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Post-endocytic sorting of adrenergic and opioid receptors: new mechanisms and functions.

Shanna L. Bowman and **Manojkumar A. Puthenveedu**

Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213

Abstract

The endocytic pathway tightly regulates the activity of G protein-coupled receptors (GPCRs). Much of our understanding of this relationship between GPCR endocytic trafficking and signaling comes from studies done on catecholamine and opioid receptors. After ligand-induced endocytosis, a key sorting step in the endosome determines whether receptors are recycled back to the cell surface, leading to recovery of signaling, or are degraded in the lysosome, leading to desensitization. Recycling of GPCRs, unlike that of many other proteins, is an active process driven by specific sequences on the receptor and proteins that interact with this sequence. Recent data suggest that sequence-dependent recycling plays complex roles in regulating both the timing and location of GPCR signaling. This chapter will describe our current understanding of the mechanisms regulating GPCR sorting in the endosome, and discuss emerging ideas on their role in GPCR signaling, focusing on adrenergic and opioid receptors as prototypes.

1. Introduction: The endosome as a sorting station for internalized GPCRs.

Endocytic trafficking is a fundamental cellular process that regulates GPCR function. GPCR activation on the cell surface results in their removal from the cell surface by clathrin-mediated endocytosis[1–3]. This was first recognized by studies measuring ligand-induced desensitization of signaling, which required receptor phosphorylation, binding of beta-arrestins, and endocytosis. Endocytosed GPCRs are transported to the endosome, where a critical sorting step determines the further fate of GPCRs[4–6]. They may be either recycled to the cell surface, or be degraded in the lysosome. An established consequence of this sorting is that it directly controls the number of receptors on the cell surface, causing either recovery of sensitivity of the cell to the signal or downregulation of signaling, respectively[7,8]. Recent evidence, however, suggests that this post-endocytic sorting might have much more complex roles in regulating the function of many GPCRs, including the prototypic adrenergic and opioid receptors.

The early endosome serves as the primary sorting station for most internalized proteins including GPCRs. How endosomes sort receptors, considering the amount of membrane cargo that traffic through these dynamic organelles, is a fundamental question with many implications. There are four main pathways for internalized proteins out of the endosome (Figure 1). Proteins may be recycled by bulk membrane flow, sorted to the lysosome to be

degraded, recycled via a specialized regulated “sequence-dependent” recycling pathway, or transported to the Golgi apparatus.

Many proteins, like the transferrin receptor (TfR), are recycled back to the cell surface as part of bulk membrane flow, apparently without any specific requirements. This “bulk recycling” was first described over 20 years ago, by Maxfield and colleagues, who labeled and tracked the membrane and fluid phase compartments of endosomes[9,10]. They observed that the endosome extruded narrow tubules with a large surface area (i.e., membranes) but low volume (i.e., fluids). Continued fission and recycling of these tubules provided a geometric basis for how nutrient receptors could be recycled as part of bulk membrane flow, simply because they are membrane proteins, leaving their soluble ligands in the lumen of the endosome to be eventually degraded in the lysosome[9–11]. Several membrane-modifying proteins responsible for membrane tubulation have been identified. Such geometric sorting likely occurs extensively along the endocytic pathway, iteratively recycling receptors from the early, recycling, and the late endosomes.

Considering that recycling is thought to be a “default” fate for membrane proteins, it is interesting that GPCRs are not commonly sorted via bulk recycling. Many GPCRs, like the delta opioid receptor (DOR), and signaling receptors like the EGF receptor, are degraded in the lysosome[12,13]. Degradation of these proteins takes advantage of geometric sorting. These proteins are packaged into vesicles that bud off into the interior of the endosomes, essentially partitioning the protein-containing vesicles into the fluid phase of these endosomes, which eventually mature into or fuse with lysosomes[14–16]. For GPCRs that recycle, this is a regulated process that requires specific protein sequences and interactions[17–30]. Mutation of these sequences direct GPCRs to the lysosome, reiterating that their recycling is not simply a function of them being membrane proteins[17,22,25]. This chapter will discuss our current understanding of GPCR sorting between the degradative and recycling pathways, using adrenergic and opioid receptors as model receptors.

2. Mechanisms of sequence-dependent GPCR sorting

2.1 GPCRs are sorted into lysosomes by multiple mechanisms

The first conceptual step in GPCR sorting to the lysosome is to segregate receptors away from bulk recycling, by packaging them into intralumenal vesicles (ILVs). The process of generating vesicles that pinch off to the interior of the vesicles, called involution, is a topologically highly interesting process, as this involves the generation of vesicles away from the cytoplasm, unlike with the “classical” coat-mediated vesicle transport processes like endocytosis. Most of our understanding of protein sorting to lysosomes comes from studies done in yeast. Genetic and biochemical studies of protein sorting to the vacuole, a compartment homologous to the mammalian lysosome, have identified the ESCRT proteins as the primary protein machinery responsible for this process. The current views on the mechanisms of how ESCRT proteins induce negative curvature and generate ILVs are discussed in structural detail in several recent authoritative reviews[31–34].

The main sorting signal that targets proteins to the lysosome is the addition of ubiquitin to cytoplasmic lysine residues of proteins. Early evidence for the importance of ubiquitination

in GPCR trafficking came from studies on the yeast GPCR Ste2, for which ubiquitination promoted transport to the vacuole. Since then, ubiquitination has been shown to promote lysosomal targeting of many membrane proteins in mammalian cells, including the EGF receptor[13,35]. In the case of many of these proteins, ubiquitin interacts with the ubiquitin-interacting motif (UIM) of Hrs, an endosomal protein often termed ESCRT-0. Hrs then transfers these proteins to Tsg101, an ESCRT-I component that also has a UIM. This localizes the cargo in ESCRT domains, which allows them to be incorporated in ILVs. While many GPCRs are known to be ubiquitinated, the role of these interactions in its lysosomal targeting has been shown only for CXCR4[36]. The role of ubiquitination in GPCR trafficking is discussed much more extensively elsewhere in this book.

Receptor ubiquitination or ESCRTs, however, are not required for sorting all GPCRs to lysosomes. A mutant of the DOR lacking all cytoplasmic lysines is endocytosed and trafficked to the lysosome[12]. This still requires Hrs and Tsg101, suggesting ubiquitin-independent binding of GPCRs with ESCRT[37]. The protease activated receptor PAR1, on the other hand, can be sorted to the lysosome independent of both ubiquitination and ESCRTs[38]. Instead, this sorting depends on the endosomal protein sorting nexin-1 (SNX1)[39]. Additionally, members of the family of GPCR-associated sorting proteins (GASP1 and GASP2) and Beclin-2 have been implicated in degradation of DOR[40,41]. While both SNX1 and GASP proteins interact with several GPCRs, including DOR, the exact mechanism by which they sort GPCRs into the lysosome is still not well understood[42]. Our current understanding is that these proteins may play a role in downregulation of receptors under conditions of chronic agonist stimulation.

2.2 GPCRs Contain Diverse Recycling Sequences

It has been appreciated for over 30 years that adrenergic receptors recycle to the surface, based on the reappearance of activity after agonist-induced desensitization[2,43,44]. Over the years, however, it has become clear that the simple model of bulk recycling cannot explain GPCR recycling, mostly from research on the beta adrenergic receptors. The recycling of the beta-2 adrenergic receptor (B2AR) depends on a specific sequence on its C-terminus that interacts with post-synaptic density 95/disc large/zonula occludins-1 (PDZ) -domain containing proteins[17]. Similar sequences that conform to classical type I PDZ-ligand sequences have been identified on several GPCRs, including the related beta-1 adrenergic receptor (B1AR) and the kappa opioid receptor (KOR), over the past decade[19,22,29]. For many of these receptors, these sequences are required for the recycling of these receptors, and are also sufficient, as transplanting these sequences onto a non-recycling receptor like the DOR allows the receptor to recycle[45]. Alpha adrenergic receptors also contain PDZ ligands, although they are more diverse, and their role in recycling is less well understood[46]. One critical feature of these PDZ-ligand sequences is that they need to be on the C-terminal tail of the receptors. The terminal peptide binds in an antiparallel fashion in a hydrophobic cleft formed by a beta strand, a loop, and an alpha helix in the PDZ protein, with the free carboxyl group occupying a hydrophobic pocket[47]. In addition, sequence-comparisons have identified many internal PDZ ligands on GPCRs that might be involved in receptor recycling[48,49].

How do PDZ-ligand sequences mediate adrenergic receptor recycling? For the B2AR, a complement of proteins that bind the PDZ ligand, including NHERF-1, NHERF-2, PDZK1, and MAGI-3, was identified soon after the identification of the sequence[17,50]. Similarly, several proteins, such as PIST, MAGI-2, MAGI-3, PSD-95, and SAP97 that bind the PDZ ligand sequence of the B1AR have also been identified[19,20,29]. It is interesting that these two related receptors bind distinct complements of PDZ proteins. SAP97 has been implicated as the main protein that mediates B1AR recycling, by directly interacting with the PDZ ligand and recruiting the A-Kinase Anchoring Protein-79 (AKAP-79) which phosphorylates the B1AR on a residue that contributes to recycling[29]. Similarly, B2AR also interacts with AKAP-12 (Gravin), which might recruit c-Src to this complex[51].

However, while these protein interactions have been delineated, we have only recently started to understand how GPCRs are recycled by these interactions. Some of the key breakthroughs came from recent advances in live cell imaging that allow for direct visualization of receptor sorting and recycling in the endosome. These studies showed that the recycling of the B2AR is mediated by specific microdomains on the endosome, distinct from those that mediate bulk recycling[52]. These sequence-dependent recycling microdomains are marked by a specialized actin cytoskeleton and specific membrane modifying proteins such as sorting nexins and retromer[53,54]. This was exciting, since it challenged the traditional view that all recycling tubule populations at the early endosome have the same sorting kinetics and trafficking machinery[52]. A variety of proteins have been localized to this specific actin/sorting nexin/retromer tubular (ASRT) microdomain on the endosome[52,54,55]. A global analysis of plasma membrane proteins after depletion of SNX27 or retromer components showed a reduction in surface levels of over a 100 proteins, suggesting that this is a general recycling pathway used by many proteins[56].

Evidence suggests that the primary role of the PDZ interactions is to link the receptor to these ASRT domains. Disrupting these interactions or depleting components of the endosomal actin cytoskeleton, retromer complex, or SNX27 inhibited B2AR recycling, similar to mutating the PDZ ligand or depleting the PDZ proteins[52–54]. Conversely, replacing the PDZ domain with an actin-binding domain from ezrin was sufficient to confer recycling to DOR and B2AR[57]. The facts that actin-binding is required and sufficient for recycling, and that several GPCRs have the ability to bind to PDZ-domain proteins, suggests a conserved role of the PDZ-linked actin cytoskeleton in endosomal sorting of GPCRs.

However, why and how GPCRs are excluded from bulk recycling is still an open question. Current data suggest a kinetic basis for this exclusion (Figure 2). Estimation of diffusion rates showed that the mobility of the B2AR on endosomes was highly restricted[52]. This might make it difficult for B2AR to diffuse into and populate the bulk recycling tubules, which undergo fast fission. The actin cytoskeleton on the ASRT domains stabilizes these domains and delays fission of these tubules. This might allow enough time for the slow-diffusing B2AR to diffuse into these tubules, where they will be concentrated by interactions with the ASRT proteins. Such a “kinetic sorting” mechanism in fact might provide a common mechanism for sorting in many different membrane compartments. Validating this model at a mechanistic level is an important future direction in our understanding of GPCR biology.

In contrast to beta adrenergic receptors, the opioid receptor family exhibits very diverse trafficking characteristics. The KOR requires a PDZ domain for post-endocytic recycling, similar to the adrenergic receptors[22]. DOR, as mentioned above, does not recycle and is degraded in lysosomes following agonist-induced endocytosis[12,58]. Interestingly, the mu-opioid receptor (MOR), recycles following agonist-induced internalization, but does not require a PDZ ligand sequence, like KOR and the adrenergic receptors. MOR contains a unique, seven amino acid recycling sequence in its C-terminal tail, LENLEAE. Mutation of this sequence re-routes MOR to the lysosome following endocytosis, and fusion of this sequence to the C-terminal tail of DOR is sufficient to promote its rapid recycling, and prevent lysosomal degradation of DOR[25].

Although MOR's recycling sequence was discovered a decade ago, the exact mechanism of how this sequence promotes MOR sorting and recycling remains unknown. However, a number of MOR binding partners that regulate MOR trafficking have been found. The actin-binding protein, filamin A, has been shown to interact with the C-terminal tail of MOR, and this interaction is thought to reduce MOR agonist-induced internalization[59]. Additionally, the dendritic spine protein, spinophilin, interacts with MOR in the striatum, and interestingly, knockout of spinophilin reduces sensitivity to morphine-induced analgesia[60]. Further, agonist-induced internalization of MOR is significantly reduced in spinophilin knockout cells[60]. Interestingly, spinophilin interacts with DOR, as well as MOR, and this interaction requires the third intracellular loop, the G protein coupling domain of GPCRs, as well as the first eighteen amino acids of the C-terminal tail, conserved between the two opioid receptors[61]. Additionally, this interaction seems to enhance ERK signaling through DOR, but not MOR, suggesting that the interaction with spinophilin may modulate sensitivity of MOR and DOR differentially[61]. Moreover, single nucleotide polymorphisms within the G protein-coupling domain of the third extracellular loop of MOR are also associated with calmodulin binding and an increase in basal MOR activity[62]. Interestingly, spinophilin also interacts with alpha-2 adrenergic receptors and D2 dopamine receptors, also through the third intracellular loop of these receptors[63,64], suggesting that spinophilin may regulate several GPCRs, potentially through G protein coupling. The additional requirement of a conserved region of MOR and DOR C-terminal tails for spinophilin binding suggests a potential role in membrane trafficking, while the exact mechanism remains unknown.

Even though GPCR recycling uses diverse sequences and proteins, it is likely that there is a common mechanism that mediates the recycling of most GPCRs, which can be accessed by specific "recycling adaptors" that link receptors to this machinery. In support of this, disruption of Hrs inhibits the recycling of most GPCRs that recycle in a sequence-dependent manner. This role of Hrs seems to be independent of its role in ESCRT-mediated degradation of proteins, as the other components of the ESCRT machinery such as Tsg101 do not produce this phenotype[65]. Further, Hrs mediates its effect on B2AR recycling via its Vps27-Hrs-STAM (VHS) domain, which does not play an active role in lysosomal sorting[65]. Additional support for this idea comes from the identification of mutations on the B2AR that converts the receptor into a bulk recycling protein. A sequence on the proximal part of the C-terminal tail on B2AR (EKENKLL) that resembles, but is distinct from, an acidic dileucine sequence, was required for Hrs-dependence and sequence-dependence of B2AR recycling[66]. Similarly, mutating a phosphorylation site on B2AR, adjacent to the EKENKLL

sequence, that is phosphorylated by Protein Kinase A (PKA), converts B2AR recycling to be independent of actin and the PDZ ligand sequence[67,68]. Identification of these specific sequences indicates that there is a specific machinery that retains B2AR on the endosome (which might constitute step 1 in the hierarchical sorting model , actively excluding it from bulk recycling. As of now, there are no known interacting partners to either of these sites. Once future studies identify candidates, we will be able to dissect out the mechanisms and function of this hierarchical sorting of GPCRs.

Another argument for common machineries mediating GPCR recycling is the involvement of general vesicle trafficking machineries in this process. For example, Rab GTPases regulate many steps in vesicle trafficking of GPCRs, such as vesicle budding, tethering, and docking[69]. Rab5 is a marker for the early endosome to which many GPCRs, such as the B2AR, endothelin A and B receptors, and the thyrotropin-releasing hormone receptor, localizes following agonist-induced endocytosis[70–73]. Overexpression of dominant negative Rab5 mutants interferes with endocytic trafficking of B2AR, the D2 dopamine receptor, neurokinin-1 receptor (NK1R), CXC chemokine receptor 2, lysophosphatidic acid-coupled/EDG-2 receptor, and the cannabinoid receptor 2[72,74–78]. Rab5 may be involved in resensitization of B2AR and NK1R[72,75]. Further, Rab4 is thought to control rapid recycling from Rab5/Rab4 early endosomes to the plasma membrane, while Rab11 mediates a slower recycling pathway[79]. Both these have been implicated in the recycling of GPCRs as well as a variety of non-GPCR cargo, including TfR. For example, a dominant negative Rab4 or depletion of Rab4 by RNA interference inhibits rapid B2AR recycling following agonist-induced endocytosis[72,67]. MOR is also thought to recycle in a Rab4 dependent manner, but also recycles through a slower Rab11-mediated pathway[80]. Other GPCRs are thought to traffic through the slow, Rab11-dependent pathway, such as the cannabinoid receptor 2, angiotensin II type I receptor, and the M4 muscarinic acetylcholine receptor[78,81,82]. Together, this suggests that, in addition to regulation of GPCR recycling by C-terminal recycling sequences and their respective binding partners, GPCR post-endocytic trafficking is also subject to regulation by Rab GTPase activity at different endosomal compartments.

3. Regulation of sequence-dependent recycling by modifying steps in hierarchical sorting

Recent evidence suggests that intracellular signaling cascades can control endosomal sorting of GPCRs. This provides new explanations for how cells might coordinate the diverse cellular responses mediated by different GPCRs at physiological time scales. B2AR recycling is regulated by protein kinase A (PKA), a signaling kinase downstream of B2AR activation[67,68]. Further, B2AR signaling can homologously regulate its recycling through PKA phosphorylation of the C-terminal tail of B2AR. These two PKA sites on the C-terminal tail of B2AR regulate a switch in the type of recycling tubules that B2AR is sorted into at the early endosome. Increased PKA phosphorylation of B2AR, following sustained adrenergic signaling, restricts B2AR to the ASRT domain on the endosome. Conversely, non-phosphorylated B2AR can enter the non-ASRT, or bulk recycling tubules, traversed by nutrient receptors, like the transferrin receptor (TfR)[68]. This suggests a hierarchical sorting mechanism that allows a cell to fine-tune its responses to extracellular signals (Figure 3A).

For example, in the case of sustained adrenergic signaling, restriction of B2AR to the sequence-dependent pathway by PKA phosphorylation allows a cell to quickly slow down B2AR resensitization by decreasing recycling from the endosome. It is possible that a similar mechanism exists for B1AR, considering the described role of PKA phosphorylation in B1AR recycling and resensitization[29,51], but the exact mechanisms have not been addressed in detail.

Considering that the ASRT domains contain a variety of protein complexes whose functions are highly regulated, these potentially provide additional control points for regulating GPCR recycling even beyond a simple switch between bulk and ASRT-dependent recycling. For example, cortactin, one of the key components of the actin cytoskeleton in ASRT domains, is regulated by c-Src phosphorylation[83]. This phosphorylation increases the rate of vesicle scission from these domains[84]. Because modifying vesicle scission affects the surface delivery of all cargo proteins that use this pathway[56], it is possible that this provides a general mechanism for controlling recycling of all these proteins. Such hierarchical steps of recycling provide a number of checkpoints for signaling receptors. They can first be sorted at the early endosome according to the recycling sequence on their C-terminal, then phosphorylation of GPCRs themselves at their C-terminal tails, or components of the ASRT domains, by kinases allows the cell to alter recycling kinetics in response to diverse physiological situations (Figure 3B).

Recent work suggests that MOR recycling can also be regulated by phosphorylation by kinases downstream of GPCR activation. Much like B2AR, MOR recycling in striatal neurons is decreased by forskolin, which activated cAMP, although PKA was not directly tested in these neurons[85]. MOR recycling is also regulated through a heterologous pain signaling pathway in sensory neurons. Pain signaling, through activation of the neurokinin-1 receptor (NK1R) by substance P, increases MOR recycling in sensory neurons through PKC phosphorylation of two residues on the C-terminal tail of MOR, serine 363 (S363) and threonine 370 (T370)[86]. This is consistent with physiological and pharmacological data that PKC inhibition reduces MOR resensitization, and that PKC modulation can change behavioral correlates of opioid tolerance[87,88]. Interestingly, the phosphorylation state at these sites can be differentially regulated by diverse opioid agonists. S363 is constitutively phosphorylated, whether or not the receptor is bound to an opioid drug. T370, on the other hand is phosphorylated when the receptor is activated by high-efficacy opioids, like [D-Ala2, N-Me-Phe4, Gly5-ol]-Enkephalin (DAMGO) and fentanyl, but not morphine[89]. Further, substance P has also been shown to induce phosphorylation at MOR T370[90]. This heterologous regulation of MOR recycling further suggests the possibility that hierarchical sorting of GPCRs, through signaling regulation, allows the cell to control GPCR resensitization by regulating recycling kinetics in response to different physiological stimuli.

4. Relevance of sequence-dependent GPCR recycling in the endosome

The exact role of endocytic trafficking in GPCR function has been often highly debated in the past. After activation, receptors are desensitized by phosphorylation, and need to be dephosphorylated to be resensitized and made competent for ligand binding and signaling. Evidence suggests that both phosphorylation and dephosphorylation can occur at the cell

surface. Endocytic trafficking might play roles in modifying the kinetics of this process, although the direction in which endocytosis drives resensitization might vary depending on the receptor and conditions[6,30]. Nevertheless, the long-standing model for GPCR function was that they signal primarily at the cell membrane through their heterotrimeric G proteins, after which they are desensitized. Based on this, a straightforward role for endosomal GPCR sorting, that sorting of receptors into the sequence-dependent pathway caused rapid recycling and delivery of receptors to the cell surface and led to recovery of cellular responsiveness to the same signal, has been appreciated for a while[91].

Recent evidence suggests, however, that sequence-dependent recycling plays more complex roles in tuning both spatial and temporal characteristics of GPCR signaling. GPCRs can signal through several non-G protein signaling pathways - for example through the GPCR adaptor, beta arrestin[92]. Some GPCRs recruit and signal through arrestin at the endosome. Sustained signaling through G proteins at the endosome following GPCR endocytosis has also been demonstrated for some GPCRs[93–95]. The parathyroid hormone receptor (PTHR) continues to signal after receptor endocytosis, and different agonists differ in their ability to induce this type of signaling. Interestingly, PTHRs signaling at the endosome were also shown to associate with Gαs, challenging the traditional view that G protein coupling and signaling occurs primarily at the cell surface[93,96]. Further, internalized thyroid stimulating hormone receptors (TSHR) exhibit coupling to Gs and cyclic AMP (cAMP) production following internalization[94].

For “conventional” GPCRs like the B2AR, however, G protein signaling at endosomes has remained controversial, in part because the signaling profiles of B2AR are much faster compared to receptors like the PTHR, and because traditional signaling readouts could not discriminate between the cell surface and subcellular compartments as signaling sources. Recent breakthroughs have generated a GFP-tagged nanobody biosensor that specifically recognizes the activated form of the Gα stimulatory protein (Gαs). This sensor, which provides spatial resolution, showed that B2AR can activate Gαs at early endosomes[97]. This presents the first clear demonstration that even conventional GPCRs, where the focus has largely been on signaling from the cell surface, can induce G protein signaling cascades from endosomal compartments. Following up on this work, the von Zastrow group has also shown that cell surface and endosomal G protein signaling activated distinct transcriptional profiles[98], suggesting that spatial encoding of where the cAMP signaling was generated could produce diverse cellular responses. While still early, this emerging body of work provides a new perspective on the role of endocytic trafficking in GPCR function. Direct visualization of active conformations of B2AR and Gs proteins on the endosome suggests a transformative idea that endocytosis and subsequent endosomal sorting of GPCRs, in addition to deciding whether GPCRs are degraded at the lysosome or recycled to the cell surface, could also regulate diverse intracellular signaling cascades at the early endosome following agonist activation.

5. Summary and perspectives

The post-endocytic sorting of internalized adrenergic and opioid receptors between the recycling and degradative pathways determines whether receptors are delivered back to the

cell surface or degraded in the lysosome. GPCR recycling is an active process that requires specific sequences on the receptor tails. Several proteins that interact with these sequences and drive receptors into the sequence-dependent recycling pathway have been identified. Considering that many membrane proteins can recycle as part of bulk membrane flow, how and why GPCRs are excluded from bulk recycling is a clear area that needs further investigation.

A well-appreciated role for post-endocytic sorting is that it controls receptor signaling by determining the number of signaling receptors that are recycled to the cell surface. Accumulating data in the recent years, however, suggest that post-endocytic sorting plays more complex roles in regulating receptor function. Recycling of GPCRs is mediated by specific microdomains on the endosome that are physically separate from domains that mediate bulk membrane recycling. GPCR recycling domains also serve as organizing centers for specific signaling complexes. Importantly, signals originating from the surface and endosomes induce distinct downstream responses, suggesting that GPCR signaling is spatially encoded. The cytoplasmic interactions of GPCRs, by determining receptor localization in these microdomains, might directly determine this spatial encoding, and therefore might provide control points for the cell to precisely modulate both the spatial and temporal characteristics of signaling. We have only recently begun to identify signaling pathways that modify these interactions and regulate receptor signaling. As future studies identify more examples of signaling-mediated regulation of these machineries, especially in physiologically relevant systems, we will be able to build a better picture of how cross-talk between multiple signaling pathways allow cells to generate an integrated response in complex signaling environments.

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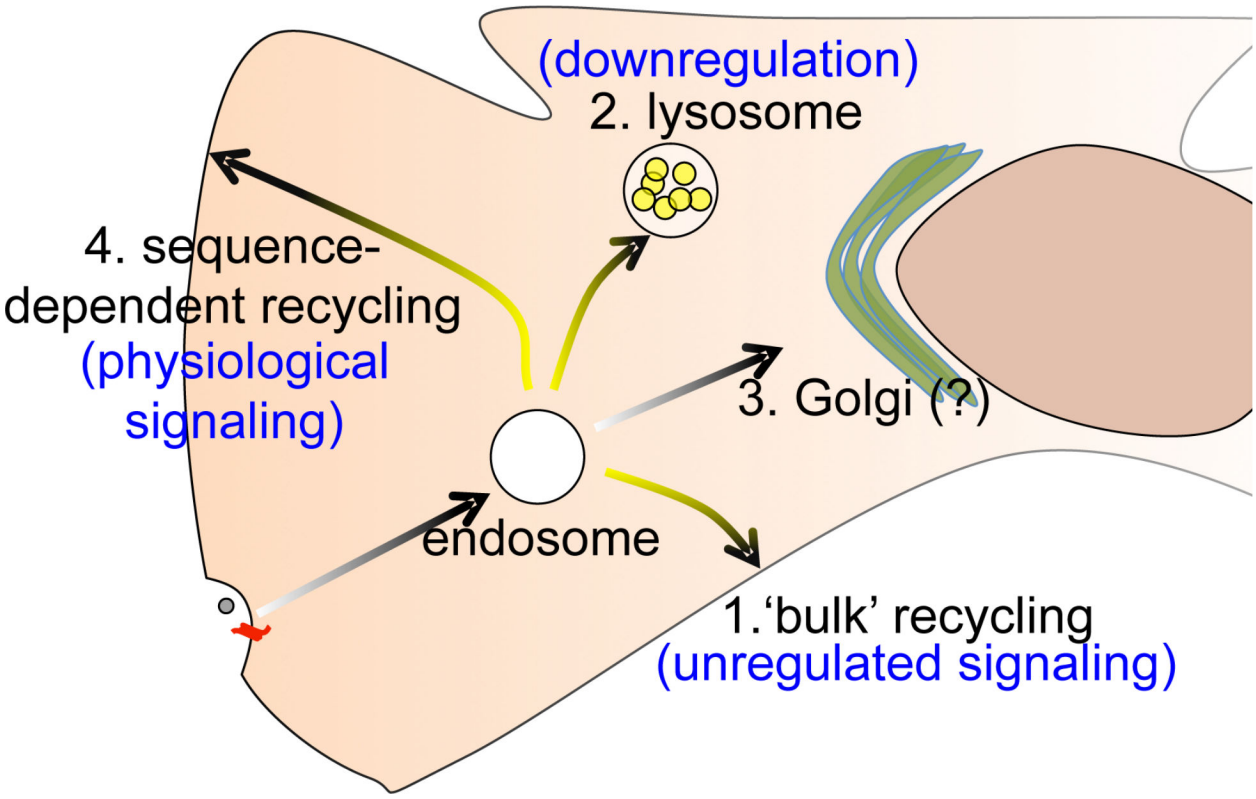

Figure 1.

Potential pathways for endocytosed GPCRs from the endosome. Sequence-dependent recycling is the physiological scenario for recovery of signaling, while sorting to the lysosome leads to degradation and downregulation. Bulk recycling pathway, taken normally by nutrient receptors but not GPCRs, potentially leads to unregulated signaling for GPCRs. While some proteins can travel to the Golgi apparatus, this is not established as a common route for GPCRs.

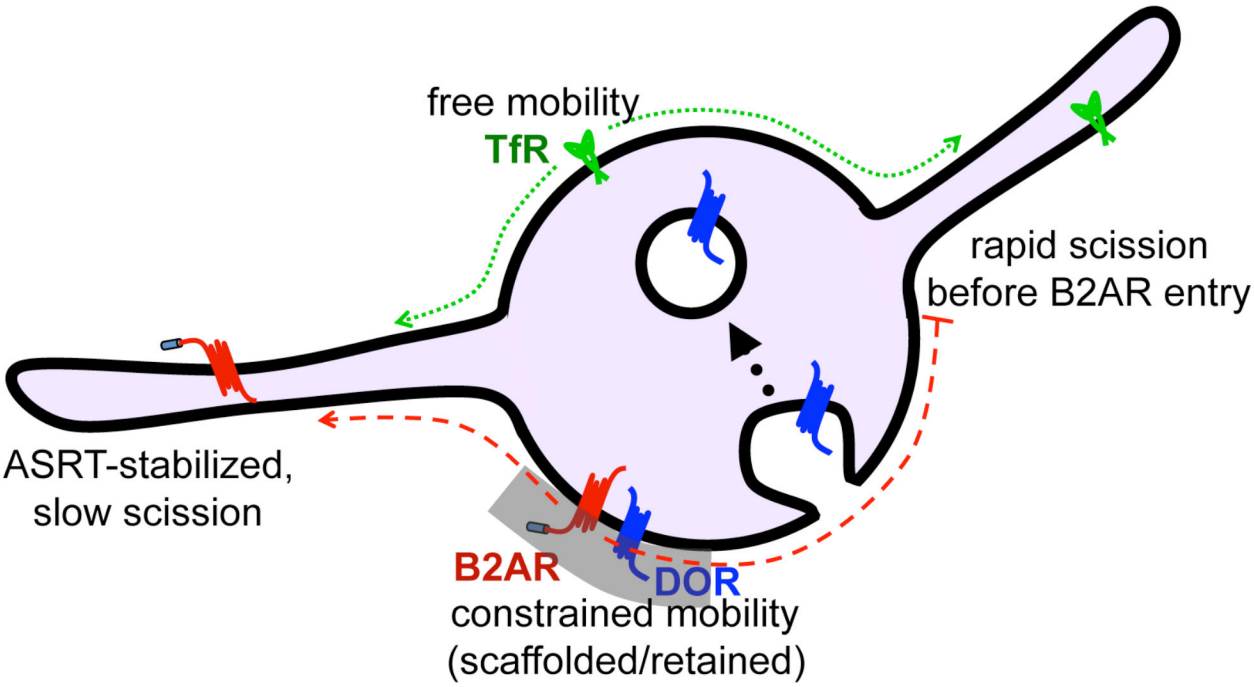

Figure 2.

A kinetic model for sorting in the endosome. The mobility of sequence-dependent recycling proteins or degraded proteins on the endosome are constrained by an unknown mechanism. This slow mobility reduces their entry into bulk recycling tubules which form and undergo scission rapidly. Bulk recycling proteins have unconstrained mobility on the endosome, which allows them to enter the short-lived bulk recycling tubules. ASRT domains are stabilized by the actin cytoskeleton, which provides sequence-dependent recycling proteins enough time to diffuse into these tubules. Degraded proteins are captured by the ESCRT machinery and packaged into intralumenal vesicles.

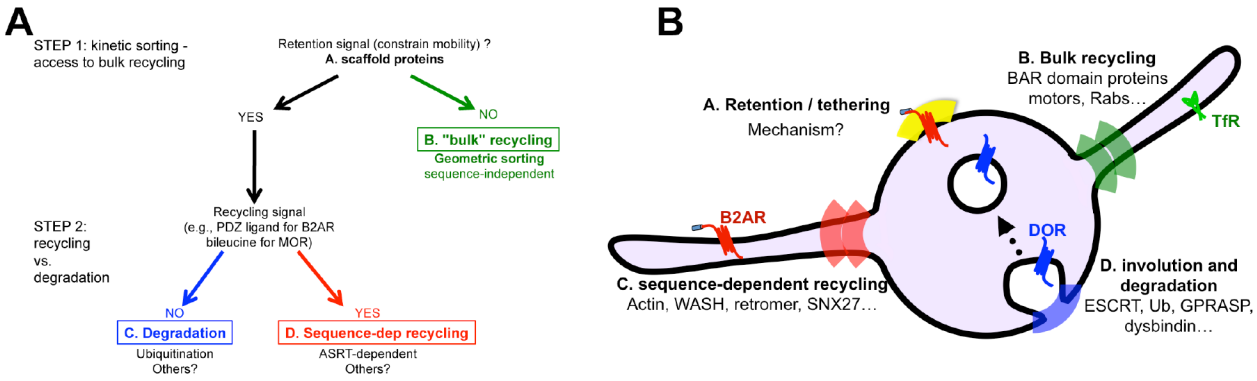

Figure 3.

A hierarchical model for GPCR sorting in the endosome. A) The first step in sorting is an unknown mechanism that tethers GPCRs on the body of the endosome, excluding them from entering bulk recycling tubules. This could be a consequence of decreased mobility, as in the kinetic sorting model described in Figure 2. Bulk recycling proteins are not tethered by this mechanism, allowing them to recycle by geometric sorting. Tethered proteins are either recycled, if they have a recycling sequence, or degraded, if they contain one of multiple signals including ubiquitination. B) The proposed protein complexes on the endosome that mediate these hierarchical steps in sorting are shown.