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Regulation of α_{2B} -Adrenergic Receptor Export Trafficking by Specific Motifs

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Abstract

Intracellular trafficking and precise targeting to specific locations of G protein-coupled receptors (GPCRs) control the physiological functions of the receptors. Compared to the extensive efforts dedicated to understanding the events involved in the endocytic and recycling pathways, the molecular mechanisms underlying the transport of the GPCR superfamily from the endoplasmic reticulum (ER) through the Golgi to the plasma membrane are relatively less well defined. Over the past years, we have used α_{2B} -adrenergic receptor (α_{2B} -AR) as a model to define the factors that control GPCR export trafficking. In this chapter, we will review specific motifs identified to mediate the export of nascent α_{2B} -AR from the ER and the Golgi and discuss the possible underlying mechanisms. As these motifs are highly conserved among GPCRs, they may provide common mechanisms for export trafficking of these receptors.

1. EXPORT TRAFFICKING OF GPCRs

The transport of cell surface proteins (such as receptors, channels, and transporters) has been considered as a constitutive process. However, over the past decade, many studies have shown that their transport is regulatable, in a cell type- and cargo-specific manner, and can be mediated through non-classical routes.^{1–5} As the largest superfamily of cell surface receptors involved in signal regulation in cells, the precise function of G protein-coupled receptors (GPCRs) is under control by spatial–temporal regulation of their intracellular trafficking which dictates the amount of receptor expression at their functional destinations and the magnitude of the cellular response to a given signal. Over the past decades, most studies on GPCR trafficking have focused on their internalization, recycling, and degradation pathways.^{6–9} However, the molecular mechanisms underlying the anterograde transport of newly synthesized GPCRs from the endoplasmic reticulum (ER) to the cell surface have just begun to be revealed.

It has become increasingly apparent that similar to the endocytic pathway, the anterograde trafficking of GPCRs is a complicated and highly regulated cellular process which is orchestrated by the structural features of the receptors and by many regulatory proteins. Specifically, (1) GPCR transport to the cell surface is regulated by extracellular stimuli^{10–12} and mediated through multiple pathways^{13–15}; (2) GPCR export from the ER and the Golgi

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is dictated by highly conserved motifs^{16–29}; (3) dimerization and post-translational modifications (such as N-linked glycosylation) also play important roles in GPCR export from the ER to the cell surface³⁰; (4) GPCR export is modulated by a multitude of regulatory proteins such as Rab GTPases, ER chaperones, and receptor activity-modifying proteins, which may facilitate receptor maturation, stabilize receptor conformation, and promote receptor delivery to the plasma membrane.^{13–15,31–42}

α_2 -Adrenergic receptors (α_2 -ARs) are prototypic GPCRs that have three subtypes, α_{2A} -AR, α_{2B} -AR, and α_{2C} -AR, all of which have an important role in regulating sympathetic nervous system, both peripherally and centrally. All three α_2 -ARs have similar structural features: the third intracellular loop (ICL3) is quite large with more than 170 amino-acid residues, whereas other loops and the termini are relatively short with less than 25 residues (Fig. 1). Over the past several years, we have mainly used α_{2B} -AR as a representative to investigate the export trafficking of GPCRs. We have identified several highly conserved motifs, in both termini and intracellular loops, which are essential for the receptors to exit from the ER and the Golgi apparatus and subsequent transport to the cell surface. The locations of these motifs in α_{2B} -AR are summarized in Fig. 1. In this chapter, we will review the roles of these motifs in export trafficking of α_{2B} -AR, as well as other GPCRs, and discuss the possible underlying mechanisms.

2. REGULATION OF α_{2B} -AR EXPORT FROM THE ER

Similar to many other plasma membrane proteins, the life of GPCRs begins in the ER where they are synthesized. Once correctly folded and properly assembled, nascent receptors are able to pass the ER quality-control system and exit from the ER, beginning their long journey of intracellular trafficking. The receptors then move through several successive intracellular compartments, which include the ER–Golgi intermediate compartment, the *cis*/medial/*trans*-Golgi apparatus, and the *trans*-Golgi network (TGN), *en route* to the cell surface. As the first step in intracellular trafficking of GPCRs, export from the ER is the rate-limiting step in receptor targeting to the cell surface and markedly affects the kinetics of receptor maturation.⁴³ Our studies have identified a single Leu residue in the first intracellular loop (ICL1), a triple Arg motif (3R) in the ICL3, and a hydrophobic motif F(x)₆IL as well as a positively charged motif R(x)₃R(x)₄R in the C-terminus, all of which are required for α_{2B} -AR export from the ER.

2.1 Single Leu Residue in the ICL1

The ICL1 of α_{2B} -AR is very short (Fig. 1) containing Leu48 in the center. This single Leu residue is remarkably conserved among the GPCR superfamily (Fig. 2): about 85% of the family A GPCRs in humans and 83% in all species have this single Leu residue.⁴⁴ We found that deleting the ICL1 or mutating Leu48 residue in the ICL1 caused extensive ER accumulation of α_{2B} -AR, indicating that Leu48 plays a crucial role in α_{2B} -AR exit from the ER. Because mutation of Leu48 to Ile, Val, Tyr, and Trp similarly abolished α_{2B} -AR export from the ER while substitution of Leu48 for Phe preserved α_{2B} -AR transport, the function of the Leu48 residue in modulating α_{2B} -AR export is mediated by its specific physiochemical and structural features including overall size of the side chain, spacing

between the bulky portion of the side chains and the α -carbon, and polarity. In addition to α_{2B} -AR export, mutation of the Leu residue in the ICL1 also significantly attenuated the cell surface expression of β_2 -AR, α_{1B} -AR, and angiotensin II type 1 receptor (AT1R).⁴⁴ Based on the crystal structures of GPCRs, the side chain of this conserved Leu residue inserts into the transmembrane bundle, suggesting that this Leu residue is most likely involved in receptor folding to a specific conformation which is crucial for receptor export from the ER.

2.2 The Triple Arg Motif (3R) in the ICL3

Protein export from the ER is exclusively mediated through the COPII-coated vesicles. In order to be efficiently exported in COPII vesicles, cargo proteins, particularly transmembrane proteins, may use ER export motifs in their cytoplasmic C-termini to bind to the components of COPII vesicles, particularly Sec24 subunits. There are four Sec24 isoforms (Sec24A, Sec24B, Sec24C, and Sec24D) identified in human cells, and these can be further divided into Sec24A/B and Sec24C/D subclasses based on the sequence homology. Interaction of ER export motifs with Sec24 enhances the recruitment of the cargo on ER exit sites and facilitates cargo export from the ER. Although the diacidic, dihydrophobic, and dibasic motifs have all been identified to function as ER export motifs in non-GPCR membrane proteins,^{45–57} the diacidic motifs are the most well characterized to control ER export of several proteins, including vesicular stomatitis viral glycoprotein (VSVG), cystic fibrosis transmembrane conductance regulator, and potassium channels. It has also been described that different ER export motifs may preferentially interact with certain Sec24 isoforms. For example, the FF motif may bind to all four Sec24 isoforms, whereas the IxM, LxxLE, and DxE export signals can interact with different Sec24 isoforms.⁵⁵

We have identified three basic Arg residues (3R) located in the ICL3 as a novel ER export code to direct α_{2B} -AR exit from the ER.²⁸ Similar to the single Leu residue in the ICL1, triple basic residues are also highly conserved in the ICL3 of GPCRs. The 3R motif has several important properties that are very similar to well-characterized ER export motifs in non-GPCR membrane proteins. First, the 3R motif mediates α_{2B} -AR interaction with Sec24 isoforms as determined in GST fusion protein pull-down and co-immunoprecipitation assays using exogenously expressed, endogenous and purified Sec24. Thus, the 3R motif represents the first motif in the GPCR superfamily which is able to physically associate with components of the COPII vesicles. More interestingly, the interaction of the 3R motif with Sec24C/D isoforms was much stronger than Sec24A/B isoforms (Fig. 3A), supporting the notion that the ER sorting signals may have selectivity toward distinct Sec24 isoforms. Based on the studies showing that mutation of the 3R motif to Ala, Gln, or Glu markedly attenuated or abolished the interaction, while substitution of the 3R motif to Lys preserved the interaction (Fig. 3B), we can conclude that the interaction is likely ionic.

Second, the export function of the 3R motif is independent of its position within α_{2B} -AR. The localization of the 3R motif in the ICL3 of α_{2B} -AR is in marked contrast to other Sec24-interacting ER export motifs identified thus far which are exclusively localized in the C-terminal regions of membrane proteins. We found that the addition of the 3R motif to the C-terminus not only promoted the cell surface export of α_{2B} -AR but also completely rescued

the defective cell surface transport of mutated α_{2B} -AR, in which the 3R motif of the ICL3 was mutated. These data strongly indicate that the 3R motif, either in the ICL3 or in the C-terminus, can function as an export signal to direct receptor exit from the ER.

Third, export function of the 3R motif is transferable to other proteins. It has been well defined that linear ER export motifs are capable of conferring their transport function to other proteins which are normally expressed in the ER^{46,47,53} as long as they are accessible to the COPII transport machinery. For example, the export function of the diacidic motifs can be conferred to CD8 glycoprotein. We demonstrated that transplantation of an ICL3 fragment containing the 3R motif was able to translocate CD8 glycoprotein to the cell surface and mutation of the 3R motif abolished this function. These data further support that the 3R motif is an independent and linear ER export signal.

It is interesting to note that dibasic motifs such as KKxx and RxR motifs have been demonstrated to function as ER retention or retrieval signals for ER-resident proteins which is likely mediated through interacting with members of the COPI vesicles.⁵⁸ The RxR motif has also been found to be responsible for ER retention of γ -aminobutyric acid type B receptor.⁵⁹ These data suggest that basic residues may have multiple or even opposing effects on GPCR trafficking.

2.3 The Hydrophobic Motif F(x)₆LL in the C-Terminus

Similar to many other GPCRs, the C-terminal tail of α_{2B} -AR consists of a putative amphipathic 8th α -helix in the membrane-proximal region and a nonstructural membrane-distal region (Fig. 1). Our studies have demonstrated that dileucine residues I443/L444 together with the F436 residue in the membrane-proximal portion are essential for α_{2B} -AR export from the ER and transport to the cell surface⁶⁰ (Fig. 1); and the function of F436 and I443/L444 in mediating α_{2B} -AR export cannot be fully substituted by any other hydrophobic residues.²⁶ Furthermore, the F(x)₆LL motif (where x can be any residue and L leucine or isoleucine) is highly conserved in the membrane-proximal C-termini of many family A GPCRs.⁶⁰ Indeed, further studies have shown that this motif is also required for ER export of α_{1B} -AR, β_2 -AR, AT1R, and M1-muscarinic receptor.^{19,26}

The function of the C-terminus, particularly the membrane-proximal 8th α -helical portion, in regulating cell surface transport of the receptors has been described for a number of GPCRs. Mutagenesis studies of the membrane-proximal C-termini have led to the identification of LL motif and several other hydrophobic motifs such as E(x)₃LL, FN(x)₂LL(x)₃L, F(x)₃F(x)₃F, L(x)₃F(x)₃F, and Y(x)₃F(x)₃F. These motifs are required for the ER export and cell surface transport of α_{1B} -AR, serotonin 5HT1A and 5-HT1B receptors, dopamine D1 receptor, vasopressin V2 and V3 receptors, and neuropeptide Y2 receptor.^{18–24,60–62}

Although F(x)₆LL and other hydrophobic motifs are well demonstrated to mediate GPCR export from the ER, the underlying molecular mechanisms remain elusive. Because these motifs are located in the membrane-proximal C-terminal α -helical region, it is reasonable to assume that mutation of these motifs will most likely disrupt proper receptor folding in the ER; therefore, mutated receptors are unable to export from the ER. This possibility is

supported by the fact that mutating the motifs caused the receptors to lose their ligand-binding abilities, and the defective transport of the mutated receptors can be rescued by pharmacological and molecular chaperones.^{24,26,59,61} However, it remains to be determined if wild-type receptors, once arrested in the ER by mutating these hydrophobic motifs, are still able to bind to their ligands. Furthermore, we have demonstrated that the LL motif mediates β_2 -AR interaction with Rab8,³⁶ a small GTPase involved in the post-Golgi transport, suggesting that the hydrophobic motifs in the C-termini of GPCRs may have multiple functions (see Section 3.3).

2.4 The Positively Charged Motif R(x)₃R(x)₄R in the C-Terminus

To elucidate the molecular mechanisms responsible for the function of the C-termini in GPCR transport from the ER to the cell surface, we searched for proteins interacting with the C-terminus of α_{2B} -AR by using peptide-conjugated affinity matrix combined with proteomics. This strategy identified tubulin directly interacting with α_{2B} -AR.⁶³ Subsequent studies revealed that tubulin also bound to the C-termini of α_{2A} -AR and AT1R, but not β_2 -AR⁶⁴ (Fig. 4A and B). Mutagenesis analysis of the C-terminus identified R437, R441, and R446, which form the motif R(x)₃R(x)₄R in the membrane-proximal region, as responsible for tubulin interaction. Importantly, mutation of these three Arg residues abolished receptor transport to the cell surface, and receptor mutants were extensively arrested in the ER. These data provide evidence indicating that the cargo GPCRs may directly contact with the microtubule network to coordinate their own ER-to-cell surface traffic.

Based on the structural homology modeling using high-resolution crystal structure of β_2 -AR,⁶⁵ the positively charged residues R437/R441/R446 in the α_{2B} -AR C-terminus likely project from the same side on the cytosolic face (Fig. 4C). This structural feature of positively charged residues is shared by many family A GPCRs. These basic residues appear to cluster on helix 8 in an amphipathic pattern, spaced apart by one or two residues, to ensure their presentation on the same face of the helical structure. It is possible that these highly conserved positively charged residues in the C-termini of GPCRs have multiple functions, including regulating proper receptor folding in the ER and subsequent export from the ER through interaction with tubulin.

It is well known that the microtubule network modulates many intracellular trafficking processes including ER export and ER-to-Golgi transport.^{66–70} Microtubules are characterized by hollow tubes of polymerized α - and β -tubulin dimers. Both α - and β -tubulin contain the highly acidic EE_xEEY/F motif in the flexible CT. This motif does not play a major role in maintaining the microtubule structure. Instead, this motif coats the outer surface of microtubules to mediate microtubule interaction with many proteins, including motor proteins, and to regulate microtubule dynamics. For example, microtubule plus-end tracking proteins, such as cytoplasmic linker protein 170, use highly basic grooves in the CAP-Gly domain to target the tubulin C-terminal acidic motif.^{71,72} The α_{2B} -AR-binding domain was also mapped to the tubulin C-terminus, as the tubulin mutant lacking the C-terminus prepared by limited proteolysis did not interact with α_{2B} -AR (Fig. 4D).⁶³ These data indicate that the interaction of α_{2B} -AR with tubulin is ionic in nature. These data also

suggest that the microtubule association with GPCRs and many other proteins likely share similar structural features.

3. REGULATION OF α_{2B} -AR POST-GOLGI TRANSPORT

The Golgi/TGN compartment is often referred to as the “sorting center” where newly synthesized proteins are sorted to be delivered to their final cellular destinations such as endosomes, lysosomes, and the plasma membrane. As discussed above, protein exit from the ER is mediated through COPII-coated vesicles and directed by ER export signals. Similarly, post-Golgi transport can be mediated through clathrin-coated transport vesicles, and specific motifs can enhance cargo recruitment onto the vesicles. Clathrin-coated vesicles are composed of clathrin and various adaptor proteins, including heterotetrameric adaptor protein (AP) complexes, GGAs (Golgi-associated, γ -adaptin homologous, ARF-interacting proteins), and hepatocyte growth factor receptor substrate (Hrs). The tyrosine-based motifs, NPxY and Yxx $\emptyset$ (where x can be any residue and $\emptyset$ is a hydrophobic residue), and the dileucine-based motifs, [D/E]xxxL[L/I] and DxxLL, are well-defined endosomal sorting signals which mediate cargo protein interaction with the AP complex of TGN-derived transport vesicles and sort the cargo into the TGN-to-endosomes pathway.^{73–75} Whereas Yxx $\emptyset$ and [D/E]xxxL[L/I] motifs are recognized by the AP complexes, DxxLL is recognized by GGAs.⁷⁶ Although clathrin/AP-coated vesicles have been demonstrated to mediate endocytosis of agonist-occupied GPCRs from the plasma membrane to the endosomes, the vesicles involved in the transport of newly synthesized GPCRs from the Golgi/TGN to the plasma membrane remain unknown.

3.1 The GGA-Binding Motif in the ICL3

There are three GGA isoforms in humans (GGA1, GGA2, and GGA3) with identical domain organizations. The GGA protein is composed of the N-terminal VHS (the Vps27, Hrs, Stam) domain followed by the GAT (GGAs and TOM1) domain, the hinge region, and the C-terminal GAE (γ -adaptin ear) domain. Each domain of GGAs has been shown to interact with specific proteins to coordinate their trafficking functions. The VHS domain has been well defined to interact with the DxxLL-type sorting motifs of cargo proteins such as cation-dependent and cation-independent mannose 6-phosphate receptors,^{76–80} sortilin,⁸¹ sorting-protein-related receptor,⁸² low-density lipoprotein receptor-related proteins,^{83,84} and β -secretase.⁸⁵ These highly coordinated VHS-DxxLL signal interactions specifically sort cargo proteins into the TGN-to-endosome pathway.⁸⁶ The GAT domain has been identified to interact with GTP-bound ARF1, and this interaction, together with PIP4, provides molecular anchors for the recruitment of GGAs onto the TGN.^{87–91} The hinge region interacts with clathrin which is responsible for the recruitment of clathrin onto the TGN, leading to the formation of clathrin-coated vesicles. The C-terminal GAE domain of GGAs associates with a number of accessory proteins.^{92–94}

Our recent studies have shown that shRNA-mediated depletion of individual GGAs strongly arrested α_{2B} -AR in the TGN and significantly reduced receptor cell surface expression (unpublished data). We further demonstrated that GGAs physically associated with α_{2B} -AR through specific domains. The GGA-binding domain was mapped to the ICL3 of the receptor, and progressive deletion of the ICL3 identified that the fragment R285-E369

mainly mediated α_{2B} -AR interaction with GGAs (Fig. 1). Surprisingly, the α_{2B} -AR-binding domains were identified to different regions of all three GGAs, specifically the GGA1 hinge, the GGA2 GAE, and the GGA3 VHS domains (unpublished data). These studies demonstrate novel functions of the GGA family proteins in the TGN-to-plasma membrane transport of GPCRs which is likely mediated through specific interactions.

3.2 The YS Motif in the N-Terminus

Similar to the C-termini, the N-termini are also important in the export trafficking of GPCRs. For example, the deletion of the N-termini facilitates the cell surface expression of α_{1D} -AR and α_{2C} -AR, suggesting that the N-termini may contain signals retaining the receptors in the ER.⁹⁵ A hydrophobic sequence in the N-terminus of α_{2C} -AR was then proven to function as an ER retention motif,⁹⁶ which provides a mechanism responsible for the intracellular accumulation of α_{2C} -AR in some cell types.

We found that the YS motif in the membrane-proximal N-terminal region is required for the transport of α_{2B} -AR to the cell surface. Substitution of Y12 and S13 residues significantly reduced the cell surface expression of α_{2B} -AR and the mutated receptors were retained in the Golgi apparatus,²⁷ suggesting that the YS motif mediates α_{2B} -AR export at the level of the Golgi. The YS motif only exists in the membrane-proximal N-termini of all three α_2 -AR family members, and indeed, it exerts a similar function on α -AR trafficking.²⁷ 2A Therefore, the YS motif may function as an export signal specifically modulating the Golgi export of the members of α_2 -AR subfamily.

As the N-terminus is positioned toward the lumen of ER and Golgi during the export process, the YS motif is not able to directly interact with components of transport machinery in the cytoplasm. Furthermore, the fact that YS mutant receptors are able to exit from the ER to reach the Golgi compartment suggests that they are properly folded. Although it is clear that the defective transport induced by mutation of the YS motif is unlikely caused by misfolding, the exact underlying mechanism remains unknown.

3.3 The Rab8-Binding Motif in the C-Terminus

Rab8 has been extensively investigated in protein transport from the TGN under polarized conditions. For GPCRs, Rab8 modulates the post-Golgi transport of rhodopsin in *Xenopus*⁹⁷ and the internalization of metabotropic glutamate receptor subtype 1.⁹⁸ We have demonstrated that Rab8 GTPase regulates the post-Golgi transport of α_{2B} -AR and β_2 -AR in several cell lines and primary neurons which is mediated through physical interactions.³⁶ More interestingly, distinct motifs in the C-termini of α_{2B} -AR and β_2 -AR were identified to interact with Rab8, and these motifs probably dictate differential regulation of these two receptors by Rab8.³⁶ The dileucine LL motif was shown to be required for β_2 -AR interaction with Rab8, whereas multiple residues in the fragments TVFN and PWTQTGW of the C-terminus were identified to modulate α_{2B} -AR interaction with Rab8. The fact that mutation of the LL motif selectively influences Rab8 interaction with β_2 -AR, but not α_{2B} -AR,³⁶ suggests that it may have different roles in the regulation of post-Golgi transport of distinct GPCRs. It is possible that, similar to the function of the diacidic ExD motif in VSVG transport,⁹⁹ a single LL motif may modulate export trafficking of some, but not all, GPCRs

(e.g., β_2 -AR) at multiple intracellular compartments. In addition to regulating ER export, it may also coordinate GPCR exit from the Golgi/TGN.

4. CONCLUDING REMARKS

It is apparent that export trafficking of α_2 B-AR is a selective process which is under control at multiple levels by a number of specific motifs embedded within the receptors. Receptor export from a specific intracellular compartment or transport along a specific pathway may be coordinated by several different motifs, whereas one motif may have multiple functions that influence receptor export trafficking at distinct transport steps. Although the precise mechanisms underlying the function of these motifs in regulating receptor export remains largely to be determined, these motifs may be involved in proper receptor folding/assembly to ensure that the receptors are able to pass through the ER quality-control system, mediate receptor interaction with specific components of the transport machinery which will enhance receptor recruitment onto specific vesicles and sort the receptors to specific transport pathways, and/or modulate receptor interaction with some regulatory proteins involved in the export processing. Further investigation, including search for proteins interacting with these motifs in the cytoplasm, particularly components of the transport machinery or other trafficking-related regulatory proteins, will help elucidate the molecular mechanisms of α_2 B-AR export trafficking. Although above-mentioned motifs are mainly identified in α_2 B-AR, the export function of these motifs has been determined in several other GPCRs. Importantly, these motifs are highly conserved among a group or family of GPCRs, and thus they may provide common mechanisms for export trafficking of these receptors. As it has become increasingly appreciated that defective export trafficking of GPCRs is linked directly to the pathogenesis of several human diseases, understanding the mechanisms of GPCR cell surface transport may provide an important foundation for the development of new therapeutic strategies in treating diseases involving abnormal GPCR trafficking.

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REFERENCES

1. Sun Y, Chiu TT, Foley KP, Bilan PJ, Klip A. Myosin Va mediates Rab8A-regulated GLUT4 vesicle exocytosis in insulin-stimulated muscle cells. *Mol Biol Cell*. 2014; 25(7):1159–1170. [PubMed: 24478457]
2. Lu W, Man H, Ju W, Trimble WS, MacDonald JF, Wang YT. Activation of synaptic NMDA receptors induces membrane insertion of new AMPA receptors and LTP in cultured hippocampal neurons. *Neuron*. 2001; 29(1):243–254. [PubMed: 11182095]
3. Ward DT, Hammond TG, Harris HW. Modulation of vasopressin-elicited water transport by trafficking of aquaporin2-containing vesicles. *Annu Rev Physiol*. 1999; 61:683–697. [PubMed: 10099706]
4. Patterson MA, Szatmari EM, Yasuda R. AMPA receptors are exocytosed in stimulated spines and adjacent dendrites in a Ras-ERK-dependent manner during long-term potentiation. *Proc Natl Acad Sci USA*. 2010; 107(36):15951–15956. [PubMed: 20733080]
5. Brewer PD, Habtemichael EN, Romenskaia I, Mastick CC, Coster AC. Insulin-regulated Glut4 translocation: membrane protein trafficking with six distinctive steps. *J Biol Chem*. 2014; 289(25):17280–17298. [PubMed: 24778187]

6. von Zastrow M. Mechanisms regulating membrane trafficking of G protein-coupled receptors in the endocytic pathway. *Life Sci.* 2003; 74(2–3):217–224. [PubMed: 14607249]
7. Marchese A, Paing MM, Temple BR, Trejo J. G protein-coupled receptor sorting to endosomes and lysosomes. *Annu Rev Pharmacol Toxicol.* 2008; 48:601–629. [PubMed: 17995450]
8. Wolfe BL, Trejo J. Clathrin-dependent mechanisms of G protein-coupled receptor endocytosis. *Traffic.* 2007; 8(5):462–470. [PubMed: 17376169]
9. Reiter E, Ahn S, Shukla AK, Lefkowitz RJ. Molecular mechanism of beta-arrestin-biased agonism at seven-transmembrane receptors. *Annu Rev Pharmacol Toxicol.* 2012; 52:179–197. [PubMed: 21942629]
10. Yin H, Li Q, Qian G, et al. Rab1 GTPase regulates phenotypic modulation of pulmonary artery smooth muscle cells by mediating the transport of angiotensin II type 1 receptor under hypoxia. *Int J Biochem Cell Biol.* 2011; 43(3):401–408. [PubMed: 21095238]
11. Li Y, Wang G, Lin K, et al. Rab1 GTPase promotes expression of beta-adrenergic receptors in rat pulmonary microvascular endothelial cells. *Int J Biochem Cell Biol.* 2010; 42(7):1201–1209. [PubMed: 20417717]
12. Bie B, Zhang Z, Cai YQ, et al. Nerve growth factor-regulated emergence of functional delta-opioid receptors. *J Neurosci.* 2010; 30(16):5617–5628. [PubMed: 20410114]
13. Wu G, Zhao G, He Y. Distinct pathways for the trafficking of angiotensin II and adrenergic receptors from the endoplasmic reticulum to the cell surface: Rab1-independent transport of a G protein-coupled receptor. *J Biol Chem.* 2003; 278(47):47062–47069. [PubMed: 12970354]
14. Filipeanu CM, Zhou F, Fugetta EK, Wu G. Differential regulation of the cell-surface targeting and function of beta- and alpha1-adrenergic receptors by Rab1 GTPase in cardiac myocytes. *Mol Pharmacol.* 2006; 69(5):1571–1578. [PubMed: 16461589]
15. Wang G, Wu G. Small GTPase regulation of GPCR anterograde trafficking. *Trends Pharmacol Sci.* 2012; 33(1):28–34. [PubMed: 22015208]
16. Dong C, Filipeanu CM, Duvernay MT, Wu G. Regulation of G protein-coupled receptor export trafficking. *Biochim Biophys Acta.* 2007; 1768(4):853–870. [PubMed: 17074298]
17. Zhang X, Dong C, Wu QJ, Balch WE, Wu G. Di-acidic motifs in the membrane-distal C termini modulate the transport of angiotensin II receptors from the endoplasmic reticulum to the cell surface. *J Biol Chem.* 2011; 286(23):20525–20535. [PubMed: 21507945]
18. Carrel D, Hamon M, Darmon M. Role of the C-terminal di-leucine motif of 5-HT1A and 5-HT1B serotonin receptors in plasma membrane targeting. *J Cell Sci.* 2006; 119(Pt 20):4276–4284. [PubMed: 17003106]
19. Sawyer GW, Ehlert FJ, Shults CA. A conserved motif in the membrane proximal C-terminal tail of human muscarinic m1 acetylcholine receptors affects plasma membrane expression. *J Pharmacol Exp Ther.* 2010; 332(1):76–86. [PubMed: 19841475]
20. Guo Y, Jose PA. C-terminal di-leucine motif of dopamine D(1) receptor plays an important role in its plasma membrane trafficking. *PLoS One.* 2011; 6(12):e29204. [PubMed: 22206002]
21. Donnellan PD, Kimbembe CC, Reid HM, Kinsella BT. Identification of a novel endoplasmic reticulum export motif within the eighth alpha-helical domain (alpha-H8) of the human prostacyclin receptor. *Biochim Biophys Acta.* 2011; 1808(4):1202–1218. [PubMed: 21223948]
22. Bermak JC, Li M, Bullock C, Zhou QY. Regulation of transport of the dopamine D1 receptor by a new membrane-associated ER protein. *Nat Cell Biol.* 2001; 3(5):492–498. [PubMed: 11331877]
23. Robert J, Auzan C, Ventura MA, Clauser E. Mechanisms of cell-surface rerouting of an endoplasmic reticulum-retained mutant of the vasopressin V1b/V3 receptor by a pharmacological chaperone. *J Biol Chem.* 2005; 280(51):42198–42206. [PubMed: 16210325]
24. Schulein R, Hermosilla R, Oksche A, et al. A dileucine sequence and an upstream glutamate residue in the intracellular carboxyl terminus of the vasopressin V2 receptor are essential for cell surface transport in COS.M6 cells. *Mol Pharmacol.* 1998; 54(3):525–535. [PubMed: 9730911]
25. Dong C, Nichols CD, Guo J, Huang W, Lambert NA, Wu G. A triple Arg motif mediates alpha2B-adrenergic receptor interaction with Sec24C/D and export. *Traffic.* 2012; 13(6):857–868. [PubMed: 22404651]

26. Duvernay MT, Dong C, Zhang X, Zhou F, Nichols CD, Wu G. Anterograde trafficking of G protein-coupled receptors: function of the C-terminal F(X)6LL motif in export from the endoplasmic reticulum. *Mol Pharmacol*. 2009; 75(4):751–761. [PubMed: 19118123]
27. Dong C, Wu G. Regulation of anterograde transport of alpha2-adrenergic receptors by the N termini at multiple intracellular compartments. *J Biol Chem*. 2006; 281(50):38543–38554. [PubMed: 17038316]
28. Dong C, Nichols CD, Guo J, Huang W, Lambert NA, Wu G. A triple arg motif mediates alpha(2B)-adrenergic receptor interaction with Sec24C/D and export. *Traffic*. 2012; 13(6):857–868. [PubMed: 22404651]
29. Hirota N, Yasuda D, Hashidate T, et al. Amino acid residues critical for endoplasmic reticulum export and trafficking of platelet-activating factor receptor. *J Biol Chem*. 2010; 285(8):5931–5940. [PubMed: 20007715]
30. Terrillon S, Bouvier M. Roles of G-protein-coupled receptor dimerization. *EMBO Rep*. 2004; 5(1):30–34. [PubMed: 14710183]
31. McLatchie LM, Fraser NJ, Main MJ, et al. RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. *Nature*. 1998; 393(6683):333–339. [PubMed: 9620797]
32. Colley NJ, Baker EK, Stamnes MA, Zuker CS. The cyclophilin homolog ninaA is required in the secretory pathway. *Cell*. 1991; 67(2):255–263. [PubMed: 1913822]
33. Dwyer ND, Troemel ER, Sengupta P, Bargmann CI. Odorant receptor localization to olfactory cilia is mediated by ODR-4, a novel membrane-associated protein. *Cell*. 1998; 93(3):455–466. [PubMed: 9590179]
34. Ferreira PA, Nakayama TA, Pak WL, Travis GH. Cyclophilin-related protein RanBP2 acts as chaperone for red/green opsin. *Nature*. 1996; 383(6601):637–640. [PubMed: 8857542]
35. Achour L, Scott MG, Shirvani H, et al. CD4-CCR5 interaction in intracellular compartments contributes to receptor expression at the cell surface. *Blood*. 2009; 113(9):1938–1947. [PubMed: 19064722]
36. Dong C, Yang L, Zhang X, et al. Rab8 interacts with distinct motifs in alpha2B- and beta2-adrenergic receptors and differentially modulates their transport. *J Biol Chem*. 2010; 285(26):20369–20380. [PubMed: 20424170]
37. Filipeanu CM, Zhou F, Claycomb WC, Wu G. Regulation of the cell surface expression and function of angiotensin II type 1 receptor by Rab1-mediated endoplasmic reticulum-to-Golgi transport in cardiac myocytes. *J Biol Chem*. 2004; 279(39):41077–41084. [PubMed: 15252015]
38. Dong C, Zhang X, Zhou F, et al. ADP-ribosylation factors modulate the cell surface transport of G protein-coupled receptors. *J Pharmacol Exp Ther*. 2010; 333(1):174–183. [PubMed: 20093398]
39. Dong C, Zhou F, Fugetta EK, Filipeanu CM, Wu G. Endoplasmic reticulum export of adrenergic and angiotensin II receptors is differentially regulated by Sar1 GTPase. *Cell Signal*. 2008; 20(6):1035–1043. [PubMed: 18378118]
40. Dong C, Wu G. Regulation of anterograde transport of adrenergic and angiotensin II receptors by Rab2 and Rab6 GTPases. *Cell Signal*. 2007; 19(11):2388–2399. [PubMed: 17716866]
41. Xia Z, Gray JA, Compton-Toth BA, Roth BL. A direct interaction of PSD-95 with 5-HT2A serotonin receptors regulates receptor trafficking and signal transduction. *J Biol Chem*. 2003; 278(24):21901–21908. [PubMed: 12682061]
42. Zhang X, Wang G, Dupre DJ, et al. Rab1 GTPase and dimerization in the cell surface expression of angiotensin II type 2 receptor. *J Pharmacol Exp Ther*. 2009; 330(1):109–117. [PubMed: 19357319]
43. Petaja-Repo UE, Hogue M, Laperriere A, Bhalla S, Walker P, Bouvier M. Newly synthesized human delta opioid receptors retained in the endoplasmic reticulum are retrotranslocated to the cytosol, deglycosylated, ubiquitinated, and degraded by the proteasome. *J Biol Chem*. 2001; 276(6):4416–4423. [PubMed: 11054417]
44. Duvernay MT, Dong C, Zhang X, Robitaille M, Hebert TE, Wu G. A single conserved leucine residue on the first intracellular loop regulates ER export of G protein-coupled receptors. *Traffic*. 2009; 10(5):552–566. [PubMed: 19220814]
45. Wang X, Matteson J, An Y, et al. COPII-dependent export of cystic fibrosis transmembrane conductance regulator from the ER uses a di-acidic exit code. *J Cell Biol*. 2004; 167(1):65–74. [PubMed: 15479737]

46. Ma D, Zerangue N, Lin YF, et al. Role of ER export signals in controlling surface potassium channel numbers. *Science*. 2001; 291(5502):316–319. [PubMed: 11209084]
47. Votsmeier C, Gallwitz D. An acidic sequence of a putative yeast Golgi membrane protein binds COPII and facilitates ER export. *EMBO J*. 2001; 20(23):6742–6750. [PubMed: 11726510]
48. Zuzarte M, Rinne S, Schlichthorl G, Schubert A, Daut J, Preisig-Muller R. A di-acidic sequence motif enhances the surface expression of the potassium channel TASK-3. *Traffic*. 2007; 8(8): 1093–1100. [PubMed: 17547699]
49. Mikosch M, Kaberich K, Homann U. ER export of KAT1 is correlated to the number of acidic residues within a triacidic motif. *Traffic*. 2009; 10(10):1481–1487. [PubMed: 19659502]
50. Giraudo CG, Maccioni HJ. Endoplasmic reticulum export of glycosyltransferases depends on interaction of a cytoplasmic dibasic motif with Sar1. *Mol Biol Cell*. 2003; 14(9):3753–3766. [PubMed: 12972562]
51. Farhan H, Reiterer V, Korkhov VM, Schmid JA, Freissmuth M, Sitte HH. Concentrative export from the endoplasmic reticulum of the gamma-aminobutyric acid transporter 1 requires binding to SEC24D. *J Biol Chem*. 2007; 282(10):7679–7689. [PubMed: 17210573]
52. Miller EA, Beilharz TH, Malkus PN, et al. Multiple cargo binding sites on the COPII subunit Sec24p ensure capture of diverse membrane proteins into transport vesicles. *Cell*. 2003; 114(4): 497–509. [PubMed: 12941277]
53. Nishimura N, Balch WE. A di-acidic signal required for selective export from the endoplasmic reticulum. *Science*. 1997; 277(5325):556–558. [PubMed: 9228004]
54. Nishimura N, Bannykh S, Slabough S, et al. A di-acidic (DXE) code directs concentration of cargo during export from the endoplasmic reticulum. *J Biol Chem*. 1999; 274(22):15937–15946. [PubMed: 10336500]
55. Mancias JD, Goldberg J. Structural basis of cargo membrane protein discrimination by the human COPII coat machinery. *EMBO J*. 2008; 27(21):2918–2928. [PubMed: 18843296]
56. Mossessova E, Bickford LC, Goldberg J. SNARE selectivity of the COPII coat. *Cell*. 2003; 114(4): 483–495. [PubMed: 12941276]
57. Wendeler MW, Paccaud JP, Hauri HP. Role of Sec24 isoforms in selective export of membrane proteins from the endoplasmic reticulum. *EMBO Rep*. 2007; 8(3):258–264. [PubMed: 17255961]
58. Jackson MR, Nilsson T, Peterson PA. Identification of a consensus motif for retention of transmembrane proteins in the endoplasmic reticulum. *EMBO J*. 1990; 9(10):3153–3162. [PubMed: 2120038]
59. Margeta-Mitrovic M, Jan YN, Jan LY. A trafficking checkpoint controls GABA(B) receptor heterodimerization. *Neuron*. 2000; 27(1):97–106. [PubMed: 10939334]
60. Duvernay MT, Zhou F, Wu G. A conserved motif for the transport of G protein-coupled receptors from the endoplasmic reticulum to the cell surface. *J Biol Chem*. 2004; 279(29):30741–30750. [PubMed: 15123661]
61. Robert J, Clauser E, Petit PX, Ventura MA. A novel C-terminal motif is necessary for the export of the vasopressin V1b/V3 receptor to the plasma membrane. *J Biol Chem*. 2005; 280(3):2300–2308. [PubMed: 15528211]
62. Walther C, Lotze J, Beck-Sickinger AG, Morl K. The anterograde transport of the human neuropeptide Y2 receptor is regulated by a subtype specific mechanism mediated by the C-terminus. *Neuropeptides*. 2012; 46(6):335–343. [PubMed: 23020974]
63. Duvernay MT, Wang H, Dong C, Guidry JJ, Sackett DL, Wu G. Alpha2B-adrenergic receptor interaction with tubulin controls its transport from the endoplasmic reticulum to the cell surface. *J Biol Chem*. 2011; 286(16):14080–14089. [PubMed: 21357695]
64. Zhang X, Wang H, Duvernay MT, Zhu S, Wu G. The angiotensin II type 1 receptor C-terminal Lys residues interact with tubulin and modulate receptor export trafficking. *PLoS One*. 2013; 8(2):e57805. [PubMed: 23451270]
65. Rosenbaum DM, Cherezov V, Hanson MA, et al. GPCR engineering yields high-resolution structural insights into beta2-adrenergic receptor function. *Science*. 2007; 318(5854):1266–1273. [PubMed: 17962519]
66. Driskell OJ, Mironov A, Allan VJ, Woodman PG. Dynein is required for receptor sorting and the morphogenesis of early endosomes. *Nat Cell Biol*. 2007; 9(1):113–120. [PubMed: 17173037]

67. Mizuno M, Singer SJ. A possible role for stable microtubules in intracellular transport from the endoplasmic reticulum to the Golgi apparatus. *J Cell Sci.* 1994; 107(Pt 5):1321–1331. [PubMed: 7929638]
68. Presley JF, Cole NB, Schroer TA, Hirschberg K, Zaal KJ, Lippincott-Schwartz J. ER-to-Golgi transport visualized in living cells. *Nature.* 1997; 389(6646):81–85. [PubMed: 9288971]
69. Ralston E, Ploug T, Kalhovde J, Lomo T. Golgi complex, endoplasmic reticulum exit sites, and microtubules in skeletal muscle fibers are organized by patterned activity. *J Neurosci.* 2001; 21(3): 875–883. [PubMed: 11157074]
70. Saraste J, Svensson K. Distribution of the intermediate elements operating in ER to Golgi transport. *J Cell Sci.* 1991; 100(Pt 3):415–430. [PubMed: 1808196]
71. Slep KC, Vale RD. Structural basis of microtubule plus end tracking by XMAP215, CLIP-170, and EB1. *Mol Cell.* 2007; 27(6):976–991. [PubMed: 17889670]
72. Mishima M, Maesaki R, Kasa M, et al. Structural basis for tubulin recognition by cytoplasmic linker protein 170 and its autoinhibition. *Proc Natl Acad Sci USA.* 2007; 104(25):10346–10351. [PubMed: 17563362]
73. Johnson KF, Kornfeld S. The cytoplasmic tail of the mannose 6-phosphate/insulin-like growth factor-II receptor has two signals for lysosomal enzyme sorting in the Golgi. *J Cell Biol.* 1992; 119(2):249–257. [PubMed: 1400571]
74. Boucher R, Larkin H, Brodeur J, Gagnon H, Theriault C, Lavoie C. Intracellular trafficking of LRP9 is dependent on two acidic cluster/dileucine motifs. *Histochem Cell Biol.* 2008; 130(2):315–327. [PubMed: 18461348]
75. Hou JC, Suzuki N, Pessin JE, Watson RT. A specific dileucine motif is required for the GGA-dependent entry of newly synthesized insulin-responsive aminopeptidase into the insulin-responsive compartment. *J Biol Chem.* 2006; 281(44):33457–33466. [PubMed: 16945927]
76. Puertollano R, Aguilar RC, Gorshkova I, Crouch RJ, Bonifacino JS. Sorting of mannose 6-phosphate receptors mediated by the GGAs. *Science.* 2001; 292(5522):1712–1716. [PubMed: 11387475]
77. Doray B, Bruns K, Ghosh P, Kornfeld S. Interaction of the cation-dependent mannose 6-phosphate receptor with GGA proteins. *J Biol Chem.* 2002; 277(21):18477–18482. [PubMed: 11886874]
78. Zhu Y, Doray B, Poussu A, Lehto VP, Kornfeld S. Binding of GGA2 to the lysosomal enzyme sorting motif of the mannose 6-phosphate receptor. *Science.* 2001; 292(5522):1716–1718. [PubMed: 11387476]
79. Shiba T, Takatsu H, Nogi T, et al. Structural basis for recognition of acidic-cluster dileucine sequence by GGA1. *Nature.* 2002; 415(6874):937–941. [PubMed: 11859376]
80. Misra S, Puertollano R, Kato Y, Bonifacino JS, Hurley JH. Structural basis for acidic-cluster-dileucine sorting-signal recognition by VHS domains. *Nature.* 2002; 415(6874):933–937. [PubMed: 11859375]
81. Nielsen MS, Madsen P, Christensen EI, et al. The sortilin cytoplasmic tail conveys Golgi-endosome transport and binds the VHS domain of the GGA2 sorting protein. *EMBO J.* 2001; 20(9):2180–2190. [PubMed: 11331584]
82. Nielsen MS, Gustafsen C, Madsen P, et al. Sorting by the cytoplasmic domain of the amyloid precursor protein binding receptor SorLA. *Mol Cell Biol.* 2007; 27(19):6842–6851. [PubMed: 17646382]
83. Takatsu H, Katoh Y, Shiba Y, Nakayama K. Golgi-localizing, gamma-adaptin ear homology domain, ADP-ribosylation factor-binding (GGA) proteins interact with acidic dileucine sequences within the cytoplasmic domains of sorting receptors through their Vps27p/Hrs/STAM (VHS) domains. *J Biol Chem.* 2001; 276(30):28541–28545. [PubMed: 11390366]
84. Doray B, Knisely JM, Wartman L, Bu G, Kornfeld S. Identification of acidic dileucine signals in LRP9 that interact with both GGAs and AP-1/AP-2. *Traffic.* 2008; 9(9):1551–1562. [PubMed: 18627575]
85. He X, Chang WP, Koelsch G, Tang J. Memapsin 2 (beta-secretase) cytosolic domain binds to the VHS domains of GGA1 and GGA2: implications on the endocytosis mechanism of memapsin 2. *FEBS Lett.* 2002; 524(1–3):183–187. [PubMed: 12135764]

86. Bonifacino JS, Traub LM. Signals for sorting of transmembrane proteins to endosomes and lysosomes. *Annu Rev Biochem.* 2003; 72:395–447. [PubMed: 12651740]
87. Dell'Angelica EC, Puertollano R, Mullins C, et al. GGAs: a family of ADP ribosylation factor-binding proteins related to adaptors and associated with the Golgi complex. *J Cell Biol.* 2000; 149(1):81–94. [PubMed: 10747089]
88. Puertollano R, Randazzo PA, Presley JF, Hartnell LM, Bonifacino JS. The GGAs promote ARF-dependent recruitment of clathrin to the TGN. *Cell.* 2001; 105(1):93–102. [PubMed: 11301005]
89. Wang J, Sun HQ, Macia E, et al. PI4P promotes the recruitment of the GGA adaptor proteins to the trans-Golgi network and regulates their recognition of the ubiquitin sorting signal. *Mol Biol Cell.* 2007; 18(7):2646–2655. [PubMed: 17494868]
90. Lefrancois S, McCormick PJ. The Arf GEF GBF1 is required for GGA recruitment to Golgi membranes. *Traffic.* 2007; 8(10):1440–1451. [PubMed: 17666033]
91. Shiba T, Kawasaki M, Takatsu H, et al. Molecular mechanism of membrane recruitment of GGA by ARF in lysosomal protein transport. *Nat Struct Biol.* 2003; 10(5):386–393. [PubMed: 12679809]
92. Wasiak S, Legendre-Guillemain V, Puertollano R, et al. Enthoprotin: a novel clathrin-associated protein identified through subcellular proteomics. *J Cell Biol.* 2002; 158(5):855–862. [PubMed: 12213833]
93. Kalthoff C, Groos S, Kohl R, Mahrhold S, Ungewickell EJ. Clint: a novel clathrin-binding ENTH-domain protein at the Golgi. *Mol Biol Cell.* 2002; 13(11):4060–4073. [PubMed: 12429846]
94. Page LJ, Sowerby PJ, Lui WW, Robinson MS. Gamma-synergisin: an EH domain-containing protein that interacts with gamma-adaptin. *J Cell Biol.* 1999; 146(5):993–1004. [PubMed: 10477754]
95. Hague C, Chen Z, Pupo AS, Schulte NA, Toews ML, Minneman KP. The N terminus of the human alpha1D-adrenergic receptor prevents cell surface expression. *J Pharmacol Exp Ther.* 2004; 309(1):388–397. [PubMed: 14718583]
96. Angelotti T, Daunt D, Shcherbakova OG, Kobilka B, Hurt CM. Regulation of G-protein coupled receptor traffic by an evolutionary conserved hydrophobic signal. *Traffic.* 2010; 11(4):560–578. [PubMed: 20059747]
97. Moritz OL, Tam BM, Hurd LL, Peranen J, Deretic D, Papermaster DS. Mutant rab8 impairs docking and fusion of rhodopsin-bearing post-Golgi membranes and causes cell death of transgenic *Xenopus* rods. *Mol Biol Cell.* 2001; 12(8):2341–2351. [PubMed: 11514620]
98. Esseltine JL, Ribeiro FM, Ferguson SS. Rab8 modulates metabotropic glutamate receptor subtype 1 intracellular trafficking and signaling in a protein kinase C-dependent manner. *J Neurosci.* 2012; 32(47):16933–16942a. [PubMed: 23175844]
99. Nishimura N, Plutner H, Hahn K, Balch WE. The delta subunit of AP-3 is required for efficient transport of VSV-G from the trans-Golgi network to the cell surface. *Proc Natl Acad Sci USA.* 2002; 99(10):6755–6760. [PubMed: 11997454]

Figure 1.
Specific motifs required for α_{2B} -AR export trafficking (see text for detail).

GPCRs	ICL1	GPCRs	ICL1	GPCRs	ICL1
5HT1A	ACNRHLRTPT	DRD2	SREKALQTTT	MTR1A	YRNKKLRNAG
5HT1B	YRTRKLHTPA	DRD3	LKERALQTTT	MTR1B	LRNRKLRNAG
5HT1D	LLTRKLRHTPA	DRD4	ATERALQTPPT	OPSG	MKFKKLRHPL
5HT1E	GTTKKLRHQA	DRD5	VRSRHLRANM	OPSB	LRYYKKLRQPL
5HT1F	IVTRKLRHHPA	HRH1	RSEKLRHTVG	OPSR	MKFKKLRHPL
5HT2A	SLEKKLRQAT	HRH2	GLNRRLRNLT	OPSD	VQHKKLRTPPL
5HT2B	SLEKKLRQAT	HRH3	VADSSLRTQN	AT1R	YFYMKLRKTV
5HT2C	SMEKKLRHNAT	SSR3	CFVKKLRQPS	CXCR4	GYQKKLRSM
5HT4R	CWDRQLRKIK	TAAR5	SYFKALHTPT	CCR1	VQYKRLKNMT
5HT6R	CTQPALRNTS	TAAR8	LHFKQLHSPT	CCR2	INCKKLRKLT
5HT7	CFVKKLRQPS	TAAR9	LHFKQLHTPT	CCR3	IKYRRLRIMT
α_{1A} -AR	ACHRHLSVT	ACM1	KVNTELKTVN	CCR5	INCKRLKSMT
α_{1B} -AR	ACNRHLRTPT	ACM2	KVNRHLQTVN	CCR4	PKYKRLRSM
α_{1D} -AR	ACNRHLQTVT	ACR4	KVNRQLQTVN	CCR7	IYFKRLKTMT
α_{2A} -AR	FTSRALKAPQ	MC3R	VRNGNLHSPM	CCR8	VVCKKLRST
α_{2B} -AR	LTSRSLRAPQ	MC4R	AKKNLHSPM	XCR1	VKYESLES
α_{2C} -AR	LTSRALRAPQ	MC5R	VKNLHSPM	GASR	GLSRRLRTVT
β_1 -AR	AKTPRLQTLT	MSHR	AKNRNLHSPM	GHSR	SRFRELRSTT
β_2 -AR	AKFERLQTVT	AA1R	KVNQALRDAT	PRLHR	ARVRLHNV
β_3 -AR	AWTPRLQMT	AA2A	WLNSNLQNV	OPN3	YKQRLRTPT
DRD1	IRFRHLRSKV	AA2B	GTANTLQTP	OPN4	CRSRSLRTPA

Figure 2.

The conserved Leu residues in the ICL1 of GPCRs.⁴⁴ 5HT, 5-hydroxytryptamine receptor; AR, adrenergic receptor; DR, dopamine receptor; HR, histamine receptor; SSR3, somatostatin receptor type 3; TAAR, trace amine-associated receptor; ACM, muscarinic acetylcholine receptor; MCR, melanocortin receptor; MSHR, melanocyte-stimulating hormone receptor; AR, adenosine receptor; MTR, melatonin receptor; OPSG, green-sensitive opsin; OPSB, blue-sensitive opsin; OPSR, red-sensitive opsin; OPSD, rhodopsin; AT1R, angiotensin II type 1A receptor; CXCR4, CXC chemokine receptor type 4; CCR, C–C chemokine receptor; XCR1, chemokine XC receptor 1; GASR, gastrin/cholecystokinin type B receptor; GHSR, growth hormone secretagogue receptor type 1; PRLHR, prolactin-releasing peptide receptor; OPN, opsin.

Figure 3.

Interaction of the ICL3 of α_{2B} -AR with different Sec24 isoforms. (A) Interaction of the ICL3 with Sec24A, Sec24B, Sec24C, and Sec24D isoforms as determined in GST fusion protein pull-down assays. Each Sec24 isoform tagged with green fluorescent protein was expressed in HEK293 cells, and total cell lysates were incubated with GST or GST-ICL3 fusion proteins. (B) Effect of the combinatorial mutation of three Arg residues at positions 361, 362, and 363 (3R) to three Ala (3A), three Glu (3E), three Gln (3Q), and three Lys (3K) on the ICL3 interaction with Sec24D. The ICL3 fragment G349-E369 and its mutants were generated as GST fusion proteins and their interaction with Sec24D were determined. *The data are adapted from Ref. 28.*

Figure 4.

Interaction of the C-termini of GPCRs with tubulin. (A) Alignment of the C-termini (CT) of four GPCRs. (B) Interaction of the C-termini with purified tubulin measured in GST fusion protein pull-down assays. (C) Homology modeling of α_{2B} -AR based on the crystal structure of β_2 -AR. (D) Tubulin S lacking the C-terminus does not bind to the α_{2B} -AR C-terminus.

The data are adapted from Refs. 63,64.