 in facilitating CRFR1- and 5-HT\(_2A\)R-stimulated ERK1/2 phosphorylation does not require interactions with the PDZ binding motifs of these receptors and knockdown of endogenous SAP97 also reduces CRFR2-mediated ERK1/2 phosphorylation (Dunn et al., 2013; Dunn et al., 2014). Since CRFR2 does not encode a PDZ binding motif, it is possible that SAP97 may play a global role in regulating GPCR-mediated ERK1/2 activity independent of receptor interactions.

**PSD-93 (DLG2) and SAP102 (DLG3):** Post-synaptic density protein of 93 kDa (PSD-93) contains three PDZ domains, an SH3 domain, and a GK domain (Fig. 1). Not a great deal is known about the role of PSD-93 in regulating GPCRs, but PSD-95 and PSD-93 have previously been demonstrated to compensate for one another (Sun and Turrigiano, 2011). Therefore, it is likely that both PSD-93 and PSD-95 may play similar roles with respect to GPCR regulation. PSD-95 and PSD-93 have been identified to interact with the somatostatin receptor 1 (SSTR1) and SSTR4 (Christenn et al., 2007) and have both been shown to inhibit NMDAR endocytosis (Lavezzari et al., 2003). Future studies are needed to examine the role of PSD-93 in the regulation of GPCR trafficking to determine whether its function overlaps with both PSD-95 and SAP97. Synapse-associated protein of 102 kDa (SAP102) contains three PDZ domains, an SH3 domain, and a GK domain (Fig. 1). SAP102 has been demonstrated to regulate adenosine A\(_{2A}\) receptor (A\(_{2A}\)R) mobility and promote A\(_{2A}\)R-mediated ERK signaling (Thurner et al., 2014). SAP102 has
additionally been identified to regulate the trafficking of AMPA and NMDA receptors. Thus, it is of interest in the future to determine whether SAP102 plays a role similar to that of other MAGUK proteins in the regulation of GPCR activity.

**DLG5:** DLG5 differs from the common topology of the PSD-95 subfamily of MAGUKs with the inclusion of an amino terminal caspase activation and recruitment domain (CARD), similar to CARMAs, and a fourth PDZ domain (de Mendoza et al., 2010) (Fig. 1). CARMA3 has been implicated in facilitating GPCR-induced activation of NFκB via lysophosphatidic acid, endothelin-1 and angiotensin II (Scudiero et al., 2014). Although there doesn’t appear to be any examples of DLG5 in the direct regulation of GPCRs, DLG5 has been implicated in regulating synaptogenesis by enhancing the membrane localization of the transmembrane protein N-cadherin (Wang et al., 2014). DLG5 has also been demonstrated to scaffold atypical protein kinase C (PKC) isoforms and this provide a mechanism by which DLG5 contributes to the regulation of GPCR-mediated signaling (Nechiporuk et al., 2013).

**Other GPCR-interacting PDZ Domain-Containing MAGUK Proteins**

**Membrane Palmitoylated Proteins and CASK (PALS3, LIN-2):** Membrane palmitoylated proteins (MPP1/p55, MPP2, MPP3, MPP4, MPP5/PALS1, MPP6/PALS2, and MPP7) are unified by the inclusion of a PDZ domain, SH3 domain, and GK domain (Fig. 1). Additionally, all but MPP1 have two amino terminal L27 domains, with MPP5 also including an amino terminal coiled-coil (CC) domain. MPP1-2 and MPP5-7 also include a HOOK domain between their SH3 and GK domains. Although MPP proteins are a relatively abundant group of MAGUK proteins, very little is known about their regulation of GPCR function. MPP3 has been demonstrated to promote the membrane stability of 5-HT_{2c}R and prevent receptor desensitization (Gavarini et al., 2006). MPP1 has additionally been implicated in membrane organization, raft formation, and receptor tyrosine
kinase-mediated ERK signaling (Lach et al., 2012; Biernatowska et al., 2013). Thus, it is plausible that MPPs may generally promote the membrane organization of integral proteins including GPCRs.

Ca\textsuperscript{2+}/Calmodulin-activated serine/threonine kinase (CASK) is very similar in topology to the MPPs with protein domains that include a catalytically active Ca\textsuperscript{2+}/calmodulin-dependent kinase (CaMK) domain at the amino terminal followed by two L27 domains, a PDZ domain, a SH3 domain, and a GK domain (te Velthuis et al., 2007; Mukherjee et al., 2008) (Fig. 1). CASK forms a tripartite complex with PDZ domain-containing Mint1 and Veli proteins, but the role of Mint1 and Veli proteins in the regulation of GPCRs remains undetermined (Butz et al., 1998). Like MPP3, CASK has been shown to interact with 5-HT\textsubscript{2c}R (Gavarini et al., 2006; Bécamel et al., 2002; Bécamel et al., 2004). Although the functional consequence of this interaction on 5-HT\textsubscript{2c}R trafficking and signaling remains to be tested, CASK has been implicated in regulating the trafficking of the NMDAR and AMPAR, partly via its regulation of SAP97 conformation and receptor interactions (Jeyifous et al., 2009; Lin et al., 2013). Interestingly, CASK has been demonstrated to interact with PKA, PKC\textsubscript{\varepsilon} and regulator of G protein signaling 4 (RGS4), which may suggest a role for CASK in regulating GPCR-mediated signaling (Hong and Hsueh, 2006).

**MAGI PDZ Protein Family**

Membrane-associated guanylate kinase with inverted orientation (MAGI) proteins include three proteins with an amino terminal PDZ domain followed by a GK domain, two tryptophan-tryptophan (WW) domains, and five more PDZ domains (Fig. 1). MAGI proteins differ from other MAGUK proteins in the exclusion of an SH3 domain (Dobrosotskaya et al., 1997). MAGI-1 colocalizes with brain angiogenesis inhibitor 1 receptor (BAI-1R) at the cell membrane via an interaction with the receptor carboxyl-terminal tail, and MAGI-3 interacts with BAI-1R to promote
ERK phosphorylation (Shiratsuchi et al., 1999; Stephenson et al., 2013). MAGI-3 promotes ERK and RhoA signaling mediated by the lysophosphatidic acid receptor 2 (LPA2R), but antagonizes ERK1/2 activation in response to the activation of either β1AR or β2AR (Zhang et al., 2007; He et al., 2006; Yang et al., 2010). MAGI-2 interacts with the β1AR via its first PDZ domain and functions to promote β1AR endocytosis without affecting β1AR-mediated cAMP signaling (Xu et al., 2001). In contrast, MAGI-2 interactions with the vasoactive intestinal peptide receptor 1 (VPAC1) and functions to both inhibit VPAC1 endocytosis and suppress VPAC1-mediated cAMP signaling (Gee et al., 2009). MAGI-2 also promotes the cell surface expression of metabotropic glutamate receptor 1a (mGluR1a) via its association with the PDZ domain-containing protein tamalin (Sugi et al., 2007). Thus, similar to what has been reported for PSD-95 family PDZ proteins, the MAGI family of PDZ proteins contributes to the regulation of the endocytosis and cell signaling of a number of GPCRs, but the functional effects of these protein interactions has differential effects depending upon the GPCR studied.

NHERF Family of PDZ Proteins

**NHERF1 (EBP50):** Na+/H+ Exchanger Regulatory Factor 1 (NHERF1), or ezrin/radixin/moesin (ERM)-Binding Protein 50 (EBP50), is a relatively small PDZ domain-containing protein characterized by two PDZ domains and a carboxyl terminal ezrin-binding domain (Fig. 2). NHERF1 represents one of the earliest PDZ proteins to be shown to interact with a GPCR (Hall et al., 1998). NHERF1 regulates Na+/H+ exchange via its interaction with β2AR without altering cAMP signaling, and has since been demonstrated to regulate a number of GPCRs. NHERF1 regulates the recycling of the β2AR and its binding to the receptor is disrupted by G protein-coupled receptor kinase phosphorylation of the β2AR at serine residue 411 (Cao et al., 1999). However, NHERF1 is reported to inhibit recycling of the parathyroid 1 receptor (PTH1R) (Wang
et al., 2007). NHERF1 also inhibits PTH1R desensitization and endocytosis, a function that appears to involve NHERF1-dependent inhibition of β-arrestin2 recruitment to the PTH1R (Wang et al., 2007; Wang et al., 2009). NHERF1 expression also enhances PTH1R-mediated cAMP signaling and couples PTHR1 to the activation of $\text{G}_{\alpha_\text{q}}$ (Wang et al., 2007; Wheeler et al., 2008; Wang et al., 2010). NHERF1 expression enhances cell surface expression of the κ opioid receptor inhibiting down-regulation and promoting receptor recycling (Li et al., 2002). In contrast, NHERF1 increases thromboxane receptor β (TPβ) cell surface expression by blocking the internalization of the receptor (Rochdi and Parent, 2003). An additional mechanism by which NHERF1 may increase GPCR membrane targeting is via its competition with the cystic fibrosis transmembrane conductance regulator-associated ligand (CAL) to antagonize CAL-mediated retention of GPCRs in the Golgi (Bauch et al., 2014).

In contrast to the role of NHERF1 in antagonizing the endocytosis of the PTHR1 and TPβ, NHERF1 is reported to facilitate the endocytosis of a number of GPCRs. NHERF1 enhances CCR5 endocytosis and β-arrestin1 recruitment, thereby promoting the activation of ERK, Rho, and FAK signaling pathways, as well as potentially contribute to CCR5-mediated HIV-1 entry (Hammad et al., 2010; Kuang et al., 2012). NHERF1 overexpression also rescues the endocytosis of an internalization-defective platelet-activating factor receptor (PAFR) and antagonizes PAFR-mediated inositol phosphate formation (Dupré et al., 2012). Agonist activation of the P2Y$_{12}$ receptor results in the β-arrestin-dependent recruitment of NHERF1 to the receptor and promotes the formation of a P2Y$_{12}$ receptor/NHERF1 complex that does not require PDZ-binding motif interactions (Nisar et al., 2012). NHERF1 also regulated frizzled family receptor activity (Wheeler et al., 2011). Thus, NHERF1 appears to play an integral, but complex, role in regulating the endocytosis and recycling of a variety of different GPCRs.
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**NHERF2:** The topology of NHERF2 is quite similar to NHERF1 as it shares 44% sequence homology with NHERF1 and contains two PDZ domains and a carboxyl terminal ezrin-binding domain (Ardura and Friedman, 2011) (Fig. 2). Similar to NHERF1, NHERF2 contributes to the regulation of the PTH1R (Mahon et al., 2002; Wang et al., 2010). NHERF2 functions to antagonize PTHR1 coupling to G\(\alpha_s\)-coupling, while concomitantly promoting the coupling of PTH1R to both the activation of G\(\alpha_q\) and G\(\alpha_i\) (Mahon et al., 2002; Wang et al., 2010). NHERF2 also interacts directly with PLC\(\beta\) to enhance P2Y\(_1\) receptor-mediated Ca\(^{2+}\) signaling (Fam et al., 2005). Similarly, NHERF2 interacts with PLC\(\beta3\) and the LPA\(_2\)R allowing for the formation of a protein complex that directly links the receptor to PLC\(\beta3\)-mediated inositol phosphate signaling (Choi et al., 2010; Oh et al., 2004). NHERF2 and mGluR5 show overlapping expression in mouse brain at postsynaptic neuronal sites and astrocytic processes and NHERF2 prolongs the mGluR5-mediated Ca\(^{2+}\) response (Paquet et al., 2006).

**PDZK1 (NHERF3) and PDZK2 (NHERF4):** PDZK1, formerly known as NHERF3, differs from NHERF1 and NHERF2 in structural topology by having four PDZ domains and no carboxyl terminal Ezrin-binding domain (Fig. 2). Nevertheless, PDZK1 has been implicated in regulating a subset of GPCRs. PDZK1 promotes the formation of a complex between SSTRs and PLC\(\beta3\), similar to what is observed for the LPA\(_2\)R (Choi et al., 2010; Oh et al., 2004), thereby facilitating somatostatin-stimulated PLC activation, Ca\(^{2+}\) mobilization, and ERK1/2 phosphorylation (Kim et al., 2012). PDZK1 also functions to enhance human prostacyclin receptor (hIPR) cell surface localization and cAMP signaling and contributes to endothelial cell migration and angiogenesis (Turner et al., 2011). PDZK1 inhibits 5-HT\(_{2A}\)R endocytosis and siRNA knockdown of PDZK1 results in reduced 5-HT\(_{2A}\)R-mediated inositol phosphate accumulation, but is not involved in 5-HT\(_{2A}\)R-stimulated ERK1/2 phosphorylation (Walther et al., 2015). However, PDZK1 interactions with 5-HT\(_{2A}\)R do not appear to be required for its regulation of 5-HT\(_{2A}\)R activity. In contrast,
although PDZK1 does not regulate CRFR1-mediated cAMP accumulation, unlike what is observed for the 5-HT$_2A$R, PDZK1 facilitates CRFR1-mediated ERK1/2 phosphorylation. Similar to PDZK1, PDZK2 also has four PDZ domains and has been shown to regulate hIPR (Reid et al., 2012). Agonist activation of the hIPR increases PDZK2 association and results in PKA- and PKC-mediated phosphorylation of PDZK2 (Reid et al., 2012). Like PDZK1, PDZK2 also enhances hIPR cell surface expression and cAMP accumulation (Reid et al., 2012). Taken together, PDZK1 and PDZK2 appear to be important for regulating the trafficking of an increasing subset of GPCRs and may bias toward increased G$_{a_q}$ signaling, similar to what is observed for both NHERF1 and NHERF2.

**PDZ Proteins that Regulate Golgi Trafficking**

**GIPC (TIP-2, Synectin):** Regulator of G protein signaling G$_{a}$-binding protein (RGS-GAIP)-interacting protein carboxyl terminus (GIPC) is a PDZ domain-containing protein with one PDZ domain that is implicated in the sorting of nascent proteins from the Golgi network (Liu et al., 2001) (Fig. 2). In regards to GPCRs, GIPC has been shown to target the D$_2$R to endosomes and the Golgi apparatus (Jeanneteau et al., 2004). Furthermore, GIPC expression suppresses D$_3$R G$_{a_i}$-coupling and prevents the D$_3$R degradation (Jeanneteau et al., 2004). GIPC also plays a role in regulating both human luteinizing hormone receptor (hLHR) and LPA$_1$R trafficking (Hirakawa et al., 2003; Varsano et al., 2012). The interaction of GIPC with the LPA$_1$R is essential for LPA$_1$R trafficking from APPL-positive signaling endosomes to EEA1-positive early endosomes (Varsano et al., 2012). Additionally, GIPC links the LPA$_1$R to the Akt signaling pathway, cell proliferation, and cell motility (Varsano et al., 2012). GIPC also contributes to the suppression of $\beta_1$AR-mediated ERK activation, but does affect $\beta_1$AR-stimulated cAMP accumulation (Hu et al., 2003).
CAL (GOPC, PIST): CAL is also named Golgi-associated coiled-coil and PDZ domain-containing protein (GOPC), due to its common subcellular localization within the trans-Golgi network and structural topology consisting of two coiled-coil domains and one PDZ domain (Fig. 2). CAL is selectively localized to the trans-Golgi network in neurons, as well as other cell types, and interacts with Rab6a, a small GTPase implicated in Golgi-related trafficking pathways (Chen et al., 2012; Bergbrede et al., 2009; Valente et al., 2010). CAL reduces plasma membrane expression and recycling of the β1AR, and interferes with both β1AR-mediated ERK signaling and postendocytotic receptor degradation via the lysosome (He et al., 2004; Koliwer et al., 2015). CAL overexpression retains the SSTR5 in the Golgi apparatus, thereby reducing SSTR5 cell surface expression (Wente et al., 2005; Bauch et al., 2014). Additionally, CAL colocalizes with mGluR1a following agonist activation and its over-expression decreases mGluR1a-stimulated ERK signaling (Zhang et al., 2008). CAL is suggested to regulate mGluR5a function by increasing the expression of the receptor by a mechanism that involves the inhibition of mGluR5a ubiquitination (Cheng et al., 2010). Taken together, it appears CAL could have a regulatory role over the subcellular localization of a subset of GPCRs, perhaps by contributing to the post-translational modification of nascent and mature proteins that ultimately influence the sorting and trafficking fate.

Additional GPCR-interacting PDZ Proteins

Spinophilin (Neurabin-2) and Neurabin-1: Both spinophilin/neurabin-2 and neurabin-1 contain an amino terminal actin-binding domain, a PP1γ-binding domain, a single PDZ domain, and a coiled-coil domain, with neurabin-1 also containing a carboxyl terminal SAM domain (Kelker et al., 2007) (Fig. 2). Spinophilin has been shown to interact with both the D2R and α2AR (Smith et al., 1999; Richman et al., 2001; Brady et al., 2003; Wang and Limbird, 2002; Wang et al., 2004).
However, these interactions appear to be mediated by the third intracellular loop domains of these GPCRs, as opposed to interactions with PDZ binding motifs. Spinophilin functions to promote the membrane localization and inhibit the endocytosis and desensitization of $\alpha_2$ARs by competing for $\beta$-arrestin2 binding (Wang et al., 2004). The interaction between spinophilin and $\alpha_2$AR is prevented by PKA-mediated phosphorylation of spinophilin that results in increased agonist-stimulated $\alpha_2$AR endocytosis (Xu et al., 2008). $\beta_2$AR activation also stimulates PKA-mediated spinophilin phosphorylation to increase $\alpha_2$AR-endocytosis (Cottingham et al., 2013). Conversely, spinophilin appears to promote RGS2-mediated inhibition of $\alpha_2$AR-evoked Ca$^{2+}$ signaling and RGS2-mediated modulation of $\alpha_1$AR-NMDAR crosstalk (Wang et al., 2005; Liu et al., 2006). In spinophilin knockout mice, $\alpha_2$AR exhibits increased G protein-coupling and sensitized responses to $\alpha_2$AR agonists (Lu et al., 2010; Cottingham et al., 2012). Both spinophilin and neurabin-1 are implicated in the D1R-dependent regulation of AMPAR, as well as long-term depression and potentiation, respectively (Allen et al., 2006). Spinophilin promotes prostacyclin receptor signaling via $\mathrm{G}_\alpha_5$ and influences both m1AChR and m3AChR activity by enhancing RGS8-mediated inhibition of the $\mathrm{G}_\alpha_{i}$-coupled signaling (Ma et al., 2012; Fujii et al., 2008; Kurogi et al., 2009). Similarly, spinophilin recruits RGS4 to the m3AChR, and like RGS8, RGS4 antagonizes m3AChR inositol phosphate signaling (Ruiz de Azua et al., 2012). Spinophilin also promotes $\mu$-opioid receptor ($\mu$OR)-mediated signaling via $\mathrm{G}_\alpha_i$, but inhibits $\mu$OR-mediated ERK activation, while facilitating $\mu$OR endocytosis (Charlton et al., 2008; Fourla et al., 2012).

The interaction between spinophilin and opioid receptors appears to occur via the opioid receptor third intracellular loop and a conserved region of the carboxyl termini, proximal to the seventh transmembrane domain (Fourla et al., 2012). Interestingly, this region appears to correlate with a small helical region identified in many Class A Rhodopsin-like GPCRs as helix 8 (Huynh et al., 2009). This domain is suggested to run perpendicularly to the other 7 helical transmembrane domains and is initiated by an N-P-x-x-Y motif (Huynh et al., 2009). In examining
the amino acid sequences of Class A Rhodopsin-like GPCRs with this motif, a possible internal class I PDZ-binding motif, as characterized by a S/T-x-Φ motif, may be present near this region (Trejo, 2005). Furthermore, homologous regions are found within α2ARs and D2R, which also interact with spinophilin via the third intracellular loop domain. Notably, a recent study has identified helix 8 of D2R to associate with the PDZ domain of GIPC (Sensoy and Weinstein, 2015). Future studies could look to investigate whether secondary interactions with spinophilin may occur within the α2ARs and D2R carboxyl terminal/helix 8, and whether these interactions require spinophilin’s PDZ domain.

**Shank Proteins:** SH3 and multiple ankyrin repeat domains (Shank1-3) proteins are unified by the inclusion of multiple ankyrin repeat domains, a SH3 domain, a PDZ domain, and a sterile alpha motif (SAM) domain, however Shank2 lacks the ankyrin repeats (Fig. 2). Shank1B enhances mGluR1/5-mediated ERK1/2 and Ca2+-dependent signaling, and Shank3 is important for mGluR5-mediated ERK1/2 and CREB phosphorylation and subsequent mGluR5-mediated LTD (Sala et al., 2005; Verpelli et al., 2011). Furthermore, Shank3 prevents mGluR1-mediated inhibition of NMDAR via its association with Homer1A (Bertaso et al., 2010; Guo et al., 2004). Similarly, Shank1/3 modulates mAChR1- and D2R-mediated inhibition of L-type Ca2+-channels via Homer proteins (Olson et al., 2005). In regards to GPCR trafficking, Shank influences the clustering and subcellular localization of mGluR5 and calcium-independent alpha-latrotoxin/latrophilin 1 receptor (CL1) (Tu et al., 1999; Tobaben et al., 2000). Interestingly, a Shank/Homer1A complex can suppress NMDAR and AMPAR clustering and surface expression (Sala et al., 2003). Shank1 directly interacts with dynamin-2, which may provide insight into a mechanism of action in preventing GPCR-mediated crosstalk mechanisms and receptor surface expression (Okamoto et al., 2001). Future studies could look to investigate the role of Shank proteins in regulating GPCR trafficking and the crosstalk between GPCRs and ion channels.
**Par3 and Par6:** Partitioning defective (Par or PARD) proteins have been implicated in cellular polarization and Par3 and Par6 are PDZ domain-containing members of the Par family (Fig. 2) (Macara, 2004). Par3 is implicated as having a role in synaptogenesis as a consequence of its interaction with the BAI-1R (Duman et al., 2013). Additionally, Par3 has been shown to increase bradykinin receptor interactions with \( \text{PLC}_\beta \) (Choi et al., 2010). Interestingly, both Par3 and Par6 interact and catalyze the activation of \( \text{PLC}_\beta \) downstream of heterotrimeric G proteins and form a complex with atypical PKCs (Cai et al., 2005; Joberty et al., 2000). Taken together, these observations suggest that Par3 and Par6 may contribute the regulation of GPCR-mediated \( \text{G}_\alpha_q \) signaling, as well as feedback receptor desensitization by atypical PKCs.

**MUPP1:** Multiple PDZ protein 1 (MUPP1) is one of the largest PDZ domain-containing proteins and is comprised of an amino terminal L27 domain followed by thirteen PDZ domains (Fig. 2). The interaction of MUPP1 with melatonin 1 receptor (MT1R) facilitates MT1R \( \text{G}_\alpha_i \)-coupling resulting in the inhibition of adenylyl cyclase activity (Guillaume et al., 2008). MUPP1 has also been shown to promote \( \text{GABA}_\beta \) receptor-mediated \( \text{Ca}^{2+} \) signaling, although MUPP1 knockdown prolongs the decay of the odorant receptor OR2AG1-mediated \( \text{Ca}^{2+} \) response (Balasubramanian et al., 2007; Dooley et al., 2009). In regards to GPCR trafficking, MUPP1 increases the cell surface expression of the 5-HT2A-R (Jones et al., 2009). Additionally, MUPP1 promotes the targeting of SSTR3 to tight junctions, thereby influencing transepithelial permeability (Liew et al., 2009; Vockel et al., 2010). Given that MUPP1 influences NMDA-dependent AMPA trafficking and clustering, it is likely that MUPP1 also regulates the trafficking of GPCRs that encode PDZ-binding motifs thereby contributing to GPCR-dependent regulation of synaptic activity (Krapivinsky et al., 2004).
Tamalin (GRASP): Tamalin, or general receptor for phosphoinositides (GRP1)-associated scaffold protein (GRASP), encodes a PDZ domain, a leucine zipper, and a class I PDZ-binding motif on the distal carboxyl terminal (Kitano et al., 2002; Kitano et al., 2003) (Fig. 2). Tamalin promotes the plasma membrane localization of mGluR1a, as well as the neuritic targeting of mGluR5 in hippocampal neurons (Kitano et al., 2002). Tamalin also interacts with mGluR2, mGluR3 and the GABA<sub>B2</sub>R, but the functional consequence of these interactions remain to be determined (Kitano et al., 2002). In the absence of mGluRs, or potentially other GPCR binding-partners, tamalin displays an auto-inhibitory confirmation caused by the interaction between the tamalin PDZ domain and tamalin PDZ-binding motif (Sugi et al., 2007). Upon mGluR1a binding to the tamalin PDZ domain, the tamalin PDZ-binding motif is free to associate with MAGI-2 to further enhance the membrane localization of mGluR1a (Sugi et al., 2007). PDZ-GEF1/2 also contain PDZ-binding motifs and future studies could look to determine whether they similarly exhibit auto-regulation (Ogawa et al., 2007; Kuiperij et al., 2003; Kuiperij et al., 2006).

nNOS: Neuronal nitric oxide synthase (nNOS) contains an amino terminal PDZ domain, a flavodoxin-like domain, and a flavin adenine dinucleotide (FAD)-binding domain (Fig. 2). nNOS, in conjunction with RGS17, has been demonstrated to complex with multiple GPCRs, including: μOR, δOR, 5-HT<sub>1A</sub>R, 5-HT<sub>2A</sub>R, α<sub>2A</sub>AR, D<sub>1</sub>R, D<sub>2</sub>R, m2AChR, m4AChR, mGluR2, mGluR5, and cannabinoid receptor 1 (Sánchez-Blázquez et al., 2012). Activation of these receptors leads to the nNOS/NO-dependent recruitment of PKCγ and Raf-1 to many of these GPCRs. nNOS also facilitates crosstalk between μOR and NMDAR (Rodríguez-Muñoz et al., 2008; Sánchez-Blázquez et al., 2010; Garzón et al., 2011). Interestingly, nNOS interacts with both PSD-95 and PSD-93, and is targeted to the neuromuscular junction via its interaction with PDZ protein α-
syntrophin (Brenman et al., 1996; Adams et al., 2010). Although this nNOS interaction with PSD-95 is suggested to regulate NMDAR activity (Christopherson et al., 1999), it is yet to be determined whether these PDZ/PDZ protein interactions regulate GPCR function.

**Syntrophins:**  α-syntrophin, β₁-syntrophin and β₂-syntrophin all have an amino-terminal PH domain interrupted by a PDZ domain, followed by another PH domain and a syntrophin unique (SU) calmodulin-binding domain (Fig. 2) (Adams et al., 1995; Ahn et al., 1996; Chen et al., 2006). These syntrophins interact with α₁DAR and collectively facilitate the functional expression of the receptor at the membrane, promoting α₁DAR-mediated phosphatidylinositol hydrolysis, ERK1/2 phosphorylation and Ca²⁺ mobilization (Chen et al., 2006; Lyssand et al., 2008; Lyssand et al., 2010; Lyssand et al., 2011). Neither γ₁-syntrophin nor γ₂-syntrophin comparably bind α₁DAR despite containing one PDZ domain and a PH domain, and their potential role in GPCR regulation remains uncertain (Chen et al., 2006). α-syntrophin can additionally scaffold the PDZ protein nNOS and notably binds Gβγ subunits via its PDZ domain (Brenman et al., 1996; Adams et al., 2010; Zhou et al., 2005).

**PICK1:** The protein interacting with C kinase 1 (PICK1) protein encodes one PDZ domain and an arfaptin homology domain/BAR (Bin/Amphiphysin/Rvs) domain involved in cell membrane interactions (Katsushima et al., 2013) (Fig. 2). PICK1 promotes the intracellular clustering of the prolactin-releasing peptide receptor, influences plasma membrane expression of the growth hormone-releasing hormone receptor (GHRHR) and antagonizes GHRHR-mediated cAMP signaling (Lin et al., 2001; Katsushima et al., 2013). PICK1 regulates PKC phosphorylation of mGluR7a, regulates the pre-synaptic clustering of mGluR7 and mediates stable mGluR7 cell surface expression (Dev et al., 2000; Boudin et al., 2000; Suh et al., 2008). mGluR7a knock-in
mice lacking a PDZ binding motif exhibit deficits in hippocampal-dependent spatial memory and are highly susceptible to the convulsant drugs, and the disruption of the mGluR7a-PICK1 complex induces epilepsy-like seizures (Zhang et al., 2008; Bertaso et al., 2008). Taken together, it appears PICK1 may be important for regulating the trafficking of a subset of GPCRs and may prove important in regulating GPCR-mediated signaling pathways. Notably, PICK1 can both homodimerize and heterodimerize with another PDZ domain-containing protein, syntenin-1 (Staudinger et al., 1997; Koroll et al., 2001).

Syntenin-1: Syntenin-1 contains two PDZ domains (Fig. 2) and has been found to self-associate, as well as heterodimerize with PICK1 and form a complex with mGluR7 (Koroll et al., 2001; Enz and Croci, 2003; Hrbec et al., 2002). Although PICK1 regulates mGluR7 phosphorylation, clustering, and membrane expression, it is not yet clear what role syntenin-1 may play in this regulation (Dev et al., 2000; Boudin et al., 2000; Suh et al., 2008). Nonetheless, syntenin-1 has been demonstrated to enhance the membrane expression of GPR37 (Dunham et al., 2009). In regards to signaling, syntenin-1 interacts with frizzled-7 (Fzd7) and promotes c-Jun phosphorylation, CDC42 activation, and PKCα recruitment to the membrane (Luyten et al., 2008). Syntenin-1 can also heterodimerize with syntenin-2, although little is known about the role of syntenin-2 in GPCR regulation (Koroll et al., 2001).

SNX27: Sorting nexin-27 (SNX27) differs from other sorting nexins through the inclusion of an amino terminal PDZ domain, followed by a Phox homology (PX) domain and a Ras-associating domain (Fig. 2). SNX27 interacts with both 5-HT_{4A}R and β_{2}AR in early endosome antigen 1 (EEA1)-positive early endosomes (Joubert et al., 2004; Lauffer et al., 2010). Moreover, SNX27 is involved in regulating the recycling of the β_{2}AR, β_{1}AR, and SSTR5, thereby preventing receptor
degradation (Lauffer et al., 2010; Temkin et al., 2011; Nakagawa and Asahi, 2013; Bauch et al., 2014). The regulation of β2AR recycling by SNX27 is dependent upon PX domain-mediated associations with the endosomal membrane (Lauffer et al., 2010). Furthermore, SNX27 interacts with the endosomal WASH complex to target the β2AR to the retromer tubule for efficient recycling (Temkin et al., 2011). Taken together, it appears SNX27 is capable of promoting the endosomal sorting and recycling of a subset of GPCRs, a role that may be generalizable to several other PDZ motif-encoding GPCRs.

**PDZ-GEFs (RAPGEFs, CNrasGEF, RA-GEF):** PDZ domain-containing guanine nucleotide exchange factors (PDZ-GEF1 and PDZ-GEF2) share approximately 56% sequence homology and include one or two cyclic nucleotide-binding domains, respectively, an N terminal Ras GEF domain, a PDZ domain, a Ras-associating domain, and Ras GEF catalytic domain within their molecular structure (Kuiperij et al., 2003; Kuiperij et al., 2006) (Fig. 2). Similar to the PDZ domain-containing protein tamalin, PDZ-GEF1/2 have also been reported to contain a class I PDZ-binding motif at their carboxyl termini, suggesting a capability for homo/hetero-oligomerization with PDZ domain-containing proteins, or perhaps even auto-regulatory capability via self-association (Ogawa et al., 2007; Kuiperij et al., 2003; Kuiperij et al., 2006). Our current understanding of PDZ-GEF2 regulation of GPCRs is poor, but PDZ-GEF1 couples the β1AR to the activation of Ras (Pak et al., 2002). Furthermore, PDZ-GEF1 is essential for coupling the pituitary adenylate cyclase-activating polypeptide type I receptor to the ERK1/2 signaling pathway and the subsequent activation of neuritogenesis, with no effect on cAMP accumulation (Emery et al., 2013).
RGS Proteins with PDZ domains (PDZ-RhoGEF, LARG, RGS3, and RGS12): PDZ-RhoGEF and leukemia-associated RhoGEF (LARG) are members of the regulators of G protein signaling (RGS) homology domain-containing RhoGEF (RH-RhoGEF) subfamily and include an amino terminal PDZ domain, a RGS-homology domain, a RhoGEF domain, and a pleckstrin-homology (PH) domain (Fig. 2). LARG transduces Gαq/12/13 activation into Rho activation via GPCRs such as the Mas receptor, G2 accumulation receptor, mACH1R, AT1R, sphingosine-1 phosphate receptor 2, histamine H1 receptor, thromboxane A2 receptor, and endothelin 1 receptor (Booden et al., 2002; Ying et al., 2006; Chiu et al., 2012; Del Galdo et al., 2013; Medlin et al., 2010; Pfreimer et al., 2012; Artamonov et al., 2013). Similarly, PDZ-RhoGEF is proposed contribute to gastrin-releasing peptide receptor-mediated activation of Rho/ROCK pathway via Gα13 (Patel et al., 2014). Finally, both PDZ-RhoGEF and LARG have been implicated in sustaining Rho activation following thrombin and LPA receptor activation (Chikumi et al., 2002; Wang et al., 2004; Yamada et al., 2005). Interestingly, both proteins appear capable of homo- and hetero-dimerization (Chikumi et al., 2004).

RGS12 contains one PDZ domain, a phosphotyrosine-binding domain (PTB), a RGS domain, two Ras-binding domains, and a GoLoco motif (Fig. 2). The RGS12 PDZ domain binds to the interleukin-8 receptor B PDZ binding motif, but the functional consequence of this interaction is not well defined (Snow et al., 1998). Notably, RGS12 has been suggested to couple D2R to inward rectifier potassium channels Kir3.1/3.2 (Oxford and Webb, 2004). Regulator of G protein signaling 3 (RGS3) contains a membrane-targeting C2 domain, one PDZ domain, and an RGS domain (Fig. 2). RGS3 has been identified to inhibit Gαq- and Gαi-mediated signaling by acting as a GTPase-activating protein (Scheschonka et al., 2000). RGS3 antagonizes Gαq/11 signaling via pheromone P factor receptor and mAChR3 activation and RGS3 promotes Ca2+ oscillatory behaviour during submaximal mAChR3 activation (Ladds et al., 2007; Anger et al., 2004; Wang et al., 2002; Anger et al., 2007; Karakoula et al., 2008; Tovey and Willars, 2004).
RGS3 also antagonizes follicle-stimulating hormone receptor- and luteinizing hormone receptor-mediated inositol phosphate and cAMP accumulation (Castro-Fernandez et al., 2004). Furthermore, RGS3 has been demonstrated to suppress Ga-mediated signaling pathways via μOR, mAChR1, complement C5a receptor, and β2AR, and even promote a Gαs bias for β2AR (Potenza et al., 1999; Anger et al., 2007; Nishiura et al., 2009; Chakir et al., 2011). In contrast, RGS3 was shown to inhibit gonadotropin-releasing hormone receptor-stimulated inositol phosphate signaling via Gq, but had no effect on cAMP signaling (Neill et al., 1997; Neill et al., 2001; Castro-Fernandez et al., 2002; Castro-Fernandez and Conn, 2002; Karakoula et al., 2008). Interestingly, RGS3 palmitoylation is increased following GRHR activation (Castro-Fernandez et al., 2002). Curiously, truncated RGS3 isoforms that have been reported to lack the amino terminal and PDZ domain have also demonstrated a role in influencing GPCR activity, including S1PR1-3, AT1R, ET1R, GRHR, 5-HT1AR, and mAChR2/3 (Druey et al., 1996; Cho et al., 2003; Castro-Fernandez et al., 2003; Jaén and Doupnik, 2005; Anger et al., 2004; Anger et al., 2007). Distinguishing the role of LARG, PDZ-RhoGEF, RGS3, and RGS12 PDZ domain interactions, as opposed to RGS domain interactions with heterotrimeric G proteins, in the regulation of GPCR signaling remains a challenge.

Role PDZ Proteins in GPCR-regulated Physiology

PSD-95 Family of MAGUK PDZ Proteins: The PDZ domain-containing MAGUK proteins play an essential role in human neurophysiology and development. This is demonstrated in mouse knockout studies, where PSD-95 and PSD-93 double-knockout mice exhibit severe deficiencies in AMPA currents, and SAP97 knockout mice show neonatal lethality (Caruana and Bernstein, 2001; Howard et al., 2010). Of particular interest is the observation that PSD-95 is essential for hallucinogenic and atypical antipsychotic actions of 5-HT2AR and 5-HT2CR (Abbas et al., 2009). In addition to being involved atypical antipsychotic actions (Abbas et al., 2009), PDZ protein
interactions with GPCRs also appear important regulating stress and anxiety responses (Magalhaes et al., 2010). Pre-activation of the CRFR1 receptor sensitizes 5-HT2A-R-stimulated IP formation dependent upon intact PDZ binding motifs in both receptors, receptor endocytosis and recycling (Magalhaes et al., 2010). Furthermore, the phenomenon can be blocked by a Tat-tagged fusion protein corresponding to the last 15 amino acids of the CRFR1 tail. In addition, pre-treatment of mice with sub-threshold doses of CRF into the prefrontal cortex sensitizes mouse anxiety responses to DOI treatment (Magalhaes et al., 2010). Thus, it is possible that PDZ protein interactions may serve as a good pharmaceutical target for the treatment of disease.

SAP102 is important during early synaptic development and SAP97 appears to be important in SSTR1-mediated growth cone dynamics, as evidenced by colocalization within the growth cone (Kim and Sheng, 2004; Elias et al., 2006; Cai et al., 2008). However, this role may not be limited to SAP97 and may include additional PDZ domain-containing proteins (Cai et al., 2008). PSD-95 plays a functional role in synaptic plasticity and contributes to GPCR-mediated regulation of both long-term potentiation, and long-term depression (Xu, 2011). Notably, SAP97 also modulates the ability to regulate AMPA and NMDA receptors by promoting synaptic trafficking of these receptors (Howard et al., 2010). Acute overexpression of SAP97 in hippocampal slice cultures restored synaptic transmission in PSD-95/PSD-93 double knockout mice, and long-term overexpression of SAP97 throughout development led to enhancements in synaptic transmission in vivo (Howard et al., 2010). This regulation of NMDAR- and AMPAR-mediated synaptic transmission is likely to also involve a role of GPCRs. PSD-95 is reported to have an important role in regulating the trafficking dynamics of D1R in striatal neurons, and this regulatory role may contribute to L-DOPA-induced dyskinesia (Porras et al., 2012). Thus, the role of PSD-95 in regulating D1R dynamics may be complicated by its ability to disrupt the formation of D1R/NMDAR complexes, a function which potentially may be directly associated with its role in the regulation of synaptic activity (Zhang et al., 2009).
β₁AR allows it to form a complex with the NMDAR and this may contribute to the regulation of synaptic activity by adrenergic ligands (Hu et al., 2000).

**Other PDZ Proteins:** There are a number other examples of PDZ proteins regulating GPCR-mediated regulation of physiological functions. In the immune system it has been found that the interaction of NHERF1 with complement component C3a receptor is required for C3a-mediated mast cell degranulation, NFκB-activation and chemokine production (Subramanian et al., 2012). CCR5 functions as a co-receptor for HIV-1 viral entry into mammalian cells by functioning as a cofactor for the entry of the virus (Henrich and Kuritzkes, 2013). NHERF1 interactions with CCR5 function to enhance actin filament rearrangement of host cells: a function that is essential to allow post-cell entry HIV-1 replication (Hammad et al., 2010; Kuang et al., 2012). PDZK1 interactions with hIPR selectively facilitate hIPR-dependent activation of endothelial migration and vascular angiogenesis *in vitro* (Turner et al., 2011). MUPP1, the largest of the PDZ domain-containing adaptor protein promotes the targeting of SSTR3 to tight junctions and consequently influences trans-epithelial permeability of skin cells (Liew et al., 2009; Vockel et al., 2010). Tamalin plays an important role in regulating mGluR signaling and tamalin knockout mice exhibit differences in their acute and adaptive responses to morphine administration. (Ogawa et al., 2007). Similarly, nNOS mediates a mechanism of crosstalk between μOR and NMDA receptors to regulate opioid tolerance and analgesia (Rodríguez-Muñoz et al., 2008; Sánchez-Blázquez et al., 2010; Garzón et al., 2011). PICK1 interactions with mGluR7a have been shown to be important for pre-synaptic mGluR7a clustering, and mGluR7a knock-in mice lacking a PDZ binding motif exhibit deficits in hippocampal-dependent spatial memory and the disruption of the mGluR7a-PICK1 complex induces epileptic-like seizures (Boudin et al., 2000; Zhang et al., 2008; Bertaso et al., 2008). α-syntrophin and β₂-syntrophin knockout mice display normal systolic blood pressure and resting heart rate, however a double knockout prevents α₁DAR-mediated blood pressure responses and
exhibits a distinct hypotonic phenotype at rest, thereby demonstrating the capability for PDZ protein compensation \textit{in vivo} (Lyssand et al., 2008).

\textbf{Concluding Remarks}

GPCRs are influential in the regulation of every aspect of human physiology. Therefore, any advancement in the understanding of how they can be regulated could contribute to the design and development of new pharmacological treatment and prevention strategies for a multitude of human diseases (Bockaert et al., 2010; Heng et al., 2013). Accordingly, it is becoming clear that PDZ proteins play an important role in the regulation of GPCR signaling and trafficking. Considering it is estimated that 20\% of GPCRs have PDZ-binding motifs, and over 800 GPCRs have been identified in the human genome, it is safe to assume that this field is still in its infancy (Lee and Zheng, 2010; Fredriksson et al., 2003). Nevertheless, our growing understanding of the functional specificities and redundancies in PDZ regulation of GPCRs may lead to the development of new pharmacological compounds for precise modulation of GPCR activity. Such a strategy could be pertinent in the pharmacological treatment of a multitude of human pathologies including but not limited to mental illnesses, cystic fibrosis, and osteoporosis (Abbas et al., 2009; Magalhaes et al., 2010; Holcomb et al., 2014; Mahon, 2012).
Acknowledgements

S. S. G. F. holds a Tier I Canada Research Chair in Molecular Neurobiology and is a Career Investigator of the Heart and Stroke Foundation of Ontario, Canada. H. A. D. is the recipient of a Jonathan and Joshua Memorial Scholarship in Mental Health Research.
MOL Manuscript # 98509

Authorship Contribution:

Henry A. Dunn and Stephen S. G. Ferguson wrote the manuscript.
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Footnotes:
This work is supported by a Canadian Institutes of Health Research grant [MOP-62738] to S. S. G. F.
**Figure 1:** Molecular topology of protein-protein interaction domains found in MAGUK family PDZ proteins. CaMKII, Ca\(^{2+}\)/Calmodulin-dependent kinase domain; CARD, Caspase activation and recruitment domain; CC, coiled-coiled domain; GK, Guanylate kinase-like domain; L27, L27 domain; PDZ, PDZ domain; SH3, Src homology 3 domain WW, tryptophan-tryptophan domain.

**Figure 2:** Molecular topology of other PDZ domain-containing proteins that interact with GPCRs. ABD, actin binding domain; ANK, ankyrin repeat domain; AH, Arfaptin homology domain; C2, C2 domain; CC, coiled-coiled domain; cNBD, Cyclic nucleotide binding domain; EBD, Ezin-binding domain; FAD, FAD-binding domain; FDX, flavodoxin-like domain; G, Golocco motif; GEF-CD, Ras GEF catalytic domain; GEF-N, Ras-like GEF, N-terminal domain; L27, L27 domain; PDZ, PDZ domain; PB1, Phox/Bem1 domain; PH, pleckstrin homology domain; PHA/B, interrupted pleckstrin homology domain; PP1, PP1-binding domain; PTB, phosphotyrosine-binding domain; PX, Phox-homolgy domain; RA, Ras association domain; RB, Ras-binding domain; RGS, RGS domain; RGSL, RGS-like domain; RhoGEF, RhoGEF domain; SAM, Sterile alpha motif; SU, syntrophin unique domain; SH3, Src homology 3 domain.
Table 1: Effect of PDZ proteins on GPCR trafficking

<table>
<thead>
<tr>
<th>PDZ Protein</th>
<th>Trafficking Function</th>
<th>GPCR</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSD-95</td>
<td>↓ endocytosis</td>
<td>β1AR, 5-HT2AR</td>
<td>Hu et al., 2000; Xia et al, 2003</td>
</tr>
<tr>
<td></td>
<td>↑ recycling</td>
<td>D1R</td>
<td>Sun et al., 2009</td>
</tr>
<tr>
<td></td>
<td>↑ membrane localization</td>
<td>GPR30</td>
<td>Akama et al., 2013; Broselid et al., 2014</td>
</tr>
<tr>
<td></td>
<td>↑ endocytosis</td>
<td>5-HT3CR, D1R</td>
<td>Gavarini et al., 2006; Zhang et al., 2007</td>
</tr>
<tr>
<td>SAP97</td>
<td>↓ endocytosis</td>
<td>CRFR1, 5-HT2AR</td>
<td>Dunn et al., 2013; Dunn et al., 2014</td>
</tr>
<tr>
<td></td>
<td>↑ recycling</td>
<td>β1AR</td>
<td>Gardner et al., 2007</td>
</tr>
<tr>
<td>SAP102</td>
<td>↓ mobility</td>
<td>A2AR</td>
<td>Thurner et al., 2014</td>
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<tr>
<td>MPP3</td>
<td>↓ membrane localization</td>
<td>5-HT2CR</td>
<td>Gavarini et al., 2006</td>
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<tr>
<td>MAGI-2</td>
<td>↓ endocytosis</td>
<td>VPAC1</td>
<td>Gee et al., 2009</td>
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<tr>
<td></td>
<td>↑ endocytosis</td>
<td>β1AR</td>
<td>Xu et al., 2001</td>
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<tr>
<td></td>
<td>↑ membrane localization</td>
<td>mGluR1a</td>
<td>Sugi et al., 2007</td>
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<td>NHERF1</td>
<td>↓ endocytosis</td>
<td>β2AR, TPβ1</td>
<td>Wang et al., 2007; Hochli and Parent, 2003</td>
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<tr>
<td></td>
<td>↑ recycling</td>
<td>β2AR, hκ-OR</td>
<td>Cao et al., 1999; Li et al., 2002</td>
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<td></td>
<td>↑ membrane localization</td>
<td>SSTR5, PTH1R</td>
<td>Bauch et al., 2014; Wheeler et al., 2008</td>
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<tr>
<td></td>
<td>↑ microvilli localization</td>
<td>5-HT4AR</td>
<td>Joubert et al., 2004</td>
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<td>↑ cytoskeletal localization</td>
<td>Fzd4</td>
<td>Wheeler et al., 2011</td>
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<td></td>
<td>↑ endocytosis</td>
<td>CCR5, PAFR, P2Y12R</td>
<td>Hammond et al., 2010; Dupré et al., 2012; Nisar et al., 2012</td>
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<td>PDZK1</td>
<td>↓ endocytosis</td>
<td>5-HT2AR</td>
<td>Walther et al., 2015</td>
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<td>PDZK2</td>
<td>↑ membrane localization</td>
<td>hIPR</td>
<td>Turner et al., 2011</td>
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<td>Shank1</td>
<td>↑ clustering</td>
<td>mGluR5, CL1</td>
<td>Yu et al., 1999; Tobaben et al., 2000</td>
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<td>Spinophilin</td>
<td>↓ endocytosis</td>
<td>α2AR</td>
<td>Brady et al., 2003</td>
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<td>↑ endocytosis</td>
<td>μOR</td>
<td>Charton et al., 2008</td>
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<td>MUPP1</td>
<td>↑ membrane localization</td>
<td>5-HT2AR</td>
<td>Jones et al., 2009</td>
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<td>↑ tight junction localization</td>
<td>SSTR3</td>
<td>Liew et al., 2009</td>
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<td>Tamalin</td>
<td>↑ membrane localization</td>
<td>mGluR1</td>
<td>Kitano et al., 2002; Sugii et al., 2007</td>
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<td></td>
<td>↑ neurite localization</td>
<td>mGluR5</td>
<td>Kitano et al., 2002</td>
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<td>Syntrophins</td>
<td>↑ membrane localization</td>
<td>α1DAR</td>
<td>Chen et al., 2006; Lyssand et al., 2008; Lyssand et al., 2011</td>
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<td>PICK1</td>
<td>↑ intracellular clustering</td>
<td>GPR10</td>
<td>Lin et al., 2001; Madsen et al., 2012</td>
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<td>↑ recycling</td>
<td>GHKHR</td>
<td>Katsushima et al., 2013</td>
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<td>↑ membrane localization</td>
<td>GPR37</td>
<td>Dunham et al., 2009</td>
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<td>SNX27</td>
<td>↑ recycling</td>
<td>β2AR, β1AR, SSTR5</td>
<td>Lauffler et al., 2010; Temkin et al., 2011; Nakagawa and Asahi, 2013; Bauch et al., 2014</td>
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<td>GIPC</td>
<td>↑ endosome/golgi localization</td>
<td>D2R, D3R</td>
<td>Jeanneau et al., 2004</td>
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<td>↑ trafficking to early endosome</td>
<td>LPA1R</td>
<td>Varsano et al., 2012</td>
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<td>↑ membrane stability</td>
<td>hLHR</td>
<td>Hirakawa et al., 2003</td>
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<tr>
<td>CAL</td>
<td>↓ membrane localization</td>
<td>β1AR, SSTR5</td>
<td>He et al., 2004; Koliwer et al., 2015; Bauch et al., 2014</td>
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<tr>
<td></td>
<td>↓ recycling</td>
<td>β1AR</td>
<td>Koliwer et al., 2015</td>
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<td></td>
<td>↓ golgi localization</td>
<td>SSTR5</td>
<td>Wente et al., 2005; Bauch et al., 2014</td>
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Table 2: Effect of PDZ proteins on GPCR Signaling

<table>
<thead>
<tr>
<th>PDZ Protein</th>
<th>Signaling Function</th>
<th>GPCR</th>
<th>Reference</th>
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<tbody>
<tr>
<td>PSD-95</td>
<td>↑ IP₃, c-fos, desensitization of Ca²⁺, or = cAMP</td>
<td>5-HT₂AR, 5-HT₄AR, 5-HT₃AR, D₁R</td>
<td>Asano et al., 2009; Abeida et al., 2009; Guerra et al., 2006; Zhang et al., 2007; Sun et al., 2009</td>
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<td>SAP97</td>
<td>↑ IP₃, ERK</td>
<td>5-HT₂AR, CRFR1, CRFR2, 5-HT₃AR</td>
<td>Dong et al., 2014; Dong et al., 2013; Dunn et al., 2014</td>
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<td>SAP102</td>
<td>ERK</td>
<td>A₂OR</td>
<td>Thanos et al., 2014</td>
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<td>MPP3</td>
<td>desensitization of Ca²⁺</td>
<td>5-HT₃OR</td>
<td>Gavirini et al., 2006</td>
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<td>MAGI-2</td>
<td>cAMP</td>
<td>VPAC1</td>
<td>Lee et al., 2007</td>
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<td>NHERF1</td>
<td>desensitization of cAMP, Cα₁ coupling and activation, ERK, FAK, Rho, β-catenin</td>
<td>PTH₁R, PTH₁R, PTH₁R, CCR5, CCR5, Fzd²/⁴</td>
<td>Wang et al., 2007; Wheeler et al., 2006; Wang et al., 2007; Wang et al., 2010; Hammad et al., 2010; Kuo et al., 2012; Kuo et al., 2012; Wheeler et al., 2011</td>
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<td>NHERF2</td>
<td>Gαᵢ₂, coupling and PLC activation, Gα₉, coupling and ↓ AC activation, PLC interaction, IP₃ and ERK, Ca²⁺</td>
<td>PTH₁R, PTH₁R, P₂Y₁₀, LPA₁R, LPA₁R, P₂Y₁₀, mGlur5</td>
<td>Wang et al., 2010; Mahon et al., 2006; Wang et al., 2010; Mahon et al., 2006; Fan et al., 2005; Choi et al., 2010; Oh et al., 2004; Fan et al., 2005; Pappat et al., 2006</td>
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<td>PDZK1</td>
<td>PLC interaction and activation, IP₃, Ca²⁺, Cα₁ coupling, ERK</td>
<td>SSTR₅, SSTR₅, 5-HT₂AR, SSTR₅, 5-HT₂AR, CRFR₁</td>
<td>Kim et al., 2012; Kim et al., 2012; Walther et al., 2015; Kim et al., 2012; Turner et al., 2011; Walther et al., 2015; Turner et al., 2011</td>
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<td>PDZK2</td>
<td>CAMP</td>
<td>hIPR</td>
<td>Reid et al., 2012</td>
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<td>Shank1</td>
<td>Ca²⁺ and ERK</td>
<td>mGlur1/5</td>
<td>Tsuboi et al., 2005</td>
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<td>Shank3</td>
<td>ERK and CREB phosphorylation</td>
<td>mGlur5</td>
<td>Vorpahl et al., 2011</td>
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<td>Par3</td>
<td>PLC interaction</td>
<td>B,R</td>
<td>Cho et al., 2010</td>
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<tr>
<td>Spinophilin</td>
<td>Ca²⁺, Gαᵢ coupling, CAMP, Gα₁ coupling, or ↑ ERK, Gα₁ coupling, ERK</td>
<td>α₁AR, M,R,M,R, α₁AR, A,R, IPR, μOR, δOR, δOR</td>
<td>Wang et al., 2005; Kit et al., 2008; Yonoge et al., 2004; Yoshida et al., 2013; Lu et al., 2010; Chen et al., 2012; Me et al., 2012; Chotrun et al., 2009; Fourla et al., 2012; Chotrun et al., 2009; Fourla et al., 2012; Fourla et al., 2012; Fourla et al., 2012</td>
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<td>MUPP1</td>
<td>Gα₁ coupling, Ca²⁺, Ca²⁺ decay, or ↑ ERK, Gα₁ coupling, ERK</td>
<td>MT,R, GABA₅, OR2AG1</td>
<td>Malheiro et al., 2006; Belostrablon et al., 2007; Doxey et al., 2009</td>
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<td>nNOS</td>
<td>PKC interaction</td>
<td>μOR, 5-HT₂AR, 5-HT₂AR, D₂R, M₃R, CB₁R</td>
<td>Lysandrou et al., 2008; Lysandrou et al., 2011</td>
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<td>Syntrophins</td>
<td>IP₃, Ca²⁺ and ERK</td>
<td>α₁AR</td>
<td>Balaguer et al., 2004</td>
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<td>PICK1</td>
<td>CAMP</td>
<td>GHRHR</td>
<td>Balaguer et al., 2004</td>
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<td>Syntenin-1</td>
<td>c-Jun, CDC42, and PKCα</td>
<td>Fzd7</td>
<td>Lyon et al., 2006</td>
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<td>GIPC</td>
<td>Gα₁ coupling, Akt, ERK</td>
<td>D₁R, LPA₁R, β₁AR</td>
<td>Jouondieu et al., 2004; Versano et al., 2012; Hs et al., 2003</td>
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<td>CAL</td>
<td>ERK</td>
<td>mGlur1, β₁AR</td>
<td>Wang et al., 2010; Patel et al., 2010</td>
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<td>PDZ-GEF1</td>
<td>Ras, ERK</td>
<td>β₁AR, PAC₁</td>
<td>Patel et al., 2004; Enany et al., 2013</td>
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<td>LARG</td>
<td>Rho</td>
<td>AT₁R, S₁PR₂, ET₁R, M₃R, GPR₁3, H₁R, TP, MAS₁</td>
<td>Boulanger et al., 2004; Yang et al., 2008; Kim et al., 2010; De Galis et al., 2010; Mathieu et al., 2010; Pham et al., 2012; Albenouwers et al., 2012</td>
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<td>PDZ-RhöGEF</td>
<td>Rho</td>
<td>GRPR</td>
<td>Patel et al., 2004</td>
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<td>RGS3</td>
<td>Gα₉ and Gα₁₁ activation, IP₃</td>
<td>M₃R, GRHR, LHR, FSHR, PAR1</td>
<td>Landis et al., 2007</td>
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<td></td>
<td>DAG</td>
<td>M₃R, ET₁R, M₃R, C₅aR, ET₁R</td>
<td>Anger et al., 2004; Touhy and Williams, 2004; Kono et al., 2005; Castro-Fernandez et al., 2004; Neil et al., 1997; Neil et al., 2001; Castro-Fernandez and Castro, 2009; Castro-Fernandez et al., 2002; Chen et al., 2014; Kersten et al., 2007; Touhy and Williams, 2004; Duin et al., 1989</td>
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<td></td>
<td>Ca²⁺, ERK, Akt, CAMP</td>
<td>M₃R, M₃R, LHR, FSHR, μOR, D₂R, β₁AR</td>
<td>Anger et al., 2007; Wang et al., 2002; Nozaka et al., 2006; Duin et al., 1989; Anger et al., 2007; Castro-Fernandez et al., 2004</td>
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<td></td>
<td>Ca²⁺, mediated signaling, Gα₉-mediated signaling</td>
<td>M₃R, M₃R, LHR, FSHR, μOR, D₂R, β₁AR</td>
<td>Patel et al., 1988; Czar et al., 2011</td>
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<td>Ca²⁺, mediated signaling</td>
<td>M₃R, M₃R, LHR, FSHR, μOR, D₂R, β₁AR</td>
<td>Czar et al., 2011</td>
</tr>
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</table>
Figure 1

- PSD-95:
  - PDZ
  - PDZ
  - PDZ
  - SH3
  - GK

- SAP97:
  - L27
  - PDZ
  - PDZ
  - SH3
  - GK

- PSD-93:
  - PDZ
  - PDZ
  - PDZ
  - SH3
  - GK

- SAP102:
  - PDZ
  - PDZ
  - PDZ
  - SH3
  - GK

- DLG5:
  - CARD
  - PDZ
  - PDZ
  - PDZ
  - PDZ
  - SH3
  - GK

- CARMA3:
  - CARD
  - CC
  - PDZ
  - SH3
  - GK

- MPP3:
  - L27
  - L27
  - PDZ
  - SH3
  - GK

- CASK:
  - CaMK
  - L27
  - L27
  - PDZ
  - SH3
  - GK

- MAGI-1:
  - PDZ
  - GK
  - WW
  - WW
  - PDZ
  - PDZ
  - PDZ
  - PDZ

- MAGI-2:
  - PDZ
  - GK
  - WW
  - WW
  - PDZ
  - PDZ
  - PDZ
  - PDZ

- MAGI-3:
  - PDZ
  - GK
  - WW
  - WW
  - PDZ
  - PDZ
  - PDZ
  - PDZ