Advances in membrane trafficking and endosomal signalling of G protein-coupled receptors

Aylin C. Hanyaloglu*

Institute of Reproductive and Developmental Biology, Dept. Surgery and Cancer, Imperial College London, UK

*Correspondence: Dr. Aylin Hanyaloglu

Address: Institute of Reproductive and Developmental Biology, Dept. Surgery and Cancer, Hammersmith Campus, Imperial College London, Du Cane Road, London, W12 0NN, UK

Email: a.hanyaloglu@imperial.ac.uk

Tel: +44(0)20 759 42128

Keywords: GPCR, endosomal signalling, trafficking, internalization, recycling, degradation

Abstract

The integration of GPCR signalling with membrane trafficking, as a single orchestrated system, is a theme increasingly evident with the growing reports of GPCR endosomal signalling. Once viewed as a mechanism to regulate cell surface heterotrimeric G protein signalling, the endocytic trafficking system is complex, highly compartmentalized, yet deeply interconnected with cell signalling. The organization of receptors in to distinct plasma membrane signalosomes, biochemically distinct endosomal populations, endosomal microdomains and its communication with distinct subcellular organelles such as the Golgi, provides multiple unique signalling platforms that are critical for specifying receptor function physiologically and pathophysiologically. In this chapter I discuss our emerging understanding in the endocytic trafficking systems employed by GPCRs and their novel roles in spatial control of signalling. Given the extensive roles that GPCRs play *in vivo*, these evolving models are starting to provide mechanistic understanding of distinct diseases and provide novel therapeutic avenues that are proving to be viable targets.

1. Introduction

The primary mechanism for GPCRs to transduce communication from extracellular signals is via coupling to distinct heterotrimeric G proteins. Classically this signal activation stems from individual cell surface receptors activating a single cognate G protein pathway that converges on to core downstream signal pathways. However, given the central physiological importance of this superfamily of signalling receptors and the large number of distinct receptors (<800), an outstanding question for many years was to understand how such a limited number of pathways can mediate diverse responses in distinct tissues. From studies over the past three decades it is now accepted that the GPCR superfamily mediates diverse functions *in vivo* by activation of pleiotropic signalling mechanisms. These altered models of GPCR signalling exhibit systems that are intricately regulated and exhibit extensive signal crosstalk and diversity. Such mechanisms for pleiotropic signalling include; the ability of a GPCR to activate multiple heterotrimeric G protein pathways that may differ across distinct tissues; that GPCRs can associate as homomeric and heteromeric complexes that can alter its pharmacology and signal activation, to the ability of receptors to potentially activate signalling independently of their G proteins. Despite this increased insight into GPCR function, how cells decode this complex signalling into downstream specific responses is poorly understood (Stallaert et al. 2011; Ferre et al. 2014; Rankovic et al. 2016). A key recent contributor not only to this signal pleiotropy, but also as a 'decoding' mechanism, is endocytic membrane trafficking. In fact, the mechanisms that underlie GPCR signal complexity and specificity, point to a significant role for endocytic trafficking in these processes. Viewed classically as a means to facilitate signal termination from the cell surface, we now appreciate endocytic trafficking is highly integrated with the GPCR signal network, providing an intricate system to organize and direct receptor signalling. In this chapter, I will describe how early events in endocytosis not only contributes to creating organized functional signal microdomains at both the plasma membrane and endosomal membrane, but as a system to provide spatial bias in signal activation and regulation. I will

also provide an updated overview of how endocytic trafficking has progressed our understanding of the fundamental mechanisms driving GPCR signal activity *in vivo*.

2. Updates in our understanding of early endocytic events on GPCR signal regulation

2.1. Archetypal mechanisms of GPCR signal desensitization and internalization

Following receptor activation at the cell surface and initiation of distinct signalling cascades, cell surface GPCR signalling is well-known to be regulated at a temporal level via endocytosis. Rapid ligand-induced internalization, contributes to acute signal termination or desensitization of G-protein signalling (Figure 1). The archetypal model for internalization of many GPCRs occurs primarily via clathrin-coated pits (CCPs) and involves a mechanism whereby arrestins 2 and 3, play a central role (Moore et al. 2007; Barki-Harrington and Rockman 2008). In this model, a family of adaptor proteins, the arrestins, are recruited from the cytoplasm to the activated and phosphorylated GPCR, by members of the GPCR kinase (GRK) family, resulting in uncoupling the receptor from its cognate G protein leading to signal desensitization at the G protein level. Arrestin also drives receptor clustering in to CCPs, via its ability to bind both GPCR and CCP proteins, namely the β-subunit of the adaptor protein 2 (AP2) and heavy chain of clathrin. Once GPCRs are concentrated in CCPs, the scission of clathrin-coated vesicles from the plasma membrane involves the large GTPase dynamin, which is recruited to the CCP, resulting in GPCR internalization to the endosomal compartment (Figure 1) (Luttrell and Lefkowitz 2002; Pierce and Lefkowitz 2001). GPCRs greatly differ in how they utilize this GRK/arrestin/CCP model for its desensitization and internalization. Receptor-specific engagement with this system helps to understand how diversity in GPCR function can be generated with limited G protein pathways, and the impact for specific receptor systems *in vivo*. Early studies examining the role of arrestins in GPCR internalization described a classification of ‘class A’ and ‘class B’ GPCRs that differed in their temporal engagement and arrestin preference, with the former group preferring to recruit arrestin-2 and only exhibiting a transient association at the plasma

membrane and CCPs (e.g. β2-adrenergic receptor (β2AR)), whereas the latter recruited both equally and co-internalized with their receptors, e.g V2 vasopressin receptor. Mechanistically, it was identified that C-terminal Ser/Thr phosphorylation clusters were essential for providing a sustained arrestin association. Such phosphorylation barcodes of GPCR tails may underlie why a receptor may differ in internalization properties and arrestin-utilization across cell types, even with the same ligand, e.g. morphine-activated mu-opioid receptor, but also that distinct ligands can induce distinct patterns of receptor phosphorylation. Whilst not all arrestin-dependent GPCRs may fit into these two groups, these features of GPCR/arrestin associations in these two classes have facilitated recent fundamental molecular understanding of arrestin-dependent G protein coupling at the receptor and contrasting roles in G protein signal activation, which will be discussed further in section xx.

Arrestins have been in the spotlight in this field for some time due to the increasing number of fundamental roles they play in GPCR activity that go beyond receptor desensitization and clathrin-mediated internalization, including a scaffold for non-G protein signalling pathways and endosomal G protein signalling. These proteins represent a key mechanism for GPCR signal diversification and their function as signalling scaffolds, distinct from G protein signalling, and is one area of strategic focus in drug discovery. These adaptor proteins mediate G-protein-independent GPCR signalling by acting as scaffolds for a variety of signalling proteins. The primary pathway studied for arrestin-dependent signalling is the MAPK cascade, specifically activation of ERK1/2 (Reiter et al. 2012). However, proteomic analysis of the arrestin signalosome has identified a vast array of proteins that can interact and/or be activated by these multi-functional scaffolding proteins, such as AKT, p38, JNKs, STATs, mediating downstream functions such as cell growth, cell survival, apoptosis, contractility, cell migration and cytoskeletal reorganization (Xiao et al. 2007; Xiao et al. 2010). While it was thought that arrestin 2 and 3 played redundant functions, there is extensive evidence that each arrestin is regulated by a distinct manner, interacts with distinct

protein partners and thus has specialized cellular functions (Srivastava et al. 2015). The current model is that GPCRs can couple to this G protein-independent form of signalling without detectable G protein signalling, however, a recent study has challenged this. Employing cell lines where each G protein or arrestin was deleted via gene editing approaches, except for the Gαi family whereby the Gαi/o inhibitor pertussis toxin was used to generate a 'G protein zero' state (Grundmann et al. 2018). Interestingly, arrestin recruitment and receptor internalization to distinct GPCRs was still evident in the absence of functional G protein. However, for the GPCRs tested in this study ERK signalling was entirely dependent on G protein in contrast genetic ablation of arrestins 2/3 had either no effect or partially impacted ligand-induced ERK signalling. The authors aptly express caution in future application of arrestin-dependent signalling, yet acknowledge limitation of the HEK 293 cell type employed in the interpretation of these findings in distinct tissues. Many of the receptors employed exhibited, for the most part, a transient ERK signalling profile exclusively G protein-dependent, such as the beta2-adrenergic receptor (class A arrestin profile), and also confirmed recently by a distinct study (O'Hayre et al. 2017). Whereby the AT1 angiotenisn receptor (class B arrestin profile), the loss of arrestin did impact the signalling profile, although loss of G protein completely abolished ERK signalling from this receptor (Grundmann et al. 2018). These important findings may also suggest that arrestin can act as a scaffold (confirmed in this study) for signalling molecules, but that the ability of arrestin-mediated signalling still requires an activated G protein for some receptors as an upstream step in this pathway. Further, it also emphasizes that different upstream pathways can feed in to MAPK signalling, and thus highlights caution for using ERK as a readout for biased signalling. Biased signalling between G protein and arrestin-dependent activity is currently of high interest in drug discovery (Rankovic et al. 2016). Although this recent study (Grundmann et al. 2018) may shed caution for certain GPCRs on use of ERK as a biased signal readout, and perhaps if this a valid target for screening; for certain GPCRs it has been shown physiologically, using arrestin knockout animal models, that ligand-directed signalling to the arrestin-dependent pathway is beneficial or, underlie unwanted side effects of full

agonists/antagonists. For example, biased ligands to the μ-opioid receptor (MOR) that activate G protein, but not arrestin-dependent signalling, are thought to have potent analgesic properties without the anti-respiratory, and dysfunctional gastrointestinal tract side effects (DeWire et al. 2013). This is also good example of a receptor where morphine-dependent activation of arrestin exhibits distinct properties between HEK 293 cells and neurons.

Although clathrin-dependent internalization of GPCRs via the GRK and arrestin-model contributes to rapid desensitization, there are several studies indicating that these common internalization and desensitization mechanisms have expanding roles in spatio-temporal control of GPCR signalling. For example, there is known heterogeneity in CCP function, where different GPCRs can be organized within distinct subsets of CCPs to dictate subsequent post-endocytic fate between recycling or lysosomal pathways (Mundell et al. 2006; Lakadamyali et al. 2006). Such divergent sorting of GPCRs at the plasma membrane was mechanistically dependent on GRK/arrestin (for CCPs directing receptors to the plasma membrane recycling pathway) and receptor phosphorylation by second messenger kinases (for lysosomal/degradative sorting). Specialization of CCPs have the potential to function as platforms for signalling microdomains, that could recruit molecules distinct from ligand-bound activated cell surface localized receptor outside of the CCPs, and/or the tight temporal control of G protein activation. This is supported by the ability of GPCRs to regulate their own residency time in CCPs. For the β2AR), interaction with scaffold proteins, namely postsynaptic density 95 (PSD95)/discs large (Dlg)/zonula occludens-1 (Zo-1) (PDZ) proteins, tether receptors to cortical actin and extend the occupancy time of a receptor in a CCP by delaying the recruitment of dynamin (Puthenveedu and von Zastrow 2006). However, MOR, which does not have a PDZ ligand within its intracellular domain, modulates its residency time by delaying the scission activity rather than recruitment of dynamin and involves a mechanism whereby receptor ubiquitination dictates CCP residency time (Henry et al. 2012; Roman-Vendrell et al. 2012) . The downstream function of altering CCP residency time of a

GPCR in CCPs has been demonstrated for the cannabinoid receptor type 1 (CB1). For CB1, CCP residency time provides a mechanism to regulate mitogenic signals in a highly defined spatial-temporal manner, via formation of arrestin signalling scaffolds, with downstream actions in regulating expression of genes involved in contrasting pro-survival and pro-apoptotic functions (Delgado-Peraza et al. 2016; Flores-Otero et al. 2014). Interestingly, a recent study has demonstrated that arrestin signalosomes at the CCP may not require the GPCR. Ligand-dependent activation of β1-adrenergic receptor (β1AR) can robustly recruit arrestin to CCPs without detectable receptor localized to CCPs. This CCP-bound arrestin was shown to activate a prolonged MAPK signal profile from CCPs. How this receptor recruits arrestin to CCPs without receptor is unknown, although it is proposed that arrestin is initially recruited to the activated receptor, and akin to the G protein cycle, rapidly dissociates in its active form to CCPs (Eichel et al. 2016). However, it is tempting to speculate that involvement of PDZ proteins known to bind to this receptor's C-terminal tail (e.g. PSD95, MAGI-2, GIPC (He et al. 2006)) that could regulate β1AR clustering and/or facilitate a plasma membrane scaffold between receptor external to the CCP and activated arrestin in CCPs.

Specialization of clathrin at the plasma membrane within the confines of coated pits may also extend to larger, distinct clathrin structures called clathrin lattices or plaques (Figure 1). Although these structures have been observed by cells biologists for many years there is little information on the role of these structures, in particular to GPCR (Lampe et al. 2014; Grove et al. 2014). But may function as signalling microdomains. Plaques and lattices have been observed in multiple cell types and are distinct from CCPs morphologically, the latter being smaller (100-200nm diameter) and spherical, their stability, including the persistent association of dynamin. GPCRs that have been observed to be organized in such structures include the β2AR, β1AR, MOR and the chemokine receptor CCR5 with evidence suggesting they contribute to a sustained signal profile, consistent with the proposed microdomain function (Lampe et al. 2014; Grove et al. 2014). Indeed, the ligand-dependent recruitment of

arrestin in CCPs without □1AR, was also observed in larger clathrin structures corresponding to lattices or plaques (eichel, 2016).

Although clathrin-associated adaptors such as arrestin and AP2 represent core CCP machinery utilized by many kinds of receptors, it is unclear whether there are additional players that can direct receptors into distinct pits. Although organization of receptors across CCPs may be initiated via the differential phosphorylation of GPCR intracellular domains (Mundell et al. 2006), it remains to be determined whether this dictates any adaptor protein specificity in addition to the arrestins. Certainly, GPCR internalization can be regulated, both positively and negatively, by additional post-translational modifications such as ubiquitination and palmitoylation, suggesting additional adaptor proteins are likely involved; indeed, arrestins are known to recruit E3 ligases to GPCRs (Girnita et al. 2005; Shenoy et al. 2009; Shenoy 2014). How arrestin can mediate such numerous functions, even when considering the early events of endocytic trafficking alone, may be rationalized by an elegant study employing biosensors detecting multiple and specific conformational changes upon receptor activation. While the study demonstrated multiple conformational signatures of arrestin with pharmacologically distinct ligands to the same receptor (Lee et al. 2016), one can certainly conceive that there could be multiple extracellular and intracellular factors *in vivo* that would induce multiple arrestin conformations, and hence specific biological functions.

While internalization of receptors via CCPs is a dominant endocytic pathway in mammalian cells and certainly has been the primary endocytic mechanism studied for many different kinds of receptors, there are additional dynamin-mediated, but clathrin-independent pathways that GPCRs internalize via, either in addition to, or in preference to CCP-mediated endocytosis. One recent pathway identified, termed FEME (fast endophilin-mediated endocytosis) is mediated by the protein endophilin-A, which binds to activated receptors and key endocytic molecules dynamin and syntaptojamin, yet co-traffics with its cargo into FEME vesicles (Figure 1). Although initially characterized as a CCP adaptor protein, it was found to

be found only on a minimal number of CCPs, and genetic ablation of all three endophilin isoforms had no impact on CCP-mediated endocytosis. Different receptor types have been shown to employ FEME. Interestingly, endophilins were known interacting partners for certain GPCRs, namely the B1AR. Given the observation that this receptor does not occupy arrestin-occupied CCPs may be in part due to the rapid clathrin-independent endocytosis of some receptors via FEME; indeed, arrestin is not observed in endophilin-positive structures following B1AR activation (Boucrot et al. 2015). Boucrot et. al., (Boucrot et al. 2015) also identified a subset of distinct GPCRs that could bind endophilin including D3 and D4 dopamine receptors, M4 muscarinic receptor. The profile of FEME compared to CME, is that it is rapid (seconds), forms tubulo-vesicular structures containing endophilinA and preferentially occurs at the leading edge of cells, due to involvement of Class I PI3 kinases, production of PI(3,4, 5)P3, and its subsequent dephosphorylation into PtdIns(3,4)P_2 by PTEN to recruit endophilin and bind lamellipodin. This form of endocytosis highlights the interlinked nature of membrane trafficking and signalling as FEME can only be triggered by receptor-dependent activation of signalling pathways. The role for such targeted and rapid receptor-mediated internalization at present is unknown, but likely to be unveiled in the near future.

The plasma membrane of cells is highly organized and specialized environment for receptors, and exemplified by the presence of lipid rafts and caveolae in many cell types. These are defined as planar microdomains of the plasma membrane enriched in specific lipids and proteins such as cholesterol, sphingolipids and glycosylphosphatidylinositol (GPI)-anchored proteins or for caveolae, caveolin proteins, forming flask, or ‘cup-shaped’ pits ~60-80nm in diameter (Bastiani and Parton, 2010) (Figure 1). Lipid rafts are known to be enriched in signalling proteins and receptors and thus proposed as a platform for organizing certain receptors with its signalling machinery including heterotrimeric G-proteins, effector enzymes such as adenylate cyclase, and regulators of G-protein signalling (Hiol et al. 2003) and even a platform for enabling receptor crosstalk and/or heteromerization. Localization of

GPCRs to lipid rafts can occur in a constitutive or ligand-dependent manner. And disruption of lipid raft formation can abolish signalling from certain GPCRs including the NK1 receptor (Monastyrskaya et al. 2005) and the serotonin 1A receptor (Pucadyil and Chattopadhyay 2004).

Despite the technical challenges involved in the biochemical study of lipid rafts (for review please see (Pike 2009), the identification that caveolae contain caveolin proteins Cav1, -2, and/or -3 (the latter being skeletal and cardiac muscle-specific) (Song et al. 1996), greatly advanced understanding of these microdomains. However, like lipid rafts, they can also act as GPCR signalosomes, both as a means to spatially desensitize and also locally amplify signal responses. GPCRs, G-protein subunits, effector enzyme systems and even arrestins have been shown to be sequestered by caveolae (Oh and Schnitzer 2001; Chini and Parenti 2004) (kubale-V Vrecl. However, the primary G protein signalling pathway thought to be regulated within caveolae through interaction with Cav1 is Gαq and Ca^{2+} signalling whereby Cav1 prolongs Gαq activation, and even may direct certain Gαq-coupled receptors to caveolae (Sengupta et al. 2008; Oh and Schnitzer 2001; Calizo and Scarlata 2012). However, other studies have demonstrated that in cardiac myocytes is important for the compartmentalization of cAMP signalling from the β2AR and its ability to couple to both Gαs and Gαi in myocytes (MacDougall et al., 2012; Wright et al., 2014)(Xiang and Kobilka 2003; Macdougall et al. 2012; Wright et al. 2014), a physiologically and pathophysiologically important mechanism as in heart failure there is a loss of caveolae and Cav3 and subsequent loss of compartmentation of β2AR/cAMP signalling (Nikolaev et al. 2010; Feiner et al. 2011). In addition to its role as a signalosome, certain GPCRs have reported to internalize via caveolae, although the molecular detail in how this process occurs is not well understood (Shvets et al. 2014). However, one interesting role of caveolae is as a mechanosensor and a recent study employing super-resolution imaging of caveloae under osmotic pressure to visualize caveloae deformation indicated that distinct G protein

pathways are differentially sensitive to osmotic stress eliminates the ability of caveolae to stabilize calcium signals mediated through Gαq, it does not affect cAMP signals mediated through Gαi (Yang and Scarlata 2017).

3. Post-endocytic sorting pathways for GPCRs

In this section I will give a brief overview of the trafficking pathways employed by GPCRs following endocytosis, and their subsequent trafficking to opposing cellular fates; plasma membrane recycling and lysosomal degradation. Work over the past two decades to uncover the mechanisms that drive receptors to these distinct fates demonstrates that GPCR endocytic trafficking is tightly regulated, via a multi-step mechanism. We have previously proposed such complexity enables system plasticity, or reprogramming of receptor fate/s with altering extracellular stimuli (Hanyaloglu and von Zastrow 2008), however, the impact of these altered endocytic fates on GPCR signalling has been primarily focused on more 'classical' signalling models, i.e. impact on cell surface signal responses. Thus, the subsequent primary focus will be on more recent studies on the role of internalized receptors in generating signal responses from different endomembranes, and even endosomal microdomains, and the latest insights in to the physiological, pathophysiological and pharmacological impact will be discussed.

3.2 Post-endocytic sorting of GPCRs to the regulated recycling pathway

Following GPCR endocytosis, receptor trafficking to the early endosome (EE) is the compartment that all cargo is first thought to be sorted to different subcellular destinations. For GPCRs a number of mechanisms dictate their cellular fate between opposing plasma membrane recycling or lysosomal-mediated degradation, resulting in resensitization, or continuous signalling, versus a more transient signal response (degradation). The significance of these divergent post-endocytic routes to both physiology and disease highlights their importance in shaping GPCR activity (Dunn and Ferguson 2015; Hanyaloglu and von Zastrow 2008). Moreover, there is significant evidence that the biological role of

these pathways extends beyond regulation of classical G protein signalling profiles (Hanyaloglu and von Zastrow 2008). The body of research that has deciphered the cellular machinery directing the fate of these receptors, clearly demonstrates that these pathways are tightly regulated at multiple levels during sorting. GPCRs targeted to a recycling pathway back to the plasma membrane generally require a sequence-directed mechanism involving specific cis-acting sorting sequences in their intracellular C-terminal tails (C-tail) (Hanyaloglu and von Zastrow 2008; Trejo 2005). This was first identified for the archetypal GPCR the β2AR, where it was shown that this form of recycling differs to cargo that recycle in the absence of sorting sequences, which occur in a "default" manner via bulk membrane flow (Hirakawa et al. 2003a; Cao et al. 1999). For the β2AR, the recycling sequence encoded in the distal part of its C-terminal tail conferred to a type 1 PDZ binding sequence or 'PDZ ligand'. Removal or mutation of this site inhibits recycling and reroutes the receptor to the degradative pathway. Furthermore, addition of the β2AR PDZ ligand to the δ-opioid receptor (DOR) that is sorted primarily to a degradative pathway, altered its post-endocytic trafficking fate from degradative to the recycling pathway. Thus, these recycling sequences are, in general, found to be both necessary and sufficient in directing receptors back to the plasma membrane (Cao et al. 1999; Gage et al. 2001) and intriguingly was the first indication that post-endocytic trafficking fate of a GPCR, and consequently activity, could be reprogrammed with changes in the cellular environment. These initial studies with the β2AR suggested the interacting PDZ-domain containing protein partner responsible was Ezrin–Radixin–Moesin (ERM)-binding phosphoprotein-50 (EPB50) also called Na^+/H^+ exchange regulatory factor 1 (NHERF-1). However, subsequent studies identified that this receptor, and other GPCRs with PDZ ligands, e.g., the β1AR, can interact with its own subset of PDZ proteins (He et al. 2006), and that such unique GPCR/PDZ interactions may define distinct functions for the receptor. However, EPB50 was primarily a plasma membrane, or juxta-membrane localized protein, and so focus was directed to a unique PDZ protein, sorting nexin-27 (SNX27), that could bind PI3P, a lipid enriched in the EE membrane. SNX27 was demonstrated to be essential for post-endocytic trafficking of β2AR to the recycling pathway (Lauffer et al. 2010).

Since the identification of a recycling sequence in the β2AR, there have been numerous GPCRs identified that require receptor recycling sequences in their C-tails. Interestingly, these recycling sequences are not all PDZ type 1 ligands, with some corresponding to unknown interacting protein partners, thus this high diversity in recycling sequences across GPCRs suggests that they bind specific cytoplasmic proteins (Marchese et al. 2008; Hanyaloglu and von Zastrow 2008).

If GPCRs are sorted to a regulated recycling pathway, exhibit different recycling sequences and potentially different interacting protein partners to achieve this, then are there distinct post-endocytic trafficking pathways and/or common machinery that mediate this? A collection of studies indicates that both common and receptor specific mechanisms are involved. Importantly, these have also highlighted that regulated GPCR recycling was not a one-step mechanism occurring via a protein/PDZ-interaction with the GPCR recycling sequence, but a complex, multi-step system, involving multiple sequences in the receptor C-tail, post-translational modifications in the receptor and the receptors own signalling all playing an integral part (Irannejad et al. 2013; Hanyaloglu and von Zastrow 2008). Examples of core endocytic machinery that process receptors to the regulated recycling pathway include the early endosome-localized adaptor protein hepatocyte growth factor regulated tyrosine kinase substrate (Hrs), also termed Vps27 (part of ESCRT 0-see section 3.3). Hrs was first characterized for its role in the first steps of lysosomal sorting but is also essential for regulated recycling of the β2AR, MOR and calcitonin-receptor-like receptor (Hanyaloglu et al. 2005; Hasdemir et al. 2007). Given its well established role in regulating lysosomal sorting of cargo for distinct cargo, loss of Hrs results in retention of receptors within the EE compartment, suggesting this adaptor protein acts as a sorting point between these divergent endocytic fates. Hrs-dependent recycling is mediated via the N-terminal VHS domain of Hrs, a domain only characterized by its conserved presence in yeast Hrs, Vps27, Hrs, and the Hrs-interacting partner STAM (Hanyaloglu et al. 2005; Hasdemir et al. 2007; Huang et al. 2009). The VHS domain was a poorly understood domain in terms of its role in

regulating membrane trafficking, and due to the role of Hrs as a scaffolding protein that does not associate directly with receptors, it was proposed that this domain may associate with as yet unknown additional proteins (Hanyaloglu et al. 2005). For some GPCRs arrestins may also act as core sorting proteins. For the N-formyl peptide receptor, receptor recycling, but not internalization is highly dependent on β-arrestin (Vines et al. 2003), although for other GPCRs it may negatively regulate rapid recycling and promote slow sorting via the trans-Golgi network (TGN), leading to receptor downregulation (Abdullah et al. 2016). A core endosomal complex that has gained recent attention in regulating GPCR sorting to the recycling pathway is the retromer complex. This macromolecular complex was known for its role in endosome-TGN transport, but identification of its association with the WASH (Wiskott-Aldrich Syndrome Protein and SCAR Homolog) complex has identified additional roles in receptor sorting to the plasma membrane recycling pathway, and specifically organization of receptors in to endosomal tubules that mediate regulated, or PDZ-dependent, recycling (Puthenveedu et al. 2010). Interestingly, the PDZ protein SNX27 acts as a 'bridge' between the receptor and the retromer complex, via the WASH protein complex (Temkin et al. 2011; McGarvey et al. 2016). This not only highlights the complex nature of regulated recycling, but there are mechanisms for a cell to regulate receptors between the two modes of recycling, regulated and default, via physical organization into biochemically defined tubules emanating from the same single endosome. Tubules mediating regulated-recycling cargo require endosomal actin, WASH and retromer and were also kinetically distinct from those tubules mediating recycling of cargo in a default manner, which were more transient for fast-diffusing bulk recycling receptors compared to regulated-recycling (Irannejad et al. 2013). That a GPCR could potentially 'switch' between these two modes of recycling was first demonstrated via a study that identified specific receptor C-tail sequences mediating Hrs/Vps27 dependent recycling. An acidic dileucine-like sequence located upstream of the β2AR PDZ ligand was essential to confer Hrs sensitivity. Mutation of this sequence did not affect ability of the receptor to recycle but switched the recycling to a PDZ-independent, default pathway (Hanyaloglu and von Zastrow 2007). Subsequent studies have

also identified additional molecular requirements critical for switching between bulk/default and sequence-dependent recycling modes for the same receptor. PKA phosphorylation on the proximal region of the β2AR C-terminal tail, induced by agonist-dependent activation of the Gαs-cAMP pathway, inhibits entry into the sequence-dependent recycling tubule (Vistein and Puthenveedu 2013). Interestingly, recycling of the β1AR is also regulated by PKA phosphorylation of its intracellular C-tail but in an opposing manner to β2AR, whereby PKA is required for receptor recycling (Nooh and Bahouth 2017). Such complexity in a recycling system, when there is a more simpler default alternate recycling pathway, indicates a requirement for these recycling GPCRs to enable flexible reprogramming of the receptor to multiple distinct fates (Hanyaloglu and von Zastrow 2008), and that these can be altered as cells are exposed to dynamic extracellular environment (West and Hanyaloglu 2015). The downstream biological significance of this sequence-directed recycling pathway is underscored by demonstration of its direct involvement in regulating cardiac myocyte contractility, where regulated recycling was essential for G protein switching of the β2AR from Gαs to Gαi (Xiang and Kobilka 2003), while for the μ-opioid receptor (MOR) there may be a role for this trafficking pathway in opiate tolerance (Roman-Vendrell et al. 2012). Many GPCRs require sequence-directed recycling for signal regulation, and therefore the inherent flexibility in reprogramming trafficking fates that this pathway affords has driven strategies to potentially screen for novel compounds that could favor between default and regulated recycling (Nooh et al. 2016).

While the above studies have identified the complexity of sequence-directed recycling to enable flexible programming of receptor post-endocytic fate, these receptors primarily internalize to common early endocytic compartments and in part, utilize common core early endosomal machinery. Despite the mechanisms diversifying GPCR signalling, outlined in section 2, these also converge on common downstream pathways. Thus, an outstanding question is what is the role of these different GPCR recycling sequences, and potentially

distinct interacting partners? One function identified is that these sequences can direct receptors to physically and biochemically distinct endosomal compartments. The EE is classically viewed as both the primary platform for receiving, organizing, and sorting diverse sets of cargo. However, certain GPCRs (human luteinizing hormone receptor, LHR, follicle stimulating hormone receptor and β1AR) that undergo sequence-directed recycling are internalized to a distinct endosomal compartment we have termed the very early endosome (VEE) (Jean-Alphonse et al. 2014). Unlike GPCRs such as the β2AR that are well known to rapidly internalize and sort from EEs, these distinct GPCRs internalized to a physically smaller endosome compartment devoid of EE markers EE antigen 1, PI3P, and independent of the GTPase Ras-related protein, Rab5. However, the adaptor protein containing PH domain, PTB domain, and Leucine zipper motif (APPL1) was present on a subpopulation of these VEEs (Jean-Alphonse et al. 2014; Sposini et al. 2017). Mechanistically the targeting of VEE-localized GPCRs was dependent on receptor interactions with the PDZ protein Gαi-interacting protein C terminus (GIPC) (Hirakawa et al. 2003a), early during endocytosis. A loss of interaction between LHR with GIPC inhibited receptor recycling due to the rerouting of receptor to EEs (Jean-Alphonse et al. 2014). This not only demonstrates that sequence-directed sorting of GPCRs can occur from compartments other than the EE, but may also suggest that 'recycling sequences' are too limited description for its role/s, and these receptor C-tail sites may encode multiple functions at distinct points throughout the endocytic life cycle of the receptor. We have recently demonstrated the underlying mechanim mediating regulated GPCR recycling from the VEE. Recycling of LHR, FSHR and β1AR, from VEEs all required APPL1, which is the first reported role ascribed to this multi-functional adaptor protein. Again, receptor signalling was an essential upstream step in directing rapid receptor recycling as LHR-mediated activation of cAMP/PKA signalling was necessary for recycling, but in contrast to the above studies where the target of phosphorylation has been the receptor, for recycling from the VEE PKA phosphorylation of APPL1 on serine (Ser410) was essential (Sposini et al. 2017).

3.3 Sorting of GPCRs to the degradative pathway

Sorting of internalized GPCRs to a degradative fate results in permanent signal termination or transient signal profile. For GPCRs that are rapidly targeted to this pathway, disruption of receptor sorting would result in overactive, or persistent signalling, which for certain GPCRs such as the chemokine receptor CXCR4 is associated with invasive breast cancer (Marchese et al. 2008). Furthermore, lysosomal sorting is a key part of receptor downregulation for recycling receptors that are chronically activated and is thought to play a role in drug tachyphylaxis (acute loss of pharmacological response) or tolerance (Tappe-Theodor et al. 2007; Hanyaloglu and von Zastrow 2008). The pharmacological and clinical significance has thus driven studies to identify the molecular mechanisms targeting GPCRs to this pathway. Following internalization to EEs, GPCRs sorted from this compartment for degradation are trafficked to Rab7 late endosomes leading to involution of receptors to form multivesicular bodies (MVBs), where subsequent fusion with lysosomes results in receptor degradation. The canonical pathway for sorting of many types of membrane cargo, not just GPCRs, is via cargo ubiquitination at lysine residues and engagement with the endosomal sorting complex required for transport (ESCRT) pathway for its degradation. ESCRT are a series of 4 distinct protein complexes (ESCRT-0, I, II, III) with the first 3 containing proteins with ubiquitin binding domains (UBDs), which sequentially capture and retain ubiquitinated cargo from the EE-localized ESCRT0, to involution of cargo in MVBs. Disassembly of ESCRT complexes from the maturing MVB occurs via the AAA-ATPase Vps4 in order to facilitate additional rounds of MVB sorting (Rusten et al. 2011; Henne et al. 2013). Ubiquitination and ESCRT-dependent degradation has been well-described for certain GPCRs such as the CXCR4, protease-activated receptor 2 (PAR2) and β2AR. Distinct ubiquitin E3 ligases mediate receptor ubiquitination during early endocytosis, either associating directly with E3 ligases or via arrestin, and depending on the GPCR, may or may not be required for internalization (Kennedy and Marchese 2015). Although several (>30 GPCRs) have been reported to be ubiquitinated, GPCRs either do not require this ubiquitination, or certain components of the ESCRT machinery, for its lysosomal

degradation, such as DOR (Henry et al. 2011; Hislop et al. 2011; Dores and Trejo 2015). As ubiquitin is not the sorting signal for such receptors, they require association with additional machinery for lysosomal targeting that directly interact with GPCR C-tails, such as GPCR associated sorting protein-1 (GASP-1) and the autophagy protein Beclin-2, prior to ESCRT targeting (He et al. 2013). However, subsequent studies have indicated that DOR ubiquitination, via the E3 ligase AIP4, may play a role in later steps of lysosomal sorting, specifically for efficient receptor involution or transfer to intraluminal vesicles of MVBs to enable proteolysis of the receptor endodomain (Henry et al. 2011; Hislop et al. 2009). This has also been demonstrated for the MOR, which reprograms its sorting from a sequence-dependent recycling to degradative fate, whereby receptor ubiquitination, specifically in the first intracellular loop (which interestingly also defines CCP residency time (Henry et al. 2012)) promotes receptor involution into MVBs, but is not necessary for lysosomal sorting per se (Hislop et al. 2011). Targeting GPCRs to degradation does not always require ubiquitination of the receptor, but instead ubiquitination of associated adaptor proteins, namely arrestin (Mosser et al. 2008). Diversity in GPCR lysosomal sorting mechanisms is further demonstrated by GPCRs such as the protease activated receptor 1 (PAR1) that require neither receptor ubiquitination, ESCRT 0, I or GASP-1 for its degradation, but does require the ESCRT III complex and Vps4. This receptor is directed to lysosomes via the ubiquitous adaptor protein ALG-interacting protein X (ALIX). ALIX is ubiquitinated by E3 ubiquitin ligases that form a complex at the endomembrane with the arrestin-domain containing proteins (ARRDCs) ARRDC1 and ARRDC3 (Dores et al. 2012a; Dores et al. 2015; Dores et al. 2012b). More recently, the defective lysosomal sorting of PAR1 and sustained signalling in invasive breast cancer is directly linked with loss of ARRDC3 levels, as re-expression of this protein alone in human breast cancer cells lines was sufficient to restore normal ALIX-dependent lysosomal sorting of PAR1, thus attenuating thrombin-mediated JNK signalling and a reduction of these cancer cells to exhibit an invasive capacity (Arakaki et al. 2018).

As discussed for sorting of GPCRs to the regulated recycling pathway (section 3.2), there is evidence that lysosomal sorting can be driven by GPCR signalling. For example, degradation of the dopamine D2 and D3 receptors are mediated via phosphorylation of the receptor at specific residues by the second messenger kinase PKC (Zhang et al. 2016; Cho et al. 2013). Interestingly, the Gαs subunit of heterotrimeric G proteins has been demonstrated to have a direct role in post-endocytic trafficking of GPCRs, independent of its GTPase activity (Rosciglione et al. 2014). Heterotrimeric G proteins have been shown to localize to multiple cellular compartments in addition to the plasma membrane, including the endomembranes. Depletion of Gαs was found to inhibit the degradation of distinct GPCRs (CXCR4, DOR, and the angiotensin 1A receptor) known to sort to this pathway via both ubiquitin-dependent and -independent pathways, by inhibiting receptor involution in to MVBs. Despite its identified role at a late stage in lysosomal sorting, Gαs associated with early stage lysosomal sorting proteins GASP-1, dysbindin and Hrs (ESCRT) at the EE. Given that CXCR4 does not employ a GASP-1/ dysbindin pathway for its degradation, this may suggest Gαs interacts with additional proteins (Beas et al. 2012), but also highlights an adaptor role for this signalling protein in the lysosomal sorting of receptors via multiple mechanisms (Rosciglione et al. 2014). Whether other G protein family members could have similar roles in direct sorting of receptors remains to be determined, however, this role of heterotrimeric G proteins in membrane trafficking may not be restricted to GPCRs, as Gαs was recently found to contain a UIM in its N-terminus, and through this ability to bind ubiquinated cargo could mediate the sorting of tyrosine kinase receptors, specifically the EGF receptor to involute in MVBs (Li et al. 2017). What these studies also highlight is that this evident multi-functionality of core GPCR machinery, such as heterotrimeric G proteins, must be considered when interpreting studies employing genome editing or knockdown approaches with these proteins. Overall the complex nature, and diverse mechanisms of lysosomal sorting could also suggest that, as for receptors targeted to regulated recycling pathways, it provides a platform for flexible reprogramming of GPCR fate. Indeed, disruption

of the latter stages of lysosomal sorting results in MVB/endosomal retention, while inhibition at earlier steps, upstream of ESCRT0, can reroute receptors to a recycling pathway, as shown for PAR1 and CXCR4 where inhibition of its ubiquitination via the tyrosine kinase Her-2/ErB2 results in increased surface expression of these receptors in breast cancer and promotion of tumor progression (Marchese et al. 2008). In non-cancer cells, reprogramming GPCR post-endocytic fate could be advantageous in tightly regulating cellular sensitivity such as in development (Mukai et al. 2010; Cadigan 2010).

4. The endomembrane system provides multiple signalling platforms for GPCRs

As described above, endocytic trafficking pathways provide a mechanism to define complex cell surface responses by shaping the signalling profile generated from the plasma membrane. However, studies over the past decade have built a model whereby these endocytic mechanisms and compartments also contribute to this complexity by acting as distinct signalling platforms (Figure 2). This next section will describe our current understanding of how the endomembrane can directly activate signalling across distinct endosomal compartments and even via microdomains within a single endosome.

4.1 The early endosome (EE) as a platform for heterotrimeric G protein signalling for GPCRs

The endocytic network provides a system that not only regulates temporal activity of cell surface signalling receptors, but can also extend the signalling activity, giving access to new substrates with specific subcellular localization and provide specialized compartments for unique signalling responses. Such unique signalling functions in turn have distinct downstream physiological responses. The first examples of GPCR endosomal signalling was from receptors that exhibit a sustained association with arrestin and co-internalize as a complex, or Class B arrestin phenotype described above, whereby arrestin-mediated signalling was shown to sequester MAPK signalling molecules in the cytoplasm (McDonald et al. 2000; DeFea et al. 2000; Terrillon and Bouvier 2004). However, pivotal studies on the Gαs-coupled receptors the thyrotropin-stimulating hormone receptor (TSHR) and the

parathyroid hormone receptor (PTHR) have led to the categorization of GPCR/G protein signalling in to 2 phases: a transient plasma membrane signalling phase and a sustained endomembrane signalling phase. These receptors continue to activate, or exhibit persistent cAMP signalling after internalization, demonstrated by the use of cAMP biosensors (Calebiro et al. 2009; Ferrandon et al. 2009). For PTHR, endosomal cAMP signalling is enhanced by arrestin and attenuated by the retromer complex, essential for its recycling, whereby endosomal acidification plays a key role in this deactivating switch (Ferrandon et al. 2009; Feinstein et al. 2011; Gidon et al. 2014; McGarvey et al. 2016) (Figure 2). Subsequently, elegant structural studies have demonstrated that the β-arrestins, known to uncouple receptors from G proteins at the plasma membrane, simultaneously associate with the Gαs/βγ heterotrimer, and GPCRs that exhibit persistent arrestin associations, via C-tail serine/threonine phosphorylation sites, as a 'megaplex' (Thomsen et al. 2016), that represent a distinct receptor/arrestin conformation from those that mediate G protein desensitization at the plasma membrane (Cahill et al. 2017; Kumari et al. 2017).

These prior studies were able to demonstrate that the signalling machinery to generate such persistent cAMP signals, i.e., Gαs and transmembrane adenylate cyclases, are present in EEs. However, the first direct evidence that a GPCR can activate heterotrimeric G proteins from endosomes came from development of nanobody biosensors that detect the active, nucleotide-free form of Gαs (nanobody37) or the ligand-activated conformation of the β2AR (nanobody80), and were originally generated as tools to aid β2AR crystallization in distinct conformations (Irannejad et al. 2013; Pardon et al. 2014). Follow on studies employing these tools have demonstrated that endosomal signalling of the β2AR is highly organized at the level of the individual endosome. The alpha-arrestin member ARRDC3 associates with β2AR on EEs whereby it negatively regulates receptor entry in to sequence-directed recycling tubules, characterized by SNX27 and retromer, and thus results in increased endosomal signalling (Figure 2). The authors suggest that ARRDC3 regulates endosomal

residency time (Tian et al. 2016). However, it could also suggest a more direct role of ARRDC3 in driving endosomal signalling, as shown for the β-arrestins. Whether ARRDC3 can associate with G proteins and form similar megaplexes is unknown, although it does associate with members of ESCRT-0 (Tian et al. 2016), which are known to form microdomains at flat clathrin patches on the endomembrane (Raiborg et al. 2002). Interestingly, a subsequent study on β2AR endosomal signalling revealed a further layer of complexity in the control of endosomal β2AR signalling. While the localization of the nanbody that recognizes the active β2AR (nanobody80) was detected throughout the endosome membrane, active Gαs via nanobody37 was observed specifically in SNX27/WASH recycling tubules, suggesting the presence of active G protein endosomal microdomains (Bowman et al. 2016) (Figure 2A). Given the role of ARRDC3 in promoting endosomal signalling, this suggests that the functional site of ARRDC3 in terms of regulating GPCR/Gαs signalling is at microdomains within the regulated recycling tubule, considering ARRDC3 was found to alter associations of β2AR with SNX27 (Tian et al. 2016). Whether there are other pathways in addition to Gαs/cAMP that β2AR may activate at the endomembrane, as revealed by the diffuse endosomal distribution of nanobody80, including default recycling tubules, is as yet unknown. However, the phosphatidylinositol 3-kinase (Vps34) that is responsible for production of PI3P, regulates β2AR recycling and signalling (Varandas et al. 2016). Of note, in hepatocellular carcinoma, β2AR activation of Akt disrupts the Vps34/Beclin-1 autophagic complex, altering glucose metabolism and leading to drug resistance (Wu et al. 2016).

Endosomal G protein signalling is also relevant for heterotrimeric G protein pathways in addition to Gαs, as has been demonstrated for the kisspeptin receptor, a Gαq/11-coupled GPCR that exhibits a persistent calcium signalling profile entirely dependent on receptor internalization, and may in part underlie the enhanced activity of an activating mutation, R386P, which causes central precocious puberty (Min et al. 2014; Bianco et al. 2011).

Furthermore, the PAR1 and purinergic P2Y1 receptors can activate p38 within endosomes via the transforming growth factor-β–activated protein kinase-1 binding protein 1 (TAB1) pathway in a receptor ubiquitin-dependent manner, whereby TAB1 is recruited to the ubiquitinated receptor via its UBD (Grimsey et al. 2015). However, many receptors couple to more than one G protein pathway, thus if a receptor exhibits endosomal signalling are all its pathways mediated at the endosome? This question has been recently addressed with the Class C GPCR the calcium-sensing receptor that exhibits pleiotropic G protein signalling. In addition to being the first report of a Class C GPCR to exhibit endosomal signalling, this receptor's ability to signal through Gαi and Gαq/11 exhibited differential requirements for endosomal signalling with the latter pathway requiring receptor internalization (Gorvin et al. 2018).

Recently, Gβγ was shown to have a key role in regulating endosomal signalling from the PTHR. Gβγ is well established to activate distinct effectors at the plasma membrane (Figure 1B), in this study however, Gβγ released from Gαi, can stimulate endosomal adenylate cyclase 2 activity to prolong nuclear cAMP and PKA activity via an interesting mechanism involving crosstalk with the β2AR cAMP signalling pathway (Jean-Alphonse et al. 2016). The source of Gβγ is proposed to be from plasma membrane β2AR/Gαi, demonstrating an interesting communication between plasma membrane and endomembrane signalling. Section 2.2 describes how GPCR crosstalk may be a common feature of GPCR signalling, yet this study is the first example of how such receptor crosstalk can impact GPCR/G protein endosomal signalling. Both β2AR and PTHR are expressed in osteoblasts, and this work provides mechanistic information of prior studies suggesting the promotion of bone mineralization of PTH by β2AR activation (Hanyu et al. 2012; Jean-Alphonse et al. 2016). Thus, different receptors at different locations in the cell can impact endosomal signalling and this also highlights that endosomes are not independent signalling stations. Further, the endosomal signalling machinery is not confined to the endomembrane only, but can be

recruited from the cytoplasm. A recent study on corticotropin-releasing hormone receptor 1 suggested that soluble, and not transmembrane, adenylate cyclases were the essential counterparts for the production of endosomal cAMP signalling (Inda et al. 2016). Although the mechanism of recruitment for these soluble enzymes is still unknown, the use of different sources of cAMP could explain how cells elicit specific cellular responses via the same signalling pathway.

4.2 Additional endosomal compartments as GPCR signalosomes

EEs are only one compartment that GPCRs can traffic to during their post-endocytic sorting, and as described in Section 3, the endomembrane systems are complex yet highly interconnected. While the majority of studies have demonstrated endosomal signalling from EEs, more recent studies have demonstrated intracellular signalling of GPCRs from multiple subcellular organelles. For the purpose of this chapter I will next focus on primarily on those compartments requiring receptor endocytosis to access signalling, and will only briefly mention for completeness recent studies reporting intracellular signalling from other organelles.

4.2.1-Compartmentalized endomembrane signalling-the Very Early Endosomes

The recently reported VEE (described in section 3.2)(Jean-Alphonse et al. 2014) also plays a key role in direct spatial control of receptor signalling and a mechanism to generate compartmental bias in heterotrimeric G protein signalling not only between the plasma membrane and EEs described in the studies above, but also compartmental bias in GPCR signalling across distinct ‘flavors’ of endosomes. We first reported that GPCRs which traffic to the VEE exhibit a sustained temporal profile of ERK signalling that requires both internalization and targeting to the VEE (Jean-Alphonse et al. 2014). Loss of association with the PDZ protein GIPC, reroutes the receptor to EEs resulting in a transient ERK profile, while inhibition of internalization completely inhibits ERK activation. Furthermore, addition of the LHR distal C-tail to an EE localized GPCR, a C-tail truncated form of the V2 vasopressin

receptor (V2R), reroutes the receptor to the VEE and in turn alters the ERK signalling from a transient to a sustained ERK signalling profile (Jean-Alphonse et al. 2014). More recently, we have demonstrated that acute Gαs/cAMP signalling activated by LHR primarily occurs from the VEE compartment and not the plasma membrane (Sposini et al. 2017), as has been demonstrated for the D1 dopamine receptor, where internalization is necessary to mediate acute dopaminergic signalling in striatal neurons (Kotowski et al. 2011). This, in contrast to the EE localized GPCRs discussed above where the G protein endosomal signalling contributes to the prolonged or sustained, but not acute, cAMP signalling. Surprisingly, APPL1, which was essential for driving GPCR recycling from the VEEs, also negatively regulates this endosomal G protein signalling, without impacting ERK signalling. By visualizing active G protein on LHR occupied VEEs indicated that this signalling occurs from distinct VEE populations both positive and negative for APPL1 and even across microdomains within these small endosomes. In contrast to its role in recycling, APPL1 must be in its unphosphorylated form to negatively regulate endosomal signalling, further indicating that the VEE compartment is a heterogenous one functionally both across individual VEEs and perhaps even within a single VEE (Sposini et al. 2017) (Figure 2C).

For LHR, the downstream role of VEE targeting and the G protein and ERK signalling from this compartment remains to be determined, although these membrane trafficking pathways are conserved, and the VEE/APPL1 recycling of LHR was also evident in primary human endometrial stromal cells that endogenously express this receptor (Sposini et al. 2017). Further, LH does induce a sustained ERK signalling profile in human ovarian granulosa cells that negatively regulates expression of aromatase (an enzyme key in converting androgens to estrogens) (Casarini et al. 2012), a signalling profile that may result from localization to and trafficking from VEEs. Furthermore, transgenic expression of the same cAMP biosensors used to study TSHR, indicated that the mouse LHR exhibits also a sustained cAMP profile in ovarian follicles. Use of chemical inhibitors to block internalization demonstrated a loss of LH-mediated resumption in meiosis (Lyga et al. 2016), although

whether the VEE is involved is unclear as rodent and human LHRs differ in their ability to internalize, associate with GIPC and undergo regulated recycling (Galet et al. 2004; Galet et al. 2003; Hirakawa et al. 2003b; Nakamura et al. 2000).

4.2.2-Signalling of GPCRs from further intracellular membrane compartments

The endomembrane compartment includes not on the early stages of the pathway, i.e VEE and EEs but is interconnected with late endosomes/MVBs and also with the biosynthetic compartment and the Golgi. Therefore, it is conceivable that these have the potential to provide additional signal platforms. The strongest evidence to date that late endosomes, or MVBs, could represent GPCR signalling platforms is their requirement for Wnt-mediated signalling. Wnts activate the Frizzled members of the GPCR superfamily. Rather than attenuate receptor signalling, the sequestration or involution of the enzyme glycogen synthase kinase 3 (GSK3) with the Wnt/Frizzled receptor was essential for sustained WNT signalling, illustrating a signalling role of MVBs independent of receptor degradation (Figure 2D). This results in a decrease of active GSK3 causing the accumulation of newly synthesized β-catenin and its subsequent transportation to the nucleus to activate gene transcription (Dobrowolski et al. 2012; Taelman et al. 2010).

A recent study has extended the endosomal signalling properties of the TSHR in thyroid cells to the TGN. Employing tools such as, fluorescently labelled TSH to track the trafficking of the endogenous TSH receptor, FRET based sensors of cAMP/PKA activity and nanobody37 to direct visualize the compartment that Gαs activation, the authors identified that while the ligand/receptor complex passed through the EE, it accumulated in perinuclear tubulovesicular structures positive for markers of the TGN and retromer, where active Gαs was also recruited to following stimulation with TSH (Figure 2B). The effector of this compartmentalized cAMP signalling activated by TSHR from the TGN was identified to be specifically PKA II isoform, and interestingly this organization of internalized TSHR to TGN

was necessary for TSH mediated phosphorylation of CREB and activation of early response genes. Intriguingly, the β1AR has also been shown to generate Gαs/cAMP signals from the Golgi, however, unlike TSHR this active receptor is not internalized receptor from the plasma membrane but is a pre-existing receptor pool. Given we have also shown that β1AR employs the VEE/APPL1 endosomes for its regulation (Sposini et al. 2017), highlights that compartmentalized G protein signalling of internalized GPCRs can occur from multiple sites; EEs, VEEs and the TGN, and that the same receptor may activate signalling from more than source with perhaps distinct functional consequences. The impact to cell function, physiology and whether we can harness these properties of GPCRs therapeutically, will be discussed next.

4.3 The physiological and therapeutic significance of GPCR endosomal signalling

The studies to date have clearly demonstrated that the endocytic system can temporally control plasma membrane signalling and as platforms for spatial control signalling directly from intracellular compartments; enabling a cell to control signalling with exquisite precision and diversify GPCR activity even from a single heterotrimeric G protein pathway. However, key questions remain unanswered. What is the physiological significance of GPCR plasma membrane versus endosomal signalling, and how do cells mechanistically decode similar signals (e.g. Gαs-cAMP, ERK) from plasma membrane versus endomembrane, or as described above, even between different types of endosomes, to manifest in discrete downstream responses? The physiological relevance for the persistent endosomal cAMP signalling, first observed for the TSHR and PTHR, was also demonstrated in these seminal studies. For TSHR, pituitary TSH acts on the thyroid gland resulting in secretion of the hormones thyroxine, which is converted to triiodothyronine, that is key in regulating metabolism. Sustained endosomal cAMP signalling was demonstrated for TSHR in 3D cultures of thyroid follicles (Calebiro et al. 2009). TSH-mediated cAMP signalling in the thyroid is known to reorganize the actin cytoskeleton via the PKA effector vasodilator-

stimulated phosphoprotein (VASP), which regulates actin polymerization. Cytoskeletal modulations by TSH in the thyroid cell are implicated in the reuptake of thyroglobulin and in the induction of thyroid-specific genes. Accordingly, chemical inhibition of TSHR internalization, impaired the ability of TSH to activate VASP and modulate actin depolymerization (Calebiro et al. 2009). For the PTHR, which has critical roles in regulating Ca^{2+} homeostasis, sustained signalling induced by distinct PTH ligands impacts on trabecular bone volume yet induces greater increases in cortical bone turnover by its actions on the bone and kidney, with clinical implications for using PTH analogs in osteoporosis due to their sustained signalling properties (Ferrandon et al. 2009; Okazaki et al. 2008). The spatial organization of second messenger signalling is likely to be important in a wider physiological context. Indeed, the downstream roles of sustained endosomal cAMP signalling has been also been reported for other GPCRs including; the glucagon-like peptide 1 receptor with a requirement in glucose-stimulated insulin secretion (Kuna et al. 2013), the V2R with roles in regulating renal water and sodium transport (Feinstein et al. 2013) and the pituitary adenylate cyclase activating polypeptide type 1 receptor to regulate neuronal excitability (Merriam et al. 2013). However, two recent elegant studies have not only demonstrated the physiological importance of endosomal GPCR signalling, but that this population of receptors is a viable and efficacious therapeutic target. Both the neurokinin1 receptor, whose endogenous ligand is substance P, and the Class B GPCR the calcitonin-receptor-like receptor (CLR) activated by calcitonin gene related peptide (CGRP). Both these GPCRs were shown to exhibit endosomal G protein/second messenger and MAPK signalling, and that if this endosomal signalling was disrupted in vivo prevented nociception in spinal neurons. Moreover, these groups were then able to demonstrate that blocking the intracellular endosomal population of receptors, via lipid conjugation of receptor specific antagonists with cholestenol, and injection intrathecally to animals, blocked responses to nociceptive stimuli (Jensen et al. 2017; Yarwood et al. 2017). It is clear from these studies that it is technically possible to target different populations of receptors at a spatial level, and perhaps will steer future drug targeting opportunities for ligands that do not just bias between

two signal pathways, but directs the receptor to distinct subcellular locations to achieve the desired signal profile.

Clues on how cells decode signals at a spatial level have been provided from studies of β2AR endosomal signalling. Through the use of optogenetic adenylate cyclase probes targeted to either plasma membrane, endosome or cytoplasm, the functional role of this endosomal Gαs-cAMP signalling is to activate distinct downstream transcriptional responses (Tsvetanova and von Zastrow 2014), demonstrating that endosomally generated cAMP is differentially translated by the cell. Furthermore, the exquisite precision that the spatial control of β2AR signalling affords has been further demonstrated by Bowman and colleagues. Assessing the same downstream endosomally-driven transcriptional responses identified in (Tsvetanova and von Zastrow 2014), the authors identified that β2AR must reside in regulated, and not default, recycling tubules to activate this downstream transcriptional response, consistent with their findings that active Gαs signalling occurs exclusively in these microdomains ((Bowman et al. 2016) and Figure 2). Given that GPCRs such as the β2AR where only a fraction of the sustained cAMP produced by this receptor is endosomally driven, it is proposed that a key function of regulated recycling over default recycling is to modulate the degree of signalling between surface and endosomal signals, and subsequently downstream responses (Bowman et al. 2016). Indeed, a distinct study exposes the sensitivity of β2AR endosomal signals to specific control of certain genes, whereby the endosomal signal saturates at a level well below the cell's transcriptional capacity, and that it is the number of receptor-containing endosomes that modulates the endosomal signalling profile. Thus, ligands with distinct efficacies, internalization kinetics and upstream signal profiles to the same receptor exert very similar endosomal signals and subsequent downstream responses, proposing a role for GPCR endosomal signalling as a biological 'noise' filter (Tsvetanova et al. 2017). It remains to be determined if this applies to GPCRs with a higher dependency on endosomal signalling, such as PTHR or LHR, and

where there are known opposing ligand-dependent differences in sustained endosomal signalling.

5. Future Perspectives

Our view of endocytic trafficking in regulating GPCR signalling has evolved from a system that regulates cellular and tissue sensitivity of a ligand to a model where trafficking and signalling is highly integrated at multiple levels. Our more recent understanding has been rapidly advanced by development of novel tools and imaging technologies that applied to this area has challenged the archetypal view of signal regulation for this superfamily of signalling receptors.

The physiological role of GPCR endosomal signalling has been demonstrated for several GPCRs, and it is likely we will see further examples where these mechanisms are perturbed in disease. Certainly, with some GPCRs such as the calcium-sensing receptor, the involvement of endosomal signalling has emerged from the clinic, as it was disease causing mutations in the endocytic machinery that mediate clathrin-mediated endocytosis of this receptor, the sigma subunit of AP2, that led to discovery for spatially directed G protein signalling from this GPCR (Gorvin et al. 2018). It also illustrates that even minor perturbations in trafficking systems, such as altering endosomal compartment, or loss/gain of an adaptor protein that regulates endosomal signalling, could have profound effects on cellular signalling. Given these receptors represent an established and successful therapeutic target, GPCR endosomal signalling has the potential to impact on not only how compounds are assessed but also provide novel therapeutic avenues to ‘fine-tune’ receptor activity. The translational road from cell biology to drug development is often slow but with the growing appreciation of not only pleiotropy in GPCR signalling, but also in creating compounds with pathway selectivity, and critically that there is evidence now that in doing so as a valid and physiological significant strategy, provides a precedence for value in such

approaches. Thus, it is very possible that future pharmacological targeting of GPCR endocytic signalling systems is likely be expedited.

Acknowledgements

This work was supported by grants from the Wellcome Trust (WT085099MA), Genesis Research Trust (P15844) to A.C.H.

Abbreviations

AIP1, actin interacting protein 1; AKT (PKB), protein kinase B; ALIX, ALG-interacting protein X; AMSH, associated molecule with the SH3 domain of STAM; AP2, adaptor protein 2; APPL1, adaptor protein containing PH domain, PTB domain, and Leucine zipper motif; ARRDC, arresting domain containing; B1AR, B2AR, beta adrenergic receptor 1 or 2; CB1, cannabinoid receptor 1; CCP, clathrin coated pit; CXCR4, chemokine receptor 4 ; DOR, δ-opioid receptor; EE, early endosome; EGF, epidermal growth factor; EGFR, EGF receptor; EPB 50, ERM binding phosphoprotein 50; ERM, Ezrin Radixin Moesin; ESCRT, endosomal sorting complex required for transport; GAP, GTPase activating protein; GASP-1, GPCR associated sorting protein-1; GDP, guanosine diphosphate; GEF, GDP exchange factor; GIPC, Gαi-interacting protein C terminus; GPCR, G protein coupled receptors; GRK, GPCR kinase; GSK3, glycogen synthase kinase 3; GTP, guanosine triphosphate; HGF, hepatocyte growth factor; Hrs, HGF regulated tyrosine kinase substrate; LHR, luteinizing hormone receptor; MOR, μ-opioid receptor; MVB, multi vesicular body; PAR, protease activated receptor; PDZ, postsynaptic density 95/disc large/zonula occludens-1; PI3P, phosphatidylinositol-3 phosphate; PKA, protein kinase A; PKC, protein kinase C; PSD, postsynaptic density protein 95; PTHR, parathyroid hormone receptor; RGS, regulator of G protein signalling; SNX27, sorting nexin 27; STAM, signal transducing adaptor protein
STAT, signal transducer and activator of transcription; TAB1, TGF-beta activated kinase 1 binding protein; TGN, trans-Golgi network; TM, transmembrane; TSHR, thyroid stimulating

hormone receptor; UBD, ubiquitin binding domain; UBPY, ubiquitin-specific processing protease Y; V2R, V2 vasopressin receptor ; VASP, vasodilator stimulated phosphoprotein; VEE, very early endosome; Vps, vacuolar protein sorting; WASH, Wiskott-Aldrich Syndrome Protein and SCAR Homolog; Wnt, Wingless-related integration site

References

Abdullah N, Beg M, Soares D, Dittman JS, McGraw TE (2016) Downregulation of a GPCR by beta-Arrestin2-Mediated Switch from an Endosomal to a TGN Recycling Pathway. Cell reports 17 (11):2966-2978. doi:10.1016/j.celrep.2016.11.050

Arakaki AKS, Pan WA, Lin H, Trejo J (2018) -The a-arrestin ARRDC3 suppresses breast carcinoma invasion by regulating G protein-coupled receptor lysosomal sorting and signaling. The Journal of biological chemistry. doi:10.1074/jbc.RA117.001516

Barki-Harrington L, Rockman HA (2008) Beta-arrestins: multifunctional cellular mediators. Physiology 23:17-22. doi:10.1152/physiol.00042.2007

Beas AO, Taupin V, Teodorof C, Nguyen LT, Garcia-Marcos M, Farquhar MG (2012) Galphas promotes EEA1 endosome maturation and shuts down proliferative signaling through interaction with GIV (Girdin). Molecular biology of the cell 23 (23):4623-4634. doi:10.1091/mbc.E12-02-0133

Bianco SD, Vandepas L, Correa-Medina M, Gereben B, Mukherjee A, Kuohung W, Carroll R, Teles MG, Latronico AC, Kaiser UB (2011) KISS1R intracellular trafficking and degradation: effect of the Arg386Pro disease-associated mutation. Endocrinology 152 (4):1616-1626. doi:10.1210/en.2010-0903

Boucrot E, Ferreira AP, Almeida-Souza L, Debard S, Vallis Y, Howard G, Bertot L, Sauvonnet N, McMahon HT (2015) Endophilin marks and controls a clathrin-independent endocytic pathway. Nature 517 (7535):460-465. doi:10.1038/nature14067

Bowman SL, Shiwarski DJ, Puthenveedu MA (2016) Distinct G protein-coupled receptor recycling pathways allow spatial control of downstream G protein signaling. The Journal of cell biology 214 (7):797-806. doi:10.1083/jcb.201512068

Cadigan KM (2010) Receptor endocytosis: Frizzled joins the ubiquitin club. EMBO J 29 (13):2099-2100. doi:10.1038/emboj.2010.132

Cahill TJ, 3rd, Thomsen AR, Tarrasch JT, Plouffe B, Nguyen AH, Yang F, Huang LY, Kahsai AW, Bassoni DL, Gavino BJ, Lamerdin JE, Triest S, Shukla AK, Berger B, Little Jt,

Antar A, Blanc A, Qu CX, Chen X, Kawakami K, Inoue A, Aoki J, Steyaert J, Sun JP, Bouvier M, Skiniotis G, Lefkowitz RJ (2017) Distinct conformations of GPCR-beta-arrestin complexes mediate desensitization, signaling, and endocytosis. Proceedings of the National Academy of Sciences of the United States of America 114 (10):2562-2567. doi:10.1073/pnas.1701529114

Calebiro D, Nikolaev VO, Gagliani MC, de Filippis T, Dees C, Tacchetti C, Persani L, Lohse MJ (2009) Persistent cAMP-signals triggered by internalized G-protein-coupled receptors. PLoS biology 7 (8):e1000172. doi:10.1371/journal.pbio.1000172

Calizo RC, Scarlata S (2012) A role for G-proteins in directing G-protein-coupled receptor-caveolae localization. Biochemistry 51 (47):9513-9523. doi:10.1021/bi301107p

Cao TT, Deacon HW, Reczek D, Bretscher A, von Zastrow M (1999) A kinase-regulated PDZ-domain interaction controls endocytic sorting of the beta2-adrenergic receptor. Nature 401 (6750):286-290. doi:10.1038/45816

Casarini L, Lispi M, Longobardi S, Milosa F, La Marca A, Tagliasacchi D, Pignatti E, Simoni M (2012) LH and hCG action on the same receptor results in quantitatively and qualitatively different intracellular signalling. PLoS ONE 7 (10):e46682. doi:10.1371/journal.pone.0046682

Chini B, Parenti M (2004) G-protein coupled receptors in lipid rafts and caveolae: how, when and why do they go there? Journal of molecular endocrinology 32 (2):325-338

Cho DI, Zheng M, Min C, Kwon KJ, Shin CY, Choi HK, Kim KM (2013) ARF6 and GASP-1 are post-endocytic sorting proteins selectively involved in the intracellular trafficking of dopamine D(2) receptors mediated by GRK and PKC in transfected cells. British journal of pharmacology 168 (6):1355-1374. doi:10.1111/bph.12025

DeFea KA, Zalevsky J, Thoma MS, Dery O, Mullins RD, Bunnett NW (2000) beta-arrestin-dependent endocytosis of proteinase-activated receptor 2 is required for intracellular targeting of activated ERK1/2. The Journal of cell biology 148 (6):1267-1281

Delgado-Peraza F, Ahn KH, Nogueras-Ortiz C, Mungrue IN, Mackie K, Kendall DA, Yudowski GA (2016) Mechanisms of Biased beta-Arrestin-Mediated Signaling

Downstream from the Cannabinoid 1 Receptor. Molecular pharmacology 89 (6):618-629. doi:10.1124/mol.115.103176

DeWire SM, Yamashita DS, Rominger DH, Liu G, Cowan CL, Graczyk TM, Chen XT, Pitis PM, Gotchev D, Yuan C, Koblish M, Lark MW, Violin JD (2013) A G protein-biased ligand at the mu-opioid receptor is potently analgesic with reduced gastrointestinal and respiratory dysfunction compared with morphine. The Journal of pharmacology and experimental therapeutics 344 (3):708-717. doi:10.1124/jpet.112.201616

Dobrowolski R, Vick P, Ploper D, Gumper I, Snitkin H, Sabatini DD, De Robertis EM (2012) Presenilin deficiency or lysosomal inhibition enhances Wnt signaling through relocalization of GSK3 to the late-endosomal compartment. Cell reports 2 (5):1316-1328. doi:10.1016/j.celrep.2012.09.026

Dores MR, Chen B, Lin H, Soh UJ, Paing MM, Montagne WA, Meerloo T, Trejo J (2012a) ALIX binds a YPX(3)L motif of the GPCR PAR1 and mediates ubiquitin-independent ESCRT-III/MVB sorting. The Journal of cell biology 197 (3):407-419. doi:10.1083/jcb.201110031

Dores MR, Lin H, N JG, Mendez F, Trejo J (2015) The alpha-arrestin ARRDC3 mediates ALIX ubiquitination and G protein-coupled receptor lysosomal sorting. Molecular biology of the cell 26 (25):4660-4673. doi:10.1091/mbc.E15-05-0284

Dores MR, Paing MM, Lin H, Montagne WA, Marchese A, Trejo J (2012b) AP-3 regulates PAR1 ubiquitin-independent MVB/lysosomal sorting via an ALIX-mediated pathway. Molecular biology of the cell 23 (18):3612-3623. doi:10.1091/mbc.E12-03-0251

Dores MR, Trejo J (2015) GPCR sorting at multivesicular endosomes. Methods in cell biology 130:319-332. doi:10.1016/bs.mcb.2015.05.006

Dunn HA, Ferguson SS (2015) PDZ Protein Regulation of G Protein-Coupled Receptor Trafficking and Signaling Pathways. Molecular pharmacology 88 (4):624-639. doi:10.1124/mol.115.098509

Eichel K, Jullie D, von Zastrow M (2016) beta-Arrestin drives MAP kinase signalling from clathrin-coated structures after GPCR dissociation. Nature cell biology 18 (3):303-310. doi:10.1038/ncb3307

Feiner EC, Chung P, Jasmin JF, Zhang J, Whitaker-Menezes D, Myers V, Song J, Feldman EW, Funakoshi H, Degeorge BR, Jr., Yelamarty RV, Koch WJ, Lisanti MP, McTiernan CF, Cheung JY, Bristow MR, Chan TO, Feldman AM (2011) Left ventricular dysfunction in murine models of heart failure and in failing human heart is associated with a selective decrease in the expression of caveolin-3. J Card Fail 17 (3):253-263. doi:10.1016/j.cardfail.2010.10.008

Feinstein TN, Wehbi VL, Ardura JA, Wheeler DS, Ferrandon S, Gardella TJ, Vilardaga JP (2011) Retromer terminates the generation of cAMP by internalized PTH receptors. Nature chemical biology 7 (5):278-284. doi:10.1038/nchembio.545

Feinstein TN, Yui N, Webber MJ, Wehbi VL, Stevenson HP, King JD, Jr., Hallows KR, Brown D, Bouley R, Vilardaga JP (2013) Noncanonical control of vasopressin receptor type 2 signaling by retromer and arrestin. The Journal of biological chemistry 288 (39):27849-27860. doi:10.1074/jbc.M112.445098

Ferrandon S, Feinstein TN, Castro M, Wang B, Bouley R, Potts JT, Gardella TJ, Vilardaga JP (2009) Sustained cyclic AMP production by parathyroid hormone receptor endocytosis. Nat Chem Biol 5 (10):734-742. doi:10.1038/nchembio.206

Ferre S, Casado V, Devi LA, Filizola M, Jockers R, Lohse MJ, Milligan G, Pin JP, Guitart X (2014) G protein-coupled receptor oligomerization revisited: functional and pharmacological perspectives. Pharmacological reviews 66 (2):413-434. doi:10.1124/pr.113.008052

Flores-Otero J, Ahn KH, Delgado-Peraza F, Mackie K, Kendall DA, Yudowski GA (2014) Ligand-specific endocytic dwell times control functional selectivity of the cannabinoid receptor 1. Nature communications 5:4589. doi:10.1038/ncomms5589

Gage RM, Kim KA, Cao TT, von Zastrow M (2001) A transplantable sorting signal that is sufficient to mediate rapid recycling of G protein-coupled receptors. The Journal of biological chemistry 276 (48):44712-44720. doi:10.1074/jbc.M107417200

Galet C, Hirakawa T, Ascoli M (2004) The postendocytotic trafficking of the human lutropin receptor is mediated by a transferable motif consisting of the C-terminal cysteine and an upstream leucine. Mol Endocrinol 18 (2):434-446. doi:10.1210/me.2003-0293

Galet C, Min L, Narayanan R, Kishi M, Weigel NL, Ascoli M (2003) Identification of a transferable two-amino-acid motif (GT) present in the C-terminal tail of the human lutropin receptor that redirects internalized G protein-coupled receptors from a degradation to a recycling pathway. Mol Endocrinol 17 (3):411-422. doi:10.1210/me.2002-0161

Gidon A, Al-Bataineh MM, Jean-Alphonse FG, Stevenson HP, Watanabe T, Louet C, Khatri A, Calero G, Pastor-Soler NM, Gardella TJ, Vilardaga JP (2014) Endosomal GPCR signaling turned off by negative feedback actions of PKA and v-ATPase. Nature chemical biology 10 (9):707-709. doi:10.1038/nchembio.1589

Girnita L, Shenoy SK, Sehat B, Vasilcanu R, Girnita A, Lefkowitz RJ, Larsson O (2005) {beta}-Arrestin is crucial for ubiquitination and down-regulation of the insulin-like growth factor-1 receptor by acting as adaptor for the MDM2 E3 ligase. The Journal of biological chemistry 280 (26):24412-24419. doi:10.1074/jbc.M501129200

Gorvin CM, Rogers A, Hastoy B, Tarasov AI, Frost M, Sposini S, Inoue A, Whyte MP, Rorsman P, Hanyaloglu AC, Breitwieser GE, Thakker RV (2018) AP2? Mutations Impair Calcium-Sensing Receptor Trafficking and Signaling, and Show an Endosomal Pathway to Spatially Direct G-Protein Selectivity. Cell reports 22 (4):1054-1066. doi:10.1016/j.celrep.2017.12.089

Grimsey NJ, Aguilar B, Smith TH, Le P, Soohoo AL, Puthenveedu MA, Nizet V, Trejo J (2015) Ubiquitin plays an atypical role in GPCR-induced p38 MAP kinase activation on endosomes. The Journal of cell biology 210 (7):1117-1131. doi:10.1083/jcb.201504007

Grove J, Metcalf DJ, Knight AE, Wavre-Shapton ST, Sun T, Protonotarios ED, Griffin LD, Lippincott-Schwartz J, Marsh M (2014) Flat clathrin lattices: stable features of the plasma membrane. Molecular biology of the cell 25 (22):3581-3594. doi:10.1091/mbc.E14-06-1154

Grundmann M, Merten N, Malfacini D, Inoue A, Preis P, Simon K, Ruttiger N, Ziegler N, Benkel T, Schmitt NK, Ishida S, Muller I, Reher R, Kawakami K, Inoue A, Rick U, Kuhl T, Imhof D, Aoki J, Konig GM, Hoffmann C, Gomeza J, Wess J, Kostenis E (2018) Lack of beta-arrestin signaling in the absence of active G proteins. Nature communications 9 (1):341. doi:10.1038/s41467-017-02661-3

Hanyaloglu AC, McCullagh E, von Zastrow M (2005) Essential role of Hrs in a recycling mechanism mediating functional resensitization of cell signaling. The EMBO journal 24 (13):2265-2283. doi:10.1038/sj.emboj.7600688

Hanyaloglu AC, von Zastrow M (2007) A novel sorting sequence in the beta2-adrenergic receptor switches recycling from default to the Hrs-dependent mechanism. The Journal of biological chemistry 282 (5):3095-3104. doi:10.1074/jbc.M605398200

Hanyaloglu AC, von Zastrow M (2008) Regulation of GPCRs by endocytic membrane trafficking and its potential implications. Annual review of pharmacology and toxicology 48:537-568. doi:10.1146/annurev.pharmtox.48.113006.094830

Hanyu R, Wehbi VL, Hayata T, Moriya S, Feinstein TN, Ezura Y, Nagao M, Saita Y, Hemmi H, Notomi T, Nakamoto T, Schipani E, Takeda S, Kaneko K, Kurosawa H, Karsenty G, Kronenberg HM, Vilardaga JP, Noda M (2012) Anabolic action of parathyroid hormone regulated by the beta2-adrenergic receptor. Proceedings of the National Academy of Sciences of the United States of America 109 (19):7433-7438. doi:10.1073/pnas.1109036109

Hasdemir B, Bunnett NW, Cottrell GS (2007) Hepatocyte growth factor-regulated tyrosine kinase substrate (HRS) mediates post-endocytic trafficking of protease-activated receptor 2 and calcitonin receptor-like receptor. The Journal of biological chemistry 282 (40):29646-29657. doi:10.1074/jbc.M702974200

He C, Wei Y, Sun K, Li B, Dong X, Zou Z, Liu Y, Kinch LN, Khan S, Sinha S, Xavier RJ, Grishin NV, Xiao G, Eskelinen EL, Scherer PE, Whistler JL, Levine B (2013) Beclin 2 functions in autophagy, degradation of G protein-coupled receptors, and metabolism. Cell 154 (5):1085-1099. doi:10.1016/j.cell.2013.07.035

He J, Bellini M, Inuzuka H, Xu J, Xiong Y, Yang X, Castleberry AM, Hall RA (2006) Proteomic analysis of beta1-adrenergic receptor interactions with PDZ scaffold proteins. J Biol Chem 281 (5):2820-2827. doi:10.1074/jbc.M509503200

Henne WM, Stenmark H, Emr SD (2013) Molecular mechanisms of the membrane sculpting ESCRT pathway. Cold Spring Harbor perspectives in biology 5 (9). doi:10.1101/cshperspect.a016766

Henry AG, Hislop JN, Grove J, Thorn K, Marsh M, von Zastrow M (2012) Regulation of endocytic clathrin dynamics by cargo ubiquitination. Dev Cell 23 (3):519-532. doi:10.1016/j.devcel.2012.08.003

Henry AG, White IJ, Marsh M, von Zastrow M, Hislop JN (2011) The role of ubiquitination in lysosomal trafficking of delta-opioid receptors. Traffic 12 (2):170-184. doi:10.1111/j.1600-0854.2010.01145.x

Hiol A, Davey PC, Osterhout JL, Waheed AA, Fischer ER, Chen CK, Milligan G, Druey KM, Jones TL (2003) Palmitoylation regulates regulators of G-protein signaling (RGS) 16 function. I. Mutation of amino-terminal cysteine residues on RGS16 prevents its targeting to lipid rafts and palmitoylation of an internal cysteine residue. The Journal of biological chemistry 278 (21):19301-19308. doi:10.1074/jbc.M210123200

Hirakawa T, Galet C, Kishi M, Ascoli M (2003a) GIPC binds to the human lutropin receptor (hLHR) through an unusual PDZ domain binding motif, and it regulates the sorting of the internalized human choriogonadotropin and the density of cell surface hLHR. J Biol Chem 278 (49):49348-49357. doi:10.1074/jbc.M306557200

Hirakawa T, Galet C, Kishi M, Ascoli M (2003b) GIPC binds to the human lutropin receptor (hLHR) through an unusual PDZ domain binding motif, and it regulates the sorting of

the internalized human choriogonadotropin and the density of cell surface hLHR. The Journal of biological chemistry 278 (49):49348-49357. doi:10.1074/jbc.M306557200

Hislop JN, Henry AG, Marchese A, von Zastrow M (2009) Ubiquitination regulates proteolytic processing of G protein-coupled receptors after their sorting to lysosomes. J Biol Chem 284 (29):19361-19370. doi:10.1074/jbc.M109.001644

Hislop JN, Henry AG, von Zastrow M (2011) Ubiquitination in the first cytoplasmic loop of mu-opioid receptors reveals a hierarchical mechanism of lysosomal down-regulation. J Biol Chem 286 (46):40193-40204. doi:10.1074/jbc.M111.288555

Huang SH, Zhao L, Sun ZP, Li XZ, Geng Z, Zhang KD, Chao MV, Chen ZY (2009) Essential role of Hrs in endocytic recycling of full-length TrkB receptor but not its isoform TrkB.T1. The Journal of biological chemistry 284 (22):15126-15136. doi:10.1074/jbc.M809763200

Inda C, Dos Santos Claro PA, Bonfiglio JJ, Senin SA, Maccarrone G, Turck CW, Silberstein S (2016) Different cAMP sources are critically involved in G protein-coupled receptor CRHR1 signaling. The Journal of cell biology 214 (2):181-195. doi:10.1083/jcb.201512075

Irannejad R, Tomshine JC, Tomshine JR, Chevalier M, Mahoney JP, Steyaert J, Rasmussen SG, Sunahara RK, El-Samad H, Huang B, von Zastrow M (2013) Conformational biosensors reveal GPCR signalling from endosomes. Nature 495 (7442):534-538. doi:10.1038/nature12000

Jean-Alphonse FG, Bowersox S, Chen S, Beard G, Puthenveedu MA, Hanyaloglu AC (2014) Spatially restricted G protein-coupled receptor activity via divergent endocytic compartments. Journal of Biological Chemistry 289 (7):3960-3977. doi:10.1074/jbc.M113.526350

Jean-Alphonse FG, Wehbi VL, Chen J, Noda M, Taboas JM, Xiao K, Vilardaga J-P (2016) β2-adrenergic receptor control of endosomal PTH receptor signaling via Gβγ. Nature chemical biology. doi:10.1038/nchembio.2267

Jensen DD, Lieu T, Halls ML, Veldhuis NA, Imlach WL, Mai QN, Poole DP, Quach T, Aurelio L, Conner J, Herenbrink CK, Barlow N, Simpson JS, Scanlon MJ, Graham B, McCluskey A, Robinson PJ, Escriou V, Nassini R, Materazzi S, Geppetti P, Hicks GA, Christie MJ, Porter CJH, Canals M, Bunnett NW (2017) Neurokinin 1 receptor signaling in endosomes mediates sustained nociception and is a viable therapeutic target for prolonged pain relief. Sci Transl Med 9 (392). doi:10.1126/scitranslmed.aal3447

Kennedy JE, Marchese A (2015) Regulation of GPCR Trafficking by Ubiquitin. Progress in molecular biology and translational science 132:15-38. doi:10.1016/bs.pmbts.2015.02.005

Kotowski SJ, Hopf FW, Seif T, Bonci A, von Zastrow M (2011) Endocytosis promotes rapid dopaminergic signaling. Neuron 71 (2):278-290. doi:10.1016/j.neuron.2011.05.036

Kumari P, Srivastava A, Ghosh E, Ranjan R, Dogra S, Yadav PN, Shukla AK (2017) Core engagement with beta-arrestin is dispensable for agonist-induced vasopressin receptor endocytosis and ERK activation. Molecular biology of the cell 28 (8):1003-1010. doi:10.1091/mbc.E16-12-0818

Kuna RS, Girada SB, Asalla S, Vallentyne J, Maddika S, Patterson JT, Smiley DL, DiMarchi RD, Mitra P (2013) Glucagon-like peptide-1 receptor-mediated endosomal cAMP generation promotes glucose-stimulated insulin secretion in pancreatic beta-cells. Am J Physiol Endocrinol Metab 305 (2):E161-170. doi:10.1152/ajpendo.00551.2012

Lakadamyali M, Rust MJ, Zhuang X (2006) Ligands for clathrin-mediated endocytosis are differentially sorted into distinct populations of early endosomes. Cell 124 (5):997-1009. doi:10.1016/j.cell.2005.12.038

Lampe M, Pierre F, Al-Sabah S, Krasel C, Merrifield CJ (2014) Dual single-scission event analysis of constitutive transferrin receptor (TfR) endocytosis and ligand-triggered beta2-adrenergic receptor (beta2AR) or Mu-opioid receptor (MOR) endocytosis. Molecular biology of the cell 25 (19):3070-3080. doi:10.1091/mbc.E14-06-1112

Lauffer BE, Melero C, Temkin P, Lei C, Hong W, Kortemme T, von Zastrow M (2010) SNX27 mediates PDZ-directed sorting from endosomes to the plasma membrane. The Journal of cell biology 190 (4):565-574. doi:10.1083/jcb.201004060

Lee MH, Appleton KM, Strungs EG, Kwon JY, Morinelli TA, Peterson YK, Laporte SA, Luttrell LM (2016) The conformational signature of beta-arrestin2 predicts its trafficking and signalling functions. Nature 531 (7596):665-668. doi:10.1038/nature17154

Li X, Letourneau D, Holleran B, Leduc R, Lavigne P, Lavoie C (2017) Galphas protein binds ubiquitin to regulate epidermal growth factor receptor endosomal sorting. Proceedings of the National Academy of Sciences of the United States of America 114 (51):13477-13482. doi:10.1073/pnas.1708215114

Luttrell LM, Lefkowitz RJ (2002) The role of beta-arrestins in the termination and transduction of G-protein-coupled receptor signals. Journal of cell science 115 (Pt 3):455-465

Lyga S, Volpe S, Werthmann RC, Gotz K, Sungkaworn T, Lohse MJ, Calebiro D (2016) Persistent cAMP Signaling by Internalized LH Receptors in Ovarian Follicles. Endocrinology 157 (4):1613-1621. doi:10.1210/en.2015-1945

Macdougall DA, Agarwal SR, Stopford EA, Chu H, Collins JA, Longster AL, Colyer J, Harvey RD, Calaghan S (2012) Caveolae compartmentalise beta2-adrenoceptor signals by curtailing cAMP production and maintaining phosphatase activity in the sarcoplasmic reticulum of the adult ventricular myocyte. Journal of molecular and cellular cardiology 52 (2):388-400. doi:10.1016/j.yjmcc.2011.06.014

Marchese A, Paing MM, Temple BR, Trejo J (2008) G protein-coupled receptor sorting to endosomes and lysosomes. Annu Rev Pharmacol Toxicol 48:601-629. doi:10.1146/annurev.pharmtox.48.113006.094646

McDonald PH, Chow CW, Miller WE, Laporte SA, Field ME, Lin FT, Davis RJ, Lefkowitz RJ (2000) Beta-arrestin 2: a receptor-regulated MAPK scaffold for the activation of JNK3. Science 290 (5496):1574-1577

McGarvey JC, Xiao K, Bowman SL, Mamonova T, Zhang Q, Bisello A, Sneddon WB, Ardura JA, Jean-Alphonse F, Vilardaga JP, Puthenveedu MA, Friedman PA (2016) Actin-Sorting Nexin 27 (SNX27)-Retromer Complex Mediates Rapid Parathyroid Hormone Receptor Recycling. The Journal of biological chemistry 291 (21):10986-11002. doi:10.1074/jbc.M115.697045

Merriam LA, Baran CN, Girard BM, Hardwick JC, May V, Parsons RL (2013) Pituitary adenylate cyclase 1 receptor internalization and endosomal signaling mediate the pituitary adenylate cyclase activating polypeptide-induced increase in guinea pig cardiac neuron excitability. The Journal of neuroscience : the official journal of the Society for Neuroscience 33 (10):4614-4622. doi:10.1523/JNEUROSCI.4999-12.2013

Min L, Soltis K, Reis AC, Xu S, Kuohung W, Jain M, Carroll RS, Kaiser UB (2014) Dynamic kisspeptin receptor trafficking modulates kisspeptin-mediated calcium signaling. Molecular endocrinology 28 (1):16-27. doi:10.1210/me.2013-1165

Monastyrskaya K, Hostettler A, Buergi S, Draeger A (2005) The NK1 receptor localizes to the plasma membrane microdomains, and its activation is dependent on lipid raft integrity. The Journal of biological chemistry 280 (8):7135-7146. doi:10.1074/jbc.M405806200

Moore CA, Milano SK, Benovic JL (2007) Regulation of receptor trafficking by GRKs and arrestins. Annu Rev Physiol 69:451-482. doi:10.1146/annurev.physiol.69.022405.154712

Mosser VA, Jones KT, Hoffman KM, McCarty NA, Jackson DA (2008) Differential role of beta-arrestin ubiquitination in agonist-promoted down-regulation of M1 vs M2 muscarinic acetylcholine receptors. Journal of molecular signaling 3:20. doi:10.1186/1750-2187-3-20

Mukai A, Yamamoto-Hino M, Awano W, Watanabe W, Komada M, Goto S (2010) Balanced ubiquitylation and deubiquitylation of Frizzled regulate cellular responsiveness to Wg/Wnt. EMBO J 29 (13):2114-2125. doi:10.1038/emboj.2010.100

Mundell SJ, Luo J, Benovic JL, Conley PB, Poole AW (2006) Distinct clathrin-coated pits sort different G protein-coupled receptor cargo. Traffic 7 (10):1420-1431. doi:10.1111/j.1600-0854.2006.00469.x

Nakamura K, Liu X, Ascoli M (2000) Seven non-contiguous intracellular residues of the lutropin/choriogonadotropin receptor dictate the rate of agonist-induced internalization and its sensitivity to non-visual arrestins. The Journal of biological chemistry 275 (1):241-247

Nikolaev VO, Moshkov A, Lyon AR, Miragoli M, Novak P, Paur H, Lohse MJ, Korchev YE, Harding SE, Gorelik J (2010) Beta2-adrenergic receptor redistribution in heart failure changes cAMP compartmentation. Science 327 (5973):1653-1657. doi:10.1126/science.1185988

Nooh MM, Bahouth SW (2017) Two barcodes encoded by the type-1 PDZ and by phospho-Ser312 regulate retromer/WASH-mediated sorting of the ss1-adrenergic receptor from endosomes to the plasma membrane. Cellular signalling 29:192-208. doi:10.1016/j.cellsig.2016.10.014

Nooh MM, Mancarella S, Bahouth SW (2016) Identification of novel transplantable GPCR recycling motif for drug discovery. Biochemical pharmacology 120:22-32. doi:10.1016/j.bcp.2016.09.011

O'Hayre M, Eichel K, Avino S, Zhao X, Steffen DJ, Feng X, Kawakami K, Aoki J, Messer K, Sunahara R, Inoue A, von Zastrow M, Gutkind JS (2017) Genetic evidence that beta-arrestins are dispensable for the initiation of beta2-adrenergic receptor signaling to ERK. Science signaling 10 (484). doi:10.1126/scisignal.aal3395

Oh P, Schnitzer JE (2001) Segregation of heterotrimeric G proteins in cell surface microdomains. G(q) binds caveolin to concentrate in caveolae, whereas G(i) and G(s) target lipid rafts by default. Molecular biology of the cell 12 (3):685-698

Okazaki M, Ferrandon S, Vilardaga JP, Bouxsein ML, Potts JT, Jr., Gardella TJ (2008) Prolonged signaling at the parathyroid hormone receptor by peptide ligands targeted to a specific receptor conformation. Proceedings of the National Academy of

Sciences of the United States of America 105 (43):16525-16530. doi:10.1073/pnas.0808750105

Pardon E, Laeremans T, Triest S, Rasmussen SG, Wohlkonig A, Ruf A, Muyldermans S, Hol WG, Kobilka BK, Steyaert J (2014) A general protocol for the generation of Nanobodies for structural biology. Nature protocols 9 (3):674-693. doi:10.1038/nprot.2014.039

Pierce KL, Lefkowitz RJ (2001) Classical and new roles of beta-arrestins in the regulation of G-protein-coupled receptors. Nature reviews Neuroscience 2 (10):727-733. doi:10.1038/35094577

Pike LJ (2009) The challenge of lipid rafts. Journal of lipid research 50 Suppl:S323-328. doi:10.1194/jlr.R800040-JLR200

Pucadyil TJ, Chattopadhyay A (2004) Cholesterol modulates ligand binding and G-protein coupling to serotonin(1A) receptors from bovine hippocampus. Biochimica et biophysica acta 1663 (1-2):188-200. doi:10.1016/j.bbamem.2004.03.010

Puthenveedu MA, Lauffer B, Temkin P, Vistein R, Carlton P, Thorn K, Taunton J, Weiner OD, Parton RG, Mark Von Z (2010) Sequence-dependent sorting of recycling proteins by actin-stabilized endosomal microdomains. Cell 143 (5):761-773. doi:10.1016/j.cell.2010.10.003

Puthenveedu MA, von Zastrow M (2006) Cargo regulates clathrin-coated pit dynamics. Cell 127 (1):113-124. doi:10.1016/j.cell.2006.08.035

Raiborg C, Bache KG, Gillooly DJ, Madshus IH, Stang E, Stenmark H (2002) Hrs sorts ubiquitinated proteins into clathrin-coated microdomains of early endosomes. Nature cell biology 4 (5):394-398. doi:10.1038/ncb791

Rankovic Z, Brust TF, Bohn LM (2016) Biased agonism: An emerging paradigm in GPCR drug discovery. Bioorg Med Chem Lett 26 (2):241-250. doi:10.1016/j.bmcl.2015.12.024

Reiter E, Ahn S, Shukla AK, Lefkowitz RJ (2012) Molecular mechanism of beta-arrestin-biased agonism at seven-transmembrane receptors. Annual review of pharmacology and toxicology 52:179-197. doi:10.1146/annurev.pharmtox.010909.105800

Roman-Vendrell C, Yu YJ, Yudowski GA (2012) Fast modulation of mu-opioid receptor (MOR) recycling is mediated by receptor agonists. The Journal of biological chemistry 287 (18):14782-14791. doi:10.1074/jbc.M111.319616

Rosciglione S, Theriault C, Boily MO, Paquette M, Lavoie C (2014) Galphas regulates the post-endocytic sorting of G protein-coupled receptors. Nature communications 5:4556. doi:10.1038/ncomms5556

Rusten TE, Vaccari T, Stenmark H (2011) Shaping development with ESCRTs. Nat Cell Biol 14 (1):38-45. doi:10.1038/ncb2381

Sengupta P, Philip F, Scarlata S (2008) Caveolin-1 alters Ca(2+) signal duration through specific interaction with the G alpha q family of G proteins. Journal of cell science 121 (Pt 9):1363-1372. doi:10.1242/jcs.020081

Shenoy SK (2014) Arrestin interaction with E3 ubiquitin ligases and deubiquitinases: functional and therapeutic implications. Handbook of experimental pharmacology 219:187-203. doi:10.1007/978-3-642-41199-1_10

Shenoy SK, Modi AS, Shukla AK, Xiao K, Berthouze M, Ahn S, Wilkinson KD, Miller WE, Lefkowitz RJ (2009) Beta-arrestin-dependent signaling and trafficking of 7-transmembrane receptors is reciprocally regulated by the deubiquitinase USP33 and the E3 ligase Mdm2. Proceedings of the National Academy of Sciences of the United States of America 106 (16):6650-6655. doi:10.1073/pnas.0901083106

Shvets E, Ludwig A, Nichols BJ (2014) News from the caves: update on the structure and function of caveolae. Current opinion in cell biology 29:99-106. doi:10.1016/j.ceb.2014.04.011

Song KS, Scherer PE, Tang Z, Okamoto T, Li S, Chafel M, Chu C, Kohtz DS, Lisanti MP (1996) Expression of caveolin-3 in skeletal, cardiac, and smooth muscle cells. Caveolin-3 is a component of the sarcolemma and co-fractionates with dystrophin

and dystrophin-associated glycoproteins. The Journal of biological chemistry 271 (25):15160-15165

Sposini S, Jean-Alphonse FG, Ayoub MA, Oqua A, West C, Lavery S, Brosens JJ, Reiter E, Hanyaloglu AC (2017) Integration of GPCR Signaling and Sorting from Very Early Endosomes via Opposing APPL1 Mechanisms. Cell reports 21 (10):2855-2867. doi:10.1016/j.celrep.2017.11.023

Srivastava A, Gupta B, Gupta C, Shukla AK (2015) Emerging Functional Divergence of beta-Arrestin Isoforms in GPCR Function. Trends in endocrinology and metabolism: TEM 26 (11):628-642. doi:10.1016/j.tem.2015.09.001

Stallaert W, Christopoulos A, Bouvier M (2011) Ligand functional selectivity and quantitative pharmacology at G protein-coupled receptors. Expert Opin Drug Discov 6 (8):811-825. doi:10.1517/17460441.2011.586691

Taelman VF, Dobrowolski R, Plouhinec JL, Fuentealba LC, Vorwald PP, Gumper I, Sabatini DD, De Robertis EM (2010) Wnt signaling requires sequestration of glycogen synthase kinase 3 inside multivesicular endosomes. Cell 143 (7):1136-1148. doi:10.1016/j.cell.2010.11.034

Tappe-Theodor A, Agarwal N, Katona I, Rubino T, Martini L, Swiercz J, Mackie K, Monyer H, Parolaro D, Whistler J, Kuner T, Kuner R (2007) A molecular basis of analgesic tolerance to cannabinoids. The Journal of neuroscience : the official journal of the Society for Neuroscience 27 (15):4165-4177. doi:10.1523/JNEUROSCI.5648-06.2007

Temkin P, Lauffer B, Jager S, Cimermancic P, Krogan NJ, von Zastrow M (2011) SNX27 mediates retromer tubule entry and endosome-to-plasma membrane trafficking of signalling receptors. Nature cell biology 13 (6):715-721. doi:10.1038/ncb2252

Terrillon S, Bouvier M (2004) Receptor activity-independent recruitment of betaarrestin2 reveals specific signalling modes. EMBO J 23 (20):3950-3961. doi:10.1038/sj.emboj.7600387

Thomsen ARB, Plouffe B, Cahill TJ, Shukla AK, Tarrasch JT, Dosey AM, Kahsai AW, Strachan RT, Pani B, Mahoney JP, Huang L, Breton B, Heydenreich FM, Sunahara RK, Skiniotis G, Bouvier M, Lefkowitz RJ (2016) GPCR-G Protein-beta-Arrestin Super-Complex Mediates Sustained G Protein Signaling. Cell 166 (4):907-919. doi:10.1016/j.cell.2016.07.004

Tian X, Irannejad R, Bowman SL, Du Y, Puthenveedu MA, von Zastrow M, Benovic JL (2016) The alpha-Arrestin ARRDC3 Regulates the Endosomal Residence Time and Intracellular Signaling of the beta2-Adrenergic Receptor. The Journal of biological chemistry 291 (28):14510-14525. doi:10.1074/jbc.M116.716589

Trejo J (2005) Internal PDZ ligands: novel endocytic recycling motifs for G protein-coupled receptors. Molecular pharmacology 67 (5):1388-1390. doi:10.1124/mol.105.011288

Tsvetanova NG, Trester-Zedlitz M, Newton BW, Riordan DP, Sundaram AB, Johnson JR, Krogan NJ, von Zastrow M (2017) G Protein-Coupled Receptor Endocytosis Confers Uniformity in Responses to Chemically Distinct Ligands. Molecular pharmacology 91 (2):145-156. doi:10.1124/mol.116.106369

Tsvetanova NG, von Zastrow M (2014) Spatial encoding of cyclic AMP signaling specificity by GPCR endocytosis. Nature chemical biology 10 (12):1061-1065. doi:10.1038/nchembio.1665

Varandas KC, Irannejad R, von Zastrow M (2016) Retromer Endosome Exit Domains Serve Multiple Trafficking Destinations and Regulate Local G Protein Activation by GPCRs. Current biology : CB 26 (23):3129-3142. doi:10.1016/j.cub.2016.09.052

Vines CM, Revankar CM, Maestas DC, LaRusch LL, Cimino DF, Kohout TA, Lefkowitz RJ, Prossnitz ER (2003) N-formyl peptide receptors internalize but do not recycle in the absence of arrestins. The Journal of biological chemistry 278 (43):41581-41584. doi:10.1074/jbc.C300291200

Vistein R, Puthenveedu MA (2013) Reprogramming of G protein-coupled receptor recycling and signaling by a kinase switch. Proceedings of the National Academy of Sciences

of the United States of America 110 (38):15289-15294. doi:10.1073/pnas.1306340110

West C, Hanyaloglu AC (2015) Minireview: Spatial Programming of G Protein-Coupled Receptor Activity: Decoding Signaling in Health and Disease. Molecular endocrinology 29 (8):1095-1106. doi:10.1210/ME.2015-1065

Wright PT, Nikolaev VO, O'Hara T, Diakonov I, Bhargava A, Tokar S, Schobesberger S, Shevchuk AI, Sikkel MB, Wilkinson R, Trayanova NA, Lyon AR, Harding SE, Gorelik J (2014) Caveolin-3 regulates compartmentation of cardiomyocyte beta2-adrenergic receptor-mediated cAMP signaling. J Mol Cell Cardiol 67:38-48. doi:10.1016/j.yjmcc.2013.12.003

Wu FQ, Fang T, Yu LX, Lv GS, Lv HW, Liang D, Li T, Wang CZ, Tan YX, Ding J, Chen Y, Tang L, Guo LN, Tang SH, Yang W, Wang HY (2016) ADRB2 signaling promotes HCC progression and sorafenib resistance by inhibiting autophagic degradation of HIF1alpha. Journal of hepatology 65 (2):314-324. doi:10.1016/j.jhep.2016.04.019

Xiang Y, Kobilka B (2003) The PDZ-binding motif of the beta2-adrenoceptor is essential for physiologic signaling and trafficking in cardiac myocytes. Proc Natl Acad Sci U S A 100 (19):10776-10781. doi:10.1073/pnas.1831718100

Xiao K, McClatchy DB, Shukla AK, Zhao Y, Chen M, Shenoy SK, Yates JR, 3rd, Lefkowitz RJ (2007) Functional specialization of beta-arrestin interactions revealed by proteomic analysis. Proceedings of the National Academy of Sciences of the United States of America 104 (29):12011-12016. doi:10.1073/pnas.0704849104

Xiao K, Sun J, Kim J, Rajagopal S, Zhai B, Villen J, Haas W, Kovacs JJ, Shukla AK, Hara MR, Hernandez M, Lachmann A, Zhao S, Lin Y, Cheng Y, Mizuno K, Ma'ayan A, Gygi SP, Lefkowitz RJ (2010) Global phosphorylation analysis of beta-arrestin-mediated signaling downstream of a seven transmembrane receptor (7TMR). Proceedings of the National Academy of Sciences of the United States of America 107 (34):15299-15304. doi:10.1073/pnas.1008461107

Yang L, Scarlata S (2017) Super-resolution Visualization of Caveola Deformation in Response to Osmotic Stress. The Journal of biological chemistry 292 (9):3779-3788. doi:10.1074/jbc.M116.768499

Yarwood RE, Imlach WL, Lieu T, Veldhuis NA, Jensen DD, Klein Herenbrink C, Aurelio L, Cai Z, Christie MJ, Poole DP, Porter CJH, McLean P, Hicks GA, Geppetti P, Halls ML, Canals M, Bunnett NW (2017) Endosomal signaling of the receptor for calcitonin gene-related peptide mediates pain transmission. Proceedings of the National Academy of Sciences of the United States of America 114 (46):12309-12314. doi:10.1073/pnas.1706656114

Zhang X, Sun N, Zheng M, Kim KM (2016) Clathrin-mediated endocytosis is responsible for the lysosomal degradation of dopamine D3 receptor. Biochemical and biophysical research communications 476 (4):245-251. doi:10.1016/j.bbrc.2016.05.104

Figure Legends

Figure 1. Archetypal model of GPCR activation, internalization and post-endocytic sorting with additional modes of GPCR internalization highlighted in pink box. The ligand activated receptor (1) is phosphorylated on multiple intracellular sites by GPCR kinases (GRKs) (2i) resulting in recruitment and binding of β-arrestin (2ii). β-arrestin uncouples receptors from their G proteins, leading to desensitization and enables receptor clustering to clathrin coated pits (CCPs) (3). Dynamin-mediated CCP scission from the plasma membrane and subsequent clathrin uncoating results in internalized receptor sorted to endosome (4). From here GPCRs sorted to divergent pathways either back to the plasma membrane leading to receptor resensitization (5a) or sorted to lysosomes for its degradation, leading to permanent signal termination (5b). GPCRs can also internalize via additional dynamin-depdenent pathways including; caveloae or FEME (fast endophilin-mediated endocytosis), and even organized in to flat clathrin plaques or lattices (see text).

Figure 2. GPCR intracellular signaling at distinct endosomal compartments. *(A)* GPCRs that transiently associate with arrestins (e.g. β2AR) are internalized to early endosomes (EEs) where active receptors are present throughout the endomembrane, although the pathways in addition to Gαs/cAMP are not known. Gαs mediated-cAMP production is elicited by receptors organized in signal microdomains including recycling tubules marked by sorting nexin 27 (SNX27), retromer and WASH complexes. α-arrestin, which associates with endosomal sorting complex required for transport 0 (ESCRT0), regulates the endosomal signaling by retention of receptors outside these tubules. *(B)* GPCRs that exhibit a sustained association with β-arrestin during internalization (e.g. V2R, PTHR) are also targeted to the EE, where they continue to activate cAMP via the interaction with both the G protein heterotrimer and β-arrestin. Interaction of GPCR with retromer and SNX27 dictates its localization into recycling tubules and terminates endosomal signaling. GPCRs such as TSHR are also able to activate Gαs/cAMP via retrograde transport to the TGN. *(C)* Certain GPCRs (e.g. LHR) are internalized into very early endosomes (VEEs), whereby a

subpopulation is marked by APPL1. The primary cAMP signaling is acutely generated from the VEEs and negatively regulated by APPL1. The receptor-mediated activation of Gαs/cAMP/PKA pathway phosphorylates APPL1 and this phosphorylated form drives receptor recycling back to the plasma membrane. *(D)* GPCRs such as Frizzled, are sorted to the lysosomal pathway and can induce a sustained Wnt-mediated β-catenin response via ESCRT-dependent involution into multi vesicular endosomes (MVE) with glycogen synthase kinase 3 (GSK3). (see text).

Figure 1

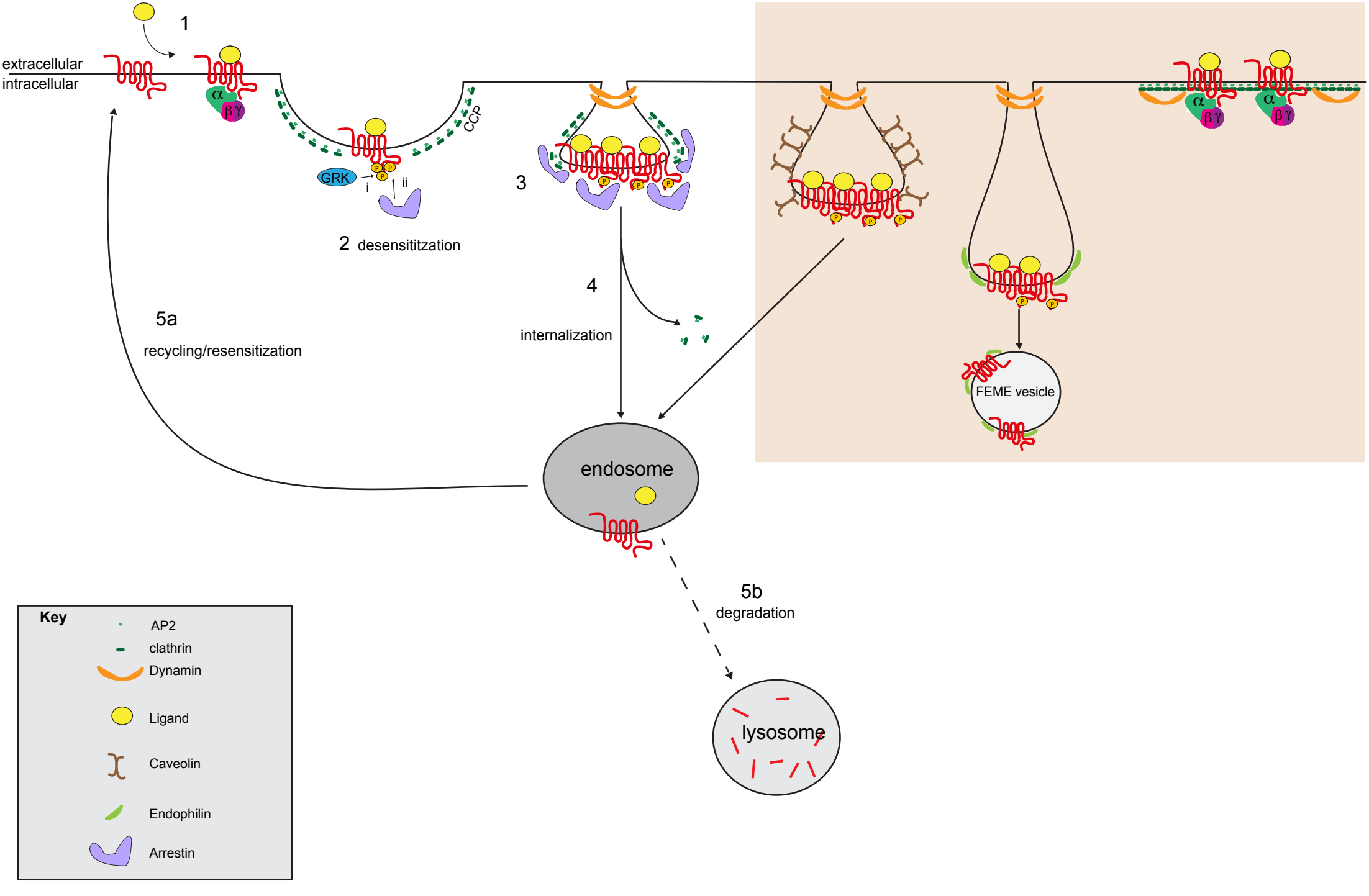
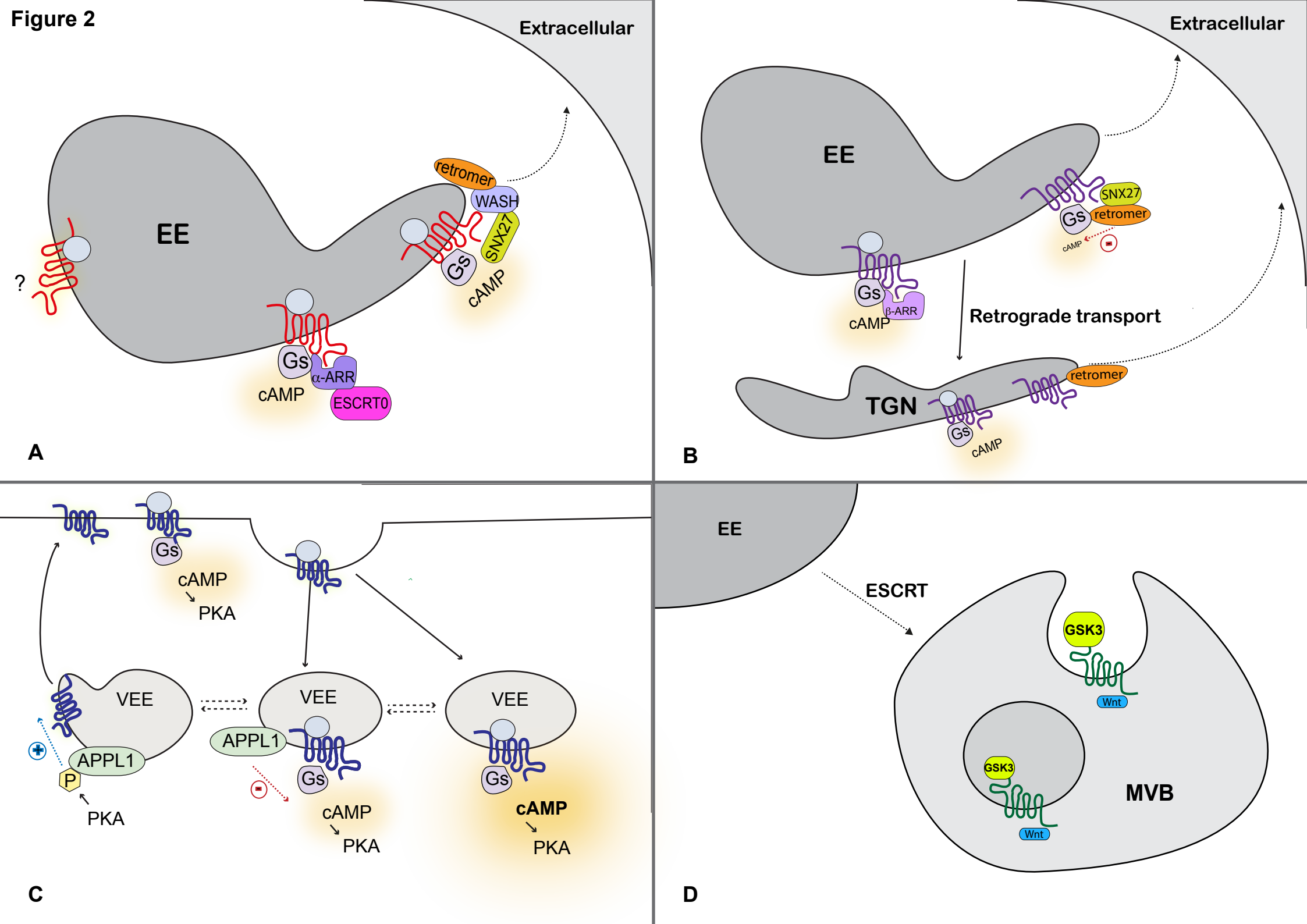

1
extracellular
intracellular
α
β γ
GRK
i
ii
CCP
2 desensititzation
3
4
internalization
5a
recycling/resensitization
FEME vesicle
endosome
5b
degradation
lysosome
Key
AP2
clathrin
Dynamin
Ligand
Caveolin
Endophilin
Arrestin

Figure 2


Extracellular
EE
?
retromer
WASH
SNX27
Gs
cAMP
Gs
α-ARR
cAMP
ESCRT0
A
Extracellular
EE
SNX27
Gs
retromer
cAMP
Gs
β-ARR
cAMP
Retrograde transport
TGN
retromer
Gs
cAMP
B
Gs
cAMP
PKA
VEE
APPL1
P
PKA
VEE
APPL1
Gs
cAMP
PKA
VEE
Gs
cAMP
PKA
C
EE
ESCRT
GSK3
Wnt
GSK3
Wnt
MVB
D