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Role of PDZ Proteins in Regulating Trafficking, Signaling, and Function of GPCRs: Means, Motif, and Opportunity

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Abstract

PDZ proteins, named for the common structural domain shared by the postsynaptic density protein (PSD95), Drosophila disc large tumor suppressor (DlgA), and zonula occludens-1 protein (ZO-1), constitute a family of 200–300 recognized members. These cytoplasmic adapter proteins are capable of assembling a variety of membrane-associated proteins and signaling molecules in short-lived functional units. Here, we review PDZ proteins that participate in the regulation of signaling, trafficking, and function of G protein-coupled receptors. Salient structural features of PDZ proteins that allow them to recognize targeted GPCRs are considered. Scaffolding proteins harboring PDZ domains may contain single or multiple PDZ modules and may also include other protein–protein interaction modules. PDZ proteins may impact receptor signaling by diverse mechanisms that include retaining the receptor at the cell membrane, thereby increasing the duration of ligand binding, as well as importantly influencing GPCR internalization, trafficking, recycling, and intracellular sorting. PDZ proteins are also capable of modifying the assembled complex of accessory proteins such as β-arrestins that themselves regulate GPCR signaling. Additionally, PDZ proteins may modulate GPCR signaling by altering the G protein to which the receptor binds, or affect other regulatory proteins that impact GTPase activity, protein kinase A, phospholipase C, or modify downstream signaling events. Small molecules targeting the PDZ protein-GPCR interaction are being developed and may become important and selective drug candidates.

I. Introduction

G protein-coupled receptors (GPCRs) form the largest family of signaling receptors that are expressed in vertebrate cells. They are responsible for transducing a strikingly vast array of extracellular signals to biological actions. GPCRs represent 2% of the human genome and are important drug targets. Effectively, these receptors are guanine nucleotide exchange factors, which when occupied by their cognate ligand, exchange guanosine diphosphate (GDP) for guanosine triphosphate (GTP) on the alpha subunit of the associated

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heterotrimeric nucleotide-binding protein. The activated alpha subunit then dissociates from the beta-gamma subunit. Signal transduction is mostly mediated by the alpha subunit but sometimes by the beta–gamma subunit pair. The two principal signaling pathways involved are by Gαs or inhibition by Gi of the adenylyl cyclase-cAMP-protein kinase A (PKA)/EPAC signaling pathway, and stimulation by Gαq of the phospholipase C (PLC)—Ca^{2+} + phosphatidylinositol pathway. GPCR desensitization provides a mechanism to protect cells against excessive stimulation, while GPCR resensitization guards cells against prolonged desensitization and hormone insensitivity. Desensitization and receptor internalization are the two primary mechanisms controlling GPCR signaling.

Although most receptors activate a single pathway, some receptors employ multiple signaling pathways. The type 1 parathyroid hormone receptor (PTH1R), for instance, in vascular smooth muscle cells, parathyroid hormone (PTH), stimulates adenylyl cyclase but not PLC (Maeda et al., 1996; Wu et al., 1993), whereas in keratinocytes (Orloff et al., 1995; Whitfield et al., 1992), cardiac myocytes (Rampe et al., 1991; Schlüter et al., 1995), and lymphocytes (Atkinson et al., 1987; Klinger et al., 1990; Whitfield et al., 1971), the PTH1R activates PLC but not adenylyl cyclase. In osteoblasts and kidney tubule cells, PTH activates both adenylyl cyclase and PLC (Abou-Samra et al., 1992; Friedman et al., 1996; Hruska et al., 1987). The origin of the cell-specific signaling remained obscure until the discovery that a PDZ adapter protein, present in some but not in all cells expressing the PTH1R switches signaling between adenylyl cyclase and PLC (Mahon et al., 2002). Increasing evidence now supports the view that cytoplasmic adapter proteins affect the signaling and trafficking of many GPCRs, and thereby their biological behavior. In this review, we describe emerging findings regarding the means by which modular PDZ proteins confer ligand- and cell-specific signaling and trafficking on select GPCRs, the corresponding recognition motifs engaged by the cognate proteins, and the physiological opportunities regulated by these interactions.

II. PDZ Proteins

PDZ proteins are soluble cytoplasmic adapter proteins that function as transient scaffolding structures to assemble multiprotein signaling complexes by virtue of highly conserved modules. The general arrangement for PDZ domains is based on the structure of PSD95, DLG, and ZO1, for which they are named. The human genome includes some 200–300 PDZ proteins. PDZ modules consist of an 80–90 amino acid sequence forming a three-dimensional globular structure that is composed of six β-sheets (βA–βF) and two α-helices (αA, αB) within the larger protein (Karthikeyan et al., 2001). Scaffolding proteins harboring PDZ domains may contain single or multiple PDZ modules, and may also include other protein–protein interaction modules (Fig. 1). The PDZ ligand of the target protein binds in an extended groove of the PDZ domain between the second β-sheet (βB) and the second α-helix (αB) in an antiparallel fashion with the terminal hydrophobic amino acid of the ligand occupying the elongated hydrophobic cavity at the top of the binding groove. Based on the terminal ligand sequence of the recognition motif, two classes of PDZ domains were initially identified (Songyang et al., 1997); three classes are now generally recognized (Table I), though additional classifications have been proposed (Tonikian et al., 2008). Although superficially similar, the three classes differ importantly in the composition of the binding

pocket and thereby in their ability to recognize distinct peptide sequences within the target ligand. Class I PDZ domains contain a conserved histidine (His^{212}) that coordinates the hydroxyl group of the —2 serine or threonine residue of the PDZ ligand (Doyle et al., 1996; Morais Cabral et al., 1996; Songyang et al., 1997). Class II ligands prefer a hydrophobic amino acid in position —2, which in turn favors a hydrophobic amino acid at the distal end of βB. The original classification of PDZ recognition motifs considered only the carboxy-terminal 3 or 4 residues (Table II). Subsequent investigation revealed the role of upstream positions 5–7 in defining the specificity of interaction with the respective PDZ protein (Zhang et al., 2006). Truncation analysis of NHERF1, for instance, points to residues as far as 18 amino acids upstream the carboxy-terminus in establishing the recognition site, which is stabilized by acid side chains (Mahon *&* Segre, 2004).

PDZ proteins may influence signaling by tethering the receptor at the cell membrane, thereby increasing ligand residence and/or modifying the assembled complex of accessory proteins, including β-arrestins. Additionally, PDZ proteins may regulate GPCR signaling by altering the G protein to which the receptor binds, RGS (GAIP), A kinase-anchoring protein (AKAP), or other regulatory proteins modulating GTPase activity, PKA, PLC, or modifying downstream signaling events. PDZ proteins may also importantly influence GPCR internalization, trafficking, recycling, and intracellular sorting.

III. GPCRs with Carboxy-Terminal PDZ Recognition Motifs

A. Family A GPCRs

Depending on how stringently one defines the consensus motif for PDZ-mediated protein interaction, a handful to a potentially large number of mammalian family A GPCRs have the ability to engage PDZ domain-containing proteins. Table III lists those family A GPCRs for which such interactions have been established most convincingly, and specifically linked to function.

1. β2-Adrenergic Receptor—Adrenergic receptors are activated by the catecholamines epinephrine (adrenalin) and norepinephrine (noradrenaline) and mediate many actions of the sympathetic nervous system, especially in the heart and cardiovascular system. Adrenergic receptors are classified as β-adrenergic, which are preferentially activated by isoproterenol (>epinephrine>norepinephrine), or α-adrenergic that exhibit selectivity for epinephrine (>norepinephrine>isoproterenol). β2-Adrenergic receptor (β2AR) and β1-adrenergic receptors (β1AR) contain PDZ-binding sequences, whereas β3-adrenergic receptors and α-adrenergic lack these motifs.

The first reported example was the β2AR, which contains a canonical type 1 PDZ motif present in its distal carboxy tail (DSLL in the human receptor). This motif binds with high affinity to PDZ domains present in NHERF/EBP50 family proteins, and binding of the β2AR specifically to NHERF1 was shown to facilitate β2AR-mediated regulation of the NHE3 sodium-proton exchanger (Hall et al., 1998a). This signaling function of PDZ-mediated protein interaction involves physical scaffolding of β2ARs in close proximity to PKA that, in turn, phosphorylates NHE3 in response to β2AR activation (Hall et al., 1998b).

The β2AR PDZ motif, in addition to its signaling function, was then found to mediate a discrete and essential trafficking function by directing receptors efficiently into the rapid recycling pathway after agonist-induced endocytosis (Cao et al., 1999). This established the first example of PDZ-directed sorting of an integral membrane protein into the recycling pathway, and also the first example of a PDZ motif whose interaction with trans-acting PDZ protein(s) is regulated by phosphorylation. Consistent with the wellestablished view that endocytic recycling of the β2AR promotes functional recovery of receptor-mediated signaling after agonist-induced desensitization (Lefkowitz et al., 1998), PDZ-dependent recycling enhanced the cellular cAMP response after prolonged B2AR stimulation (Hanyaloglu et al., 2005).

Precisely, what trans-acting PDZ protein(s) mediates β2AR recycling remained unclear for some time, and it was even proposed that the β2AR PDZ motif might drive recycling by binding to a distinct non-PDZ protein (Cong et al., 2001). Recently, the PDZ dependence of β2AR recycling has been definitively verified, and the major trans-acting localized PDZ required for this recycling process identified as protein sorting nexin 27 (SNX27). SNX27's recycling activity requires its binding to the early endosome membrane by a distinct phox-homology (PX) domain. NHERF2, but not NHERF1, further enhances the recycling efficiency of β2ARs by a mechanism that appears to involve indirect connectivity to a dynamic actin structure associated on the endosome membrane (Lauffer et al., 2010). PDZ-linked bridging of the β2AR to actin was shown to mediate a distinct trafficking function, that of prolonging the surface residence time of receptor-containing clathrin-coated pits prior to endocytic scission, by linking to the cortical actin network underlying the plasma membrane. This distinct trafficking function of the β2AR PDZ motif, in regulating endocytosis rather than recycling, is thought to contribute to trafficking specificity of GPCRs relative to other membrane proteins, whose endocytosis also requires coated pits (Puthenveedu & von Zastrow, 2006). Thus, for the β2AR, different PDZ proteins, and discrete networks of downstream protein interactions, underlie the various signaling and trafficking functions of the carboxy-terminal PDZ motif.

2. ß I-Adrenergic Receptor—The β1AR, although closely related to the β2AR, possesses a distinct type 1 PDZ motif (SVFT) that binds a largely nonoverlapping spectrum of PDZ proteins (He et al., 2006). Of these, SAP97 was shown to be required for efficient recycling of internalized receptors to the plasma membrane and, consequently, to promote functional recovery of cellular signaling following agonist-induced desensitization. Further, SAP97 was shown to bind AKAP79 and thereby link β1ARs in an organized "receptosome" complex (Gardner et al., 2007). Thus, for the β1AR, in contrast to the β2AR, the same PDZ protein interaction mediates the presently known discrete signaling and trafficking functions of its PDZ motif.

3. Serotonin (5HT) Receptors—Serotonin (5-hydroxytryptamine, 5HT) is a biologically active amine that is formed from tryptophan and serves as a neurotransmitter. Its actions are mediated by seven families of serotonin receptors (5HT1-7), several of which contain subtypes (Bohn *&* Schmid, 2010). All are GPCRs except 5HT3, which are ligand-gated ion channels.

A consensus type 1 PDZ motif present in the 5HT2CR serotonin receptor carboxy-tail regulates both receptor surface expression and signaling. This motif was found to contain two phosphorylatable residues, complicating functional interpretation of the effects of motif mutation (Backstrom et al., 2000). However, it was shown subsequently that this motif binds both PSD95 and MPP3, and these distinct PDZ proteins were found to produce opposing effects: PSD95 promotes endocytosis of 5HT2Rs and desensitization of 5HT2R signaling, whereas MPP3 binds competitively to the carboxy-terminal PDZ motif and has the opposite effect on both processes (Gavarini et al., 2006).

The nitric oxide synthases (NOS) comprise a family of closely related proteins whose main function is the production of nitric oxide (NO). The neuronal isoform nNOS (NOS-1) contains an extended type 1 PDZ domain near its amino-terminus and a canonical type 1 PDZ ligand at its carboxy-terminus (Tochio et al., 2000). The PDZ domain of nNOS is primarily involved in the regulation of nNOS localization. Remarkably, the nNOS PDZ domain is atypical in that, in addition to a typical PDZ-binding core, it contains a preformed β-finger structure that binds other PDZ domains, in particular those of PSD-95 and α1-syntrophin (Brenman et al., 1996; Hillier et al., 1999; Tochio et al., 2000). These interactions play a central role in the coupling of nNOS to *N*-methyl-D-aspartate (NMDA) receptors (Brenman et al., 1996).

The PDZ domain of nNOS also binds carboxy-terminal PDZ ligands according to the classical models. The 5HT2B receptor interacts directly with nNOS to regulate its activity (Manivet et al., 2000). Deletion of 77 amino acids of the carboxy-terminus of the 5HT2B receptor uncouples nNOS activation but not Ca^{2+} responses. Further, small peptides containing sequences identical to the carboxy-terminal 20 amino acids of the 5HT2B receptor inhibited nNOS activation (Manivet et al., 2000). These results were interpreted as evidence of a direct regulatory interaction between 5HT2BR and nNOS. However, several aspects of the results obtained with 5HT2BR suggest an alternate interoperation. For example, 20-mers ending in the sequence VSYI inhibited nNOS activation, but peptides terminating in VSYV, VSFI, or GSYI did not (Manivet et al., 2000). These results suggest either that the nNOS PDZ domain exhibits unprecedented selectivity or that other structural determinants play a dominant role in establishing the interaction between nNOS and 5HT2BR.

4. Luteinizing Hormone Receptor—Luteinizing hormone and human gonadotrophin actions are mediated by the lutropin-choriogonadotropic hormone receptor (LHCGR). It possesses a carboxy-terminal PDZ motif that binds GIPC and promotes recycling of receptors after agonist-induced internalization (Hirakawa et al., 2003). The signaling activity of cellular LHCGRs, as with β2ARs, is critically dependent on receptor sorting between recycling and degradative (lysosomal) pathways after endocytosis; hence, this recycling function of the LHCGR–GIPC interaction also affects signaling by sustaining cellular hormone responsiveness (Bhaskaran & Ascoli, 2005).

5. Kappa Opiod Receptor—Three classes of opioid receptors (delta, mu, kappa) mediate the response to a variety of endogenous peptides such as the endorphins and enkephalins as well as to exogenous compounds such as morphine.

The kappa-type opioid neuropeptide receptor (KOR) is an interesting but probably unusual example of a family A GPCR capable of engaging PDZ proteins. The KOR does not possess a canonical PDZ motif, yet has been shown to bind through its distal carboxy-tail to NHERF1 (Li et al., 2002). This interaction has been reported to affect both KOR trafficking and signaling, by promoting efficient recycling and facilitating receptor signaling via the NHE3 sodium-proton exchanger. The affinity of this atypical PDZ interaction is such that these effects are limited to, or occur preferentially in, cell types expressing NHERF1 at relatively high levels (Huang et al., 2004).

6. Lysophosphatidic Acid Receptors—Lysophosphatidic acid (LPA) is a phospholipid that is involved in many cell proliferation, differentiation, chemotaxis, cell motility, and survival. The lysophosphatidic acid receptor-2 (LPAR2) contains a canonical PDZ-binding motif (DSTL) that is remarkable in that the signaling consequence depends on which of several potential PDZ proteins it binds: NHERF2, PDZ-RhoGEF, and MAGI3 (Oh et al., 2004; Yamada et al., 2005; Zhang et al., 2007). Each of these proteins, upon engaging LPAR2, promotes signaling via different transduction pathways (Table III). These interactions occur in a mutually exclusive manner, allowing relevant cellular properties such as tumor cell invasiveness to be controlled by differential expression of cognate PDZ proteins (Lee et al., 2011).

LPA receptors are potent activators of Rho signaling pathways. An intact PDZ domain is necessary for the interactions between PDZ-RhoGEF and LARG with LPA1 receptors (Yamada et al., 2005). Further, modification of the carboxy-terminal PDZ ligand by addition of three alanines abrogated the interactions between the proteins. Overexpression of epitope-tagged PDZ domains from PDZ-RhoGEF or LARG had a dominant negative effect on LPA-induced RhoA activation. The third PDZ domain of PSD-95, which did not interact with either LPA1 or LPA2, did not affect RhoA activation.

It should be noted that PDZ domain–PDZ ligand interactions likely play a much broader role in the regulation of Rho-family GTPases. At least two dozens of the 70-odd known Rho-GEFs contain carboxy-terminal PDZ binding motifs, which can potentially interact with PDZ domain-containing scaffolding proteins (Garcia-Mata & Burridge, 2007). For example, kalirin7, a Rac1-specific GEF, terminates in the tetrapeptide STYV, a canonical Class I PDZ ligand. Kalirin7 accumulates in dendritic spines, where it colocalizes with PSD-95, the multiple PDZ protein MUPP1 (Fig. 1), and the 5-HT2A receptor, which also contains a canonical Class I PDZ ligand (Jones et al., 2009). The localization of kalirin7 to the postsynaptic density is modulated by its interactions with PDZ proteins (Jones et al., 2009). Disruption of the recruitment of kalirin7 to dendritic spines disrupts 5-HT2AR-mediated Rac activation and p21-activated kinase (PAK) phosphorylation, and impairs spine morphogenesis (Jones et al., 2009).

7. Adenosine Receptors—Adenosine modulates cardiovascular actions and notably the response to stress. A_1 adenosine receptors couple to Gi and inhibit adenylyl cyclase, whereas A2aR and A2bR engage Gs and stimulate adenylyl cyclase. A2bR also activates Gq and stimulates PLC. A_3R couples to Gi and Gq/11. The A2bR uniquely binds NHERF2 (E3KARP; Sitaraman et al., 2002). This interaction does not proceed through a canonical,

carboxy-terminal PDZ-recognition motif but is thought to recognize a putative 3-residue internal sequence, of which there are several, in the third intracellular loop. Preliminary studies indicate that mutation of this sequence reduced adenosinestimulated cAMP accumulation (Sitaraman et al., 2002).

8. Alpha-Adrenergic Receptors—The carboxy-terminus of the α1A adrenergic receptor was identified as a high-affinity target for nNOS (Schepens et al., 1997). Yeast two-hybrid methods showed that the PDZ domain of nNOS binds Class III PDZ ligands, with preference for the sequence G(D/E)XV. However, further work failed to confirm Class III PDZ specificity, as the α1BAR (carboxy-terminus: PGQF) and the α1DAR (carboxy-terminus: ETDI) interacted with nNOS with comparable affinities to that of the α1AR (Pupo & Minneman, 2002). Further work showed that carboxy-terminal truncations of the α1ARs also coimmunoprecipitated with nNOS, suggesting that PDZ–PDZ ligand interactions play a secondary role in the interactions of nNOS with α-subtype adrenergic receptors (Pupo & Minneman, 2002).

9. CXCR2 Chemokine Receptor—The CXCR2 chemokine receptor mediates chemotaxis of leukocytes and also regulates wound healing, angiogenesis, and inflammation. This GPCR possesses a C-terminal PDZ motif (STTL) that functions in both trafficking and signaling. Truncation of the PDZ motif increased the rate of receptor degradation by endocytic trafficking to lysosomes and, interestingly, did so without detectably affecting either receptor endocytosis or recycling. Evidence for a role in functional signaling came from the observation that truncation of the PDZ motif impaired the accumulation of CXCR2-expressing cells in a Boyden chamber containing the agonist CXCL8, suggesting that the PDZ motif is important for CXCR2-mediated chemotaxis (Baugher & Richmond, 2008).

10. Corticotropin-Releasing Factor Receptors—The CRHR1 (also called CRFR1) regulates pituitary hormone secretion but is also expressed in the cerebral cortex, where it mediates anxiogenic actions of CRF. This GPCR possesses a C-terminal PDZ motif (STAV) that promotes recycling of CRHR1s after CRF-induced endocytosis. Interestingly, endocytic recycling of CRHR1s was found to increase surface expression of 5HT2A serotonin receptors, which are coexpressed with CRHR1s in cortical neurons and also possess a PDZ motif (VSCV). 5HT2ARs undergo constitutive (i.e., ligand-independent) endocytosis, and the CRF-induced increase in 5HT2AR surface expression was apparently mediated in trans by PDZ-directed recycling of the CRHR1 through the same endosomes containing 5HT2ARs. This effect required intact PDZ motifs in both GPCRs, and increased 5HT2AR-mediated neural signaling in cultured cells as well as *in vivo*, as indicated by CRF-induced enhancement of the behavioral effects of the 5HT2 agonist DOI (2,5-dimethoxy-4-iodoamphetamine; Magalhaes et al., 2010).

11. Other Family A GPCRs—PDZ domain-containing protein interactions with motifs present in several other family A GPCRs have been shown to mediate various signaling functions but have not been directly linked to effects on receptor trafficking. For example, binding of the purinergic receptor P2YR1 to NHERF2 prolongs the duration of receptor-

mediated cytoplasmic calcium mobilization (Fam et al., 2005). Interaction of the melatonin receptor MTNR1A with MUPP1, mediated by a Class III PDZ motif present in the receptor's distal carboxy-tail, enhances the efficacy of receptor signaling by coupling to Gi (Guillaume et al., 2008).

Finally, there is some evidence that other family A GPCRs can bind PDZ proteins via sequences entirely distinct from consensus carboxy-terminal motifs and located more proximally in the receptor's cytoplasmic tail. Such has been suggested for the endothelin ETA receptor, in which an "internal" PDZ motif was mapped that is essential for driving the efficient recycling of receptors after agonist-induced endocytosis (Paasche et al., 2005). Based on sequence comparison and structural prediction, it was proposed that many (~30) members of GPCR family A may possess such internal PDZ-interacting sequences. Relevant trans-acting PDZ protein(s) that to these putative motifs have not been identified for any of these examples, and the PDZ protein(s) responsible for ETAR sorting into the recycling pathway remain to be defined. Accordingly, this additional group of putative PDZ-interacting GPCRs is not listed in Table I.

B. Family B GPCRs

1. PTH1R—PTH and the PTH-related protein (PTHrP) exert their biological actions on mineral ion homeostasis and bone growth and turnover through a common, PTH1R. The so-called type 2 PTH receptor principally mediates the actions of TIP39, a neuropeptide. The human PTH1R consists of 593 residues (mouse, 591) terminating in the PDZ recognition motif ETVM. Mahon and Segre discovered the interaction of PTH1R with NHERF1 and NHERF2 (Mahon et al., 2002). Binding of the PTH1R to NHERF2 was disrupted if positions 0, —2, or —3 were mutated to Ala. The PTH1R binds preferentially to PDZ1 of NHERF1 and to PDZ2 of NHERF2 (Wang et al., 2010). This finding is consistent with the greater structural homology between these two PDZ domains.

Mahon and Segre further found that the PTH1R signals predominantly through adenylyl cyclase in the absence of NHERF2, whereas in its presence, signaling switches primarily to PLC. Pertussis toxin pretreatment inhibited PLC signaling, with an accompanying increase of cAMP, by the PTH1R expressed with NHERF2 in PS120 fibroblasts. These observations suggested that PLCβ is activated by pertussis toxin-sensitive Gi/o Gβγ subunits and that adenylyl cyclase is inhibited by Gα-subunits upon PTH-induced PTH1R activation. Direct measurement of NHERF1 and NHERF2 on PTH1R G protein coupling by [^{35}S]-GTPγS binding and Gα subtype-specific immunoprecipitation revealed that PTH1R interactions with NHERF1 enhance receptor-mediated stimulation of Gαq, but have no effect on stimulation of Gαi or Gαs (Wang et al., 2010). PTH1R binding to NHERF2 enhanced PTH1R-mediated stimulation of both Gαq and Gαi, but decreased stimulation of Gαs. Consistent with these functional data, NHERF2 formed binary complexes with both Gαq and Gαi, whereas NHERF1 interacted only with Gαq. These findings establish that NHERF interactions regulate PTH1R signaling at the level of G proteins, and that NHERF1 and NHERF2 exhibit isotype-specific effects on G protein activation.

NHERF1 also importantly regulates ligand bias at the PTH1R and trafficking of the PTH1R. PTH is synthesized and secreted and circulates primarily as a full-length 84-amino acid

peptide. Cathepsin proteases in PTH glands generate amino-truncated PTH(7-84). This fragment normally present only at low levels but accumulates appreciably in certain clinical settings. Once thought to be biologically inert, PTH(7-84) is now recognized to exert important effects on both the PTH1R and the putative C-PTH receptor (Divieti et al., 2005; Murray et al., 2005). In cells lacking NHERF1, PTH (1-84) and PTH(7-84), and their shorter analogs PTH(1-34) and PTH(7-34), efficiently internalize the PTH1R (Sneddon et al., 2003). Notably, in cells expressing NHERF1, PTH(1-34) and PTH(1-84) promote PTH1R endocytosis, whereas receptor sequestration by PTH(7-34) and PTH(7-84) is eliminated. These findings suggest that NHERF1, which constitutively binds the PTH1R (Sneddon et al., 2003), stabilizes the receptor so that only full agonists induce receptor conformations capable of internalization.

NHERF1 also regulates PTH1R desensitization (Wang et al., 2009). PTH stimulation of adenylyl cyclase was desensitized by repetitive challenges in a concentration-dependent manner. However, in the presence of NHERF1, desensitization was inhibited. NHERF1 decreased PTH-induced dissociation of Gαs from the PTH1R. Reducing constitutive NHERF1 levels with short hairpin RNA restored PTH1R desensitization. Mutagenesis of NHERF1 PDZ-binding domains or deletion of the ezrin-binding domain established that both are required for inhibition of receptor desensitization. NHERF1 suppressed β-arrestin2 binding to the PTH1R. This latter finding further suggests that NHERF1 sterically interferes with β-arrestin binding to the intracellular tail of the PTH1R. This action may forestall PTH resistance and downregulation of the PTH1R.

NHERF1-null mice generated by homologous recombination of exon 1 exhibit a spectrum of mineral ion disorders (Shenolikar et al., 2002). Likewise, humans harboring NHERF1 polymorphisms display a similar presentation with conspicuous renal phosphate wasting (Karim et al., 2008). Interestingly, these variants, which are located in the linker region between PDZ1 and PDZ2, or in PDZ2, do not interfere with PTH-stimulated cAMP accumulation when heterologously expressed in kidney-like OK cells (Karim et al., 2008). Thus, the disordered phosphate transport arises from an allosteric action of NHERF1 (Li et al., 2009) on PTH1R binding or interference with a posttranslational modification (Weinman et al., 2007).

2. GLP2R—The glucagon-like peptide-2 receptor is expressed in the gastrointestinal tract and directly inhibits apoptosis and maintains mucosal integrity by stimulating cell proliferation in response to ligand activation (Drucker, 2005). The GLP2R modulates the stable association with β-arrestin2 and is required for G protein-coupled signaling, homologous desensitization, and receptor endocytosis. Interestingly, the GLP2R carboxy-terminus, which contains the PDZ-recognition sequence, ESEI (Table II), tethers the unbound receptor at the plasma membrane and directs intracellular trafficking of internalized receptors (Estall et al., 2005). The interacting proteins responsible for this activity remain to be identified.

3. Other B Family GPCRs—Several additional family B GPCRs contain carboxy-terminal PDZ ligands with 4- or 3-residue sequences. These include the calcitonin receptor (ESSA), the secretin receptor (SII), corticotrophin-releasing factor receptor (TAV), gastric

inhibitory peptide-1 receptor (ESYC), vasoactive intestinal peptide receptor (SVI), latrophilin-1 (TSL), and brain-specific angiogenesis inhibitor-1 (TEV) (Lim et al., 2002; Nishimura et al., 2000; Shiratsuchi et al., 1998). Of these, the only example yet shown to interact with a PDZ protein is the brain-specific angiogenesis inhibitor-1 (BAI1), which binds MAGI1 (Shiratsuchi et al., 1998), a PDZ protein containing six PDZ modules and a guanylate kinase domain.

Structurally related to the CTR is the calcitonin receptor-like receptor (CRLR), which bears 55% sequence homology. Whereas the CTR possesses a carboxy-terminal PDZ-interacting ligand, the CRLR does not. Both the CTR and CRLR dimerize with receptor activity-modifying proteins (RAMP) to confer ligand specificity to the CTR–RAMP or CRLR–RAMP pair (Lerner, 2006). CRLR dimerized with RAMP3, for instance, forms a high-affinity receptor for adrenomedullin (McLatchie et al., 1998). RAMP3 contains a PDZ-motif (DTLL), through which CRLR interacts with PDZ proteins such as NHERF1 (Bomberger et al., 2005). Thus, GPCRs lacking a PDZ-recognition domain may still exhibit signaling and trafficking behavior that is modulated by PDZ proteins, where an adapter protein bridges the GPCR with the PDZ protein.

C. Family C GPCRs

1. Metabotropic Glutamate Receptors—Metabotropic glutamate receptors are members of GPCR family C. They are classified into three subtypes based on primary amino acid sequence, intracellular coupling mechanisms, and pharmacology. Group I includes mGlu1R and mGlu5R, which couple primarily to Gq and are selectively activated by 3,5-dihydroxyphenylglycine (3,5-DHPG). Group II includes mGlu2R and mGlu3R that couple to Gi and are activated by aminopyrrolidine-2,4-dicarboxylate. Group III consists of mGlu4R, mGlu6R, mGlu7R, and mGluR8, and also couples to Gi but exhibits a pharmacological profile distinct from Group II and is activated by 2-amino-4-phosphonobutyrate.

mGluRs are now recognized to engage several additional PDZ proteins including PICK1, shank, tamalin, syntenin, and glutamate receptor-interacting protein (GRIP) in an isotype-specific manner. A good example of this specificity is found with mGluR2 and mGluR7. Acting through Gi, both receptors inhibit Ca^{2+} channels; mGluR2 activation blocks L/N-type Ca^{2+} channels, whereas mGluR7 inhibits P/Q-type channels. mGluR7, through its Class II PDZ ligand (NLVI), interacts with PICK1 (Fig. 1), a PDZ protein that is distributed throughout neuronal dendrites and in excitatory synaptic spines (Dev et al., 2000). PICK1 is required for surface expression of mGluR7 and for normal synaptic transmission and receptor-mediated inhibition of P/Q-type voltage-gated Ca^{2+} channels (Perroy et al., 2001, 2002). PICK1 also stabilizes the complement of receptors present in the plasma membrane (Suh et al., 2008). These signaling and trafficking functions of the mGluR7–PICK1 interaction are both thought to be mediated by physical scaffolding of receptors in perisynaptic regions of the dendritic plasma membrane. Disrupting this scaffolding of mGluR7, either using a peptide inhibitor of the mGluR7–PICK1 interaction or by mutation of the PDZ motif in mGluR7, disrupts normal excitatory signaling and results in an epilepsy-like phenotype (Bertaso et al., 2008). Models of PICK1 function in the regulation of

mGlu7R are complex. In addition to PICK1, the C-terminal tail of mGluR7 interacts with Gβγ dimers in the resting state, and these interactions prevent the inhibition of voltage-gated calcium channels in presynaptic active zones (Bertaso et al., 2006). As the local concentration of calcium increases, Ca^{2+} calmodulin binds mGlu7R, displacing Gβγ dimers and inhibiting further Ca^{2+} channel activity (Niswender & Conn, 2010). Ligand-dependent activation of mGlu7R only occurs during periods of intense synaptic activity due to the very low affinity of the receptor for glutamate (Niswender & Conn, 2010).

mGluR2 is unaffected by PICK1 but through its Class I PDZ-recognition sequence binds the multi-PDZ domain protein GRIP. The interaction of mGluR2 with GRIP and PICK1 is regulated by PKC-mediated receptor phosphorylation of the Ser present in the PDZ-binding sequence (Chung et al., 2000).

Group I mGluR5, but not mGluR1, binds NHERF2, even though both possess identical SSSL carboxy-terminal PDZ recognition ligands (Paquet et al., 2006). NHERF2 augments Gq-coupled Ca^{2+} signaling by mGluR5a, but not mGluR1a. No trafficking function of this interaction has been reported.

The scaffolding protein Homer was initially identified as a single-PDZ domain containing adapter that binds Group I mGluR (Brakeman et al., 1997). The identification of Homer as a PDZ protein was based on three main observations: (a) the presence of a conserved GLGF PDZ core-binding motif near the N-terminus, (b) the direct interaction of Homer with mGluR1 (C-terminus: SSTL) and mGlur5 (C-terminus: SSSL), and (c) the inhibition of the binding of mGluR5 to Homer by deletion of the C-terminal SSSL sequence (Brakeman et al., 1997). However, the interactions between Homer and its targets are unusual. Although Homer binds mGluR1 and mGluR5, it does not interact with mGluR2 (C-terminus: TSSL; Brakeman et al., 1997). Additional analysis of deletion mutants of mGluR5 identified a second interacting sequence present only in Group I mGluRs. This sequence, PPxxF, is characteristic of the ligands for structural domains of the Enabled/Vasp Homology 1 (EVH1) domain family (Tu et al., 1998). The N-terminal domain of Homer bound to a polyproline peptide has been crystallized (Beneken et al., 2000). No structure of the complex containing the C-terminal PDZ ligand has been reported. High resolution structural analysis shows only minor resemblance between the N-terminus of Homer and PDZ domains; therefore, it has been proposed that this region constitutes a new structural motif family related to both PDZ and EVH1 domains (Beneken et al., 2000). However, to date, the N-terminus of Homer is still indistinctly classified as either a PDZ or an EVH1 domain in research and review articles.

From a functional point of view, Homer plays a critical scaffolding role in the signaling properties of mGluR1 and mGluR5. Homer forms dimers and interacts with PSD95, ryanodine and IP3 receptors, and several other important signaling proteins (Tu et al., 1998, 1999; Xiao et al., 1998). Homer is required for clustering of Group I mGluRs in postsynaptic density areas and for mGluR-dependent calcium homeostasis (Sala et al., 2005). Homer interacts with Shank-2, a multi-PDZ protein that binds PLCβ3, which promotes efficient coupling of Group I mGluRs to calcium signaling (Hwang et al., 2005).

Finally, Homer couples mGluR signaling to Erk cascade activation (Mao et al., 2005) and promotes the development of dendritic spines (Foa & Gasperini, 2009).

Early studies searching for proteins that interact with the C-terminus of mGluRs identified a PDZ scaffolding protein termed tamalin also known as GRP1-associated scaffolding protein (GRASP; Kitano et al., 2002, 2003). This 43-kDa protein contains a typical type 1 PDZ domain that interacts with the C-terminus of Group I and Group II mGluRs (Kitano et al., 2002). Tamalin contains two additional structural motifs: a leucine zipper immediately downstream of its unique PDZ domain, and a Class I PDZ ligand at its C-terminus (Kitano et al., 2002). The leucine zipper of tamalin interacts directly with the coiled-coil domain of proteins of the cytohesin family, whereas its C-terminal PDZ ligand is involved in dimerization and interactions with other PDZ proteins (Kitano et al., 2003). Tamalin's main function appears to be related to the regulation of mGluR trafficking. Proteins of the cytohesin family are GEFs for the ARF family of small GTPases (Chardin et al., 1996; Klarlund et al., 1997; Meacci et al., 1997). Since the primary function of ARF GTPases is the regulation of intracellular membrane trafficking and endocytosis, it was inferred that tamalin plays a role in the trafficking of mGluRs to specific loci on the plasma membrane (Kitano et al., 2003). This view, however, has been recently challenged by the observation that deletion of the C-terminal PDZ ligand of mGluR1a does not alter dendrite localization of the receptor (Das & Banker, 2006).

2. $GABA_B$ Receptors—$GABA_B$ receptors ($GABA_BR$) regulate inhibitory synaptic transmission. Presynaptic $GABA_BR$ inhibit neurotransmitter release by downregulating voltage-activated calcium channels. Postsynaptic $GABA_BR$ decrease neuronal excitability by activating inwardly rectifying potassium (Kir) channels responsible for late inhibitory postsynaptic potentials. Additional neural effects of $GABA_BR$ include long-term potentiation, slow wave sleep, muscle relaxation, and antinociception (Padgett & Slesinger, 2010).

Functional $GABA_BR$ are heterodimers consisting of one molecule of $GABA_BR1$ and one molecule of $GABA_BR2$, homologous 7-transmembrane receptor proteins with specialized roles in $GABA_BR$ trafficking and function (Jones et al., 1998). Each subunit plays a particular role; $GABA_BR1$ binds the ligand, while $GABA_BR2$ couples the system to the activation of G proteins (El Far & Betz, 2002). Further, expression of $GABA_BR1$ is significantly impaired in the absence of the $GABA_BR2$ subunit or by expression of C-terminal truncated mutants of $GABA_BR2$ (Pooler et al., 2009). Recent work suggests an important role for the C-terminal PDZ ligand of $GABA_BR2$ (VSGL) in regulating the trafficking and stability of the $GABA_BR$ heterodimer (Balasubramanian et al., 2007). Mutation of the C-terminal leucine reduces the surface expression of the heterodimer accompanied by a decrease of the receptor half-life (Balasubramanian et al., 2007). *In vitro* studies using proteomic arrays identified three potential PDZ partners of $GABA_BR2$: Mupp1 (Fig. 1), PAPIN, and Erbin (Balasubramanian et al., 2007). The interactions of $GABA_BR2$ with Mupp1 and PAPIN, but not Erbin, were confirmed in live-cell models (Balasubramanian et al., 2007). Mutation of the C-terminal leucine of $GABA_BR2$ alters but does not abrogate the functional responses of the $GABA_BR$, since calcium responses are still observed in cells expressing the mutant. These reactions, however, exhibit shorter duration,

suggesting a modulatory role for receptor–PDZ protein interactions (Balasubramanian et al., 2007). Importantly, recent work examining the genetic basis of hyperexcitability in mouse congenic strains identified the *Mupp1* gene as an important regulator of sensitivity to 5-HT2CR antagonists and to $GABA_B$ agonists (Reilly et al., 2008).

D. Family F: Frizzled Receptors

Frizzled (FZD) receptors include a variable number of 7-transmembrane domain proteins that can be best described as a subset of unconventional GPCRs. Mammals express 10 distinct FZDs, suggesting extreme diversity accompanied by significant potential redundancy. Their primary function is mediating Wnt signaling programs, which range from the establishment of the basic body plan during development to the generation of organ systems and the skeleton (Schulte & Bryja, 2007). These functions are consequences of the regulation of cell growth, proliferation, fate, migration, polarization, and death by specific Wnt–FZD pairs (Logan & Nusse, 2004). Three different types of signals are transduced by Wnt–FZD interactions: the so-called canonical pathway, which involves the specific regulation of gene transcription by β-catenin; the planar cell polarity (PCP) pathway, which involves RhoA and Jun-N-terminal kinases; and aCa^{2+}-/CaMKII-/PKC-dependent pathway, whose role is still poorly understood (Logan & Nusse, 2004). Unlike other GPCRs possessing PDZ domains, FZD receptors harbor both a carboxyterminal PDZ binding sequence as well as internal PDZ-recognition motif.

It is generally accepted that most signaling events downstream of FZD receptors are mediated by adapter proteins of the Disheveled (Dvl) family. Dvl proteins contain three well-defined structural motifs: an amino-terminal Disheveled/Axin (DIX) domain, followed by a PDZ domain, and a carboxyterminal Disheveled/Egl-10/Pleckstrin-homology (DEP) domain. These structural domains play well-defined roles in the signal transduction events mediated by Dvl proteins. DIX domains target Dvl to the actin cytoskeleton (Capelluto et al., 2002) and DEP domains mediate interactions with cell membranes (Pan et al., 2004). The interactions between FZD and Dvl are mediated by the PDZ domain of the latter, which interacts with a conserved sequence (K-S/T-X-X-X-W) located immediately downstream of the 7th transmembrane domain of FZD receptors (Umbhauer et al., 2000; Wong et al., 2003). This interaction is critical for Wnt signaling functions, as PDZ deletion mutants of Dvl exhibit dominant negative behavior, and single mutations of the putative PDZ ligand act as loss-of-function mutants (Umbhauer et al., 2000; Wong et al., 2003). These interactions have recently been shown to be candidate drug targets: small peptides that mimic the PDZ ligand sequence of FZD7 display antitumor properties and interfere with Wnt signaling in a hepatocarcinoma model (Nambotin et al., 2011).

1. Internal FZD PDZ Ligands—Dvl binds to the internal PDZ motif of FZD and not the carboxyterminal site. The structural basis for this preference is uncertain. Although the Dvl–PDZ module is usually considered a Class I PDZ domain, it lacks the histidine residue in a G-H (Gly-His) position conserved in most members of the family, as described earlier (Wong et al., 2003). Solution NMR studies demonstrate that the internal PDZ ligand of FZD7 engages the carboxyterminal peptide-binding groove located between αB and βB of Dvl1–PDZ (Wong et al., 2003). Binding was abolished by replacing the three conserved

amino acids (K, T, W); single substitutions (K→M, W→G) substantially diminished the peptide affinity. The binding of internal sequences to most PDZ domains is weak. In this regard, the relative affinities of the internal PDZ ligands of FZD receptors for Dvl–PDZ domains are relatively weak in comparison to those reported for carboxy-terminal PDZ ligands with their respective targets. Whereas carboxy-terminal peptides bind cognate Class I PDZ domains with affinities of the order of 50–100 nM (Songyang et al., 1997), FZD–PDZ ligand interactions with Dvl–PDZ exhibited dissociation constants of 100 nM–2.2 μM for FZD1, FZD2, FZD3, FZD4, and FZD7 (Punchihewa et al., 2009) to as low as 10 μM for human FZD7 (Wong et al., 2003). These low-affinity interactions between the internal PDZ ligands of FZD receptors and the PDZ domain of Dvl suggest the additional involvement of other regions of FZD in the formation of stable FZD–Dvl complexes. This speculation is supported by data indicating that subsets of residues located in intracellular loops 1 and 3 of FZD1 stabilize the interaction with Dvl and are required for Wnt signaling (Cong et al., 2004).

The relatively low affinities of Dvl–PDZ for their targets further suggest the presence of a flexible conformation of the PDZ ligand-binding pocket that may accommodate interactions of ligands with diverse structures. Consistent with this theory, other Dvl–PDZ binding partners have been identified. These include Idax (inhibitor of the Dvl–axin complex, which blocks Wnt signaling; Hino et al., 2001; London et al., 2004) and the PTH1R (Romero et al., 2010). Idax binds Dvl–PDZ via the internal sequence KTXXXI (Hino et al., 2001; London et al., 2004), whereas the interactions of Dvl and the PTH1R mediated by the sequence KSWSRW lead to efficient functional coupling of the PTH1R to the activation of β-catenin (Romero et al., 2010).

Despite the preference for the internal PDZ motif, Dvl–PDZ domains are able to bind canonical carboxy-terminal PDZ ligands. The carboxy-terminus of Dapper, an endogenous Wnt signaling regulator (Cheyette et al., 2002), binds directly to Dvl1 via a mechanism analogous to canonical PDZ–PDZ ligand interactions, although with much lower affinity (16 μM; Wong et al., 2003). Importantly, the PDZ ligand of Dapper has the sequence MTTV, which is homologous to the carboxy-terminal sequences of several of the FZD receptors (ETTV in FZD1 and FZD2, ETVV in FZD4, and ETAV in FZD7; see Table IV). This suggests that some FZD receptors contain a second potential Dvl–PDZ interaction site. Nevertheless, there is no evidence indicating that the carboxy-terminal sequences of FZD receptors interact with Dvl–PDZ.

Because Dvl–PDZs mediate Wnt signaling and dysregulated β-catenin signaling plays an important role in cancer cell proliferation and metastasis (Moon et al., 2004), several laboratories have undertaken studies to determine the potential use of peptide and peptidomimetic ligands that compete for the PDZ-binding pocket of Dvl proteins. Several such compounds have been identified (Grandy et al., 2009; Mahindroo et al., 2008; Shan et al., 2005; You et al., 2008). These compounds bind Dvl–PDZ with moderate affinities (10–20 μM). Moreover, they inhibit Wnt-stimulated β-catenin activation. One compound, 3289–8625, inhibits cell proliferation in a prostate cancer cell model, albeit at very high concentrations (100 μM; Grandy et al., 2009). These studies demonstrate that the FZD–Dvl interface is a potentially useful drug target.

2. Carboxy-Terminal FZD PDZ Ligands—Table IV shows the carboxy-terminal sequences of the 10 human FZD receptors. Eight of the 10 sequences conform to the structural requirements for carboxy-terminal PDZ ligands. Simple structural considerations would predict the formation of multifunctional complexes involving FZD, Dvl, and PDZ adapters. However, this may not occur because of steric hindrance arising from the short length of the carboxy-terminus. For instance, only 13 amino acids separate the internal Dvl–PDZ-binding sequence and the carboxy-terminal PDZ-recognition motif of FZD1, FZD2, and FZD7. This distance is 29 residues in FZD4, whereas more than 40 residues separate internal and carboxy-terminal PDZ domains of FZD5, FZD8, FZD9, and FZD10. FZD3 and FZD6 lack a carboxy-terminal PDZ ligand. Because PDZ domains bind linear peptides in an extended conformation, as described earlier, it is difficult to envision canonical PDZ adapters as positive regulators of the functions of FZD1, FZD2, FZD4, and FZD7. This suggests possible participation of PDZ proteins targeting the carboxy-terminal canonical sequence in regulating FZD receptor function in specific tissues. Several PDZ partners for the putative carboxy-terminal PDZ ligands of FZD receptors have been identified (Table V). These PDZ partners regulate multiple FZD properties, ranging from trafficking and subcellular distribution to coupling of specific signaling pathways.

a. FZD Trafficking: The Golgi-associated PDZ and coiled-coil motif protein (GOPC) was among the first intracellular FZD partners to be identified (Yao et al., 2001). GOPC interacts with FZD5 via its unique PDZ domain. Its role in FZD function has not been fully established, but GOPC regulates surface expression of FZD5 (Yao et al., 2001) and of the cystic fibrosis conductance regulator (CFTR; Cheng et al., 2002). Curiously, the effects of GOPC on the surface expression of FZD5 and CFTR differ: whereas expression of GOPC promotes membrane expression of FZD5 (Yao et al., 2001), the opposite is true for the CFTR (Cheng et al., 2002, 2004).

An unexpected function of specific FZD receptors was recently discovered in *Drosophila* synaptic junction development. Wingless, the *Drosophila* ortholog of Wnt, is secreted by glutamatergic motor neurons and binds to postsynaptic D-FZD2 receptors, promoting their internalization and trafficking to the perinuclear region. These internalized receptors are then cleaved, and their carboxy-terminal segment is imported to the nucleus (Mathew et al., 2005). The *Drosophila* glutamate receptor-interacting protein (D-GRIP), a multi-PDZ protein that contains seven PDZ domains and no other known protein interaction motifs, directs the trafficking of D-FZD2 to the nucleus (Ataman et al., 2006). D-GRIP is present in the Golgi and trafficking vesicles, where it colocalizes with D-FZD2. Immunoprecipitation data demonstrate that the carboxy-terminus of D-FZD2 interacts with PDZ domains 4 and 5 of D-GRIP. Further, D-GRIP mutants and siRNA knockdowns of D-GRIP mimic the synaptic phenotypes of D-FZD2 and *wg* wingless mutants (Ataman et al., 2006). There is no evidence for this pathway in mammals.

b. PDZ Proteins and Regulation of Noncanonical Wnt Signaling: Several functional FZD–PDZ interactions regulate noncanonical Wnt signaling. For example, the multi-PDZ protein MAGI3 (Fig. 1), which interacts strongly with FZD4 and FZD7, weakly with FZD5 and FZD8, and not at all with FZD3 and FZD6, is specific for the PDZ1 domain of MAGI3

(Yao et al., 2004). Deletion of PDZ1 abrogated binding despite the presence of the remaining five PDZ domains in the pull-down construct. The results suggest that MAGI3 supports the formation of a complex with Ltap/strabismus1/Vangl2, an important regulator of noncanonical Wnt signaling (Yao et al., 2004). Because Wnt–FZD–Vangl2 signaling is essential for proper ciliogenesis in polarized epithelia (Borovina et al., 2010), the formation of this ternary complex suggests an important role for FZD4–MAGI3 interactions in this process. However, the precise nature of the Wnt–FZD–Vangl2 complex remains elusive. Vangl2 terminates in a canonical carboxy-terminal PDZ ligand (ETSV), which binds exclusively to PDZ1 of MAGI3, suggesting direct competition with FZD4 or FZD7 (Yao et al., 2004). Other reports suggest that Vangl2 can interact directly with some FZD receptors, in particular with FZD3 (Montcouquiol et al., 2006). Given that FZD3 does not contain a carboxy-terminal PDZ-binding motif, the interaction with Vangl2 is probably not directly mediated by PDZ scaffolds. In fact, the extracellular, cysteine-rich domain of FZD mediates the interactions of *Drosophila* fz and Vang/Stbm (Wu & Mlodzik, 2008). Nevertheless, certain biological functions for the FZD4/MAGI3/Vangl2 complex are suggested by studies in model systems. For instance, overexpression of MAGI3 increased the ability of FZD4 to activate c-Jun N-terminal kinase (Jnk), but had no effects on β-catenin signaling (Yao et al., 2004). Thus, the findings suggest a role for MAGI3 in the specific regulation of noncanonical Wnt signaling, though the precise role is obscure. Importantly, no data connecting MAGI3 to ciliogenesis have been reported.

3. Canonical Signaling—There is little evidence linking carboxy-terminal PDZ interactions to the regulation of canonical Wnt signaling. Until recently, the only report concerning carboxy-terminal PDZ interactions to the canonical pathway demonstrated the formation of complexes between the carboxy-terminal motifs of FZD1, FZD2, FZD4, and FZD7 with specific PDZ domains of PSD-95 and the related proteins PSD-93 and SAP-97 (Hering & Sheng, 2002). This study also demonstrated that PSD-95 forms a ternary complex with FZD2 and the adenomatous polyposis coli (APC), one of the components of the destruction complex that targets β-catenin to the proteasome. Although these observations suggest a role for PDZ proteins as scaffolds that contribute to the canonical Wnt signaling pathway, no further studies have explicitly examined this possibility.

A more recent report links NHERF1 to the regulation of canonical Wnt signaling (Wheeler et al., 2011). Here, NHERF1 inhibited canonical Wnt signaling mediated by endogenous FZD receptors in breast cancer cell lines. MCF-7 cells, which express NHERF1 at high levels, do not respond to exogenous Wnt, whereas MDA-MB231 cells, which lack NHERF1, are very sensitive. Manipulation of NHERF1 expression by a transgene or by shRNA techniques demonstrated that NHERF1 expression is responsible for these effects. The findings suggest that NHERF1 binding interferes with FZD–Dvl coupling, leading to reduced canonical signaling. FZD5 is by far the most abundant in these cells, suggesting that NHERF1 binding exerts long-range interactions that extend well beyond the carboxy-terminal tetrapeptide of FZD receptors. Immunoprecipitation and live-cell imaging results suggest that the carboxy-terminus of FZD4 binds PDZ2 of NHERF1 (Wheeler et al., 2011). NHERF1-null mice exhibited breast hyperplasia accompanied by increased proliferation and high levels of activated β-catenin, consistent with a role for NHERF1 in regulating breast

development (Wheeler et al., 2011). Finally, patient tissues displayed an inverse correlation between the expression of NHERF1 and nuclear β-catenin in primary breast tumors.

IV. PDZ Protein Regulation of GPCR Signaling

A. G Proteins

Although we focus our discussion primarily on GPCRs interacting with PDZ proteins, it should be noted that several G proteins themselves have canonical or internal PDZ-recognition sequences that bind PDZ proteins. Both NHERF1 and NHERF2, for example, bind Gαq (Rochdi et al., 2002; Wang et al., 2010), and NHERF2, but not NHERF1, interacts with Gαi. Neither NHERF1 nor NHERF2 associates with Gαs. The ability of PDZ proteins to engage G proteins underscores their ability to act as molecular routers to switch GPCR signaling pathways. Thus, although the thromboxane A_2 receptor lacks a PDZ-recognition motif and does not itself interact with NHERF1, receptor signaling is modified in the presence of NHERF1 (Rochdi et al., 2002). Here, Gαq binding and sequestration by NHERF1 diminish PLC activation and inositol phosphate accumulation.

B. PDZ Protein Regulation of GPCR Signaling by RGS

Regulators of G protein signaling (RGS) are a set of some two dozen GTPase-activating proteins (GAP) that promote the inherent GTP hydrolysis by G protein alpha subunits, thereby accelerating the inactivation of GPCR signaling by restoring the GDP-bound form. Several of these RGS protein possess PDZ and other protein–protein interaction modules in addition to the obligate RGS domain (Ishii & Kurachi, 2003). RGS12, for instance, harbors PDZ, PTB, and an RBD domain, and the RGS3 subtype PDZRGS3 includes a PDZ module.

RGS12 regulates Gαi/o/q, significantly accelerating GTP hydrolysis (Snow et al., 1998). Interestingly, the RGS12 PDZ domain most closely resembles PDZ domains of NHERF1. However, whereas NHERF1 interacts with the β2AR, RGS12 does not (Snow et al., 1998). RGS12, however, specifically recognizes the chemokine receptor CXCR2 through its Class I PDZ sequence (STTL). The platelet-derived growth factor (PDGF) receptor associates with NHERF1 to potentiate its activity (Maudsley et al., 2000). Gi-dependent PDGF receptor signaling, in turn, was reduced by RGS12 (Sambi et al., 2006).

Though GAIP, an RZ subfamily member of RGS proteins, itself does not contain a PDZ domain, its carboxy-terminal sequence (SSEA) is a canonical PDZ ligand that binds the PDZ protein GIPC (De Vries et al., 1998). GIPC specifically recognizes dopamine D2 and D3 receptors, but not D4 receptors. D2R and D3R have Class III PDZ-binding domains (Table I). In the presence of GIPC, the inhibitory action of D2 agonist-stimulated cAMP accumulation was reduced, consistent with a negative role of GIPC in Gi-mediated action (Jeanneteau et al., 2004). The authors proposed that GIPC, GAIP, and D3R form a multimeric complex, wherein GIPC links the RGS protein GAIP with D3R to promote GAIP-mediated Gi-GTP hydrolysis and terminating receptor signaling.

C. G protein receptor kinase

G protein receptor kinases (GRKs) terminate GPCR action by phosphorylating the receptor, which in turn recruits β-arrestin and initiates desensitization and internalization. Several of the seven described GRKs are expressed in a tissue-specific manner (GRK1, GRK4, GRK7), while others (GRK2, GRK3, GRK5, GRK6) are ubiquitously expressed (Pitcher et al., 1998).

NHERF1 is phosphorylated at positions 287, 289, and 290 in a serine cluster located between the PDZ2 and the ezrin-binding domain, which is required for biological activity (Weinman et al., 1998). This phosphorylation is reported to be mediated by PKA, despite the absence of a consensus PKA phosphorylation motif (RXS/T). GRK6a terminates in a 3-residue Class I PDZ ligand, TRL that mediates its specific interaction with NHERF1 (Hall et al., 1999). GRK6a mediates the constitutive phosphorylation at Ser^{289} (Hall et al., 1999). This site is a consensus GRK6 phosphorylation motif (RXXS/T). Moreover, the interaction of GRK6a is required for phosphorylation. GRK6a constructs harboring mutations of the PDZ ligand, or GRK6 isoforms lacking the carboxy-terminal PDZ ligand fail to phosphorylate NHERF1. Interestingly, GRK6a itself harbors several canonical PKA phosphorylation sites leading to the possibility that PKA phosphorylates GRK6a, which in turn phosphorylates NHERF1.

V. Conclusion

Our understanding of multiprotein interactions and how they impart many of the characteristic features of GPCRs is a subject of intense investigation and consequently a rapidly changing arena. It is now clear that many of the heretofore irreconcilable reported findings between GPCR signaling, trafficking, and function in different cells or in response to distinct ligands now can be attributed to the participation of PDZ proteins and their ability to confer ligand- and cell-specific actions on GPCRs, thereby adding to the remarkable diversity of actions a single receptor can display. Much of this work examined stable interactions of PDZ proteins with GPCRs. However, for the most part, these are low-affinity and transient interactions. To understand better, the dynamic mechanisms by which PDZ proteins assemble multiprotein complexes needs now to apply techniques that permit analyzing these short-lived interactions. Such approaches, including time-resolved FRET, FRAP, BiFC, and TIRF, take advantage of high quantum yield fluorescence proteins that permit analyzing protein-protein interactions in time and space. Moreover, the examination of the interactions of PDZ proteins with GPCRs has largely relied upon heterologous cell models and extensive overexpression. Under these circumstances, it is not surprising that many putative interactions can be detected that do not fit with described phenotypes from animal models or humans harboring spontaneous or engineered mutations in the PDZ protein. Hence, it will be critical for future work to concentrate on native cells and tissues that express constitutive levels of the GPCR and PDZ protein partner if we are to understand their true biological actions.

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Abbreviations

5HTR	serotonin receptor
AKAP	A kinase-anchoring protein
CRLR	calcitonin-like receptor
CTR	calcitonin receptor
DEP domain	Disheveled/Egl-10/Pleckstrin-homology
DIX domain	Disheveled/Axin domain
DR	dopamine receptor
Dvl	Disheveled
ETAR	endothelin type A receptor
FZD	frizzled receptor
GAIP	Galpha-interacting protein
GIPC	GAIP-interacting protein C-terminus
GLP2R	glucagon-like peptide-2 receptor
GOPC	Golgi-associated PDZ and coiled-coil motif protein
GRIP	glutamate receptor-interacting protein
GRK	G protein receptor kinase
KOR	kappa-type opioid receptor
LARG	leukemia-associated RhoGEF
LHCGR	lutropin-choriogonadotropic hormone receptor
LPAR	lysophosphatidic acid receptor
MAGI	membrane-associated guanylate kinase inverted
mGluR	metabotropic glutamate receptor
MPP	multi-PDZ protein
NHERF	Na–H exchange regulatory factor
NMDA receptor	N-methyl-D-aspartate receptor

NOS	nitric oxide synthase
P2YR	purinergic receptor
PAK	p21-activated kinase
PDZ	PSD-95, Drosophila discs large, and the adherens junction protein, ZO-1 domain
PDZ-RhoGEF	PDZ-containing Rho guanine nucleotide exchange factor
PICK	protein interacting with C kinase
PKA	protein kinase A
PX	phox homology domain
RAMP	receptor activity-modifying protein
RGS	regulators of G protein signaling
SAP97	synapse-associated protein-97
SNX27	sorting nexin 27
βAR	β-adrenergic receptor
α1CAR	α1C adrenergic receptor

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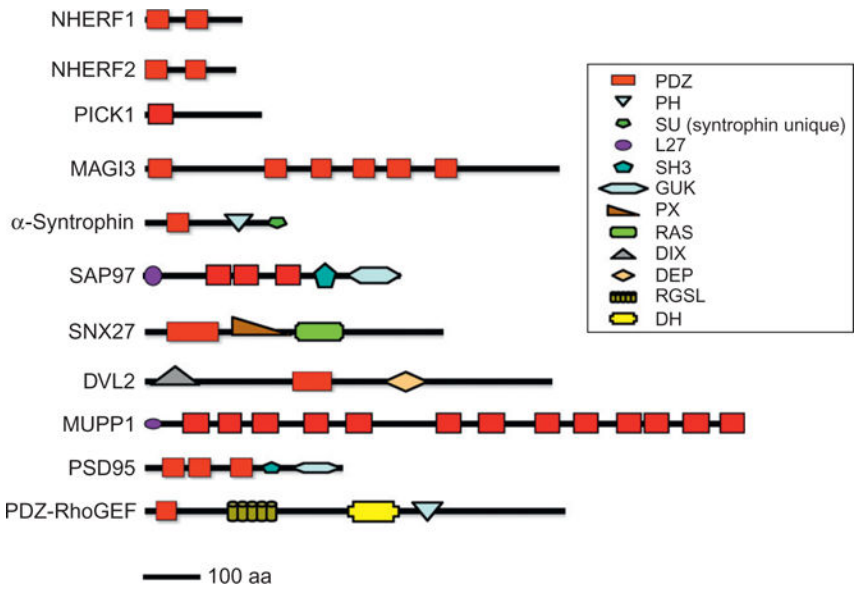

FIGURE I.

Schematic representation of select human PDZ proteins discussed in this review. PDZ and other protein modules are indicated by respective shapes and color. The relative scale is shown on the bottom. Domain name abbreviations: PDZ=PSD-95, Drosophila discs large, and the adherens junction protein, ZO 1; PH=pleckstrin homology; SU=syntrophin unique; L27=Lin2, Lin7-like; SH3=SRC homology 3; GUK=guanylate kinase; PX=phosphoinositide-binding; RAS=RAt Sarcoma; DIX=Disheveled homology; DEP=Disheveled, EGL-10, Pleckstrin; RGSL=regulator of G-protein signaling like; DH=DBL (diffuse B-cell lymphoma) homology.

TABLE I

Classes of PDZ Recognition Motif

Class	***Motif***
Class I	-[D/E]-[S/T]-X-Φ
Class II	-X-Φ-X-Φ
Class III	-X-[D/E/K/R]-X-Φ
Other	-X-X-C
	-X-Φ-[D/E]

TABLE II

Human GPCRs Expressing Class I Long C-Terminal PDZ-Binding Motifs

ID[a]	*GPCR*	*PDZ motif*
ADA1A	Alpha-1A adrenergic receptor	GEEV[b]
ADA1D	Alpha-1D adrenergic receptor	ETDI
ADRB1	Beta-1 adrenergic receptor	ESKV
ADRB2	Beta-2 adrenergic receptor	DSLL
CALCR	Calcitonin receptor	ESSA
CCR5	Chemokine receptor 5	SVGL[c]
CLTR2	Cysteinyl leukotriene receptor	ETRV
CRHR1	Corticotropin-releasing factor receptor 1	STAV
CXCR2	C-X-C chemokine receptor type 2	SVVI[b]
FZD1	Frizzled-1	ETTV
FZD2	Frizzled-2	ETTV
FZD4	Frizzled-4	ETVV
FZD7	Frizzled-7	ETAV
GIPR	Gastric inhibitory peptide-1 receptor	ESYC
GLP2R	Glucagon-like peptide 2 receptor	ESEI
GPR123	Orphan GPCR 123	ETTV
GPR124	Orphan GPCR 124	ETTV
GPR125	Orphan GPCR 125	HETT[b]
GPR135	Orphan GPCR 135	DTSL
GPR31	Orphan GPCR 31	DSYS
KOR1	Kappa opioid receptor	NKPV[b]
LHCGR	Lutropin-choriogonadotropic hormone receptor	YTEC[c]
LPAR2	Lysophosphatidic acid receptor 2	DSTL
LPAR5	Lysophosphatidic acid receptor 5	DSAL
MGLUR2	Metabotropic glutamate receptor 2	TSSL
MGLUR5	Metabotropic glutamate receptor 5	SSSL
MGLUR7	Metabotropic glutamate receptor 7	NLVI
OR2A1	Olfactory receptor 2A	ESHS
P2RY1	P2Y purinoceptor 1	DTSL
P2RY5	P2Y purinoceptor 5	DTSL
P2Y12	P2Y purinoceptor 12	ETPM
PD2R	Prostaglandin D2 receptor	ESSL
PGFRA	Platelet-derived growth factor receptor type A	DSFL
PGFRB	Platelet-derived growth factor receptor type B	DSFL
CXCR2	Prolactin-releasing peptide receptor	SVVI[b]
PTH1R	Parathyroid hormone/parathyroid hormone-related peptide receptor	ETVM
5HT2AR	Serotonin 2A receptor	VSCV
5HT2CR	Serotonin 2C receptor	ISSV

ID[a]	GPCR	PDZ motif
V2R	Vasopressin type-2 receptor	DTSS

[a] Gene nomenclature.

[b] Class II PDZ motif.

[c] Atypical sequence.

TABLE III

PDZ GPCR Partners

GPCR	*PDZ protein*	*Trafficking effect*	*Signaling effect*	*Reference (PMID)*
Family A				
β_2AR	NHERF1, NHERF2, PDZK1, SNX27	Promotes recycling (SNX27, NHERF2)	Signaling via NHE3 sodium-protein exchanger (NHERF1)	9560162 10499588 20733053
β_1AR	PSD95, SAP97, GIPC, CAL, MAGI2, MAGI3	Promotes recycling (SAP97)	Resensitization of cAMP signaling (SAP97)	16316992 17170109
5HT2CR	PSD95, MPP3	Promotes endocytosis (PSD95), inhibits endocytosis (MPP3)	Promotes desensitization (PSD95), inhibits desensitization (MPP3)	10816555 16914526
LHCGR	GIPC	Promotes recycling	Sustained hormonal responses	14507927 15821109
TSHR	HSCRIB	Inhibits endocytosis, promotes recycling	None reported	15775968
LPAR2	NHERF2, PDZ-RhoGEF, MAGI3	None reported	Potentiates LPA-induced activation of PLC-β (NHERF2), required for LPA-induced RhoA activation (PDZ-RhoGEF), promote receptor coupling to Gα12 and Erk activation (MAGI3)	15143197 15755723 16904289 21134377
P2YR1	NHERF2	None reported	Prolongs duration of the receptor-mediated Ca^{2+} response	15901899 16891310
MTNR1A	MUPP1 (type 3 PDZ)	No effect found	Required for receptor signaling via G_i	18378672
KOR	NHERF1 (atypical PDZ)	Promotes recycling	Signaling via NHE3 sodium-protein exchanger (NHERF1)	12004055 15070904
Family B				
PTH1R	NHERF1, NHERF2	Tethers receptor at cell membrane	Switches G protein signaling, regulates ERK signaling, imparts ligand bias, regulates desensitization	12075354 17599914 17884816 18272783 12920119 19188335 20562104
Family C				
mGluR5	NHERF2	None reported	Prolongs duration of the receptor-mediated Ca^{2+} response	16891310
mGluR7	PICK1	Stabilizes receptors at plasma membrane	Required for inhibition receptor-mediated inhibition of P/Q-type Ca^{2+} channels	11007882 12065412 18549785

TABLE IV

C-Terminal Sequences of Human FZD Receptors

Receptor	*Carboxy-terminal sequence*
FZD1	ETTV
FZD2	ETTV
FZD3	*GTSA*[a]
FZD4	ETVV
FZD5	LSHV
FZD6	*HSDT*
FZD7	ETAV
FZD8	LSQV
FZD9	PTHL
FZD10	PTCV

Sequences italicized denote C-termini that are not expected to interact with PDZ proteins.

[a]FZD3 and FZD6 do not contain a C-terminal PDZ-ligand.

TABLE V

PDZ Interactions of FZD Receptors

	Binding motif	***Target***	***Function***	***Reference (PMID)***
FZD1-10	Internal K-T-X-X-X-W	Dvl1, Dvl2, Dvl3	Canonical and noncanonical Wnt signaling	10990458 14636582
FZD5	Carboxyterminal L-H-S-V	GOPC	Membrane trafficking	11520064
Drosophila FZD2	Carboxyterminal A-S-H-V	*Drosophila* GRIP	Trafficking of C-terminal fragment; canonical signaling	16311339 16682643
FZD4, FZD7	Carboxyterminal E-T-X-V	MAGI-3 (strong interaction)	Formation of ternary complex with Vangl2; ciliogenesis; noncanonical signaling	15195140
FZD5, FZD8	Carboxyterminal L-S-X-V	MAGI-3 (weak interaction)	Formation of ternary complex with Vangl2; ciliogenesis; noncanonical signaling	15195140
FZD1, FZD2, FZD4, FZD7	Carboxyterminal E-T-X-V	PSD-95, PSD-93, SAP-97	Stabilization of complex with APC; canonical signaling	12067714
FZD1, FZD2, FZD4, FZD7	Carboxyterminal E-T-X-V	NHERF1	Negative modulation of canonical signaling	20802536