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 intracellular 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 ETA 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 intracellular 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).

ABRREVIATIONS: 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.
PDZ interactions with internal peptide sequences that are not adjacent to PDZ domains, as is likely to be the case for ET₄ receptor and other GPCRs.

Paasche et al. (2005) evaluated ET₄ 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 ET₄ receptor proximal ϕ-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 ET₄ receptor internal β-finger PDZ ligand. Mutations predicted to disrupt β-finger structure virtually ablated recycling of the ET₄ receptor. However, some mutations also caused significant decreases in the initial rate of ET₄ 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 β₂AR, the identity of other PDZ domain containing proteins that bind to the ET₄ 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 β₂AR 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 ET₄ 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-X₂₋₃-Y- sequences found at the end of the seventh transmembrane domain of GPCRs. The -N/D-P-X₂₋₃-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.

References


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