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Regulation of Post-Golgi Traffic of G Protein-Coupled Receptors

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Abstract

Anterograde trafficking of newly synthesized G protein-coupled receptors (GPCRs) from the endoplasmic reticulum to the cell surface represents a crucial checkpoint in controlling the amount of the functional receptors at the cell surface and the strength of signaling initiated by the receptors. In contrast to the extensively studied, well-understood endocytic and recycling pathways, the molecular mechanisms underlying the cell-surface targeting of the receptors remain poorly defined. In this chapter, I will discuss current advances in understanding post-Golgi transport of GPCRs by focusing on specific motifs or sequences that may function as sorting signals regulating export from the Golgi and subsequent transport to the plasma membrane of GPCRs.

Keywords

Anterograde transport; Cell-surface targeting; G protein-coupled receptors; Post-Golgi transport; Small GTPases; Sorting motifs

5.1 Cell-Surface Transport of Nascent G Protein-Coupled Receptors (GPCRs)

Intracellular trafficking and precise targeting to the functional destinations of GPCRs plays a crucial role in controlling the physiological functions of the receptors. Similar to many other plasma membrane proteins, the life of 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 to exit from the ER, beginning their long journey of intracellular trafficking. The nascent receptors then move through several successive intracellular compartments, which include the ER-Golgi intermediate compartment (ERGIC), the cis/medial/trans-Golgi apparatus, and the trans-Golgi network (TGN), *en route* to the cell surface where the receptors are able to bind to their cognate ligands, leading to the activation of downstream signaling molecules. Compared to the extensive efforts dedicated to understanding the events involved in the endocytic and recycling pathways over the past two to three decades (Hanyaloglu and von Zastrow 2008; Moore et al. 2007), the molecular mechanisms underlying export trafficking of the GPCR superfamily to the plasma membrane are relatively less well-defined.

It has been known that cell-surface targeting 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 (Dong et al. 2007; Achour et al. 2008; Bouschet et al. 2008). Second, recent studies have indicated that the exit of GPCRs from the ER may be directed by specific motifs embedded within the receptors (Dong et al. 2007; Dong and Wu 2006; Zhang et al. 2011; Dong et al. 2012). Third, post-translational modifications, such as N-linked glycosylation, have long been proven to be involved in the delivery of some GPCRs to the cell surface (Rands et al. 1990). Fourth, GPCR cell-surface targeting depends on the microtubule networks (Saunders and Limbird 1997) and GPCRs may directly interact with tubulin to control their cell-surface movement (Duvernay et al. 2011). Fifth, GPCR dimerization may influence proper receptor folding/assembly and the ability of receptors to pass through the ER quality-control system (Terrillon and Bouvier 2004). In this chapter, I will review the regulation of post-Golgi transport of GPCRs by focusing on specific sequences that may function as sorting signals regulating export out of the Golgi and transport from the Golgi to the plasma membrane of GPCRs (Table 5.1).

5.2 Sorting Signals for Vesicle-Mediated Post-Golgi Transport

Newly synthesized proteins are sorted at the Golgi/TGN compartments to be delivered to final cellular destinations, such as endosomes, lysosomes, and the plasma membrane. Therefore, the TGN is often referred to as the 'sorting center'. Similar to the transport along the early secretory pathway which is mediated through COPI- and COPII-coated vesicles, post-Golgi transport can be mediated through clathrin-coated transport vesicles. Clathrin-coated transport vesicles are composed of clathrin and various adaptor proteins, including heterotetrameric adaptor protein complexes (APs), GGAs (Golgi-associated, γ -adaptin homologous, ARF-interacting proteins), and hepatocyte growth factor receptor substrate (Hrs). The formation of clathrin-coated vesicles involved in the post-Golgi traffic is highly regulated and complicated process. For example, the adaptor GGA proteins have three different isoforms: GGA1, 2 and 3, all with similar functions and structural features. Each GGA consists of four functional regions. The N-terminal VHS domain binds specific sorting signals in the cytoplasmic tail of cargo proteins. The GAT domain binds the activated form of ARF1 and this interaction is responsible for targeting GGAs onto the TGN membrane. The hinge region recruits clathrin and the C-terminal GAE domain interacts with several accessory proteins (Zhu et al. 2001) (Fig. 5.1). It is known that clathrin/GGA-coated vesicles are involved in the transport from the TGN to the lysosome of selective proteins, such as nascent lysosomal hydrolases (Bonifacino and Traub 2003), whereas clathrin/AP-coated vesicles mediate endocytosis of transmembrane receptors from the plasma membrane to the endosomal compartment. Interestingly, several studies have suggested that transport from the TGN to the plasma membrane is mediated by large pleiomorphic carriers rather than small vesicles (Bonifacino and Lippincott-Schwartz 2003).

Recent studies have demonstrated that, similar to exit from the ER directed by ER export signals such as the di-acidic motifs (Sevier et al. 2000; Ma et al. 2001; Nishimura and Balch 1997; Nishimura et al. 1999; Zuzarte et al. 2007; Wang et al. 2004), protein export from the Golgi/TGN compartments can be dictated by specific motifs. For examples, there are several well-defined endosomal sorting signals, such as tyrosine-based motifs (NPxY and Yxx $\emptyset$, where x can be any residue and $\emptyset$ is a hydrophobic residue) and di-leucine-based motifs ([D/E]xxxL[L/I] and DxxLL). Whereas Yxx $\emptyset$ and [D/E]xxxL[L/I] motifs are recognized by the AP complexes, DxxLL is recognized by GGAs (Puertollano et al. 2001a, b; Hirst et al. 2000). These motifs function to sort protein transport from the TGN to the endosomal compartment (Chen et al. 1997; Lori et al. 2007; Johnson and Kornfeld 1992; Hou et al. 2006; Boucher et al. 2008). Although these endosomal sorting motifs are also found in several GPCRs (Marchese et al. 2008), their exact functions remain to be elucidated. It is

interesting to note that β_2 -AR has an ExxLL motif within the C-terminal sequence EKENKLL and this motif has been demonstrated to be involved in the regulation of recycling of internalized β_2 -AR from endosomes to the plasma membrane (Hanyaloglu and von Zastrow 2007).

It has been well demonstrated that clathrin/AP-coated vesicles mediate endocytosis of agonist-occupied GPCRs from the plasma membrane to endosomes. In contrast, the vesicles involved in the transport of newly synthesized GPCRs from the Golgi/TGN to the plasma membrane remain unknown. However, several proteins, including ARF4, Rab11, the Rab11/ARF effector FIP3, and the ARF GAP ASAP1, may form a complex on the TGN to regulate ciliary targeting of rhodopsin, a photoactivated GPCR in the retina (Mazelova et al. 2009). We have recently demonstrated that expression of GTP-bound mutant of the small GTPase ARF1, which regulates the formation of clathrin-coated vesicles from the TGN, arrested GPCRs in the Golgi compartments (Dong et al. 2010b), suggesting that ARF1-coordinated vesicles may play a role in mediating GPCR export from the Golgi.

5.3 Regulation of Post-Golgi Traffic of GPCRs by Specific Motifs and Possible Mechanisms

5.3.1 The LL Motif and Its Interaction with Rab8 GTPase in Post-Golgi Transport of β_2 -AR

Rab GTPases are the largest branch of the Ras-related GTPase superfamily, consisting of more than 60 members in mammals and 11 in yeast, and are involved in almost every step of vesicle-mediated protein transport, particularly the targeting, tethering, and fusion of transport vesicles with the appropriate acceptor membrane (Grosshans et al. 2006). Each Rab GTPase has a distinct subcellular localization pattern that correlates with the compartments between which it coordinates transport. Several recent studies have determined the roles of Rab GTPases in the anterograde transport of newly synthesized GPCRs. Specifically, Rab1, Rab2, and Rab6 are involved in GPCR transport along the early secretory pathway (Wu et al. 2003; Filipeanu et al. 2004, 2006; Zhang et al. 2009; Dong and Wu 2007; Wang and Wu 2012) and Rab8 GTPase regulates the post-Golgi transport of GPCRs (Dong et al. 2010a).

Rab8 has been extensively investigated in protein transport from the TGN to the apical/basolateral membrane under polarized conditions (Sato et al. 2007; Moritz et al. 2001; Nachury et al. 2007). Similar to Rab11 function in *Drosophila* (Li et al. 2007; Satoh et al. 2005), Rab8 modulates the post-Golgi transport of rhodopsin in *Xenopus* (Deretic et al. 1995; Moritz et al. 2001). We have recently determined the role of Rab8 in the cell-surface targeting of $\alpha_2\text{B}$ -AR and β_2 -AR in several cell lines and primary neurons (Dong et al. 2010a). Our studies have shown that attenuation of Rab8 function via expressing Rab8 mutants and shRNA reduced the cell-surface expression of $\alpha_2\text{B}$ -AR and β_2 -AR and arrested the receptors in the TGN compartment. These studies demonstrate that Rab8 is required for post-Golgi traffic of some GPCRs.

We have also demonstrated that Rab8 physically associated with both $\alpha_2\text{B}$ -AR and β_2 -AR, suggesting that the cargo GPCRs may interact with Rab GTPases to coordinate their post-Golgi traffic. More interestingly, distinct motifs in the C-termini of $\alpha_2\text{B}$ -AR and β_2 -AR were identified to interact with Rab8 and these motifs probably dictated differential regulation of these two receptors by Rab8 (Dong et al. 2010a). The di-leucine (LL) motif was shown to be required for β_2 -AR interaction with Rab8, whereas multiple residues scattered in the C-terminus were identified to modulate $\alpha_2\text{B}$ -AR interaction with Rab8.

The LL motif has been 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 (Heilker et al. 1996; Hunziker and Fumey 1994; Bonifacino and Traub 2003). We and others have recently demonstrated that the LL motif is involved in the cell-surface transport of several GPCRs, including α_{2B} -AR, α_{1B} -AR, β_2 -AR, angiotensin II type 1 receptor (AT1R), serotonin 5HT1A and 5-HT1B receptors, vasopressin V2 receptor, and M1-muscarinic receptor (M1-MR) (Duvernay et al. 2004; Schulein et al. 1998; Carrel et al. 2006; Sawyer et al. 2010). The LL motif has been postulated to modulate receptor transport from the ER as its mutants were unable to exit from the ER. Importantly, the LL motif is highly conserved in the membrane-proximal C-termini of the family A GPCRs (Duvernay et al. 2004) and therefore, it may provide a common mechanism for GPCR export from the ER compartment. The fact that mutation of the LL motif influences the interaction with Rab8 of β_2 -AR, but not α_{2B} -AR (Dong et al. 2010a), 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 ExD motif in VSVG transport (Nishimura et al. 2002), 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.

5.3.2 The C-Terminal Tails and ARF1 GTPase in Post-Golgi Transport of GPCRs

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). ARF1 plays a crucial role in both anterograde and retrograde trafficking and its traffic function is mediated through regulating the formation of transport vesicles on different intracellular compartments. In the early secretory pathway, ARF1 recruits a complex of cytosolic proteins, collectively known as coatamers and this 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 (Spang 2002). In the post-Golgi transport, ARF1 directly interacts with APs and GGAs (Fig. 5.1), initiating the formation of the clathrin-coated vesicles.

Consistent with its well-established trafficking function, ARF1 is involved in the cell-surface transport of newly synthesized GPCRs. We have recently demonstrated that expression of ARF1 mutants dramatically inhibited the cell-surface transport of all GPCRs tested, including α_{2B} -AR, β_2 -AR, AT1R, C-X-C chemokine receptor type 4 (CXCR4), and M3-MR (Dong et al. 2010b). Similarly, ARF3 mutants attenuated the cell-surface expression of α_{2B} -AR (Dong et al. 2010b), suggesting that the class I ARF GTPases, ARF1 and ARF3, play an important role in regulating the movement of GPCRs to the cell surface. More interestingly, expression of 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 (Ward et al. 2001). These data suggest that ARF1 may modulate GPCR transport at multiple transport steps and that GTP-hydrolysis of GTP-bound ARF1 GTPase plays a critical role in the post-Golgi transport of GPCRs.

Furthermore, we found that the C-terminus of α_{2B} -AR physically interacted with ARF1. As purified His-tagged ARF1 and GST-tagged C-terminus were still able to form a complex, the interaction between the receptor and ARF1 is likely direct. It is possible that the cargo GPCRs, via their C-termini, directly interact with the small GTPase ARF1 and this interaction facilitates receptor recruitment onto the ARF1-coordinated, Golgi/TGN-derived transport vesicles (Fig. 5.1).

5.3.3 The VxPx Motif and Its Interaction with ARF4 GTPase in Post-Golgi Transport of Rhodopsin

It has been known that the four highly conserved amino acids located in the C-terminus of rhodopsin plays an important role in the regulation of intracellular targeting and function of the receptor. Indeed, mutation of the C-terminal VxPx motif is clearly linked to the pathogenesis of neurodegenerative diseases, particularly the severe forms such as autosomal dominant retinitis pigmentosa. The function of the VxPx motif in regulating rhodopsin trafficking has been suggested to be mediated through interacting with Tctex-1, a light chain of cytoplasmic dynein that translocates rhodopsin-bearing vesicles along microtubules (Tai et al. 1999).

Deretic et al. have demonstrated that the VxPx motif mediates rhodopsin interaction with the small GTPase ARF4 (Deretic et al. 2005) and this interaction plays an important role in receptor sorting at the TGN into membrane carriers which transport to the rod outer segments (Deretic et al. 2005; Mazelova et al. 2009). They have also identified a complex containing ARF4 that is formed on the TGN and regulates post-Golgi transport of rhodopsin (Mazelova et al. 2009). These studies have demonstrated that the C-terminal sequence through physically association with transport machinery proteins may provide an efficient transport system for the vectorial delivery of large numbers of nascent rhodopsin. In addition, mutation of the glutamic acid residue at the position 150 in the second intracellular loop of rhodopsin (E150K) induced an accumulation of the receptor in the *cis/medial* Golgi compartments and this mutation is linked to the development of autosomal recessive retinitis pigmentosa (Zhu et al. 2006), further suggesting that rhodopsin export from the Golgi and subsequent transport to the functional destination is a regulated process. These data also suggest that defective post-Golgi traffic may be the molecular basis of retinitis pigmentosa.

5.3.4 The Acidic Motifs and Their Interaction with the p24 Family Proteins in the Post-Golgi Transport of GPCRs

The di-acidic sequences have been demonstrated to function as ER export motifs in several membrane proteins (Sevier et al. 2000; Ma et al. 2001; Nishimura and Balch 1997; Nishimura et al. 1999; Zuzarte et al. 2007; Wang et al. 2004). Their ER export functions are mediated through directly interacting with Sec24, a component of ER-derived COPII vesicles that mediate cargo transport exclusively from the ER. This interaction enhances the recruitment and concentration of the cargo carrying the di-acidic motifs into the COPII vesicles. The di-acidic motifs in the membrane-proximal C-termini have been also demonstrated to regulate the transport of AT1R and AT2R, but not β_2 -AR and α_{1B} -AR, from the ER (Zhang et al. 2011). In addition to regulating ER export, the di-acidic motifs are also involved in post-Golgi trafficking through interacting with the adaptor protein AP3 (Nishimura et al. 2002).

Luo et al. have demonstrated that protease-activated receptor-2 (PAR2) interacted with p24A, a type 1 transmembrane protein. This interaction is regulated by the small GTPase ARF1 and plays an important role in the transport of PAR-2 from the Golgi to the plasma membrane (Luo et al. 2007). Subsequently, this group demonstrates that p24 interacted with several other GPCRs, including PAR-1, μ -opioid receptor 1B, and the nucleotide receptors (P2Y₁R, P2Y₂R, P2Y₄R, and P2Y₁₁R) (Luo et al. 2011). In addition to p24, p23, another member of the p24 family protein, is also able to bind to these GPCRs. More interestingly, the conserved acidic residues located in the second extracellular loop are required for the interaction of P2Y₄R and P2Y₁₁R with p24 (Luo et al. 2011). These data strongly imply that GPCRs, via the acidic domains, may interact with Golgi-localized proteins, such as the p24 family proteins p23 and p24, to modulate their post-Golgi trafficking along the biosynthetic pathway.

5.3.5 The YS Motif in Post-Golgi Traffic of α_2 -AR

As described above the C-termini of GPCRs contain signals directing the post-Golgi transport of GPCRs and their function can be mediated by physically associating with components of the transport machinery, such as small GTPases. Similar to the C-termini, the N-termini are also involved in the regulation of anterograde trafficking of newly synthesized GPCRs. For example, the deletion of the N-termini facilitated the cell-surface export of α_{1D} -AR and α_{2C} -AR, suggesting that the N-termini may contain signals retaining the receptors in the ER (Angelotti et al. 2010; Hague et al. 2004). Indeed, a hydrophobic sequence in the C-terminus of α_{2C} -AR was proven to function as an ER retention motif (Angelotti et al. 2010), which provides a mechanism responsible for the intra-cellular accumulation of α_{2C} -AR in some types of cells.

We have found that the N-termini may play an important role in dictating GPCR export from the Golgi. We have demonstrated that single and double substitution of Y12 and S13 residues in the membrane-proximal N-terminal region significantly reduced the cell-surface expression of α_{2B} -AR (Dong and Wu 2006) and the Y12/S13 motif mutants were retained in the Golgi apparatus (Dong and Wu 2006). These data suggest that the Y12/S13 motif mediates α_{2B} -AR export at the level of the Golgi. The YS motif only exists in three α_2 -AR family members (Dong and Wu 2006) (Fig. 5.2) and indeed, it exerts a similar function on α_{2A} -AR trafficking (Dong and Wu 2006). Therefore, the YS motif may function as an export signal specifically modulating the Golgi export of the members of α_2 -AR subfamily. It should be pointed out that the N-terminal tails are positioned towards the lumen of the ER and the Golgi during the export process and the YS motif is not able to directly interact with components of transport machinery in the cytoplasm. Further investigation is needed to clarify the molecular mechanism underlying the function of YS motif in the regulation of receptor export from the Golgi.

5.4 Concluding Remarks

It has become increasingly apparent that cell-surface targeting of GPCRs is one of the important factors determining the functionality of the receptors and 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 lead to retention of the receptors in the intracellular compartments (Conn et al. 2007). As discussed above, retinitis pigmentosa is clearly linked to mutations of rhodopsin which cause the accumulation of the receptor in the Golgi compartment. Therefore, to thoroughly understand the mechanism underlying export trafficking of GPCRs will provide a foundation for the development of therapeutic strategies targeting on specific components of the transport pathway.

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Fig. 5.1.

The formation of clathrin- and GGA-coated vesicles on the TGN membrane and the possible function of the interaction of GPCRs, via their C-termini, with ARF1 in facilitating receptor recruitment onto the vesicles

		NT
α_{2A} -AR	Human	GARATP Y SLQ
	Rat	GTRATP Y SLQ
	Mouse	GTRATP Y SLQ
α_{2B} -AR	Human	MDHQDP Y SVQ
	Rat	MDHQEP Y SVQ
	Mouse	MVHQEP Y SVQ
α_{2C} -AR	Human	GPPRGQ Y SAG
	Rat	GPPPGQ Y SAG
	Mouse	VPPPGQ Y SAG

Fig. 5.2.

The conserved YS motif in the N-termini (NT) of three α_2 -AR subtypes

Table 5.1

Specific motifs and their binding partners involved in the regulation of post-Golgi traffic of GPCRs

GPCRs	Domains	Motifs	Binding partners	Refs
β_2 -AR	CT	LL	Rab8	Dong et al. (2010a)
α_{2B} -AR	CT	TVFN, PWTQTGW	Rab8	Dong et al. (2010a)
α_{2B} -AR	CT	Unknown	ARF1	Dong et al. (2010b)
Rhodopsin	CT	VxPx	Tctex-1, ARF4	Tai et al. (1999), Mazelova et al. (2009), Deretic et al. (2005)
P2Y ₄ R, P2Y ₁₁ R	ECL2	Acidic residues	p23, p24	Luo et al. (2007, 2011)
α_{2A} -AR, α_{2B} -AR	NT	YS	Unknown	Dong et al. (2007)

CT, C-terminus; *NT*, N-terminus; *ECL2*, second extracellular loop