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PERSPECTIVE

Internal PDZ Ligands: Novel Endocytic Recycling Motifs for G Protein-Coupled Receptors

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ABSTRACT

Internalization, recycling and lysosomal sorting are key processes that regulate the temporal and spatial signaling of G protein-coupled receptors (GPCRs). Interactions between GPCR intracytosolic sorting signals and adaptor proteins facilitate trafficking through the endocytic pathway. To date only a few sorting signals and molecules that regulate GPCR trafficking have been identified. A study reported in the May 2005 issue of *Molecular Pharmacology* has now identified an internal PDZ ligand motif that seems to regulate efficient recycling of the ET_A endothelin receptor. This finding now expands the diversity of GPCR sorting motifs to include internal and C-terminal PDZ ligands, tyrosine-based motifs, and lysine residues capable of being ubiquitinated.

Seven transmembrane G protein-coupled receptors (GPCRs) interact with G-proteins and signaling effectors at the plasma membrane. Several recent studies indicate that GPCR signaling can also occur from endocytic vesicles (Tohgo et al., 2003; Ahn et al., 2004). Thus, diverse signal termination mechanisms must exist to precisely regulate the temporal and spatial signaling of GPCRs and the fidelity of hormone responses. In addition to phosphorylation and arrestin binding, internalization of GPCRs contributes to signal termination by removing activated receptor from G-proteins and signaling effectors at the plasma membrane. Once internalized, activated GPCRs may continue to signal from endosomes; however, agonist eventually dissociates from the receptors. Receptors are then dephosphorylated and recycled back to the cell surface in a resensitized state competent to signal again. Trafficking of internalized GPCRs from endosomes to lysosomes and consequent receptor degradation is also an important process that terminates receptor signaling (Trejo et al., 1998). The regulation of internalization and sorting of GPCRs to recycling endosomes or lysosomal degradation compartments involves complex protein-protein interactions. Short peptide "sorting" sequences residing in the intracytosolic domains of GPCRs probably serve as recognition signals for endocytic adaptor proteins to mediate protein-protein interactions. These interactions also control the rate of receptor internalization, recycling and lysosomal receptor degradation and hence, the magnitude and duration of cellular signaling. Given the vast number of receptors in the GPCR superfamily, it is surprising that only a few sorting sequences and proteins that facilitate GPCR trafficking through the endocytic pathway have been identified (Table 1). A recent study by Paasche et al. (2005) in the current of issue of *Molecular Pharmacology* has now identified an internal PDZ ligand that serves as a novel endocytic-recycling motif for the endothelin ET_A receptor.

Most activated GPCRs are phosphorylated, bind arrestins, and then recruited to a clathrin- and dynamin-dependent pathway for internalization from the plasma membrane. Internalized GPCRs then enter a tubulovesicular endosomal sorting compartment characterized by a slightly acidic luminal content and are subsequently delivered to distinct recycling endosomes or late/multivesicular endosomes and lyso-

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ABVREVIATIONS: GPCR, G protein-coupled receptors; AR, adrenergic receptor; EBP50, ezrin/radixin/moesin-binding phosphoprotein of 50 kDa; ET, endothelin; GRK, protein-coupled receptor kinase; NHERF, Na^+/H^+ exchanger regulatory factor; nNOS, neuronal nitric-oxide synthase; PDZ, Postsynaptic density of the *Drosophila* septate junction protein Discs-large, and the epithelial tight junction protein ZO-1.

TABLE 1
GPCR sorting motifs, modifications, and molecules

	Internalization	Recycling	Lysosomal Sorting
Modifications	Phosphorylation	Dephosphorylation	Ubiquitination
Motifs	Tyrosine-based (-Y-X-X-ϕ-)	Type I PDZ ligand Internal PDZ ligand	Tyrosine-based (-Y-X-X-ϕ-)
Molecules	Arrestins	NHERF/EBP50 NSF	SNX1 GASP HRS/Vps4

GASP, G protein-coupled receptor associated protein; HRS, hepatocyte growth factor-regulated tyrosine kinase substrate; NSF, *N*-ethylmaleimide-sensitive factor; SNX1, sorting nexin1; Vps4, vacuolar protein sorting 4.

somes. Recycling of internalized membrane proteins, such as the transferrin receptor, along with major lipid constituents to the plasma membrane occurs constitutively and independent of sorting signals (Trowbridge et al., 1993; Hao and Maxfield, 2000). In contrast, recycling of certain GPCRs requires specific information residing in the intracytosolic domains of the receptor (Oakley et al., 1999; Trejo and Coughlin, 1999; Innamorati et al., 2001). One of the best-characterized GPCR endocytic-sorting motifs is the β_2-adrenergic receptor (β_2AR) tetrapeptide sequence found at the extreme C terminus of the receptor that conforms to a classic type I PDZ domain ligand. This PDZ ligand sequence is both necessary and sufficient for efficient β_2AR recycling and binds to the PDZ domain present in NHERF (Na^+/H^+ exchanger regulatory factor)/EBP50 (ezrin/radixin/moesin-binding phosphoprotein of 50 kDa) family proteins (Hall et al., 1998; Cao et al., 1999). A similar type I PDZ domain ligand sequence is found in the distal region of the β_1AR cytoplasmic tail and is capable of promoting PDZ domain-mediated protein interactions and receptor recycling (Gage et al., 2005). Several other GPCRs also possess type I or II PDZ ligand sequences located at the very C terminus of the receptor (Heydorn et al., 2004). Thus, in addition to their well characterized role in scaffolding signaling complexes, these findings raise the intriguing possibility that PDZ-mediated protein-protein interactions may have a more general function in regulating GPCR trafficking.

The current article by Paasche et al. (2005) has identified an internal PDZ ligand peptide sequence present in the ET_A receptor cytoplasmic tail that seems to regulate receptor recycling. This finding now expands the diversity of PDZ ligands present in GPCR cytoplasmic domains. In addition to binding to short C-terminal peptide sequences, a less common mode of PDZ domain binding involves internal peptide sequences that fold into a β-finger structure (Hillier et al., 1999). Intermolecular binding of a PDZ domain with an internal peptide sequence is best characterized for the interaction between neuronal nitric-oxide synthase (nNOS) and the PDZ domain of PSD-95 or syntrophin. Although the region of nNOS essential for binding to the PDZ domain of syntrophin includes the PDZ domain of nNOS itself, crystallographic studies indicate that the major contacts occur between the syntrophin PDZ domain and a nonterminal hairpin β-finger in the PDZ flanking region of nNOS (Hillier et al., 1999). In this case, the sharp β-turn replaces the C-terminal free carboxylate group normally required for PDZ binding. Although the PDZ domain of nNOS is critically important for stabilization of the β-finger structure, other proteins may display PDZ interactions with internal peptide sequences that are not adjacent to PDZ domains, as is likely to be the case for ET_A receptor and other GPCRs.

Paasche et al. (2005) evaluated ET_A receptor cytoplasmic tail truncation mutants and identified a short peptide sequence essential for efficient receptor recycling. This sequence displayed significant homology to a region found in several other proteins (including nNOS) that adopt a β-finger structure (Hillier et al., 1999). A three-dimensional model of the peptide sequence was constructed based on the nNOS β-finger structure and revealed the presence of a critical amino acid sequence conforming to the required -X-S/T-X-ϕ- motif that inserts into the PDZ binding groove and makes most of the energetically favorable critical contacts. The ET_A receptor proximal β-strand -M-S-T-V- motif is followed by diverse highly degenerate sequences thought to form a β-turn, and then more conserved sequences conforming to the distal strand of the β-finger. Site-directed mutagenesis was then used to interrogate the function of residues critical for the structural integrity of the putative ET_A receptor internal β-finger PDZ ligand. Mutations predicted to disrupt β-finger structure virtually ablated recycling of the ET_A receptor. However, some mutations also caused significant decreases in the initial rate of ET_A receptor internalization, suggesting a broader role for the internal PDZ ligand sequence in regulation of GPCR trafficking. The authors also screened several hundred GPCR cytoplasmic tail sequences and discovered the presence of internal PDZ ligand-like sequences in 27 distinct GPCRs. Thus, both C-terminal and internal PDZ ligands could possibly have diverse functions in the regulation of GPCR trafficking. Besides NHERF/EBP50, which seems to bind selectively to the β_2AR, the identity of other PDZ domain containing proteins that bind to the ET_A receptor and/or other GPCRs and function in receptor trafficking has not been determined.

It is known that arrestin binding to activated GPCRs involves phosphorylation-recognition sites and, in some cases, the stability of such interactions controls the kinetics of receptor recycling and resensitization (Oakley et al., 2001). Thus, phosphorylation of GPCRs is also likely to provide an additional level of regulation for PDZ-mediated interactions. Activated GPCRs are rapidly phosphorylated on serine and threonine residues by G protein-coupled receptor kinases (GRKs) and second-messenger regulated kinases. One of the critical residues for PDZ recognition at the -2 position is frequently a phosphorylatable residue, such as threonine, serine, or tyrosine. Indeed, phosphorylation of the -2 position serine residue of the β_2AR type I PDZ ligand sequence by GRK5 disrupts interaction with NHERF/EBP50 and receptor recycling (Cao et al., 1999). It is not known whether critical residues conforming to the internal PDZ ligand of the ET_A receptor are phosphorylated and regulate PDZ domain-mediated interactions in a similar manner.

In addition to PDZ ligand sequences, tyrosine-based motifs and lysine residues capable of being ubiquitinated regulate GPCR trafficking. Tyrosine-based motifs conforming to the -Y-X-X-ϕ- peptide sequence can function as purely endocytic signals and also have the capacity to target transmembrane proteins to lysosomes; the latter are often found at the extreme C terminus of the protein (Bonifacino and Traub, 2003). The activity of this type of sorting signal requires that the critical tyrosine be in an unphosphorylated state. A tyrosine-based sequence -Y-S-I-L- in the cytoplasmic tail of

protease-activated receptor-1 was recently shown to function in internalization and lysosomal sorting (Paing et al., 2004). Moreover, a human GPCR cytoplasmic tail sequence database screen revealed the presence of canonical tyrosine-based motifs -Y-X-X-ϕ- in 45 distinct GPCRs. It is important to distinguish the tyrosine-based -Y-X-X-ϕ- motifs from highly conserved -N/D-P-X_{2-3}-Y- sequences found at the end of the seventh transmembrane domain of GPCRs. The -N/D-P-X_{2-3}-Y- motif seems to be critical for maintaining the structural integrity of the receptor protein and unlikely to directly regulate GPCR trafficking. Last, ubiquitin modification of several GPCRs has also recently been shown to serve as a targeting signal for lysosomal sorting and receptor degradation (Marchese and Benovic, 2001; Shenoy et al., 2001; Marchese et al., 2003). Thus, like PDZ ligand sequences, tyrosine-based motifs and ubiquitin modification could have broad functions in regulation of GPCR trafficking, including endocytosis, recycling, lysosomal sorting, and perhaps basolateral sorting in polarized cells. An obvious major challenge now is to identify the protein machinery that interacts with these sorting motifs to regulate GPCR trafficking.

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