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Small GTPase regulation of GPCR anterograde trafficking

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Abstract

The physiological functions of heterotrimeric G protein-coupled receptors (GPCRs) are dictated by their intracellular trafficking and precise targeting to the functional destinations. Over the past decades, most studies on the trafficking of GPCRs have focused on the events involved in endocytosis and recycling. In contrast, the molecular mechanisms underlying anterograde transport of newly synthesized GPCRs from the endoplasmic reticulum (ER) to the cell surface have just begun to be revealed. In this review, we will discuss current advances in understanding the role of Ras-like GTPases, specifically the Rab and Sar1/ARF subfamilies, in regulating cell-surface transport of GPCRs *en route* from the ER and the Golgi.

Anterograde transport of GPCRs

The life of G protein-coupled receptors (GPCRs) begins in the endoplasmic reticulum (ER) where they are synthesized. Once correctly folded and properly assembled, GPCRs are able to pass the ER quality control system and exit from the ER, beginning the journey of intracellular trafficking. The nascent receptors then move through several successive intracellular compartments, which include the ER-Golgi intermediate compartment (ERGIC), the Golgi, and the trans-Golgi network (TGN), *en route* to the cell surface, the functional destination for most GPCRs. Compared to the extensive efforts dedicated to understanding the events involved in the endocytic and recycling pathways over the past 2–3 decades [1,2], the molecular mechanisms underlying export trafficking of the GPCR superfamily from the ER through the Golgi to the plasma membrane are relatively less well defined.

It has become clear that the anterograde transport of GPCRs is coordinated by many regulatory factors. First, GPCR export to the cell surface is regulated by multiple proteins, such as receptor activity modifying proteins (RAMPs), ER chaperones, and accessory proteins. These proteins may stabilize receptor conformation, facilitate receptor maturation, and promote receptor delivery to the plasma membrane [3–5]. Second, recent studies have indicated that the exit of GPCRs from the ER and the Golgi may be directed by specific motifs embedded within the receptors [3,6,7]. Third, post-translational modifications, such as N-linked glycosylation, have been long proven to be involved in the delivery of some

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GPCRs to the cell surface [8]. Fourth, GPCR cell-surface targeting depends on the microtubule networks [9], and a recent study demonstrates that the cargo GPCRs may directly interact with tubulin to control their cell-surface movement [10]. Fifth, GPCR dimerization may influence proper receptor folding/assembly and the ability of receptors to pass through the ER quality control mechanism [11]. In this review, we focus on emerging roles in the regulation of anterograde trafficking of GPCRs of the Ras-like small GTPases, specifically the Rab and Sar1/ARF subfamilies that have been well described to function as traffic cops to control cargo transport between various intracellular organelles (Figure 1a).

Rab GTPases in the transport of GPCRs from the ER to the cell surface

More than 60 Rab GTPases, forming the largest branch of Ras-related small GTPases, are involved in almost every step of vesicle-mediated transport, particularly the targeting, tethering, and fusion of transport vesicles [12]. Each Rab GTPase has a distinct subcellular localization pattern that correlates with the compartments between which they coordinate transport. Consistent with their well-established functions in trafficking, recent studies have revealed that several Rab GTPases are involved in anterograde transport of GPCRs, including Rab1, Rab2, and Rab6 in the ER-to-Golgi transport and Rab8 in the post-Golgi traffic (Figure 1a).

Rab1

Rab1 is localized in the ER and the Golgi and specifically modulates cargo transport from the ER to the Golgi. Consistent with this function, expression of the dominant-negative mutant Rab1N124I inhibits the maturation of rhodopsin, a photoactivated GPCR, in *Drosophila* [13]. The function of Rab1 in GPCR cell-surface transport has been recently investigated in mammalian cells. Loss-of-Rab1 function, achieved through expressing Rab1 mutants or small interfering RNA (siRNA), blocked the ER-to-cell surface transport of α_{1A}-adrenergic receptor (α_{1A}-AR), α_{1B}-AR [14], β_1-AR, β_2-AR [14–17], angiotensin II type 1 receptor (AT1R) [15,18,19], AT2R [20], and human calcium-sensing receptor (hCaR) [21]. These data indicate that the transport of these GPCRs from the ER to the cell surface is mediated through a Rab1-dependent pathway. Surprisingly, Rab1 mutants and siRNA did not alter the transport of α_{2B}-AR [15] (Table 1).

Gain-of-Rab1 function through overexpressing wild-type Rab1 also differentially modulates the export trafficking of GPCRs (Table 1). For example, Rab1 augmented the cell-surface numbers of α_{1A}-AR, α_{1B}-AR [14], AT2R [20], and hCaR [21], but did not affect the cell-surface transport of α_{2B}-AR [15], or β_1-AR [14]. The Rab1 effects on the cell-surface export of β_2-AR and AT1R are more complicated than the effects on other GPCRs. Overexpression of Rab1 enhanced the cell-surface transport of endogenous β_2-AR in endothelial cells [16] but had no effect on exogenous β_2-AR in HEK293 cells [15] or endogenous β_2-AR in cardiomyocytes [14]. Rab1 expression increased the cell-surface expression of endogenous AT1R in cardiomyocytes [18] and endothelial cells [19] without influencing exogenously expressed AT1R in HEK293 cells [15]. Nevertheless, these studies have clearly demonstrated that endogenous expression of Rab1 is a rate-limiting factor for the transport of some, but not all, GPCRs from the ER to the cell surface, and have also implied that the effects of Rab1 on the cell-surface transport of distinct GPCRs are both cell type- and receptor-specific.

Based on the studies of Rab1, the transport of GPCRs from the ER to the Golgi can be divided into three different pathways (Figure 1b). The first pathway is inhibited and facilitated by attenuating and increasing Rab1 function, respectively. This pathway is utilized by α_{1A}-AR, α_{1B}-AR, and AT1R in cardiomyocytes, β_2-AR in pulmonary microvascular endothelial cells, as well as α_{2C}-AR, AT2R, and hCaR in HEK293 cells. The

second pathway, which is used by α_{2A}-AR, β_2-AR, and AT1R in HEK293 cells, and β_1-AR and β_2-AR in cardiomyocytes, is inhibited by attenuating Rab1 function, but is not sensitive to increasing Rab1 function. The third pathway is independent of Rab1. α_{2B}-AR is only GPCR identified thus far to utilize this pathway. Although compelling evidence has indicated that different GPCRs with similar structural features use distinct pathways for their transport from the ER through the Golgi to the cell surface, the molecular mechanisms underlying the selection of a specific pathway by a given GPCR are still unknown.

Rab2 and Rab6

Rab2 and Rab6 have been proposed to coordinate the Golgi-to-ER retrograde transport [12] that is essential for the retrieval of ER/Golgi resident proteins to maintain the organelle homeostasis as well as for the recycling of components of transport machinery required for anterograde transport. Rab2 is predominantly localized to the ERGIC, the first sorting station for anterograde or retrograde transport in the early secretory pathway, and is involved in the GRGIC-to-ER transport. As a mainly Golgi-localized Rab GTPase, Rab6 modulates retrograde transport between the Golgi cisternae or from the Golgi to the ER. It has been well demonstrated that Rab2- and Rab6-coordinated transport plays an important role in anterograde transport of membrane proteins. For example, manipulation of Rab2 and Rab6 function inhibits the transport of hemagglutinin, cystic fibrosis transmembrane conductance regulator, and vesicular stomatitis viral glycoprotein (VSVG) [22].

For GPCR transport, the maturation of rhodopsin in *Drosophila* was first demonstrated to require Rab6 [23]. Recent studies have shown that Rab6 mutants attenuated the cell-surface expression of β_2-AR and AT1R (Table 2), but not α_{2B}-AR [20]. It is striking that Rab1 and Rab6, which are involved in opposing traffic between the ER and the Golgi, have similar effects on the transport of these three receptors [15]. These data suggest that Rab6-mediated retrograde transport is capable of selectively modulating anterograde trafficking of GPCRs. In contrast to Rab6, Rab2 mutants and siRNA significantly inhibits the cell-surface expression of both α_{2B}-AR and β_2-AR [24]. Consistent with its effect on the transport of VSVG, Rab2Q65L induces receptor accumulation in the ERGIC [24]. However, it remains unknown whether the inhibitory effect of Rab2 and Rab6 mutants on GPCR cell-surface export is induced by facilitating receptor retrograde transport or by disrupting normal retrograde transport machinery, which ultimately impairs the anterograde transport pathway.

Rab8

Rab8 has been extensively investigated in protein transport from the TGN to the apical/basolateral membrane under polarized conditions [25–27]. Similar to Rab11 function in *Drosophila* [28,29], Rab8 modulates the post-Golgi transport of rhodopsin in *Xenopus* [26,30]. We have recently determined the role of Rab8 GTPase in the cell-surface targeting of α_{2B}-AR and β_2-AR in several cell lines and primary neurons [31]. Attenuation of Rab8 function via expressing Rab8 mutants and shRNA reduced the cell-surface expression of α_{2B}-AR and β_2-AR, albeit at different magnitudes, and arrested the receptors in the TGN compartment. More interestingly, Rab8 physically interacted with distinct motifs in the C-termini of α_{2B}-AR and β_2-AR (Table 2), which likely dictated differential regulation of these two receptors by Rab8 [31]. These studies demonstrate that Rab8 is required for post-Golgi traffic of some GPCRs. These data also provide a good example that cargo proteins (e.g. GPCRs) may directly interact with Rab GTPases to coordinate their export trafficking.

Another intriguing finding is that the di-leucine (LL) motif, which is highly conserved in the membrane-proximal C-termini of the family A GPCRs [32], was identified as a binding site of Rab8 in β_2-AR, but not in α_{2B}-AR. The LL motif has been well described to function as a sorting signal at the TGN for basolateral cell-surface transport and at the plasma membrane

for endocytosis in clathrin-coated vesicles [33–35]. We and others have recently demonstrated that the LL motif is involved in the ER export of several GPCRs [32,36–38]. Taken together, it is possible that, similar to the function of the ExD motif in VSVG transport [39], a single LL motif may modulate export trafficking of newly synthesized 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.

Sar1 GTPase in the exit of GPCRs from the ER

The question of how GPCRs export from the ER is of fundamental importance because it represents the first step in their long journey to the cell surface, influences the kinetics of receptor maturation, and controls the amount of functional receptors at the cell surface [40]. As discussed above, GPCR export from the ER is a specific process which may be directed by specific signals embedded in the receptors. A number of sequences, mainly located in the intracellular domains, have been demonstrated to be required for GPCR export from the ER [3,6,7]. However, it remains unknown whether these putative ER export signals are capable of mediating cargo GPCR interaction with components of coat protein complex II (COPII)-coated transport vesicles or facilitating receptor recruitment onto the vesicles, as is the case for ER export motifs such as di-acidic and di-hydrophobic signals which are identified in a number of non-GPCR membrane proteins [41,42].

It has been well recognized that cargo export from the ER is exclusively mediated through the COPII-coated transport vesicles. The formation of the COPII vesicles takes place on the ER membrane at ER exit sites in which the small GTPase Sar1 plays a crucial role. Activation of Sar1 recruits the Sec23/24 complex to the ER membrane and is then clustered by the Sec13/31 complex, resulting in the formation of the COPII vesicles, whereas deactivation of Sar1 leads to the release of the COPII vesicles carrying cargo from the ER membrane [43]. Recent studies have demonstrated that transient expression of the GTP-bound mutant Sar1H79G significantly inhibits the cell-surface expression of all GPCRs tested, including AT1R, β_2-AR, α_{2B}-AR, and hCaR [44,45]. Surprisingly, Sar1H79G arrested different receptors in distinct locations [44], an effect reported on ER export of glycosylphosphatidylinositol-anchored protein, VSVG, and soluble lumenal GFP [46]. These data indicate that ER export of GPCRs is differentially modulated by efficient GTP hydrolysis of Sar1 GTPase and suggest that GPCRs can be sorted from one another at the level of ER exit sites.

ARF GTPases in the transport of GPCRs from the ER to the cell surface

Based on their amino acid sequence homology and gene organization, five ARF GTPases identified in human are divided into three classes: class I (ARF1/3), class II (ARF4/5) and class III (ARF6). It is well known that ARF1 plays a crucial role in both anterograde and retrograde trafficking, whereas ARF6 is mainly involved in endocytosis and actin cytoskeleton remodeling. Although ARF3 has not been well studied, it is generally considered that the functions of ARF1 and ARF3 are interchangeable. In contrast, the physiological roles for ARF4 and ARF5 remain poorly characterized. There is considerable evidence indicating that ARF GTPases differentially modulate anterograde transport of GPCRs (Figure 1a and Table 2).

ARF1 and ARF3

The traffic function of ARF1 is mediated through regulating the formation of different transport vesicles on different intracellular compartments. In the early secretory pathway, ARF1 recruits a complex of cytosolic proteins, collectively known as coatomers, which leads to the formation of COPI-coated vesicles that mediate cargo transport from the Golgi

to the ER, from the ERGIC to the Golgi, and between Golgi cisternae. In post-Golgi transport, ARF1 directly interacts with the adaptor proteins and GGAs (Golgi-localized γ-ear-containing ARF1-binding proteins), initiating the formation of the clathrin-coated vesicles, which are involved in protein transport between the TGN, plasma membrane, endosomes, and lysosomes.

Consistent with its well-established trafficking function, ARF1 is involved in GPCR cell-surface transport at multiple transport steps. This became evident as expression of ARF1 mutants dramatically inhibited the cell-surface transport of α_{2B}-AR, β_2-AR, AT1R, CXCR4, and M3-muscarnic receptor (M3-MR) [47]. It was also found that the GDP- and GTP-bound ARF1 mutants arrested the receptors in different intracellular compartments. Whereas the GDP-bound mutant ARF1T31N strongly blocked receptor export from the ER, the GTP-bound mutant ARF1Q71L inhibited receptor export out of the Golgi. These data are consistent with other studies showing that expression of different ARF1 mutants disrupted protein transport at different compartments [48]. In addition, expression of ARFGAP1 which facilitates the GTP hydrolysis of ARF1 also reduced α_{2B}-AR cell surface expression, further supporting an important role for ARF1 in the export trafficking of GPCRs. Similarly, ARF3 mutants attenuated the cell-surface expression of α_{2B}-AR [47]. These data suggest that the class I ARF GTPases, ARF1 and ARF3, play an important role in regulating the movement of newly synthesized GPCRs from the ER through the Golgi to the cell surface.

ARF4 and ARF5

By using crosslinking and GST-fusion protein pull-down assays, Deretic *et al.* demonstrated that ARF4 GTPase interacted with rhodopsin, specifically the C-terminal VxPx motif [49]. Mutation of this motif has been well described to contribute to the pathogenesis of neurodegenerative diseases, particularly the severe forms such as autosomal dominant retinitis pigmentosa. This interaction regulates not only the TGN sorting of rhodopsin into membrane carriers which then transport to the rod outer segments, but also ARF4 association with the TGN membrane [49,50]. Subsequently, this group identified components of the transport machinery, including ARF4, Rab11, the Rab11/ARF effector FIP3 and the ARF GAP ASAP1, which might form a complex on the TGN and regulate ciliary targeting of rhodopsin. These studies strongly demonstrate that ARF4 GTPase functions as an essential factor to modulate rhodopsin movement from the TGN to the plasma membrane in a polarized environment.

A recent study suggests that, in contrast to its function in rhodopsin trafficking in retinas, ARF4 does not play a major role in the cell-surface transport of α_{2B}-AR in HEK293 cells, because expression of ARF4 mutants did not significantly influence the cell-surface expression of the receptor [47]. However, the expression of the dominant-negative mutant ARF5N126I moderately inhibited α_{2B}-AR cell-surface transport, suggesting that ARF5, but not ARF4, is involved in α_{2B}-AR export trafficking. These data imply that the class II ARF GTPases, ARF4 and ARF5, may be able to selectively modulate the cell-surface transport of distinct GPCRs.

ARF6

As a well-characterized small GTPase involved in protein trafficking between the plasma membrane and endosomes, ARF6 has been extensively studied in the internalization of multiple GPCRs, including the luteinizing hormone/choriogonadotropin receptor, the μ-opioid receptor, the β_2-AR, the M2-muscarinic receptor (MR), and the M4-MR [51,52]. Surprisingly, Madziva and Birnbaumer found that expression of ARF6 mutants markedly inhibited the cell-surface expression of vasopressin V2 receptor (V2R), V1aR, and M2-MR [53], indicating that ARF6 may also regulate GPCR maturation. Subcellular co-localization

studies further revealed that the receptors were extensively colocalized with the ER marker calnexin in cells expressing the GTP-bound ARF6Q67L mutant, suggesting that ARF6 specifically influences the exit of the receptors from the ER. Consistent with this report, expression of ARF6Q67L inhibited α_{2B}-AR transport to the cell surface [47]. These studies suggest a novel function for ARF6 in anterograde delivery of some GPCRs.

Role of small GTPase-coordinated anterograde trafficking in the signal regulation of GPCRs

The function of GPCRs is spatially and temporally controlled by their trafficking to the functional destinations. For most GPCRs, their expression levels at the plasma membrane dictate the magnitude of cell response to hormones or drugs that bind to the receptors, and thus, manipulation of receptor exit from the intracellular compartments and subsequent transport to the cell surface will eventually influence receptor-mediated signal propagation and cellular functions. Indeed, the effects of modifying the function of small GTPases on the cell-surface transport of GPCRs as discussed above are strongly parallel to the effects on receptor-mediated signaling events, such as ERK1/2 activation, cAMP production, inositol phosphate production, cardiac myocyte hypertrophic growth, endothelial cell monolayer permeability, and phenotypic modulation of pulmonary artery smooth muscle cells [14,15,18,20,24,31,44].

For example, functional inhibition of Rab2, Rab8, ARF1, and Sar1 in HEK293 cells by expressing their mutants or siRNA, which inhibits α_{2B}-AR transport to the cell surface, impaired ERK1/2 activation by UK14304 [24,31,44,47]. In neonatal cardiomyocytes, augmentation of Rab1 function by adenoviral expression of Rab1, which specifically enhanced the cell-surface expression of AT1R and α_1-AR without influencing the transport of β_2-AR, significantly enhanced myocyte hypertrophic growth as measured by protein synthesis, cell size, and sarcomeric organization in response to stimulation with angiotensin II and phenylephrine, but not isoproterenol [14,18]. It will be interesting to investigate if augmentation of β_2-AR and AT1R expression induced by increased Rab1 expression in cultured cardiomyocytes contributes to the development of cardiac hypertrophy and heart failure observed in the transgenic mouse model overexpressing Rab1 specifically in cardiac tissue [54]. These data indicate that GPCR function can be selectively or differentially modulated through manipulating GPCR anterograde traffic. These data also provide strong evidence to suggest that small GTPase-coordinated anterograde trafficking of GPCRs *en route* from the ER and the Golgi may function as a regulatory site for selective control of not only receptor targeting to the cell surface but also receptor-mediated signaling (Figure 1a).

Concluding remarks

Over the past several years, great progress has been made in defining the role of small GTPases in the cell-surface transport of nascent GPCRs *en route* from the ER and the Golgi. The most exciting finding is that each small GTPase may differentially modulate the anterograde movement of distinct GPCRs along the secretory pathway. These studies indicate that selective export of GPCRs can be achieved at discrete intracellular levels and that the specificity/selectivity of GPCR targeting to the plasma membrane can be controlled by small GTPases. These studies, together with several newly identified sorting motifs and many accessory proteins clearly indicate that anterograde trafficking of GPCRs is a highly regulated, dynamic process.

It should be pointed out that the functions of small GTPases in the ER-Golgi-cell surface transport of GPCRs have just begun to be revealed. Future research should continue to search for more GTPases involved in the processing of nascent GPCRs and to elucidate the

possible molecular mechanisms. Probably the most challenging task is to investigate the specificity/selectivity between various small GTPases and distinct GPCRs. It will be also interesting to elucidate the molecular mechanisms underlying the selection of different anterograde transport pathways by distinct GPCRs. Furthermore, the function of different Sar1/ARF-coordinated transport vesicles in the transport regulation of the GPCR superfamily merits more attention.

It has become increasingly apparent that anterograde transport of GPCRs is directly linked to the pathogenesis of several human diseases that involve the presence of greater or fewer functional receptors at the cell surface. In particular, many GPCR trafficking-related diseases are caused by naturally occurring mutations and truncations in the receptors which prevent proper folding and lead to ER retention of the receptors [55]. There is great interest in developing means to successfully rescue those disease-causing misfolded GPCR mutants, such as through the use orthosteric and allosteric ligands [55–57], to achieve normal surface expression and signaling. In addition, it will be interesting to explore the possibility of promoting cell-surface transport of disease-associated GPCR mutants using modulators that enhance the activity of small GTPases involved in the anterograde transport of the receptors. For example, in Parkinson's disease models, Rab1 expression successfully rescues dopaminergic neuron loss caused by α-synuclein accumulation, which inhibits the ER-to-Golgi trafficking [58]. Therefore, further exploration of the roles of small GTPases in anterograde transport of nascent GPCRs will not only help elucidate the molecular mechanisms governing the GPCR targeting to the functional destination, but also provide an important foundation for developing new therapeutic means in treating human diseases involving abnormal trafficking of GPCRs.

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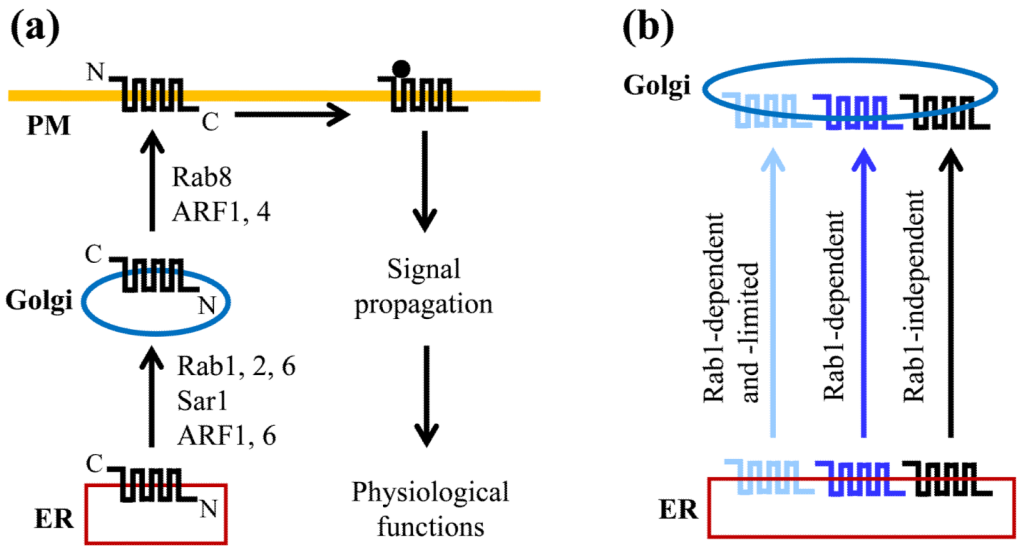

Figure 1.

Roles of small GTPases in the anterograde transport and signaling of GPCRs. **(a)** Rab1, Rab2, Rab6, Sar1, and ARF6 modulate the ER-to-Golgi traffic of GPCRs, whereas Rab8 and ARF4 regulate the Golgi-to-plasma membrane (PM) transport of GPCRs. ARF1 is involved in GPCR transport from the ER through the Golgi to the cell surface at multiple steps. Small GTPase-coordinated transport processes will dictate the cell-surface numbers of the receptors available for binding to hormones or drugs (●) and thus, will influence the magnitude of signal transduction and physiological/pharmacological responses mediated by the receptors. N, N terminus; C, C terminus. **(b)** Three possible pathways that mediate GPCR transport from the ER to the Golgi.

Table 1

Effects of Rab1 wild-type, Rab1 mutants, and siRNA-mediated knockdown of Rab1 on the cell-surface expression of GPCRs

GPCRs	Wild-type	Mutants	siRNA	Cells	Refs
α_{1A}-AR	↑ *a*	↓ *a*	ND	NRVM	[14]
α_{1B}-AR	↑ *a*	↓ *a*	↓ *b*	NRVM	[14]
α_{2B}-AR	_ *b*	_ *b*	_ *b*	HEK293	[15]
β_1-AR	_ *a*	↓ *a*	ND	NRVN	[14]
	_ *a*	↓ *a*	ND	PMVEC	[16]
β_2-AR	_ *b*	↓ *b*	↓ *b*	HEK293	[15,17]
	_ *a*	↓ *a,b*	↓ *b*	NRVM	[14]
	↑ *a,b*	↓ *a,b*	↓ *a,b*	PMVEC	[16]
AT1R	_ *b*	↓ *b*	↓ *b*	HEK293	[15]
	ND	↓ *b*	ND	HL-1 myocytes	[18]
	↑ *a*	↓ *a,b*	ND	NRVM	[18]
	↑ *a*	ND	↓ *a,b*	PASMC	[19]
AT2R	↑ *b*	↓ *b*	↓ *b*	HEK293	[20]
hCaR	↑ *b*	↓ *b*	ND	HEK293	[21]

↑, increase; ↓, decrease; , no effect; ND, not determined;

a endogenous receptors;

b exogenous receptors;

PMVEC, pulmonary microvascular endothelial cells; PASMC, pulmonary arterial smooth muscle cells; NRVM, neonatal rat ventricular myocytes.

Table 2

Roles of small GTPases in anterograde transport of GPCRs

GTPases	Regulated steps	GPCRs	GTPase binding	Refs
Rab family				
Rab1	ER-to-Golgi	(Table 1)		
Rab2	Between ER and Golgi	α_{2B}-AR		[24]
		β_2-AR		
Rab6	Between ER and Golgi	β_2-AR		[24]
		AT1R		
Rab8	Golgi-to-cell surface	α_{2B}-AR	CT	[31]
		β_2-AR	CT: LL motif	
Sar1/ARF family				
Sar1	ER exit	α_{2B}-AR		[44]
		β_2-AR		
		AT1R		
		hCaR		[45]
ARF1	ER-to-Golgi and Golgi-to-cell surface	α_{2B}-AR	CT	[47]
		β_2-AR		
		AT1R		
		CXCR4		
		M3-MR		
ARF3	Unknown	α_{2B}-AR		[47]
ARF4	Post-Golgi	Rhodopsin	CT: VxPx motif	[49,50]
ARF5	Unknown	α_{2B}-AR		[47]
ARF6	ER-to-cell surface	V2R	Receptor	[53]
		V1a-R		
		M2-MR		
		α_{2B}-AR		[47]

CT, C terminus.