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β-arrestins and G Protein-Coupled Receptor Trafficking

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Abstract

Non-visual arrestins (β-arrestin-1 and β-arrestin-2) are adaptor proteins that function to regulate G protein-coupled receptor (GPCR) signaling and trafficking. β-arrestins are ubiquitously expressed and function to inhibit GPCR/G protein coupling, a process called desensitization, and promote GPCR trafficking and arrestin-mediated signaling. β-arrestin-mediated endocytosis of GPCRs requires the coordinated interaction of β-arrestins with clathrin, adaptor protein 2 (AP2) and phosphoinositides. These interactions are facilitated by a conformational change in β-arrestin that is thought to occur upon binding to a phosphorylated activated GPCR. In this review, we provide an overview of the key interactions involved in β-arrestin-mediated trafficking of GPCRs.

Keywords

arrestin; receptor; phosphorylation; endocytosis; clathrin; adaptin; phosphoinositides

β-arrestins and GPCR trafficking

Many transmembrane signaling systems consist of specific G protein-coupled receptors (GPCRs) that transduce the binding of extracellular stimuli into intracellular signaling. GPCRs modulate the activity of numerous intracellular effectors and ultimately regulate a myriad of biological processes. To ensure that extracellular stimuli are translated into intracellular signals of appropriate magnitude and duration, most signaling cascades are tightly regulated. GPCRs are subject to three principle modes of regulation: (i) desensitization, where a receptor becomes refractory to continued stimuli; (ii) internalization, where receptors are physically removed from the cell surface by endocytosis; and (iii) down-regulation, where total cellular receptor levels are decreased (Fig. 1). GPCR desensitization is primarily mediated by second messenger dependent kinases and by GPCR kinases (GRKs). GRKs specifically phosphorylate activated GPCRs and initiate the recruitment of arrestins, which mediate receptor desensitization, endocytosis and signaling (Krupnick and Benovic 1998).

A role for β-arrestins in agonist-promoted internalization of GPCRs was first discovered in 1996 (Ferguson et al. 1996; Goodman et al. 1996). These initial studies focused on the β_2-adrenergic receptor (β_2AR) while more recent studies have demonstrated that β-arrestins

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promote the trafficking of many GPCRs as well as additional classes of receptors (Moore et al. 2007; Shenoy and Lefkowitz 2011). Mechanistic insight into this process has revealed an essential role for the coordinated interaction of β-arrestins with the GPCR (Vishnivetskiy et al. 1999, 2011), clathrin (Krupnick et al. 1997; Kang et al. 2009), adaptor protein 2 (AP2) (Laporte et al. 1999, 2000; Kim and Benovic 2002; Schmid et al. 2006; Burtey et al. 2007) and phosphoinositides (Gaidarov et al. 1999; Milano et al. 2006). Moreover, β-arrestin binding to the GPCR appears to induce a conformational change that promotes interaction with the endocytic machinery, thereby linking the binding and trafficking events (Kim and Benovic 2002; Xiao et al. 2004; Nobles et al. 2007).

General structure of β-arrestins

The four mammalian arrestins fall into two classes, visual and non-visual, and X-ray structures for all four family members have been solved. Arrestins can be divided into two major domains, the N-domain and C-domain, with each domain primarily consisting of anti-parallel β-sheets connected by short flexible loops (Fig. 2). The N- and C-domains are connected by a short “hinge region” while the C-tail is connected by a flexible linker to the C-domain and contains a short β strand that interacts with a lateral β strand of the N-domain. The overall structure is stabilized by a polar core of buried salt bridges and by a three-element interaction involving the first β strand, an α-helix in the N-domain and the C-terminal tail (Han et al. 2001; Milano et al. 2002, 2006; Kang et al. 2009; Zhan et al. 2011). The polar core is comprised of charged residues from the amino terminus (Asp-29 in β-arrestin-1), N-domain (Arg-169), C-domain (Asp-290 and Asp-297), and C-terminal tail (Arg-393) thus bringing different parts of the molecule together to maintain a basal conformation. The residues involved in formation of the polar core are highly conserved, suggesting that this structural element is critical for the function of all arrestins. Because the buried side chains of the polar core achieve neutrality by an elaborate network of electrostatic interactions, it has been suggested that disturbance of the polar core by introduction of a phosphate group from the receptor promotes structural changes that result in an active conformation of arrestin (Hirsch et al. 1999). Indeed, two of the five polar core residues, namely Arg169 and Asp290 in β-arrestin-1 (Han et al. 2001) and Arg170 and Asp291 in β-arrestin-2 (Zhan et al. 2011) are particularly important for arrestin selectivity for binding to activated phosphorylated receptors (Vishnivetshy et al. 1999).

It is believed that arrestins make an initial contact with phosphorylated receptors via adjacent lysines in the amino terminus (Vishnivetskiy et al. 2000). Biochemical data suggests that this interaction perturbs the three-element interaction, guides phosphorylated receptors to the polar core, allows the negatively charged phosphate from the receptor to interact with positively charge Arg169 (in β-arrestin-1), and ultimately causes release of the C-terminal tail from the polar core (Palczewski et al. 1991; Gurevich et al. 1998; Vishnivetskiy et al. 2000; Gurevich and Gurevich 2004). This leads to the disruption of the basal state and subsequent conformational rearrangement of arrestin. Studies monitoring arrestin conformational changes in live cells, along with other biochemical data, suggest that the arrestin amino terminus and C-terminal tail move closer upon binding to an activated receptor (Xiao et al. 2004; Charest et al. 2005). This conformational rearrangement enhances

arrestin interaction with receptors and is also thought to expose binding motifs that interact with other proteins such as clathrin and AP2 (Moore et al. 2007).

β-arrestin interaction with clathrin

Clathrin is a well-studied endocytic protein that is essential for the formation of clathrin-coated pits (CCPs), which play a central role in receptor endocytosis. Clathrin is composed of a heavy and a light chain and three clathrin molecules associate to form a propeller-shaped triskelion, which is the basic structural unit of CCPs (Kirchhausen 2000). Although most GPCRs internalize via CCPs, GPCRs do not directly bind to clathrin and thus require an adaptor protein to provide a molecular link between the receptor and CCP. While the adaptor protein AP2 plays this role for some GPCRs, β-arrestins also function as adaptors to mediate endocytosis of GPCRs (Ferguson et al. 1996; Goodman et al. 1996). Upon agonist stimulation, β-arrestin-1 was found to colocalize with clathrin and the β_2AR. Mechanistic studies reveal that β-arrestin-1 and -2 specifically bind to clathrin with a K_d of 10–60 nM (Goodman et al. 1996). The primary clathrin-binding site in β-arrestin, called a clathrin binding box or Lφxφ[D/E] motif (where φ is a bulky hydrophobic residue and x represents any polar amino acid), is localized in the carboxyl terminal region (residues 376–380 in β-arrestin-1) (Fig. 2). This motif is also found in many other clathrin-binding proteins such as AP2, AP180, amphiphysin, and epsin (Owen et al. 2004). Importantly, mutation or deletion of this motif in β-arrestin-1 effectively disrupts clathrin binding and receptor internalization (Krupnick et al. 1997; Kim and Benovic 2002; Burtey et al. 2007). Mutagenesis studies localized the β-arrestin binding site to the N-terminal domain of the clathrin heavy chain, specifically residues 89–100, with an invariant Glu89 and conserved Lys96 and Lys98 as critical resides that mediate β-arrestin interaction (Goodman et al. 1997). Hydrophobic and basic residues in this region of clathrin complement the hydrophobic and acidic amino acids within the Lφxφ[D/E] motif in β-arrestin.

Crystallographic structures of the terminal domain of the clathrin heavy chain (residues 1–363) in complex with a β-arrestin-2 peptide (ter Haar et al. 2000) as well as with full length β-arrestin-1 (Kang et al. 2009) support the predicted location of the arrestin-clathrin interface determined by mutagenesis. These structures clearly demonstrate that the Lφxφ[D/E] motif in β-arrestin interacts with a hydrophobic patch formed by the 1st and 2nd blades of the clathrin terminal domain. In addition, charged residues outside of the Lφxφ[D/E] motif form hydrogen bonds with Glu89 and Lys96 in clathrin and help to stabilize the interaction. β-arrestin-1 actually exists in two isoforms (long and short) that differ by an 8 amino acid insert between the 18th and 19th β-strands (Sterne-Marr et al. 1993; Kang et al. 2009). Interestingly, the structure of a complex between the long isoform of β-arrestin-1 (β-arrestin-1L) and clathrin revealed a second region of interaction between these proteins. This interaction was mediated by the 8 amino acid insert unique to β-arrestin-1L and a hydrophobic patch formed by 4th and 5th blades of clathrin (Kang et al. 2009) (Fig. 2). Site directed mutagenesis of the 8 amino acid insert in β-arrestin-1L identified a $[L/I]_2$GxL motif that mediates clathrin binding. Interestingly, this motif is also found in many other clathrin binding proteins, although whether it plays a broad role in clathrin binding is currently unknown.

Cell biological approaches have also been used to characterize the functional role of the clathrin binding motifs in β-arrestin-1L. β-arrestin-1L mutants lacking a single clathrin binding motif showed reduced β_2AR endocytosis while β-arrestin-1L lacking both clathrin binding motifs effectively disrupted clathrin binding and β_2AR endocytosis (Kang et al. 2009). Taken together, these studies demonstrate that β-arrestin interaction with clathrin plays an essential role in endocytosis of many GPCRs while the two independent interactions between β-arrestin-1L and clathrin likely facilitate the formation of a macromolecular complex that regulates the dynamics of receptor endocytosis.

β-arrestin interaction with AP2

Another essential component of CCPs is the adaptor protein AP2. AP2 is a heterotetrameric protein consisting of α, β2, μ2 and σ2 subunits and it functions as a clathrin adaptor and in cargo recruitment to CCPs (Owen et al. 2004). The α-adaptin and β2-adaptin subunits of AP2 function in cargo and adaptor recruitment and are composed of ear (appendage), hinge and trunk domains. The appendage domain of α-adaptin interacts with DP[F/W], FxDxF and WxxF motifs while the appendage domain of β2-adaptin interacts with [D/E]xxFxx[F/L]xxxR. The μ2 subunit of AP2 also binds cargo proteins and interacts with Yxxφ and [D/E]xxL[L/I] motifs as well as with phosphatidylinositol.

Initial studies from the Caron laboratory identified a direct interaction between β-arrestin and β2-adaptin (Laporte et al. 1999, 2000). They found that deletion of 25 amino acids from the C-terminus of β-arrestin-1 completely disrupted interaction with β2-adaptin while mutation of Arg394 or Arg396 in β-arrestin-2 (equivalent to Arg393 and Arg395 in β-arrestin-1) disrupted β2-adaptin binding. Moreover, a β-arrestin-2-R396A mutant did not co-localize with AP2 in CCPs upon receptor activation, in contrast to wild type β-arrestin-2 (Laporte et al. 2000). Additional studies revealed an essential role for Phe391 and Arg395 in β-arrestin-1 binding to β2-adaptin and showed that F391A and R395E mutants functioned as effective dominant negative mutants in β_2AR internalization assays when clathrin binding was also disrupted (Kim and Benovic 2002). Several studies also identified the residues in β2-adaptin that mediate β-arrestin binding and revealed an important role for Arg834, Trp841, Glu849, Tyr888 and Glu902 (Kim and Benovic 2002; Edeling et al. 2006; Schmid et al. 2006).

Based on extensive mutagenesis and biochemical analysis, a β2-adaptin-binding consensus sequence was defined as [D/E]xxFxx[F/L]xxxR in β-arrestins, epsin and autosomal recessive hyper-cholesterolemia protein (ARH) (Edeling et al. 2006; Schmid et al. 2006). Crystallographic studies demonstrate that the appendage domain of β2-adaptin consists of platform and sandwich subdomains. X-ray structures of β2-adaptin crystallized with synthetic peptides containing the [D/E]xxFxx[F/L]xxxR motif from either ARH (Edeling et al. 2006) or β-arrestin-1 (Schmid et al. 2006) show a molecular interface primarily formed by hydrophobic interactions between the C-terminal domain of β-arrestin-1 and the platform domain of β2-adaptin. The center of this interaction is formed by Phe388 and Phe391 in β-arrestin-1 and Tyr888 in β2-adaptin. Interestingly, the β-arrestin-1 region involved in this interaction forms the last β-strand in holo β-arrestin-1 while this region becomes α-helical when bound to β2-adaptin, at least when bound as a peptide. These results suggest a

conformational change occurs upon arrestin/adaptin binding and support previous findings that arrestin activation promotes adaptin binding (Kim and Benovic 2002). While these studies suggest a major conformational change occurs in β-arrestin when it binds to β2-adaptin, it will be important to validate such results in β-arrestin complexes with receptor and β2-adaptin.

β-arrestin interaction with phosphoinositides

Phosphatidylinositol 4,5-bisphosphate (PIP_2) is an important component in clathrin-mediated endocytosis and is mainly enriched at the plasma membrane, although it is also detected in the Golgi, endosomes and endoplasmic reticulum (Watt et al. 2002). Clathrin-mediated endocytosis can roughly be divided into five stages: nucleation, cargo selection, coat assembly, scission, and uncoating (McMahon and Boucrot 2011). PIP_2 synthesis is important for the nucleation, cargo selection and coat assembly of CCPs, while scission and uncoating of CCPs is partially dependent on the localized turnover of PIP_2 (Antonescu et al. 2011; Zoncu et al. 2007). GPCR trafficking is also dependent on PIP_2 since alteration of plasma membrane PIP_2 levels significantly affects GPCR endocytosis and recycling (Tóth et al. 2012).

Phosphoinositides also play an important role in β-arrestin-mediated trafficking of GPCRs. β-arrestin-1 and -2 contain a high affinity phosphoinositide-binding site located in the C-domain where three basic residues (Lys-233, Arg-237 and Lys-251 in β-arrestin-2) have been implicated in phosphoinositide binding (Gaidarov et al. 1999). Mutation of these three residues in β-arrestin-2 (β-arrestin-2-KRK/Q) failed to support β_2AR recruitment to CCPs and subsequent internalization suggesting that phosphoinositides are important in delivering the receptor-arrestin complex to CCPs. The β-arrestin-2-KRK/Q mutant, however, retains the ability to bind to receptor and clathrin and was recruited to the plasma membrane upon receptor activation (Gaidarov et al. 1999). The binding affinities of various phosphoinositides for β-arrestin-1 and -2 were found to have the following affinities: IP_6 (~0.08 μM) > PIP_3 (~0.3 μM) > PIP_2 (~1.4 μM) > IP_4 (~4 μM) > IP_3 (~20 μM) (Gaidarov et al. 1999). Interestingly, β-arrestins also appear to regulate the production of PIP_2 since β-arrestin-2 binds the enzyme phosphatidylinositol 4-phosphate 5-kinase (PIP5K), which functions in PIP_2 production (Nelson et al. 2008). Overexpression of a β-arrestin-2 mutant lacking PIP_2 binding abolishes PIP5K interaction and inhibits β_2AR internalization. A positive feedback mechanism was proposed where β-arrestin-2 interaction with PIP_2 facilitates β_2AR internalization by promoting interaction with PIP5K to synergistically produce more PIP_2, thereby leading to increased local concentrations of PIP_2 (Nelson et al. 2008). Taken together, these results suggest an essential role for phosphoinositides in β-arrestin mediated trafficking of GPCRs.

While PIP_2 and PIP_3 are the proposed physiological ligands for β-arrestins at the plasma membrane, inositol hexakisphosphate (IP_6), a soluble inositol polyphosphate, displays a higher binding affinity for β-arrestins than either PIP_2 or PIP_3 (Gaidarov et al. 1999). IP_6 is abundant in cells with concentrations ranging between 15–100 μM and has been proposed to regulate receptor endocytosis and receptor signaling (Sasakawa et al. 1995). Interestingly, IP_6 inhibits both β-arrestin-1 and -2 binding to an activated phosphorylated GPCR (Gaidarov

et al. 1999). Moreover, the ability of IP_6 to bind to two distinct sites on β-arrestins appears to mediate homo- and hetero-oligomerization of β-arrestin-1 and -2 (Milano et al. 2006). Mutation of either IP_6-binding site in β-arrestin-1 disrupted oligomerization while interactions with known binding partners including clathrin, AP2, and ERK2 were maintained. Moreover, subcellular localization studies showed that β-arrestin-1 oligomers and β-arrestin-1/2 hetero-oligomers are primarily cytoplasmic, whereas β-arrestin-1 monomers displayed increased nuclear localization (Milano et al. 2006; Storez et al. 2005). This suggests that IP_6 binding to β-arrestins may regulate arrestin localization and function as a negative regulator of arrestin interaction with plasma membrane and nuclear signaling proteins.

Additional interactions involved in β-arrestin-mediated trafficking of GPCRs

While β-arrestin interactions with clathrin, AP2 and phosphoinositides appear critical in arrestin-promoted endocytosis, β-arrestins also bind several additional proteins that regulate GPCR trafficking (Table 1). For example, β-arrestin-2 interacts with endothelial NO synthase which promotes S-nitrosylation of β-arrestin-2 which, in turn, enhances association with CCPs and accelerates GPCR internalization (Ozawa et al. 2008). β-arrestin-1 also interacts with N-ethylmaleimide-sensitive fusion protein (NSF), an ATPase that regulates intracellular transport. Interestingly, β-arrestin-1 interaction with NSF is ATP dependent and overexpression of NSF enhances agonist-promoted internalization of the β_2AR (McDonald et al. 1999). β-arrestin-1 interaction with Arf6·GDP and its nucleotide exchange factors, ARNO and EFA6, leads to Arf6 activation and subsequent regulation of GPCR endocytosis and recycling (Mukherjee et al. 2000; Claing et al. 2001; Macia et al. 2012). β-arrestins have also been demonstrated to recruit E3 ubiquitin ligases and de-ubiquitinases to the plasma membrane to regulate GPCR trafficking. For example, β-arrestin-2 is rapidly ubiquitinated by Mdm2 upon β_2AR stimulation and depletion of Mdm2 by siRNA or overexpression of dominant-negative Mdm2 attenuates β-arrestin-2 ubiquitination and β_2AR internalization (Shenoy et al. 2001, 2009). Moreover, β-arrestin-2 acts as an adaptor between β_2AR and the E3 ubiquitin ligase Nedd4 to facilitate β_2AR ubiquitination and trafficking (Shenoy et al. 2008; Han et al. 2013). Interestingly, β-arrestin-2 can also be modified by sumoylation at Lys-400 and inhibition of β-arrestin-2 sumoylation attenuates β_2AR internalization (Wyatt et al. 2011).

Once internalized, GPCRs are either recycled back to the plasma membrane or sorted to the lysosome and degraded (Fig. 1). The dynamic regulation of GPCR ubiquitination by ubiquitin E3 ligases and deubiquitinases plays a crucial role in endocytic sorting (Marchese and Benovic, 2011; Shenoy et al. 2001, 2008, 2009). β-arrestins function to facilitate this process and the stability of β-arrestin ubiquitination and its interaction with a GPCR appear to contribute to whether a receptor is to be recycled or degraded. For example, agonist stimulation of the β_2AR induces transient ubiquitination and complex formation with β-arrestin-2 and the receptor is rapidly dephosphorylated and recycled after internalization. In contrast, stimulation of the AT_{1a} receptor promotes sustained binding and ubiquitination of β-arrestin-2 and the receptor is effectively sorted to lysosomes and degraded (Oakley et al. 1999, 2001; Shenoy et al. 2001, 2003). While sustained ubiquitination of GPCRs is important for receptor degradation, deubiquitination of GPCRs regulates receptor recycling

back to the plasma membrane. For example, the deubiquitinases USP33 and USP20 have been shown to directly interact with β-arrestin-2 and facilitate both β-arrestin-2 and β_2AR deubiquitination. Importantly, a double knockdown of USP20 and USP33 enhances the extent of β-arrestin-2 ubiquitination and increases β_2AR degradation (Berthouze et al. 2009; Shenoy et al. 2009).

Once a GPCR is committed to the degradation pathway, it is sorted to the lysosome with the help of the ESCRT complexes (Marchese and Trejo 2013). The presence of a functional β-arrestin has been shown to be essential for effective sorting and degradation of CXCR4. Specifically, β-arrestin-1 colocalizes with atrophin interacting protein 4 (AIP4), an E3 ubiquitin ligase, on early endosomes to facilitate CXCR4 sorting and degradation. Knockdown of β-arrestin-1 inhibits CXCR4 degradation but does not affect CXCR4 ubiquitination or internalization (Bhandari et al. 2007). CXCR4 sorting is also regulated by β-arrestin-1 interaction with signal-transducing adaptor molecule-1 (STAM-1) and disruption of this interaction attenuates agonist-promoted ubiquitination of HRS and enhances sorting to lysosomes (Malik and Marchese 2010).

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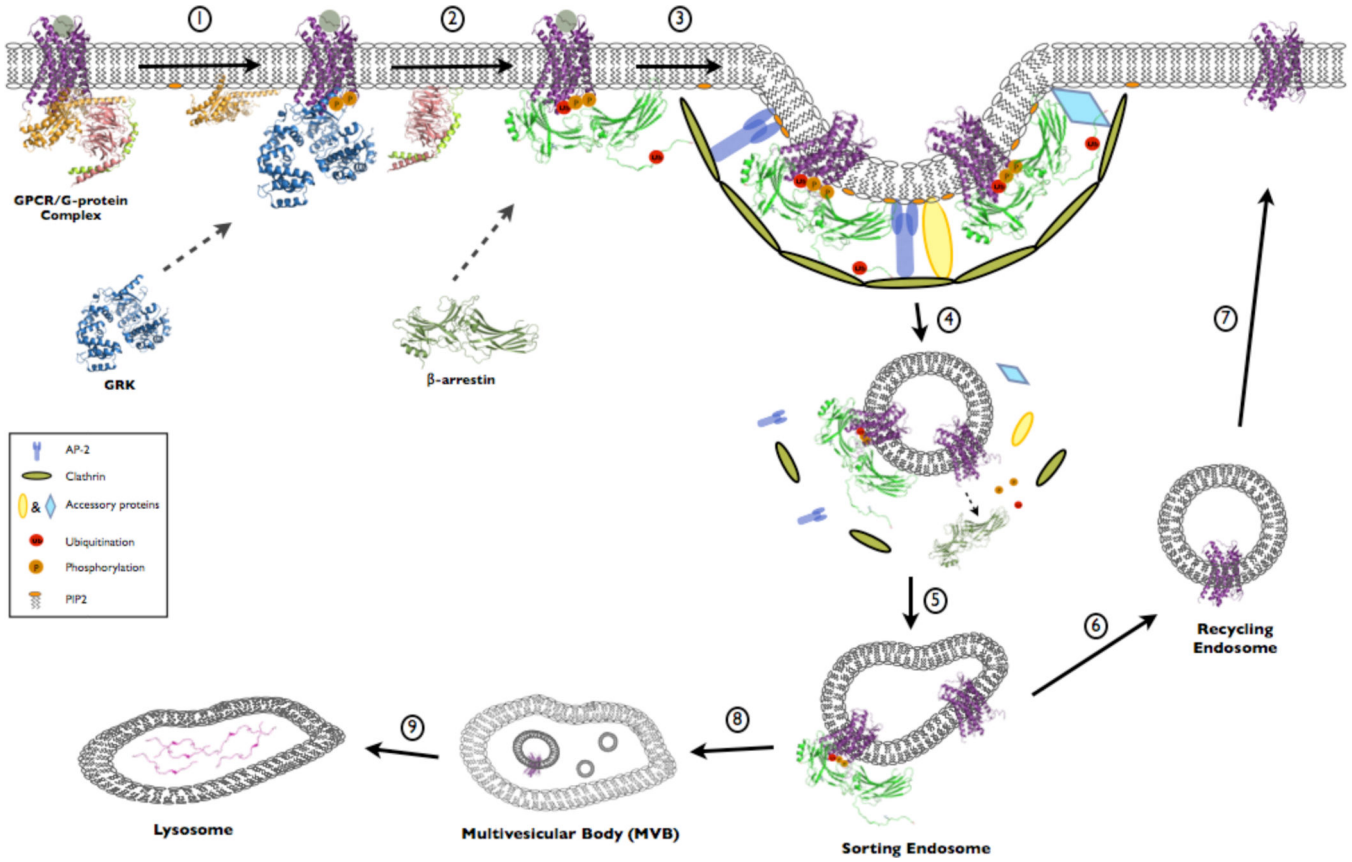

Figure 1. Role of β-arrestins in GPCR trafficking

(1) Agonist binding to a GPCR results in heterotrimeric G protein activation leading to dissociation of Gα from Gβγ subunits. This promotes GRK association with the agonist-bound GPCR which mediates receptor phosphorylation and (2) promotes β-arrestin recruitment to the receptor. (3) β-arrestin association with the phosphorylated GPCR mediates conformational changes in arrestin that promote association of the GPCR-β-arrestin complex with the endocytic machinery and subsequent endocytosis (4). GPCRs then traffic to sorting endosomes (5) and ultimately either recycle back to the plasma membrane through recycling endosomes (6 and 7) or are sorted to lysosomes where they are degraded (8 and 9).

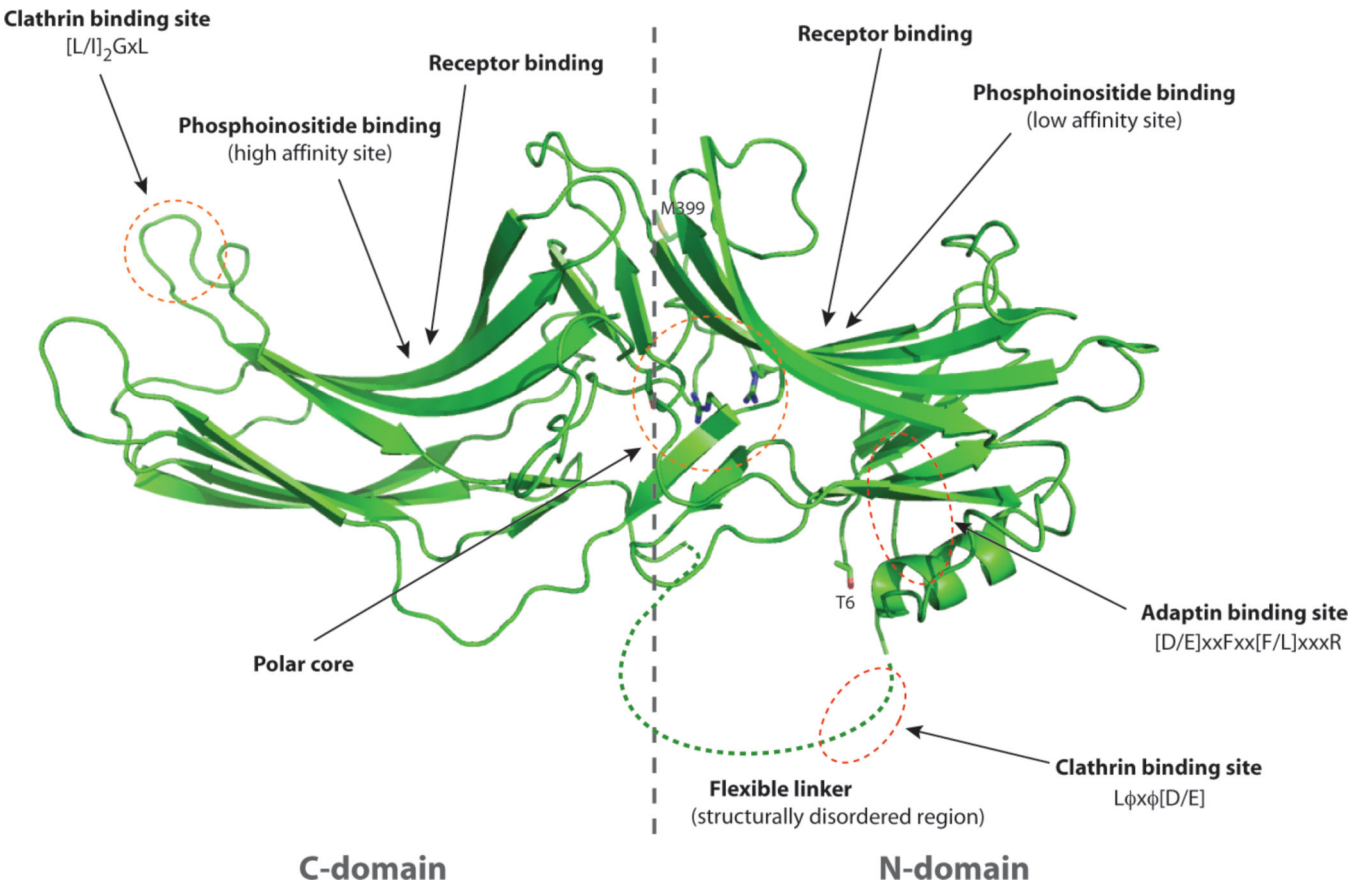

Figure 2. Secondary structure of β-arrestin-1L

Ribbon diagram of β-arrestin-1L (residues 6 to 399) indicating the N- and C-domains, the polar core and binding sites for the GPCR, phosphoinositides (high affinity site in C-domain and low affinity site in N-domain), clathrin (Lφxφ[D/E] and $[L/I]_2$GxL motifs) and β2-adaptin ([D/E]xxFxx[F/L]xxxR motif).

Table 1

Protein interactions with β-arrestins that function in GPCR trafficking

Binding Partner	Region of the binding partner interacting with arrestin	Region of arrestin interacting with the binding partner	Functions	References
β2-adaptin	The groove between α-helix 1 and the antiparallel β-sheet of the platform sub-domain	[D/E]xxFxx[F/L]xxxR in the C-terminal tail	Directly interacts with β-arrestins and facilitates GPCR endocytosis	Laporte et al. 2001 Edeling et al. 2006 Schmid et al. 2006
μ2-adaptin	-	[Y/F]VTL in the N-terminus	Preferentially interacts with β-arrestin2 and facilitates β_2AR endocytosis	Marion et al. 2007
Clathrin	Pocket formed by blades 1 and 2 (E89, K96, K98) Hydrophobic pocket formed by blades 4 and 5	LØxØ[D/E] in the C-tail $[L/I]_2$GxL (β-arrestin1L)	Directly interacts with β-arrestins and facilitates GPCR endocytosis	Goodman et al. 1997 ter Haar et al. 2000 Kang et al. 2009
PIP2	Phosphate head group	Residues 223-285 (K233, R237, K251 in β-arrestin2)	Directly interacts with β-arrestins and enhances GPCR endocytosis	Gaidarov et al. 1999 Nelson et al. 2008
PIP5K-Iα	-	Residues 240-261	Directly interacts with β-arrestin2 and facilitates β_2AR endocytosis	Nelson et al. 2008
IP6	Phosphate head group	C-domain (K233, R237, K251, K324, K326); N-domain (K157, K160, R161)	Directly interacts with β-arrestins; inhibits β-arrestin/GPCR interaction, facilitates β-arrestin homo- and hetero-oligomerization and regulates β-arrestin cellular localization	Gaidarov et al. 1999 Storez et al. 2005 Milano et al. 2006
PI3K	PIK domain	-	Regulates β_2AR endocytosis by AP2 recruitment to the β_2AR/β-arrestin complex	Naga Prasad et al. 2002
NSF	-	-	Directly interacts with β-arrestin2 and enhances β_2AR endocytosis	McDonald et al. 1999
ARF6	-	C-tail	GDP-bound form interacts with β-arrestin1; enhances GPCR endocytosis; negatively controls recycling; enhances receptor degradation	Claing et al. 2001 Houndolo et al. 2005 Macia et al. 2012
ARNO	-	-	Activates ARF6 to facilitate β-arrestin release from LH/CGR	Mukherjee et al. 2000
EFA6	-	C-tail of β-arrestin1	β-arrestin1 scaffolds ARF6-GDP and EFA6 to facilitate ARF6 activation leading to β_2AR degradation	Macia et al. 2012
Mdm2	-	N-domain	Ubiquitinates β-arrestin2 and facilitates β_2AR endocytosis	Shenoy et al. 2001
AIP4	WWI-II domains	N-domain (residues 1-260)	Interacts with β-arrestin2 on early endosomes and facilitates CXCR4 degradation	Bhandari et al. 2007
Nedd4	-	-	Interacts with β-arrestin to facilitate β_2AR ubiquitination and trafficking	Shenoy et al. 2008 Han et al. 2013
STAM-1	GAT domain	Residues 25-161	Interacts with β-arrestin 1 to regulate CXCR4 sorting	Malik and Marchese 2010
USP20	-	-	Directly deubiquitinates β-arrestin2 and β_2AR to prevent receptor degradation	Shenoy et al. 2009
USP33	-	-	Directly deubiquitinates β-arrestin2 and β_2AR to prevent receptor degradation	Berthouze et al. 2009
eNOS	-	N-terminus	Interacts with and s-nitrosylates β-arrestin2 and facilitates β-arrestin2 binding with clathrin and β-adaptin; promotes receptor internalization	Ozawa et al. 2008