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Role of β -arrestins and arrestin domain-containing proteins in G protein-coupled receptor trafficking

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Abstract

The arrestin clan can now be broadly divided into three structurally similar subgroups: the originally identified arrestins (visual and β -arrestins), the α -arrestins and a group of Vps26-related proteins. The visual and β -arrestins selectively bind to agonist-occupied phosphorylated G protein-coupled receptors (GPCRs) and inhibit GPCR coupling to heterotrimeric G proteins while the β -arrestins also function as adaptor proteins to regulate GPCR trafficking and G protein-independent signaling. The α -arrestins have also recently been implicated in regulating GPCR trafficking while Vps26 regulates retrograde trafficking. In this review, we provide an overview of the α - and β -arrestins with a focus on our current understanding of how these adaptor proteins regulate GPCR trafficking.

Keywords

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

Introduction

Arrestins were initially discovered in the visual system and include four mammalian members, two visual (arrestin-1 in rod cells and arrestin-4 in cone cells) and two non-visual (β -arrestin1 and β -arrestin2, also called arrestin-2 and arrestin-3, respectively). Arrestins are expressed in metazoans and selectively bind to the agonist-occupied phosphorylated conformation of G protein-coupled receptors (GPCRs) [1]. While arrestins were initially named on the basis of their ability to arrest or turn-off the coupling of GPCRs to heterotrimeric G proteins and thereby inhibit signaling, it is now evident that β -arrestins can also regulate GPCR trafficking as well as G protein-independent signaling [2,3]. A general scheme for β -arrestin-mediated regulation of GPCR function is depicted in Fig. 1.

Interestingly, recent studies have identified two additional families of arrestin related proteins, a group of Vps26-related proteins and α -arrestins. Vps26 is broadly expressed in eukaryotes and is a component of the retromer and has a structure similar to the visual and

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β -arrestins [4]. The α -arrestins are broadly expressed in all eukaryotes except plants and include 6 mammalian members referred to as arrestin domain-containing (ARRDC) proteins [5,6]. Computational modeling suggests that the α -arrestins also have a structure similar to the visual and β -arrestins and several studies have suggested a role for these proteins in GPCR trafficking.

β -arrestins in GPCR endocytosis

A role for β -arrestins in GPCR trafficking was initially revealed by the demonstration that overexpression of wild type β -arrestins enhanced agonist-promoted internalization of the β_2 -adrenergic receptor (β_2 AR) while expression of dominant negative β -arrestin mutants inhibited β_2 AR internalization [7]. Mechanistic insight into this process was initially provided by the finding that β -arrestin directly interacts with clathrin, a major component of clathrin-coated pits (CCPs) [8]. The primary clathrin binding site in β -arrestins was found to be a clathrin binding box (LIELD in β -arrestin1) localized in the C-terminal region [9], while a secondary clathrin binding site (LLGDL) was identified in a splice variant of β -arrestin1 [10]. Additional mechanistic insight was provided by the demonstration that β -arrestins also interact with the endocytic adaptor AP2 [11,12], which normally functions to promote the assembly of clathrin lattices and targets receptors to CCPs. This interaction is mediated by an adaptin binding motif in the C-tail of β -arrestins that binds to the β_2 -subunit of AP2 [12–16]. While the ability of β -arrestins to interact with clathrin and AP2 is essential in arrestin-mediated endocytosis of GPCRs [10,12,13], β -arrestin interaction with phosphoinositides also plays a critical role in this process [17]. Interestingly, β -arrestins contain two phosphoinositide binding sites and interaction with inositol hexakisphosphate (IP₆) regulates homo- and hetero-oligomerization and ultimately the cellular localization and function of β -arrestins [18]. The regions of β -arrestin1 involved in clathrin, AP2 and phosphoinositide binding are depicted in Fig. 2A.

Structural insight into β -arrestin-mediated trafficking

While β -arrestin-mediated endocytosis of GPCRs requires interaction with clathrin, AP2 and phosphoinositides, the temporal and spatial dynamics of these interactions are at least partially controlled by conformational changes that occur when β -arrestin binds to a phosphorylated activated GPCR [13,19,20]. Arrestins are composed of two major domains, the N- and C-domain, which primarily consist of β -sheets and connecting loops with one short α -helix (Fig. 2A). There are two major intra-molecular interactions that maintain arrestins in this basal conformation, a polar core of charged residues that connects the N-terminus, N- and C-domains and C-tail and a 3-element region that connects the N-terminus, α -helix and C-tail [1]. The binding of arrestin to an activated phosphorylated GPCR is believed to disrupt these intra-molecular constraints thereby releasing the C-tail and promoting β -arrestin interaction with clathrin and AP2. While several lines of evidence support this proposed model [13,16,19–26], the recent crystallographic structure of β -arrestin1 bound to a V2 vasopressin receptor phosphopeptide perhaps provides the most compelling evidence to date [27]. Importantly, the receptor phosphopeptide binds to Lys-10 and Lys-11, disrupting the 3-element region and displacing the C-tail of β -arrestin1. The displacement of the C-tail by the phosphopeptide as well as the large movement of connecting loops also results in a significant twist of the N- and C-domains relative to each other (Fig. 2B) [27].

Ultimately, the release of the C-tail of β -arrestin facilitates binding to clathrin and AP2 [13,16,19,20]. While the C-tail is not visible in the β -arrestin1/phosphopeptide structure [27], the β_2 -adaptin binding region in β -arrestin is thought to transition from a β -sheet in the basal state of the protein to an α -helix when associated with β_2 -adaptin [14–16]. This

conformational change has been suggested to function as a molecular switch to stabilize the activated conformation of β -arrestin [14,15,27]. Taken together, rearrangement of various domains in the active state of β -arrestin optimizes arrestin-receptor interaction and facilitates β -arrestin interaction with endocytic proteins by releasing the C-tail. Crystal structures of holo-arrestin/GPCR complexes with and without clathrin and β 2-adaptin will be needed to more fully define how arrestins discriminate activated phosphorylated GPCRs and how GPCR binding promotes association with the endocytic machinery.

Role of post-translational modifications of β -arrestin in GPCR trafficking

While the interactions described above are important for arrestin-mediated endocytosis of GPCRs, dynamic post-translational modifications of β -arrestins including phosphorylation, nitrosylation, sumoylation and ubiquitination also regulate the endocytic process. β -arrestins are basally phosphorylated in the C-tail (e.g., Ser-412 in β -arrestin1), which inhibits interaction with the endocytic machinery, and GPCR binding promotes β -arrestin dephosphorylation and facilitates receptor internalization [28]. β -arrestin2 interaction with endothelial nitric oxide synthase promotes S-nitrosylation at Cys-410 and enhances β -arrestin2 association with CCPs and accelerates GPCR internalization [29]. Interestingly, sumoylation of β -arrestin2 was recently shown to occur on Lys-400 upon β_2 AR activation and also facilitate internalization [30]. A common theme in each of these modifications is that they occur in the β -arrestin C-tail, a region involved in stabilizing intra-molecular interactions and ultimately the region that directly interacts with the endocytic machinery (Fig. 2A).

β -arrestin ubiquitination by the E3 ubiquitin ligase Mdm2 appears to be a particularly important modification in regulating arrestin function. This modification is driven by GPCR activation as first noted for the β_2 AR and ultimately plays a role in stabilizing the β -arrestin/GPCR complex [31], although β -arrestin ubiquitination does not appear to have a direct effect on receptor binding [32]. An interesting feature of this modification is that the sites of ubiquitination appear to be dictated by the specific GPCR involved. For example, β -arrestin binding to the AT_{1a} receptor promotes ubiquitination of Lys-11 and 12 in β -arrestin, binding to the V2 vasopressin receptor promotes ubiquitination of Lys-18, 107, 108, 207 and 296, and binding to the β_2 AR promotes ubiquitination that is not site-specific [32,33]. The removal of ubiquitin is also dynamic since β -arrestins bind deubiquitinases (DUBs) such as UPS20 and USP33 in a process regulated by GPCR activation. Importantly, a knockdown of USP20 and USP33 enhances β -arrestin2 ubiquitination and increases β_2 AR degradation [34,35]. Ultimately, the dynamics and site-specificity of β -arrestin ubiquitination appears to play a critical role in regulating the stability of β -arrestin/GPCR complexes as well as downstream processes such as GPCR trafficking and signaling.

Additional interactions involved in β -arrestin-promoted trafficking

The initial studies demonstrating β -arrestin ubiquitination by Mdm2 also revealed that β -arrestins regulate the ubiquitination of the β_2 AR [31]. β -arrestin2 was subsequently found to serve as an adaptor between the β_2 AR and the E3 ubiquitin ligase Nedd4 and facilitate β_2 AR ubiquitination and trafficking [36,37]. β -arrestin recruitment of E3 ubiquitin ligases appears to be a common theme and has also been shown for Smurf2, which interacts with β -arrestin2 to mediate ubiquitination of the μ -opioid receptor and regulate CCP maturation [38], and AIP4, which interacts with β -arrestin1 on early endosomes and facilitates the sorting and degradation of the chemokine receptor CXCR4 [39]. Interestingly, β -arrestins also directly interact with the E3 ubiquitin ligase parkin and while this interaction enhances β -arrestin binding to Mdm2, it ultimately decreases β -arrestin ubiquitination [40]. Thus, β -arrestins

function as adaptors for a variety of E3 ubiquitin ligases to regulate receptor ubiquitination and sorting.

β -arrestins also bind several additional proteins that regulate GPCR trafficking (Table 1). For example, β -arrestin1 interacts with N-ethylmaleimide-sensitive fusion protein (NSF) in an ATP-dependent manner and regulates β_2 AR trafficking [41]. β -arrestin1 interaction with Arf6-GDP and its nucleotide exchange factors, ARNO and EFA6, results in Arf6 activation and subsequent regulation of GPCR endocytosis and recycling [42–44]. β -arrestin1 and 2 interaction with the Arf GTPase activating protein AGAP2 facilitates β -arrestin association with the β_2 AR and regulates β_2 AR recycling [45] while interaction of PIP5K-1 α with β -arrestin2 facilitates β_2 AR endocytosis [46]. β -arrestin1 interaction with signal-transducing adaptor molecule-1 (STAM-1), a component of ESCRT-0, regulates CXCR4 sorting by regulating the ubiquitination of hepatocyte growth factor-regulated tyrosine kinase substrate (HRS) [47]. β -arrestin1 also interacts with the Na⁺/H⁺ exchanger regulatory factor 1 (NHERF1) to facilitate the agonist-promoted internalization of the P2Y₁₂ receptor [48].

While β -arrestins regulate the trafficking of many GPCRs, they also appear to function as broad-based endocytic adaptors to regulate the trafficking of many other proteins including the IGF1 receptor [49,50], type III TGF- β receptor [51], Frizzled [52], Notch [53,54], TRPV1 [55], TRPV4 [56], Aph-1a [57], VE-cadherin [58] and the Na⁺/H⁺ exchanger NHE5 [59]. Thus, β -arrestins appear to have a broad role in regulating the trafficking of many receptors and channels.

Arrestin domain-containing proteins in GPCR trafficking

Recent studies have revealed an arrestin-related family of proteins called α -arrestins [5]. The α -arrestins are expressed in all eukaryotes except plants and have been most extensively studied in *S. cerevisiae* and mammals. In *S. cerevisiae*, there are 10 family members called arrestin-related trafficking adaptors (ART 1–10) that have been shown to play a broad role in regulating the trafficking of various transporters [6]. A potential role for ARTs in GPCR trafficking has not been reported.

In mammals, there are 6 α -arrestins, named ARRDC1–5 and thioredoxin-interacting protein (TXNIP). While there have been relatively few studies characterizing a role for ARRDC proteins in regulating GPCR trafficking, ARRDC3 was identified in a screen for proteins involved in regulating β_2 AR degradation [60]. This study reported that ARRDC3 interacts with the β_2 AR in an agonist-dependent manner at the plasma membrane and serves as an adaptor to facilitate Nedd4-mediated β_2 AR ubiquitination and degradation. Moreover, mutation of the two PPXY motifs in ARRDC3 disrupted interaction with Nedd4 and attenuated β_2 AR ubiquitination and degradation [60]. Characterization of a mouse ARRDC3 knockout revealed a role for ARRDC3 in metabolism and suggested that ARRDC3 interaction with the β_2 AR and β_3 AR plays a role in this process [61]. An additional study confirmed the ability of ARRDC3 to co-immunoprecipitate with the β_2 AR and provided evidence that ARRDC3 mediates β_2 AR ubiquitination [62]. These authors also showed that the V2 vasopressin receptor co-immunoprecipitated with ARRDC4. While these results are intriguing, a more recent study reported that overexpression or depletion of ARRDC3 did not affect the ubiquitination, internalization or degradation of the β_2 AR [37]. These authors found that ARRDC3, along with ARRDC2 and ARRDC4, localized on early endosomes and proposed that these proteins serve as secondary adaptors to recruit the internalized β_2 AR/ β -arrestin/Nedd4 complex to a subset of early endosomes. Thus, ARRDC proteins appear to regulate GPCR trafficking although the detailed mechanisms remain to be more fully dissected (Fig. 1).

α -arrestins are structurally related to visual/ β -arrestins

While α -arrestins have only 11–15% amino acid homology with β -arrestins, modeling studies suggest that the α -arrestins contain an arrestin-fold structure consisting of arrestin-like N- and C-domains and an extended C-tail [6]. A recent partial structure of the N-terminal domain of TXNIP appears to be more structurally similar to Vps26, a component of the retromer that also adopts an arrestin-fold structure, than to β -arrestins [63]. While it remains to be established whether the α -arrestins are structurally related to visual/ β -arrestins, sequence analysis suggests some fundamental differences between these protein families that might differentiate their function. First, the α -arrestins appear to lack a “polar core”, which normally maintains visual/ β -arrestins in a basal conformation and is crucial for their receptor phospho-sensing activity and release of the C-tail upon receptor binding [1,27]. The lack of a “polar core” might suggest that α -arrestins would not be sensitive to the phosphorylation state of a GPCR or, at least, not in a manner similar to visual/ β -arrestins. Another distinguishing feature of the α -arrestins, except for ARRDC5, is that they contain two PPXY motifs in an extended C-tail. PPXY motifs can interact with WW-domains that are commonly found in E3 ubiquitin ligases and, as described in more detail below, the ARRDCs do interact with a number of E3 ubiquitin ligases.

ARRDC localization and interactions

While the ARTs are mainly present in the cytosol [64], the cellular localization differs among the ARRDCs. TXNIP is mainly localized in the nucleus [65] while ARRDC2, 3 and 4 are generally localized on the plasma membrane and endocytic vesicles [37,60,62,66]. ARRDC1 has been reported to be localized at the plasma membrane [37,60] or on intracellular puncta [67]. It is important to note that most of these observations have been drawn from studying ARRDCs overexpressed in heterologous cell lines. Thus, it will be important to characterize the localization of the endogenous ARRDCs.

Similar to the β -arrestins, ARRDCs appear to interact with other proteins and thereby function as adaptors. These include interactions with various HECT-domain E3 ubiquitin ligases such as WWP1, WWP2, Nedd4, and Itch/AIP4, via the WW domain on the ligase and the PPXY motifs on the ARRDC (Table 1) [37,60,62,66,68]. Similarly, many ARRDCs interact with components of the ESCRT machinery. For example, ARRDC1 interacts with ALIX and VPS4, ESCRT-I and ESCRT-III associated proteins, respectively, and Tsg101, a component of the ESCRT-I complex [67,68]. Deletion of ARRDC1 C-tail abrogates interaction with ALIX and Tsg101 but does not affect VPS4 interaction [67]. ARRDC2 also interacts with ALIX while ARRDC3 interacts with ALIX and HRS, a component of the ESCRT-0 complex, and mutation of its PPXY motifs or deletion of the C-tail disrupts these interactions [37,67]. Those observations suggest a role for the ARRDCs in regulating trafficking via their ability to interact with the ESCRT machinery.

Recent studies have also suggested that the ARRDCs can heterodimerize with β -arrestins. For example, overexpressed ARRDC3 and ARRDC4 appear to co-immunoprecipitate with endogenous β -arrestins independent of receptor activation [62]. In addition, ARRDC1 has been shown to form a heterodimer with β -arrestins, interact with Notch and Itch (AIP4), and together with β -arrestins, regulate Notch ubiquitination and degradation [54]. Overexpression of an ARRDC1 PPXY mutant inhibited Itch mediated Notch ubiquitination and degradation and also led to a significant increase in Notch/ β -arrestin1 interaction, presumably due to insufficient Notch degradation.

Conclusions

The arrestin clan can now be broadly divided into three structurally similar subgroups: the visual and β -arrestins, the α -arrestins and the Vps26 proteins. These proteins appear to largely function as adaptors to modulate protein/protein interaction and receptor sorting. Mechanistic insight for the β -arrestins suggests that this process is initially driven by conformational changes that occur upon GPCR binding that drives β -arrestin interactions with the endocytic machinery. While a role for the α -arrestins in GPCR trafficking has not been as well defined, it is evident that the α -arrestins can also function as adaptors for E3 ubiquitin ligases and the ESCRT machinery. It will be important to further dissect the interplay between α - and β -arrestins in regulating GPCR trafficking as well as gain additional structural insight into the various interactions that modulate these functions.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Gurevich VV, Gurevich EV. The structural basis of arrestin-mediated regulation of G-protein coupled receptors. *Pharmacol Ther.* 2006; 110:465–502. [PubMed: 16460808]
 2. Moore CA, Milano SK, Benovic JL. Regulation of receptor trafficking by GRKs and arrestins. *Annu Rev Physiol.* 2007; 69:451–482. [PubMed: 17037978]
 3. Shenoy SK, Lefkowitz RJ. β -Arrestin-mediated receptor trafficking and signal transduction. *Trends Pharmacol Sci.* 2011; 32:521–533. [PubMed: 21680031]
 4. Shi H, Rojas R, Bonifacino JS, Hurley JH. The retromer subunit Vps26 has an arrestin fold and binds Vps35 through its C-terminal domain. *Nat Struct Mol Biol.* 2006; 13:540–548. [PubMed: 16732284]
 5. Alvarez CE. On the origins of arrestin and rhodopsin. *BMC Evol Biol.* 2008; 8:222. [PubMed: 18664266]
 - 6•. Aubry L, Klein G. True arrestins and arrestin-fold proteins: a structure-based appraisal. *Prog Mol Biol Transl Sci.* 2013; 118:21–56. Provides a comprehensive review of the arrestin family of proteins. [PubMed: 23764049]
 7. Ferguson SS, Downey WE 3rd, Colapietro AM, Barak LS, Ménard L, Caron MG. Role of β -arrestin in mediating agonist-promoted G protein-coupled receptor internalization. *Science.* 1996; 271:363–366. [PubMed: 8553074]
 8. Goodman OB Jr, Krupnick JG, Santini F, Gurevich VV, Penn RB, Gagnon AW, Keen JH, Benovic JL. β -Arrestin acts as a clathrin adaptor in endocytosis of the β 2-adrenergic receptor. *Nature.* 1996; 383:447–450. [PubMed: 8837779]
 9. Krupnick JG, Goodman OB Jr, Keen JH, Benovic JL. Arrestin/clathrin interaction. Localization of the clathrin binding domain of nonvisual arrestins to the carboxy terminus. *J Biol Chem.* 1997; 272:15011–15016. [PubMed: 9169476]
 - 10•. Kang DS, Kern RC, Puthenveedu MA, von Zastrow M, Williams JC, Benovic JL. Structure of an arrestin2-clathrin complex reveals a novel clathrin binding domain that modulates receptor trafficking. *J Biol Chem.* 2009; 284:29860–29872. Provides the first crystal structure of an arrestin in complex with another protein (clathrin) and identifies a novel clathrin binding motif ((L/I)₂GXL) distinct from the clathrin binding box. [PubMed: 19710023]

11. Laporte SA, Oakley RH, Zhang J, Holt JA, Ferguson SS, Caron MG, Barak LS. The β 2-adrenergic receptor/ β -arrestin complex recruits the clathrin adaptor AP-2 during endocytosis. *Proc Natl Acad Sci U S A*. 1999; 96:3712–3717. [PubMed: 10097102]
12. Laporte SA, Oakley RH, Holt JA, Barak LS, Caron MG. The interaction of β -arrestin with the AP-2 adaptor is required for the clustering of β 2-adrenergic receptor into clathrin-coated pits. *J Biol Chem*. 2000; 275:23120–23126. [PubMed: 10770944]
13. Kim YM, Benovic JL. Differential roles of arrestin-2 interaction with clathrin and adaptor protein 2 in G protein-coupled receptor trafficking. *J Biol Chem*. 2002; 277:30760–30768. [PubMed: 12070169]
14. Schmid EM, Ford MG, Burtey A, Praefcke GJ, Peak-Chew SY, Mills IG, Benmerah A, McMahon HT. Role of the AP2 β -appendage hub in recruiting partners for clathrin-coated vesicle assembly. *PLoS Biol*. 2006; 4:1532–1548.
15. Edeling MA, Mishra SK, Keyel PA, Steinhauser AL, Collins BM, Roth R, Heuser JE, Owen DJ, Traub LM. Molecular switches involving the AP-2 β 2 appendage regulate endocytic cargo selection and clathrin coat assembly. *Dev Cell*. 2006; 10:329–342. [PubMed: 16516836]
16. Burtey A, Schmid EM, Ford MGJ, Rapport JZ, Scott MGH, Marullo S, Simon SM, McMahon HT, Benmerah A. The conserved isoleucine-valine-phenylalanine motif couples activation state and endocytic functions of β -arrestins. *Traffic*. 2007; 8:914–931. [PubMed: 17547696]
17. Gaidarov I, Krupnick JG, Falck JR, Benovic JL, Keen JH. Arrestin function in G protein-coupled receptor endocytosis requires phosphoinositide binding. *EMBO J*. 1999; 18:871–881. [PubMed: 10022830]
18. Milano SK, Kim YM, Stefano FP, Benovic JL, Brenner C. Nonvisual arrestin oligomerization and cellular localization are regulated by inositol hexakisphosphate binding. *J Biol Chem*. 2006; 281:9812–9823. [PubMed: 16439357]
19. Xiao K, Shenoy SK, Nobles K, Lefkowitz RJ. Activation-dependent conformational changes in β -arrestin 2. *J Biol Chem*. 2004; 279:55744–55753. [PubMed: 15501822]
20. Nobles KN, Guan Z, Xiao K, Oas TG, Lefkowitz RJ. The active conformation of β -arrestin1. direct evidence for the phosphate sensor in the N-domain and conformational differences in the active states of β -arrestins1 and -2. *J Biol Chem*. 2007; 282:21370–21381. [PubMed: 17513300]
21. Shilton BH, McDowell JH, Smith WC, Hargrave PA. The solution structure and activation of visual arrestin studied by small-angle X-ray scattering. *Eur J Biochem*. 2002; 269:3801–3809. [PubMed: 12153577]
22. Carter JM, Gurevich VV, Prossnitz ER, Engen JR. Conformational differences between arrestin2 and pre-activated mutants as revealed by hydrogen exchange mass spectrometry. *J Mol Biol*. 2005; 351:865–878. [PubMed: 16045931]
23. Hanson SM, Francis DJ, Vishnivetskiy SA, Kolobova EA, Hubbell WL, Klug CS, Gurevich VV. Differential interaction of spin-labeled arrestin with inactive and active phosphorhodopsin. *Proc Natl Acad Sci U S A*. 2006; 103:4900–4905. [PubMed: 16547131]
24. Kim M, Vishnivetskiy SA, Van Eps N, Alexander NS, Cleghorn WM, Zhan X, Hanson SM, Morizumi T, Ernst OP, Meiler J, et al. Conformation of receptor-bound visual arrestin. *Proc Natl Acad Sci U S A*. 2012; 109:18407–18412. [PubMed: 23091036]
25. Zhuang T, Chen Q, Cho MK, Vishnivetskiy SA, Iverson TM, Gurevich VV, Sanders CR. Involvement of distinct arrestin-1 elements in binding to different functional forms of rhodopsin. *Proc Natl Acad Sci U S A*. 2013; 110:942–947. [PubMed: 23277586]
- 26•. Kim YJ, Hofmann KP, Ernst OP, Scheerer P, Choe HW, Sommer ME. Crystal structure of pre-activated arrestin p44. *Nature*. 2013; 497:142–146. Together with Ref. 27, reports the first crystal structure of an arrestin in its “active” conformation. [PubMed: 23604253]
- 27•. Shukla AK, Manglik A, Kruse AC, Xiao K, Reis RI, Tseng WC, Staus DP, Hilger D, Uysal S, Huang LY, et al. Structure of active β -arrestin-1 bound to a G-protein-coupled receptor phosphopeptide. *Nature*. 2013; 497:137–141. Together with Ref. 26, reports the first crystal structure of an arrestin in its “active” conformation and also identifies the interface between a phosphorylated receptor peptide and β -arrestin1. [PubMed: 23604254]

28. Lin T, Krueger KM, Kendall HE, Daaka Y, Fredericks ZL, Pitcher JA, Lefkowitz RJ. Clathrin-mediated endocytosis of the β -adrenergic receptor is regulated by phosphorylation/dephosphorylation of β -arrestin1. *J Biol Chem*. 1997; 272:31051–31057. [PubMed: 9388255]
29. Ozawa K, Whalen EJ, Nelson CD, Mu Y, Hess DT, Lefkowitz RJ, Stamler JS. S-nitrosylation of β -arrestin regulates β -adrenergic receptor trafficking. *Mol Cell*. 2008; 31:395–405. [PubMed: 18691971]
30. Wyatt D, Malik R, Vesecky AC, Marchese A. Small ubiquitin-like modifier modification of arrestin-3 regulates receptor trafficking. *J Biol Chem*. 2011; 286:3884–3893. Demonstrates that β -arrestin1 is sumoylated in a receptor dependent manner and that this modification is crucial for GPCR endocytic trafficking. [PubMed: 21118812]
31. Shenoy SK, McDonald PH, Kohout TA, Lefkowitz RJ. Regulation of receptor fate by ubiquitination of activated β 2-adrenergic receptor and β -arrestin. *Science*. 2001; 294:1307–1313. [PubMed: 11588219]
32. Kommaddi RP, Shenoy SK. Arrestins and protein ubiquitination. *Prog Mol Biol Transl Sci*. 2013; 118:175–204. Provides a comprehensive review of the role that arrestins play in regulating ubiquitination. [PubMed: 23764054]
33. Shenoy SK, Lefkowitz RJ. Receptor-specific ubiquitination of β -arrestin directs assembly and targeting of seven-transmembrane receptor signalosomes. *J Biol Chem*. 2005; 280:15315–15324. [PubMed: 15699045]
34. Berthouze M, Venkataramanan V, Li Y, Shenoy SK. The deubiquitinases USP33 and USP20 coordinate β 2 adrenergic receptor recycling and resensitization. *EMBO J*. 2009; 28:1684–1696. [PubMed: 19424180]
35. Shenoy SK, Modi AS, Shukla AK, Xiao K, Berthouze M, Ahn S, Wilkinson KD, Miller WE, Lefkowitz RJ. β -arrestin-dependent signaling and trafficking of 7-transmembrane receptors is reciprocally regulated by the deubiquitinase USP33 and the E3 ligase Mdm2. *Proc Natl Acad Sci U S A*. 2009; 106:6650–6655. [PubMed: 19363159]
36. Shenoy SK, Xiao K, Venkataramanan V, Snyder PM, Freedman NJ, Weissman AM. Nedd4 mediates agonist-dependent ubiquitination, lysosomal targeting, and degradation of the β 2-adrenergic receptor. *J Biol Chem*. 2008; 283:22166–22176. [PubMed: 18544533]
37. Han S, Kommaddi RP, Shenoy SK. Distinct roles of β -arrestin2 and arrestin-domain-containing proteins in β adrenergic receptor trafficking. *EMBO Rep*. 2013; 14:164–171. [PubMed: 23208550]
38. Henry AG, Hislop JN, Grove J, Thorn K, Marsh M, von Zastrow M. Regulation of endocytic clathrin dynamics by cargo ubiquitination. *Dev Cell*. 2012; 23:519–532. Demonstrates that receptor ubiquitination can serve as a biochemical signal to control clathrin coated pit maturation. [PubMed: 22940114]
39. Bhandari D, Trejo J, Benovic JL, Marchese A. Arrestin-2 interacts with the ubiquitin-protein isopeptide ligase atrophin-interacting protein 4 and mediates endosomal sorting of the chemokine receptor CXCR4. *J Biol Chem*. 2007; 282:36971–36979. [PubMed: 17947233]
40. Ahmed MR, Zhan X, Song X, Kook S, Gurevich VV, Gurevich EV. Ubiquitin ligase parkin promotes Mdm2-arrestin interaction but inhibits arrestin ubiquitination. *Biochemistry*. 2011; 50:3749–3763. [PubMed: 21466165]
41. McDonald PH, Cote NL, Lin FT, Premont RT, Pitcher JA, Lefkowitz RJ. Identification of NSF as a β -arrestin1-binding protein. Implications for β 2-adrenergic receptor regulation. *J Biol Chem*. 1999; 274:10677–10680. [PubMed: 10196135]
42. Mukherjee S, Gurevich VV, Jones JC, Casanova JE, Frank SR, Maizels ET, Bader MF, Kahn RA, Palczewski K, Aktories K, Hunzicker-Dunn M. The ADP ribosylation factor nucleotide exchange factor ARNO promotes β -arrestin release necessary for luteinizing hormone/choriogonadotropin receptor desensitization. *Proc Natl Acad Sci U S A*. 2000; 97:5901–5906. [PubMed: 10811902]
43. Claing A, Chen W, Miller WE, Vitale N, Moss J, Premont RT, Lefkowitz RJ. β -arrestin-mediated ADP-ribosylation factor 6 activation and β 2-adrenergic receptor endocytosis. *J Biol Chem*. 2001; 276:42509–42513. [PubMed: 11533043]
44. Macia E, Partisani M, Paleotti O, Luton F, Franco M. Arf6 negatively controls the rapid recycling of the β 2 adrenergic receptor. *J Cell Sci*. 2012; 125:4026–4035. [PubMed: 22611259]

45. Wu Y, Zhao Y, Ma X, Zhu Y, Patel J, Nie Z. The Arf GAP AGAP2 interacts with β -arrestin2 and regulates β 2-adrenergic receptor recycling and ERK activation. *Biochem J.* 2013; 452:411–421. [PubMed: 23527545]
46. Nelson CD, Kovacs JJ, Nobles KN, Whalen EJ, Lefkowitz RJ. β -arrestin scaffolding of phosphatidylinositol 4-phosphate 5-kinase $\text{I}\alpha$ promotes agonist-stimulated sequestration of the β 2-adrenergic receptor. *J Biol Chem.* 2008; 283:21093–21101. [PubMed: 18534983]
47. Malik R, Marchese A. Arrestin-2 interacts with the endosomal sorting complex required for transport machinery to modulate endosomal sorting of CXCR4. *Mol Biol Cell.* 2010; 21:2529–2541. Provides the first demonstration that β -arrestin interaction with the ESCRT complex regulates GPCR sorting. [PubMed: 20505072]
48. Nisar SP, Cunningham M, Saxena K, Pope RJ, Kelly E, Mundell SJ. Arrestin scaffolds NHERF1 to the P2Y12 receptor to regulate receptor internalization. *J Biol Chem.* 2012; 287:24505–24515. [PubMed: 22610101]
49. Lin FT, Daaka Y, Lefkowitz RJ. β -arrestins regulate mitogenic signaling and clathrin-mediated endocytosis of the insulin-like growth factor I receptor. *J Biol Chem.* 1998; 273:31640–31643. [PubMed: 9822622]
50. Girnita L, Shenoy SK, Sehat B, Vasilcanu R, Vasilcanu D, Girnita A, Lefkowitz RJ, Larsson O. β -arrestin and Mdm2 mediate IGF-1 receptor-stimulated ERK activation and cell cycle progression. *J Biol Chem.* 2007; 282:11329–11338. [PubMed: 17303558]
51. Chen W, Kirkbride KC, How T, Nelson CD, Mo J, Frederick JP, Wang XF, Lefkowitz RJ, Blobel GC. β -arrestin 2 mediates endocytosis of type III TGF- β receptor and down-regulation of its signaling. *Science.* 2003; 301:1394–1397. [PubMed: 12958365]
52. Chen W, ten Berge D, Brown J, Ahn S, Hu LA, Miller WE, Caron MG, Barak LS, Nusse R, Lefkowitz RJ. Dishevelled 2 recruits β -arrestin 2 to mediate Wnt5A-stimulated endocytosis of Frizzled 4. *Science.* 2003; 301:1391–1394. [PubMed: 12958364]
53. Mukherjee A, Veraksa A, Bauer A, Rosse C, Camonis J, Artavanis-Tsakonas S. Regulation of Notch signalling by non-visual β -arrestin. *Nat Cell Biol.* 2005; 7:1191–1201. [PubMed: 16284625]
54. Puca L, Chastagner P, Meas-Yedid V, Israël A, Brou C. α -arrestin 1 (ARRDC1) and β -arrestins cooperate to mediate Notch degradation in mammals. *J Cell Sci.* 2013; 126:4457–4468. [PubMed: 23886940]
55. Por ED, Bierbower SM, Berg KA, Gomez R, Akopian AN, Wetsel WC, Jeske NA. β -arrestin-2 desensitizes the transient receptor potential vanilloid 1 (TRPV1) channel. *J Biol Chem.* 2012; 287:37552–37563. [PubMed: 22952227]
56. Shukla AK, Kim J, Ahn S, Xiao K, Shenoy SK, Liedtke W, Lefkowitz RJ. Arresting a transient receptor potential (TRP) channel: β -arrestin 1 mediates ubiquitination and functional down-regulation of TRPV4. *J Biol Chem.* 2010; 285:30115–30125. [PubMed: 20650893]
57. Thathiah A, Horré K, Snellinx A, Vandeweyer E, Huang Y, Ciesielska M, De Kloe G, Munck S, De Strooper B. β -arrestin 2 regulates A β generation and γ -secretase activity in Alzheimer's disease. *Nat Med.* 2013; 19:43–49. [PubMed: 23202293]
58. Gavard J, Gutkind JS. VEGF controls endothelial-cell permeability by promoting the β -arrestin-dependent endocytosis of VE-cadherin. *Nat Cell Biol.* 2006; 8:1223–1234. [PubMed: 17060906]
59. Szabó EZ, Numata M, Lukashova V, Iannuzzi P, Orłowski J. β -arrestins bind and decrease cell-surface abundance of the Na^+/H^+ exchanger NHE5 isoform. *Proc Natl Acad Sci USA.* 2005; 102:2790–2795. [PubMed: 15699339]
60. Nabhan JF, Pan H, Lu Q. Arrestin domain-containing protein 3 recruits the NEDD4 E3 ligase to mediate ubiquitination of the β 2-adrenergic receptor. *EMBO Rep.* 2010; 11:605–611. [PubMed: 20559325]
61. Patwari P, Emilsson V, Schadt EE, Chutkow WA, Lee A, Marsili A, Zhang Y, Dobrin R, Cohen DE, Larsen PR, et al. The arrestin domain-containing 3 protein regulates body mass and energy expenditure. *Cell Metab.* 2011; 14:671–683. Identifies a role for ARRDC3 in obesity and provides some evidence that this is linked to ARRDC3 interaction with β -adrenergic receptors. [PubMed: 21982743]

62. Shea FF, Rowell JL, Li Y, Chang T, Alvarez CE. Mammalian alpha arrestins link activated seven transmembrane receptors to Nedd4 family E3 ubiquitin ligases and interact with beta arrestins. *PLoS One*. 2013; 7:e50557. [PubMed: 23236378]
63. Polekhina G, Ascher DB, Kok SF, Beckham S, Wilce M, Waltham M. Structure of the N-terminal domain of human thioredoxin-interacting protein. *Acta Crystallogr D Bio Crystallogr*. 2013; 69(Pt3):333–344. [PubMed: 23519408]
64. Becuwe M, Herrador A, Haguenaue-Tsapis R, Vincent O, Léon S. Ubiquitin-mediated regulation of endocytosis by protein of the arrestin family. *Biochem Res Int*. 2012; 2012:242764. [PubMed: 22988512]
65. Patwari P, Chtkow WA, Cummings K, Verstraeten V, Lammerding J, Schreiter ER, Lee RT. Thioredoxin-independent regulation of metabolism by the α -arrestin proteins. *J Biol Chem*. 2009; 284:24996–25003. [PubMed: 19605364]
66. Oka S, Masutani H, Liu W, Horita H, Wang D, Kizaka-Kondoh S, Yodoi J. Thioredoxin-binding protein-2-like inducible membrane protein is a novel vitamin D3 and peroxisome proliferator-activated receptor (PPAR) γ ligand target protein that regulates PPAR γ signaling. *Endocrinology*. 2006; 147:733–743. [PubMed: 16269462]
67. Rauch S, Martin-Serrano J. Multiple interactions between the ESCRT machinery and arrestin-related proteins: implications for PPXY-dependent budding. *J Virol*. 2011; 85:3546–3556. [PubMed: 21191027]
68. Nabhan JF, Hu R, Oh RS, Cohen SN, Lu Q. Formation and release of arrestin domain-containing protein 1-mediated microvesicles (ARMMs) at plasma membrane by recruitment of TSG101 protein. *Proc Natl Acad Sci USA*. 2012; 209:4146–4151. [PubMed: 22315426]

Figure 1. Model of β -arrestin and ARRDC-promoted trafficking of GPCRs

(1) Agonist binding to GPCR leads to dissociation of DUBs, heterotrimeric G protein activation and GRK-mediated phosphorylation of the GPCR. (2) β -arrestin, which basally binds to E3 ubiquitin ligases such as Mdm2, interacts with the activated/phosphorylated GPCR which promotes β -arrestin ubiquitination as well as (3) E3 ligase recruitment to β -arrestin and subsequent GPCR ubiquitination. There is also some evidence that ARRDC3 facilitates GPCR ubiquitination. (4) The conformational change in β -arrestin induced by binding to activated/phosphorylated GPCRs promotes the recruitment of the GPCR/ β -arrestin complex to clathrin-coated pits and (5) mediates GPCR endocytosis to early endosomes. (6) Some endocytosed GPCRs are quickly deubiquitinated and dephosphorylated by DUBs and protein phosphatases, respectively. (7) GPCRs are then sorted to sorting endosomes and (8) then move to recycling endosomes and (9) recycle back to the plasma membrane. (10) Some GPCR/ β -arrestin complexes move to the sorting endosome without being deubiquitinated and dephosphorylated and a subset of these may be recruited by ARRDC3. (11) The ESCRT-complex and its accessory proteins are recruited to the GPCR/ β -arrestin-containing sorting endosomes to facilitate GPCR degradation in lysosomes (12 and 13).

Figure 2. Structure of β-arrestin1 annotated with interactions that mediate GPCR trafficking

A. 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 (Lx[D/E] and [L/I]₂GxL motifs) and β2-adaptin ([D/E]xxFxx[F/L]xxxR motif) (PDB: 1JSY and 3GD1) **B.** Comparison of apo-β-arrestin1 (left) and phosphopeptide-bound β-arrestin1 (right) reveals that the phosphopeptide (blue) displaces the β-arrestin C-tail and disrupts the polar core that is centered around Arg169 (4JQI).

Table 1 β -arrestin and arrestin-domain containing protein interactions that function in GPCR trafficking

Binding Partner	Functional Role	References
	β-arrestins	
β 2-adaptin	Directly interacts with β -arrestins and facilitates GPCR endocytosis	Laporte et al. 2001 Edeling et al. 2006 Schmid et al. 2006
μ 2-adaptin	Preferentially interacts with β -arrestin2 and facilitates endocytosis of the β 2AR	Marion et al. 2007
Clathrin	Directly interacts with β -arrestins and facilitates GPCR endocytosis	Goodman et al. 1996 ter Haar et al. 2000 Kang et al. 2009
PIP ₂	Directly interacts with β -arrestins and enhances GPCR endocytosis	Gaidarov et al. 1999 Nelson et al. 2008
PIP5K-1 α	Directly interacts with β -arrestin2 and facilitates β 2AR endocytosis	Nelson et al. 2008
IP ₆	Directly interacts with β -arrestins; inhibits β -arrestin/GPCR interaction; facilitates β -arrestin oligomerization; regulates β -arrestin cellular localization	Gaidarov et al. 1999 Storez et al. 2005 Milano et al. 2006
PI3K	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 endocytosis of the β 2AR	McDonald et al. 1999
ARF6	GDP-bound form interacts with β -arrestin1 and enhances GPCR endocytosis and degradation and negatively controls recycling	Claing et al. 2001 Houndolo et al. 2005 Macia et al. 2012
ARNO	Activates ARF6 and facilitates β -arrestin release from the luteinizing hormone/choriogonadotropin receptor	Mukherjee et al. 2000
EFA6	β -arrestin1 binds ARF6-GDP and EFA6 and facilitates ARF6 activation leading to β 2AR degradation	Macia et al. 2012
AGAP2	AGAP2 interacts with β -arrestin1 and 2 and facilitates β -arrestin association with the β 2AR and regulates β 2AR recycling	Wu et al., 2013
Mdm2	Ubiquitinates β -arrestin2 and facilitates the endocytosis of β 2AR	Shenoy et al. 2001 Song et al. 2007
parkin	Directly interacts with β -arrestin and facilitates Mdm2 interaction	Ahmed et al., 2011
AIP4	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
Smurf2	Interacts with β -arrestin2 and mediates μ -opioid receptor ubiquitination and trafficking	Henry et al., 2012
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
STAM-1	Interacts with β -arrestin1 to regulate CXCR4 sorting	Malik & Marchese 2010
eNOS	Interacts with and s-nitrosylates β -arrestin2 and facilitates β -arrestin2 binding with clathrin and β -adaptin; promotes receptor internalization	Ozawa et al. 2008
NHERF	Interacts with β -arrestin1 to regulate P2Y12 receptor internalization	Nisar et al. 2012
Arrestin-domain containing proteins (ARRDCs)		
Nedd4	Directly interacts with the PPXY motifs of ARRDC3 to promote β 2AR ubiquitination and degradation ARRDC3 interacts with Nedd4- β 2AR upon agonist stimulation to recruit the complex to endosomes	Nabhan et al. 2010 Han et al. 2013
β -arrestins	Form heterodimers with ARRDC1 to mediate Notch ubiquitination and degradation	Puca et al. 2013

Binding Partner	Functional Role	References
Itch/AIP4	Interacts with ARRDC3 Interacts with ARRDC2, ARRDC3 and TXNIP Interacts with ARRDC1 to mediate Notch ubiquitination and degradation	Nabhan et al. 2010 Rauch et al. 2011 Puca et al. 2013
ALIX	Interacts with ARRDC1, 2 and 3 via its proline-rich domain (PRR)	Rauch et al. 2011
HRS	Interacts with the PPXY motifs of ARRDC3	Han et al. 2013 Shea et al. 2013
Clathrin	Interacts with ARRDC3/4 under basal conditions, interaction decreases upon agonist stimulation	Shea et al. 2013
WWP1	Interacts with all five ARRDCs	Nabhan et al. 2010 Rauch et al. 2011
WWP2	Interacts with all ARRDCs except ARRDC4	Nabhan et al. 2010 Rauch et al. 2011