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The structural basis of the arrestin binding to GPCRs.

Vsevolod V. Gurevich and **Eugenia V. Gurevich**

Department of Pharmacology, Vanderbilt University, Nashville, TN 37232, USA

Abstract

G protein-coupled receptors (GPCRs) are the largest family of signaling proteins targeted by more clinically used drugs than any other protein family. GPCR signaling via G proteins is quenched (desensitized) by the phosphorylation of the active receptor by specific GPCR kinases (GRKs) followed by tight binding of arrestins to active phosphorylated receptors. Thus, arrestins engage two types of receptor elements: those that contain GRK-added phosphates and those that change conformation upon activation. GRKs attach phosphates to serines and threonines in the GPCR C-terminus or any one the cytoplasmic loops. In addition to these phosphates, arrestins engage the cavity that appears between trans-membrane helices upon receptor activation and several other non-phosphorylated elements. The residues that bind GPCRs are localized on the concave side of both arrestin domains. Arrestins undergo a global conformational change upon receptor binding (become activated). Arrestins serve as important hubs of cellular signaling, emanating from activated GPCRs and receptor-independent.

Keywords

arrestin; GPCR; phosphates; receptor specificity; signaling; protein engineering

Introduction

The functions of the first member of the arrestin family, arrestin-1[a], were discovered in the visual system. Arrestin-1 (under the name of 48 kDa protein) was found to specifically bind light-activated rhodopsin along with the visual G protein transducin and rhodopsin kinase (current systematic name GRK1 (Gurevich et al., 2012)) (Kuhn, 1978). Its binding was found (also by Kuhn's group) to be greatly enhanced by rhodopsin phosphorylation (Kuhn et al., 1984) and then shown to inhibit (arrest) light-induced rhodopsin signaling (Wilden et al., 1986). This function was later satisfactorily explained by direct competition between arrestin-1 and visual G protein transducin (Krupnick et al., 1997; Wilden, 1995), which

Correspondence to: Vsevolod V. Gurevich, Department of Pharmacology, Vanderbilt University, Nashville, TN 37232; Tel: 615-322-7070; FAX: 615-343-6532, vsevolod.gurevich@vanderbilt.edu.

[a]We use systematic names of arrestin proteins, where the number after the dash indicates the order of cloning: arrestin-1 (historic names S-antigen, 48 kDa protein, visual or rod arrestin), arrestin-2 (β-arrestin or β-arrestin1), arrestin-3 (β-arrestin2 or hTHY-ARRX), and arrestin-4 (cone or X-arrestin).

arrestin wins when rhodopsin has three or more attached phosphates (Mendez et al., 2000; Vishnivetskiy et al., 2007). The first non-visual arrestin (under the name of β-arrestin, later retroactively renamed β-arrestin1; systematic name arrestin-2) was discovered later (Lohse et al., 1990). It preferentially outcompetes cognate G protein, Gs, at the β2-adrenergic receptor (β2AR) (Lohse et al., 1992) phosphorylated by an earlier discovered β2AR kinase (βARK; systematic name GRK2 (Gurevich et al., 2012)) (Benovic et al., 1989). This evidence suggested that two-step desensitization, phosphorylation of active G protein-coupled receptor (GPCR) by a specific kinase, followed by the high-affinity binding of an arrestin protein, is the common mechanism in the GPCR family (Carman and Benovic, 1998). Even though later arrestins were shown to interact with numerous non-receptor partners (Xiao et al., 2007), their binding to active phosphorylated GPCRs, which precludes further receptor interactions with cognate G proteins, remains their key biological function. Therefore, here we focus on the molecular mechanisms of arrestin binding to GPCRs, discussing both receptor and arrestin elements involved, as well as the resulting conformational rearrangements in the arrestin molecule and their functional implications.

The mechanics of GPCR activation and receptor elements engaging arrestins

The original idea that GPCRs exist in two states, active and inactive, which was the basis of the extended ternary complex model of GPCR signaling (Samama et al., 1993), turned out to be an over-simplification. Interestingly, early structures and biophysical studies of rhodopsin (Altenbach et al., 2008; Farrens et al., 1996; Palczewski et al., 2000; Scheerer et al., 2008) and β2AR (Rasmussen et al., 2007; Rosenbaum et al., 2011) appeared to support this two-state idea: the most notable conformational rearrangement accompanying GPCR activation was the outward movement of transmembrane helices V and VI, opening a cavity on the cytoplasmic side for the signaling proteins to dock to (Fig. 1). So, GPCRs were considered as on-off switches, with one active and one inactive conformation (Samama et al., 1993). However, detailed biophysical studies of the prototypical non-visual GPCR, β2AR, showed that in the absence of an agonist it exists as an ensemble of several conformations (Manglik et al., 2015). The agonist perturbs this equilibrium; although the greatest changes were visible in the presence of a G protein. Even upon G protein binding more than one receptor conformation was present (Manglik et al., 2015). Light-activated rhodopsin is also conformationally heterogenous, with the equilibrium significantly shifted by the cognate G protein, but conformational homogeneity could not be achieved even in this situation (Van Eps et al., 2017). The fact that there are likely numerous “active” conformations of any GPCR, supported by the structural studies of receptor-G protein (Carpenter et al., 2016; Koehl et al., 2018; Liang et al., 2017; Rasmussen et al., 2011; Van Eps et al., 2018; Zhang et al., 2017) and receptor-arrestin complexes (Kang et al., 2015; Zhou et al., 2017), is consistent with the idea that by “pushing” the receptor into specific subsets of these conformations activating ligands might have functional bias: preferentially facilitate GPCR interactions with particular G proteins or GRKs/arrestins (Smith et al., 2018; Wisler et al., 2018). However, documented direct competition between G proteins and arrestins (Krupnick et al., 1997; Lohse et al., 1992; Wilden, 1995) suggests that these subsets must significantly overlap.

GRKs evolved from Ser/Thr-specific AGC kinases (Mushegian et al., 2012) and phosphorylate serine and threonine, but not tyrosine residues in their targets. Activation-dependent phosphorylation of Ser and Thr residues by GRKs in various GPCRs has been demonstrated in vivo, in cultured cells, and in vitro (Azevedo et al., 2015; Fredericks et al., 1996; Hausdorff et al., 1989; Inagaki et al., 2015; Kim et al., 2004; Li et al., 2015; Moro et al., 1993; Pals-Rylaarsdam and Hosey, 1997; Seibold et al., 1998; Seibold et al., 2000; Wilden and Kühn, 1982). Receptor-attached phosphates (Shukla et al., 2013; Zhou et al., 2017), as well as several potentially phosphorylatable (Innamorati et al., 2001; Walther et al., 2010; Wanka et al., 2018) or non-phosphorylated receptor elements were directly implicated in arrestin binding by mutagenesis in rhodopsin and non-visual GPCRs (DeGraff et al., 2002; Raman et al., 2003; Raman et al., 1999; Schmidlin et al., 2003; Wanka et al., 2018), as well as by the crystal structure of the arrestin-1-rhodopsin complex (Kang et al., 2015; Zhou et al., 2017). Interestingly, relevant phosphates could be on the receptor C-terminus (Azevedo et al., 2015; Mendez et al., 2000; Wilden and Kühn, 1982), or any intracellular loop (Celver et al., 2001; Inagaki et al., 2015; Lee et al., 2000; Mukherjee et al., 2002; Pals-Rylaarsdam et al., 1997). This varied localization of the phosphates engaged by arrestins is consistent with the finding that the only thing that really matters is the number of phosphates (Mendez et al., 2000; Vishnivetskiy et al., 2007) and spacing between the phosphates and/or negative charges that need to fit the spacing of the positively charged patches on the arrestin surface (Zhou et al., 2017).

Structural basis of the competition between arrestins and G proteins for GPCRs

Arrestins and G proteins can compete for the GPCR only if they engage some of the same elements of the receptor. Judging by the structures of GPCR complexes with G proteins (Carpenter et al., 2016; Koehl et al., 2018; Liang et al., 2017; Rasmussen et al., 2011; Van Eps et al., 2018; Zhang et al., 2017) and rhodopsin complex with visual arrestin-1 (Kang et al., 2015; Zhou et al., 2017), both types of proteins engage the cavity that opens upon receptor activation on the intracellular side between the helices that move outward (Fig. 1). Interestingly, if the part of the arrestin protein that engages this cavity (see below) is deleted, arrestin no longer competes with the G protein, so that the receptor can interact with both proteins simultaneously (Cahill et al., 2017; Kumari et al., 2016; Thomsen et al., 2016). Thus, the engagement of this receptor cavity appears to be the key prerequisite for the homologous desensitization, i.e., arrestin competition with the G proteins.

The suppression of GPCR coupling to G proteins by direct competition is the first and arguably the most established function of arrestins. All arrestins preferentially bind phosphorylated active GPCRs, and GRKs are the key kinases that specifically phosphorylate active, but not inactive, receptors. Thus, for arrestins to play the key role in homologous GPCR desensitization, and that is hardly in doubt (Carman and Benovic, 1998; Gurevich and Gurevich, 2004), G proteins, GRKs, and arrestins must bind significantly overlapping subsets of GPCR conformations. This certainly limits the potential of proposed biased signaling (Smith et al., 2018; Wisler et al., 2018), as this concept implies that some ligands must induce non-overlapping GPCR conformations preferentially coupling to G proteins or

arrestins (and in the latter case by necessity GRKs, although this aspect is often overlooked). At the moment, we have very few structural studies revealing the details of the GPCR-G protein complexes (Carpenter et al., 2016; Koehl et al., 2018; Liang et al., 2017; Rasmussen et al., 2011; Van Eps et al., 2018; Zhang et al., 2017), and only one structure of the receptor-arrestin complex (Kang et al., 2015; Zhou et al., 2017). Thus, we cannot determine which conformations are specific for G protein-coupled GPCRs and which represent arrestin-preferring conformations, as conformational differences among activated GPCRs might reflect the difference inherent to the receptors themselves, rather than the differences that predetermine the binding partner. It should also be noted that the conformations of Gs- and Gi-bound receptors appear to be different (Koehl et al., 2018; Van Eps et al., 2018) (Fig. 1), which might underlie documented bias towards particular G proteins of GPCRs that couple to more than one class of G proteins (e.g., (Violin and Lefkowitz, 2007) and references therein). It is also noteworthy that double electron-electron resonance (DEER) measurements between selected points in rhodopsin and bound arrestin-1 always yield several distances (Kang et al., 2015; Zhou et al., 2017). While the most populated ones invariably match the structure, to the delight of crystallographers, the existence of the others strongly suggests that the arrestin-receptor complex likely has several “flavors”, only one of which was so far captured in the crystal. Thus, we need a lot more structures of GPCRs in complex with their binding partners before any general conclusions can be drawn.

Receptor-binding elements of arrestins

The first attempts to determine which parts of arrestin are involved in receptor binding had been made before any structural information became available. Differential chemical modification and hydrogen-deuterium exchange of free and rhodopsin-bound arrestin-1 identified numerous arrestin-1 residues likely involved in rhodopsin binding (Ohguro et al., 1994). A cluster of positive charges was identified as a phosphate-binding element by site-directed mutagenesis (Gurevich and Benovic, 1995), and large central part of the arrestin molecule was found to determine receptor preference of arrestin-1/2 chimeras (Gurevich et al., 1995). Remarkable similarity of the three-dimensional structure of all arrestin proteins established later (Granzin et al., 1998; Han et al., 2001; Hirsch et al., 1999; Milano et al., 2002; Sutton et al., 2005; Zhan et al., 2011a) explained why all chimeras fold successfully and are functional. All arrestins turned out to be elongated two-domain molecules, with each domain consisting of seven β-strands and having a concave and convex side (Fig. 2A). Several arrestin-1 peptides were shown to compete with full-length arrestin-1 and transducin for rhodopsin (Pulvermuller et al., 2000). Later studies of a new set of the arrestin-1/2 chimeras identified two elements on the concave sides of the two arrestin domains that are largely responsible for their preferential binding to rhodopsin or M2 muscarinic acetylcholine receptor (Vishnivetskiy et al., 2004). Surprisingly, few side chains within these elements were found to be immobilized by receptor binding (Fig. 2B) or play a direct role in receptor selection (Vishnivetskiy et al., 2011) (Fig. 2A). Replacement of the “receptor-discriminator” residues with alanines showed that they play key role in the binding of all arrestins to all GPCRs tested (Vishnivetskiy et al., 2011), suggesting that their replacement has a potential to yield non-visual arrestins with modified receptor specificity. Indeed, manipulation of some of these residues significantly changed receptor specificity of the most

promiscuous non-visual subtype, arrestin-3, and generated mutants with 50–60-fold preference for some GPCRs over others, in contrast to parental arrestin-3 that binds all these receptors with comparable affinity (Gimenez et al., 2014; Gimenez et al., 2012). Interestingly, a totally different experimental approach, based on the site-directed spin labeling, also identified the concave sides of both arrestin domains as the site of receptor binding (Hanson et al., 2007; Hanson et al., 2006) (Fig. 2B). In these experiments, surface residues were replaced with cysteines, which were chemically modified with a spin label. Spin labels were fairly mobile, as could be expected of labels placed on the surface of a protein where nothing obstructs their movement, and became immobilized to various degrees within the receptor "footprint" when arrestins were bound by active phosphorylated rhodopsin (Hanson et al., 2007; Hanson et al., 2006) (Fig. 2B). In addition, the effect on rhodopsin binding of the elimination or reversal of arrestin surface charges clearly supported the localization of the receptor footprint to the concave sides of both arrestin domains (Hanson and Gurevich, 2006). An NMR study of free and receptor-bound arrestin-1 also supported this localization of the receptor-binding elements (Zhuang et al., 2013).

Interestingly, the crystal structure of the arrestin-1 complex with rhodopsin revealed a much smaller footprint than deduced on the basis of mutagenesis, EPR, and NMR experiments, mostly involving the loops in the central crest of the receptor-binding side of arrestin (Kang et al., 2015; Zhou et al., 2017) (Fig. 3). The contact site included some residues that were not previously identified as contributing to receptor discrimination, including the "finger loop" and the "middle loop" (the latter was previously termed "139-loop" in arrestin-1 (Kim et al., 2012; Vishnivetskiy et al., 2013)). Site-directed mutagenesis of these residues showed that they indeed participate in receptor binding, differentially affecting the interactions with different GPCRs and/or functional states of the same receptor (Chen et al., 2017; Prokop et al., 2017). Many residues, particularly those in the C-domain, that were found to affect receptor preference, did not appear to touch the receptor in the crystallized complex (compare Figs. 2 and 3). This apparent controversy might be explained by the heterogeneity of the complex, only one flavor of which was crystallized (see above).

Arrestin transition into the "active" state

The four vertebrate arrestin subtypes demonstrate high sequence conservation (Gurevich and Gurevich, 2006; Indrischek et al., 2017) and share virtually identical three-dimensional structure in their basal state (Granzin et al., 1998; Han et al., 2001; Hirsch et al., 1999; Milano et al., 2002; Sutton et al., 2005; Zhan et al., 2011a), although careful comparison revealed subtle differences (Sente et al., 2018). High Arrhenius activation energy of the arrestin-1 binding to rhodopsin discovered as early as in 1989 has suggested that arrestin must undergo a global conformational change in the process of receptor binding (Schleicher et al., 1989). The finding that both phosphorylated rhodopsin and polyanion heparin greatly increase the accessibility of the arrestin-1 C-terminus to protease suggested that its release might be part of this conformational change (Palczewski et al., 1991). Interestingly, Arrhenius activation energy of rhodopsin binding of the short splice variant of arrestin-1 p44 lacking the C-terminus was found to be roughly half of that of the full-length arrestin-1 (Pulvermuller et al., 1997), clearly indicating that the C-terminus release is not the only conformational change in arrestin-1 induced by the receptor binding.

Greatly reduced receptor binding of arrestin-1 (Vishnivetskiy et al., 2002) and arrestin-2 and -3 (Hanson et al., 2007) mutants with deletions in the inter-domain hinge suggested that receptor binding might require the movement of the two domains relative to each other. Two hypotheses regarding the nature of this movement were proposed: it was hypothesized to be clam-shell like, so that arrestin grabs the cytoplasmic tip of the receptor like a pincer (Gurevich and Gurevich, 2004), or the twisting of the two domains relative to each other (Modzelewska et al., 2006). DEER measurements of the distances within arrestin-1 (Kim et al., 2012), as well as non-visual arrestin-2 and -3 (Zhuo et al., 2014) in free and receptor-bound form did not detect a clam-shell-like movement of the domains upon receptor binding. However, distance measurements in all three arrestins revealed other significant conformational changes, particularly the movement of the "finger loop", "139-loop" (Kim et al., 2012; Vishnivetskiy et al., 2013) (termed "middle loop" in arrestin-2 (Shukla et al., 2013)), and the loops at the tips of both arrestin-1 domains (Kim et al., 2012) (Fig. 4). Similar conformational changes were detected upon receptor binding in non-visual arrestin-2 and -3 (Zhuo et al., 2014). There was one striking difference, though: the released C-terminus in receptor-bound arrestin-1 appeared to just flop around without moving to any preferred position relative to the rest of the molecule, which yielded broad distributions of the distances between the C-terminus and selected sites in the rest of the molecule without any noticeable peaks that would reflect preferred distances (Hanson et al., 2006; Kim et al., 2012; Vishnivetskiy et al., 2010). In contrast, in both non-visual subtypes similar distance measurements showed a preference for a particular position upon release, resulting in a distinct distance (Zhuo et al., 2014). This might be related to the fact that the released C-terminus of non-visual arrestins binds clathrin (Goodman et al., 1996) and clathrin adapotor AP2 (Laporte et al., 1999), whereas the C-terminus of arrestin-1 does not bind clathrin (Goodman et al., 1996) and binds AP2 only with fairly low affinity (Moaven et al., 2013).

Crystal structures of naturally pre-activated short splice variant of arrestin-1 (Kim et al., 2013) and C-terminally truncated arrestin-2 in complex with the phosphorylated receptor peptide (Shukla et al., 2013) suggested that one of the key changes in "active" arrestins is approximately 20° twist of the two domains relative to each other. Although in these cases the receptor was absent, subsequent structure of the arrestin-1 complex with rhodopsin (Kang et al., 2015; Zhou et al., 2017) also revealed similar domain twist and confirmed loop movements detected by the EPR studies (Kim et al., 2012; Zhuo et al., 2014). This structure also supported earlier idea that arrestin finger loop assumes α-helical conformation upon receptor binding (Szczepek et al., 2014) (Fig. 3), with the helix inserting itself into the cavity between GPCR α-helices created by helix movements upon activation (Fig. 1). The structure of the arrestin-3 trimer in the presence of an abundant cytoplasmic small molecule inositol hexakisphosphate (IP6) revealed that all three protomers in this trimer are in the active receptor-bound-like conformation, with characteristic twist of the two domains (Chen et al., 2017). The tips of the finger loops of the three molecules formed α-helices, which stabilized each other via hydrophobic interactions, similar (but not identical) to the hydrophobic interactions with rhodopsin of the α-helical tip of the finger loop of bound arrestin-1 (Chen et al., 2017; Kang et al., 2015; Zhou et al., 2017). It is also worth noting that while the mutations that preclude the formation of the α-helix in the finger loop invariably reduce arrestin-3 binding to GPCRs, the effects of the replacement of the hydrophobic leucine with

charged residues are receptor subtype-specific (Chen et al., 2017). This structure solved a long-standing mystery in the field. It was shown by different labs that arrestin-3 facilitates the activation of JNK3 (Breitman et al., 2012; Miller et al., 2001; Seo et al., 2011; Song et al., 2009), as well as ubiquitous JNK1/2 (Kook et al., 2014) independently of GPCR stimulation. However, it was unclear how arrestin can become active without GPCR binding. The structure of the arrestin-3 trimer with IP6, where there were two IP6 molecules clearly resolved in each of the three interfaces between protomers, identified one possible non-receptor activator. Interestingly, the phosphates of the IP6 molecule engage the same positive charges in the arrestin-3 (Chen et al., 2017) that are engaged by the rhodopsin-attached phosphates in arrestin-1 (Zhou et al., 2017) and the phosphates on the receptor peptide in arrestin-2 (Shukla et al., 2013) in respective complexes. However, this structure does not fully explain the molecular mechanism of arrestin-3-dependent JNK3 activation: purified arrestin-3 was shown to scaffold MKK4-JNK3 and MKK7-JNK3 modules in the absence of IP6 (Zhan et al., 2011b; Zhan et al., 2013), and a short 25-residue N-terminal peptide of arrestin-3, that was found to increase JNK phosphorylation by both MKKs and facilitate JNK3 activation in cells (Zhan et al., 2016), does not contain residues implicated in the IP6 binding or trimerization.

Thus, it appears well established that in the active state of arrestins the two domains twist relative to each other by about 20°, several loops move, and the tip of the finger loop assumes α-helical conformation. The comparison of all available structures of arrestins in their active and basal state also identified several elements on the non-receptor-binding side of the molecule that show significantly different conformations (Chen et al., 2017).

Arrestins and cellular signaling: where do non-receptor partners bind?

More than a hundred non-receptor partners of each non-visual arrestin subtype were identified (Xiao et al., 2007). However, the elements of arrestin engaging these proteins were determined in very few cases. Only the interaction sites of clathrin (Goodman et al., 1996), clathrin adaptor AP2 (Laporte et al., 1999), microtubules (Hanson et al., 2007), calmodulin (Wu et al., 2006), and critical residues engaging cAMP phosphodiesterase (Baillie et al., 2007), MEK1 (Meng et al., 2009), and cRaf1 (Coffa et al., 2011b) were identified. It appears reasonable to hypothesize that any protein that binds the arrestin-receptor complex must engage the parts that are not shielded by the receptor, i.e., interact with the non-receptor-binding side of the molecule (Gurevich and Gurevich, 2003). This notion is supported by the demonstration that all three JNK3-binding elements of arrestin-3 are localized on that side (Zhan et al., 2014), suggesting that receptor-associated arrestin-3 can also bind the kinases of this pathway, which explains the reported JNK3 phosphorylation in response to GPCR activation (McDonald et al., 2000). As numerous non-receptor partners were found to preferentially bind either active or basal arrestins (Ahmed et al., 2011; Coffa et al., 2011a; Song et al., 2006), it stands to reason that the elements that these partners engage have different conformations in these two states (Fig. 5). Thus, arrestin activation by GPCRs (or any other activators) must induce conformational changes that promote or suppress the binding of the partners with the preference for a particular arrestin conformation.

While there are different models explaining how the changes on the receptor-binding side induce rearrangements on the opposite side of the arrestin molecule (Chen et al., 2018; Latorraca et al., 2018; Scheerer and Sommer, 2017), the elements that change conformation upon activation, which were termed “arrestin switch regions” (aSw) (Chen et al., 2017) (Fig. 5), appear to be the most likely docking sites of partners that differentially interact with arrestins in their basal state and in a different conformation(s) upon GPCR binding or upon activation by other mechanisms, such as IP6 binding (Chen et al., 2017).

High-resolution structure of active arrestin-3 (Chen et al., 2017) and its comparison with other structures of active and inactive arrestins revealed several aSw regions. Arrestin switch region I (aSwI) has unique sequence in arrestin-3 (residues 89–97). It is one of the “legs” attaching the only α-helix in arrestin to the N-domain (Fig. 5). This nine-residue element in arrestin-3 contains seven prolines that create two PPXP motifs, which are consensus recognition sites for SH3 domains. In IP6-activated arrestin-3, the backbone of aSwI shifted by as much as 5.8 Å from its basal position (Chen et al., 2018; Chen et al., 2017). It remains to be elucidated whether this region is involved in binding c-Src, other Src family kinases, or any other arrestin partner with an SH3 domain, but it appears to be a legitimate suspect. Switch region II (aSwII) is shared by all four arrestin subtypes. It consists of two clearly distinct parts, the inter-domain hinge (aSwIIa) that connects the N- and C-domains and the entire β-strand XI (aSwIIb) that extends from the hinge region (Fig. 5). The aSwIIa contains an unconventional poly-proline motif (PGPQP in arrestin-2 and -3, PQP in arrestin-1) that might bind SH3 domains. ASwIIa has nearly identical conformation in all solved active arrestin structures (Chen et al., 2017; Kang et al., 2015; Shukla et al., 2013; Zhou et al., 2017), whereas its conformation in basal arrestins varies (Granzin et al., 1998; Han et al., 2001; Hirsch et al., 1999; Milano et al., 2002; Sutton et al., 2005; Zhan et al., 2011a). The most prominent feature of aSwIIb is a register-shifted β-strand XI (Fig. 5), although this β-strand likely has inherent flexibility: similar register-shift was observed in several structures of basal arrestin-2 (Han et al., 2001; Kang et al., 2009; Milano et al., 2006; Milano et al., 2002). This register shift appears to affect the binding of at least some non-receptor partners. A disulfide bond that traps β-strand XI in the register-shifted “active” position enhanced the binding of JNK3 (Chen et al., 2017). ASwIII is an extension of the lariat loop supplying two negative charges to the polar core (Hirsch et al., 1999) (Fig. 5). It becomes more dynamic upon activation of non-visual arrestin-2 and -3, as interpretable electron density corresponding to this element is lost in the active structures (Chen et al., 2017; Shukla et al., 2013). The C-terminus, which contains the binding sites of the two components of the internalization machinery, clathrin (Goodman et al., 1996) and AP2 (Laporte et al., 1999), is the aSwIV. Arrestin binding to GPCRs was shown to induce the release of the C-terminus (Hanson et al., 2006; Palczewski et al., 1991; Vishnivetskiy et al., 2010; Zhuang et al., 2013; Zhuo et al., 2014), and activated non-visual arrestins show enhanced affinity for both clathrin and AP2 (Kim and Benovic, 2002). Interestingly, in arrestin mutants that engage only receptor-attached phosphates, but do not engage the inter-helical cavity of GPCRs, the C-terminus is likely also released, as these mutants facilitate GPCR internalization (Cahill et al., 2017; Kumari et al., 2016). This is consistent with structural data suggesting that phosphorylated GPCR elements, as well as IP6, displace the C-terminus of arrestins from its basal conformation (Chen et al., 2017; Shukla et al., 2013).

It should be noted that these switches are unlikely to participate in the binding of proteins that interact with basal and active arrestins with similar affinity, which might include several upstream MAP kinases (Coffa et al., 2011a; Song et al., 2009). However, any partner that has interaction sites in both domains must be sensitive to arrestin activation, as activation-associated domain twist would change the position of these sites relative to each other (Chen et al., 2018). If the partners bind receptor-associated arrestins, their docking sites have to localize to the convex non-receptor-binding side of the two arrestin domains.

Thus, the structural studies of arrestins activated by GPCR binding or other means have suggested likely binding sites for partners that discriminate between different states of arrestins, as well as those that bind similarly to the basal and active state. However, the actual elements mediating interactions with most partners still remain to be identified. Site identification is needed not only to satisfy our scientific curiosity, but also to design signaling-biased arrestins with certain functions enhanced or disabled, which have clear potential to become valuable tools for research and therapy (Gurevich and Gurevich, 2015).

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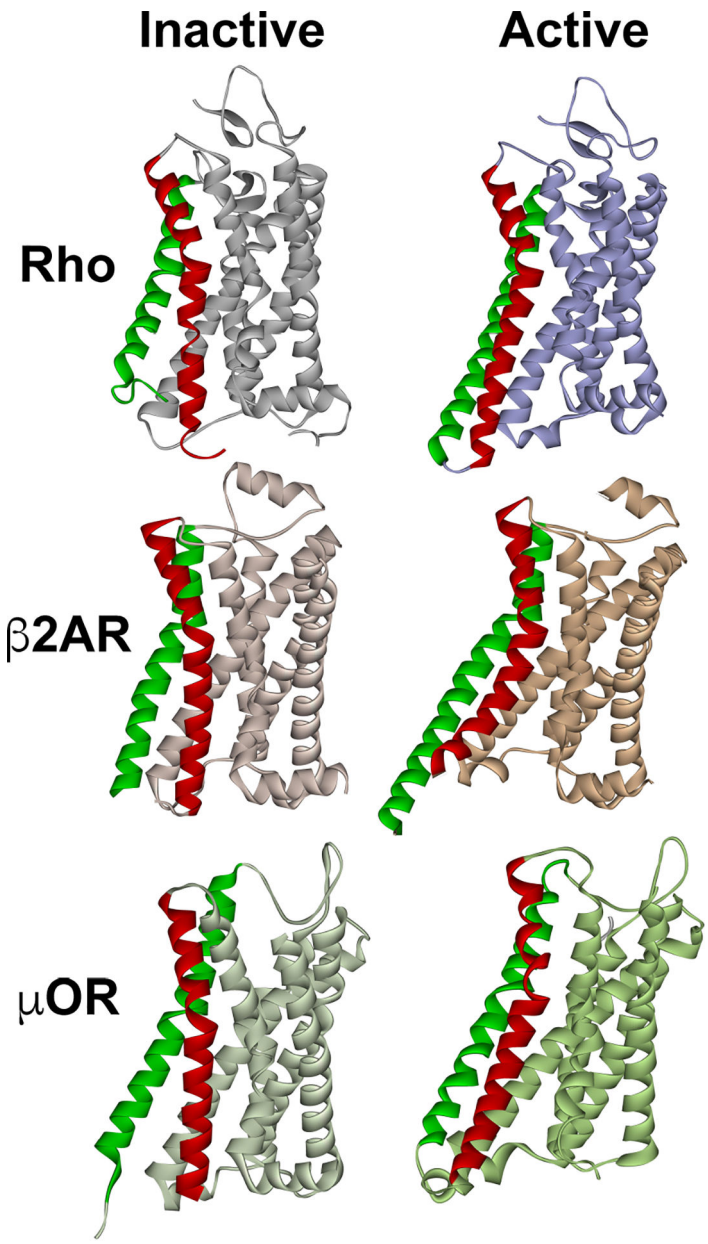

Fig. 1. The structure of inactive and active GPCRs.

The most pronounced activation-induced conformational change in all GPCRs is the outward movement of TM5 (green) and TM6 (red). In case of TM6, activation-induced shift is ~ 14 A in Gs-coupled β2AR (Rasmussen et al., 2011), but smaller in Gi-coupled receptors, ~10 A in both rhodopsin (Kang et al., 2018; Kang et al., 2015) and μ-opioid receptor (Koehl et al., 2018). The structures of inactive (dark) rhodopsin (Rho) (PDB ID: 1GZM (Li et al., 2004)), active rhodopsin (from complex with arrestin-1; PDB ID: 4ZWJ (Kang et al., 2015)), inactive β2AR (PDB ID: 2RH1 (Rasmussen et al., 2007)), active β2AR (from complex with Gs; PDB ID: 3SN6 (Rasmussen et al., 2011)), inactive μ-opioid receptor (μOR) (PDB ID: 4DKL (Manglik et al., 2012)), and active μOR (from complex with Gi; PDB ID: 6DDE (Koehl et al., 2018)) are shown. The common feature of active GPCR

structures is the cavity between helices that opens on the cytoplasmic side. In case of Gi-coupled GPCRs (Rho and μOR) the outward movement of the helices is smaller, so that this cavity is narrower than in case of Gs-coupled β2AR. The dimensions of this cavity might underlie GPCR selectivity for particular G proteins, whereas non-visual arrestins likely accommodate GPCRs that couple to all types of G proteins.

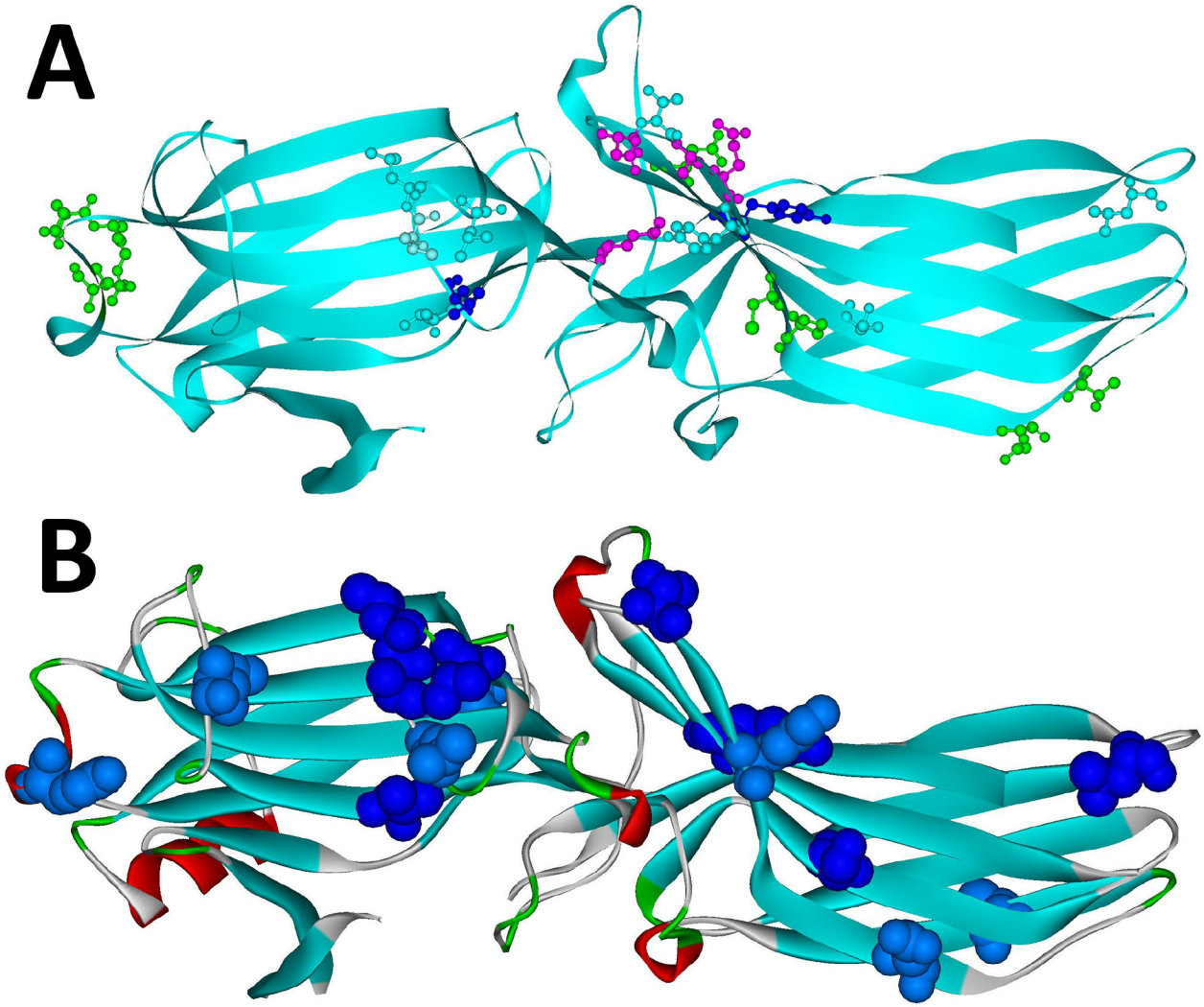

Fig. 2. Arrestin elements implicated in receptor binding.

A. The residues in positions identified as important for the receptor preference of different arrestin proteins (the structure of arrestin-2 is shown; PDB ID: 1G4M (Han et al., 2001); the residues indicated are those of arrestin-2) are shown as ball-and stick models colored as follows: light blue, those identified by reduced mobility of the spin label in receptor-bound arrestins using EPR (Val70, Leu71, Leu73, Val167, Leu191, Ser234, Thr246, Tyr249) (Hanson et al., 2007; Hanson et al., 2006); green, those identified by site-directed mutagenesis and receptor binding *in vitro* and in cells (Leu48, Glu50, Arg51, Asp240, Cys251, Pro252, Asp259, Thr261) (Gimenez et al., 2014; Gimenez et al., 2012; Prokop et al., 2017; Vishnivetskiy et al., 2011); dark blue, those identified by both of these methods (Leu68, Tyr238); magenta, those identified by direct contact with the receptor in the crystal structure of the arrestin-1 complex with rhodopsin (Lys138, Asn245, Ala247, Gln248) (Kang et al., 2015), including those confirmed by in-cell assays (Lys138, Gln248) (Prokop et al., 2017). Receptor-binding residues identified by several independent methods are localized on the concave sides of the two arrestin domains, with the highest concentration in the central crest of the receptor-binding side of arrestins. **B.** The residues that change

mobility upon receptor binding, according to SDSL-EPR study (Vishnivetskiy et al., 2011) are shown as CPK models (dark blue – strong immobilization; light blue – less pronounced immobilization). The same crystal structure of arrestin-2 (PDB ID: 1G4M) was used. Both panels were generated using Accelrys DS ViewerPro 6.0 (Dassault Systemes, BIOVIA Corp, San Diego, CA).

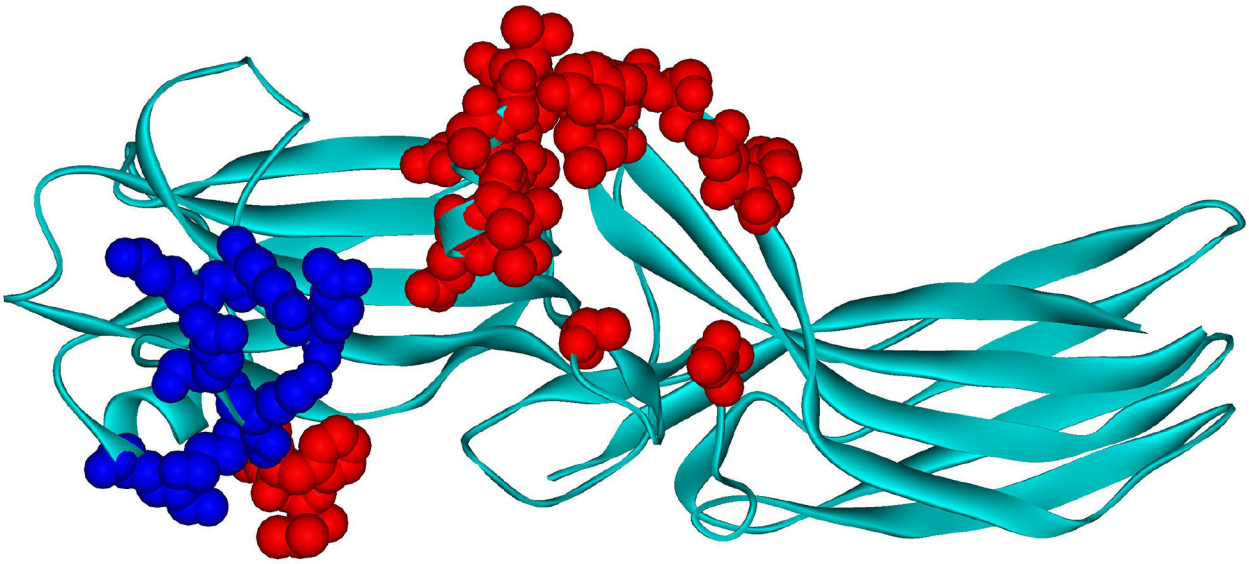

Fig. 3. Arrestin-1 residues contacting rhodopsin in co-crystal.

Mouse arrestin-1 residues that directly interact with rhodopsin are shown as CPK models (based on rhodopsin-bound mouse arrestin-1 structure from complex A, PDB ID: 4ZWJ (Kang et al., 2015)). Those that bind receptor-attached phosphates or negative charges in the rhodopsin C-terminus are colored dark blue (Zhou et al., 2017)(mouse arrestin-1 residues Lys15, Lys16, Arg19, Lys111, Lys167, Lys168, Arg172), those that interact with unphosphorylated parts of the rhodopsin molecule are colored red (Kang et al., 2015; Zhuo et al., 2014) (Val12, Ile13, Phe14, Gln70, Glu71, Ile73, Asp74, Met76, Gly77, Leu78, Arg82, Asp83, Leu84, Lys142, Leu250, Tyr251, Ser252, Asp254, Tyr255, Arg292, Thr320).

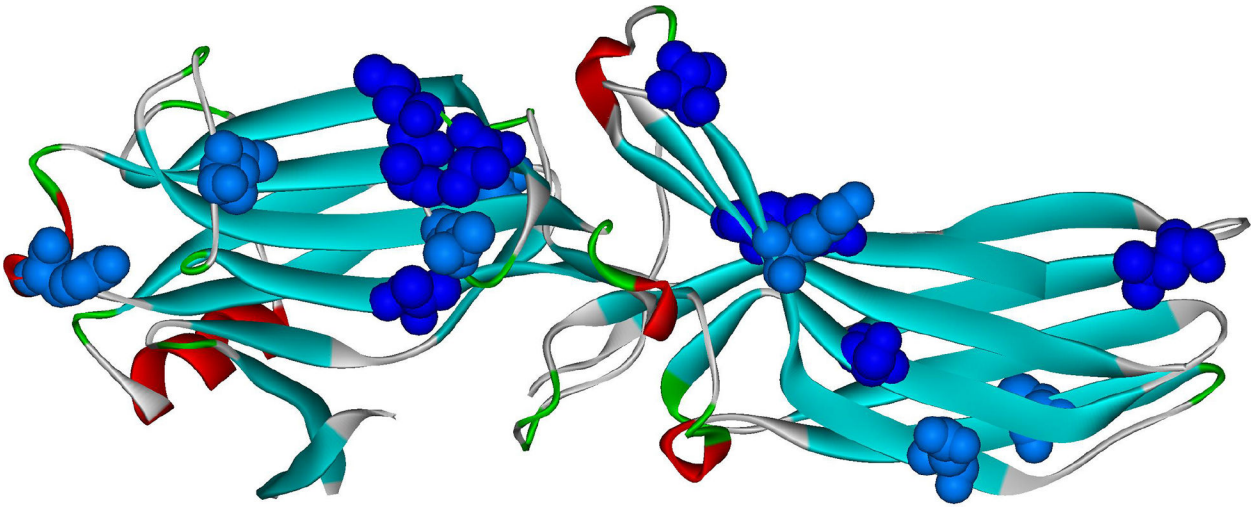

Fig. 4. Elements of arrestin-1 that move upon rhodopsin binding.

Bovine arrestin-1 structure in it basal conformation (PDB ID: 1CF1 (Hirsch et al., 1999)) is shown, with the elements that move upon rhodopsin binding (Kim et al., 2012) colored dark red. These include the finger loop (residues 67–78 (Hanson et al., 2006)), the 139-loop (Kim et al., 2012) (a.k.a. the middle loop in arrestin-2 (Shukla et al., 2013)), as well as loops at the distal tips of the N-domain (residues 155–168) and C-domain (residues 336–344).

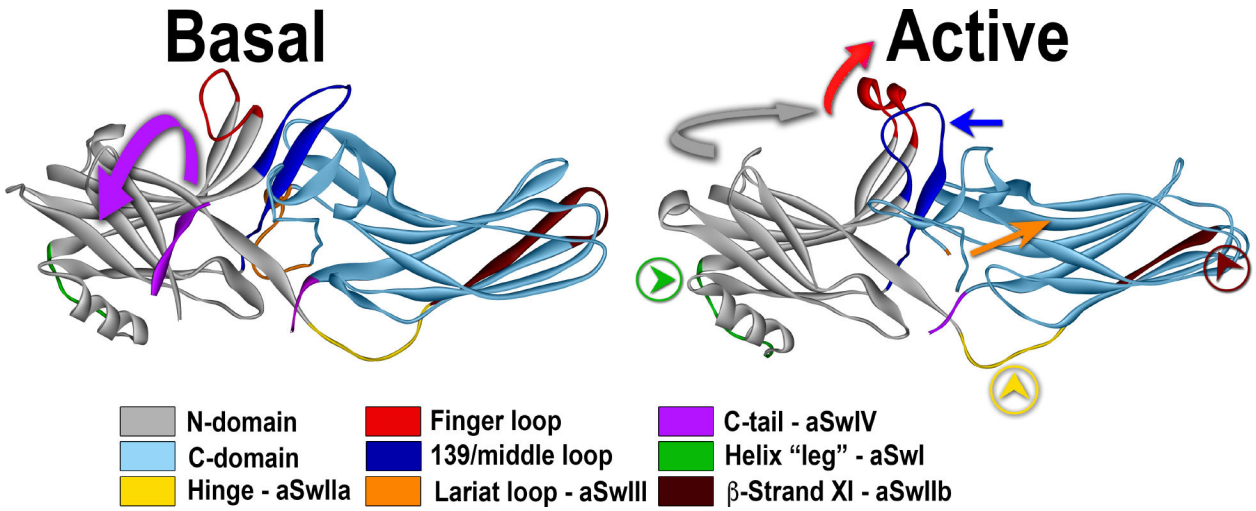

Fig. 5. Activation of arrestin-3 and switch regions.

Conformational changes upon arrestin-3 activation are shown: the release of the arrestin C-terminus (magenta; magenta arrow shows the direction of the movement; aSwIV) anchored to the body of the molecule via the three-element interaction in the basal state (PDB ID: 3P2D (Zhan et al., 2011a)), the movement of the finger loop and helix formation in its tip (red; red arrow shows the direction of the movement), the twist of the two domains relative to each other (N-domain, gray; C-domain, teal). Movements of several loops, likely creating effector docking sites, are also shown: middle loop (dark blue; dark blue arrow indicates the direction of movement); the "leg" of the α-helix I (green, with green arrowhead in a circle; aSwI), inter-domain hinge (yellow with yellow arrowhead in a circle; aSwIIa), register-shifted β-strand XI in the C-domain (dark brown; aSwIIb), and the lariat loop (orange with orange arrow; in the active structure (PDB ID: 5TV1 (Chen et al., 2017)) it is not resolved; aSwIII). All arrestin subtypes have switches II, III, and IV, whereas only arrestin-1 has polyproline motifs in the switch I. Note that only the interactions of clathrin and AP2 with the aSwIV were experimentally confirmed so far (Kim and Benovic, 2002).