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Overview of different mechanisms of arrestin-mediated signaling

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Abstract

Arrestins were discovered based on their ability to selectively bind active phosphorylated GPCRs and suppress (arrest) receptor coupling to G proteins. Subsequently non-visual arrestins were shown to be signaling proteins in their own right, activating a variety of cellular pathways. Arrestins are highly flexible proteins that can assume several distinct conformations. In receptor-bound conformation, arrestins have higher affinity for a subset of partners. This explains how receptor activation regulates certain branches of arrestin-dependent signaling via arrestin recruitment to GPCRs. However, free arrestins are also active molecular entities that act in other pathways and localize signaling proteins to particular subcellular compartments, such as cytoskeleton. These functions are regulated by the enhancement or reduction of arrestin affinity for target proteins by other binding partners and by proteolytic cleavage. Recent findings suggest that the two visual arrestins expressed in photoreceptor cells do not regulate signaling solely via binding to photopigments, but also interact with a variety of non-receptor partners, critically affecting the health and survival of photoreceptor cells. This overview describes GPCR-dependent and –independent modes of arrestin-mediated regulation of cellular signaling pathways.

Keywords

arrestin; GPCR; cell signaling; MAP kinases; ubiquitin ligases; apoptosis

Introduction

For a long time after the discovery of the ability of visual arrestin to quench rhodopsin signaling (Wilden et al., 1986), it appeared that the only function of arrestins was to bind active phosphorylated GPCRs, precluding receptor interactions with G proteins by direct competition (Krupnick et al., 1997a; Wilden, 1995). The fact that non-visual arrestins bind other proteins was only established in 1996, when the key component of the internalization machinery of coated pits, clathrin, was described as the first non-receptor binding partner of arrestins (Goodman et al., 1996). In retrospect, it is clear that this discovery opened the floodgates: clathrin adaptor AP2, c-Src and related protein kinases, MAP kinases of the JNK3, ERK1/2, and p38 activating cascades, as well as several E3 ubiquitin ligases were shown to bind non-visual arrestins. The number of reported arrestin binding partners currently exceeds 100 and keeps growing (Table 1; reviewed in (DeWire et al., 2007; Gurevich and Gurevich, 2006a)).

The functional cycle of arrestins

Arrestins were discovered as proteins that selectively bind active phosphorylated forms of their cognate GPCRs and play key role in receptor desensitization by blocking G protein coupling (Gurevich and Gurevich, 2014). In contrast to hundreds of GPCRs, mammalian arrestin family contains only four members (Gurevich and Gurevich, 2006a). The two visual subtypes, arrestin-1 (historic names S-antigen, 48 kDa protein, visual or rod arrestin) and arrestin-4 (cone or X-arrestin; for unclear reasons its gene is called “*arrestin 3*” in the HUGO database) are selectively expressed in retinal photoreceptors. The two ubiquitously expressed non-visual subtypes, arrestin-2 (a.k.a. β -arrestin or β -arrestin1) and arrestin-3 (a.k.a. β -arrestin2 or hTHY-ARRX), are present in virtually every cell. We use systematic names of arrestin proteins, with the order of cloning indicated by the number after dash.

Although arrestins bind many non-receptor signaling proteins, current concepts of the arrestin functional cycle are still dominated by its receptor binding with the first recognized functional (and likely structural) states of arrestins being “free” and GPCR-bound (Gurevich and Gurevich, 2004). Receptor binding involves a global conformational change in arrestin-1 (Schleicher et al., 1989), including the release of the arrestin C-terminus (Palczewski et al., 1991). Clathrin and its adaptor AP2 are key components of the internalization machinery of coated pits, which are used by many GPCRs as an internalization route. Thus, the finding that binding sites for both clathrin and AP2 are localized in the C-termini of the non-visual arrestins (Goodman et al., 1996; Laporte et al., 1999) was consistent with the fact that only receptor-bound arrestin recruits its partner to the coated pit (Gurevich and Gurevich, 2003). The localization of these sites also explains why the C-terminus must be “tucked” into the cavity of the N-domain in the basal state of arrestins to limit its accessibility (Fig. 1). The C-terminus of arrestin-2 expressed alone, which makes it fully accessible, inhibited GPCR internalization via competition with the arrestin-receptors complexes for clathrin and AP2 (Krupnick et al., 1997b). Ubiquitously expressed arrestin-2 and -3 were shown to interact with at least 21 different protein kinases (DeWire et al., 2007; Gurevich and Gurevich, 2006a; Kook et al., 2013), i.e., ~3.5% of ~600 protein kinases present in mammalian genome. The first studies of arrestin-mediated activation of protein kinases c-Src, JNK3, and ERK1/2 reported that arrestin-mediated signaling in these pathways was triggered by GPCR activation, i.e., by receptor-bound arrestins (Luttrell et al., 1999; Luttrell et al., 2001; McDonald et al., 2000). These findings established a new paradigm of the G protein-independent arrestin-mediated GPCR signalling, which is driven by active GPCRs after their coupling to G proteins is blocked (DeWire et al., 2007). These studies indirectly implied that arrestin molecules not associated with GPCRs are inactive. Moreover, most reviews of the subject also conveyed the impression that only non-visual arrestins have signaling functions, whereas visual arrestin-1 and -4 (Table 1) exclusively bind active phosphorylated photopigments. Numerous lines of evidence show that these are no more than myths.

Arrestins are very flexible molecules, and their ability to explore wide conformational space likely underlies their functional versatility (Gurevich and Gurevich, 2014). While differences between free and receptor-bound conformations were appreciated early on (Schleicher et al., 1989), later studies showed that microtubule-bound arrestin assumes a

conformation different from both free and receptor-bound forms (Hanson et al., 2006a). In addition to identifying critical receptor-induced conformational changes, recent biophysical studies demonstrated that arrestin molecule is highly flexible in both free and receptor-bound form (Kim et al., 2012; Zhuang et al., 2013). Consistent with the idea that receptor association increases the ability of arrestin to bind many signaling proteins (DeWire et al., 2007; Gurevich and Gurevich, 2003), NMR studies revealed that the flexibility of arrestin-1 increases upon receptor binding (Zhuang et al., 2013). Considering how many proteins were reported to bind non-visual arrestins (DeWire et al., 2007; Gurevich and Gurevich, 2006b; Xiao et al., 2007), this is hardly surprising as coupled binding and folding are key principles of protein-protein interactions (Shoemaker et al., 2000; Sugase et al., 2007). A structurally rigid protein would be unlikely to have such a varied repertoire of binding partners. Arrestins likely exist in at least four forms in the cell: free monomers; free oligomers, that have distinct size and shape for each arrestin subtype (Chen et al., 2014); bound to GPCRs, a function specific to monomeric arrestins (Hanson et al., 2007b); and associated with microtubules, the latter potentially involving both monomers and oligomers (Hanson et al., 2007b). In each form, arrestin molecules are likely to exist in a wide range of conformations, with the conformational spaces available to different forms partially overlapping.

Arrestins have binding partners that prefer every known conformational state

A key issue in arrestin biology is the functional capabilities of each conformational state. It is thus important to determine whether a binding partner preferentially interacts with a particular arrestin conformation. Since all protein-protein interactions are reversible, it is virtually impossible to force every arrestin molecule into a particular type of complex. This task became more tractable with the construction of conformationally biased arrestin mutants. The release of the C-tail is one of the changes in arrestins induced by receptor binding (Hanson et al., 2006b; Palczewski et al., 1991). In the basal arrestin conformation, the C-tail is attached to the body of the N-domain via bulky hydrophobic residues interacting with their counterparts in the β -strand I and the α -helix, which was termed the three-element interaction (Hirsch et al., 1999) (Fig. 1). This interaction can be disrupted in all arrestins by the substitution of the hydrophobic residues in the C-tail with alanines (3A mutation), which forcibly detaches the arrestin C-tail, thereby yielding a “pre-activated” mimic of the receptor-bound form (Clever et al., 2002; Gurevich, 1998). Receptor binding requires an extended inter-domain hinge, which is 12-residues long in all arrestin subtypes, with the length remarkably conserved in evolution (Gurevich and Gurevich, 2006a). The deletion of seven residues (7), leaving only five that are just sufficient to connect the two domains, greatly reduces the ability of all arrestins to interact with GPCRs (Hanson et al., 2007a; Vishnivetskiy et al., 2002), while enhancing their binding to microtubules (Hanson et al., 2007a). Thus, 7 mutants mimic the microtubule-bound state and cannot assume conformations resembling the receptor-bound form (Fig. 1).

These mutations created the tools necessary to characterize which binding partners prefer arrestins in which conformation. Comparison of the binding of several proteins to wild type (WT) arrestins (that likely can explore very wide conformational space), 3A mutants, biased

towards receptor-bound-like conformations, and 7 forms “frozen” in the basal state or, more likely, in conformations resembling microtubule-bound arrestins, provided important insights. JNK3, which was anticipated to prefer receptor-bound arrestins (McDonald et al., 2000), actually showed no clear preference, interacting equally with WT, 3A, and 7 mutants (Song et al., 2006). The subsequent finding that arrestin-3-7 facilitated JNK3 activation as effectively as WT arrestin-3 (Song et al., 2009), whereas receptor-bound-like 3A mutant did not (Breitman et al., 2012), provided additional support for that finding. Two E3 ubiquitin ligases, Mdm2 and parkin, demonstrated a clear preference for 7 forms of all arrestins over both WT and 3A versions (Ahmed et al., 2011; Song et al., 2006). In agreement with this finding, arrestins were shown to recruit Mdm2 to microtubules, directing its activity towards cytoskeleton-associated substrates (Hanson et al., 2007a). Only ERK1/2 and, to a lesser extent, its upstream activator, c-Raf1 had a strong preference for the receptor-bound form of arrestins (Coffa et al., 2011), with fairly low binding even to conformationally flexible WT forms (Song et al., 2009). Indeed, in sharp contrast to JNK3, arrestin-dependent ERK1/2 phosphorylation in the same cell was clearly regulated by GPCR activation (Breitman et al., 2012).

Collectively, these and other findings (reviewed in (Gurevich and Gurevich, 2013; Gurevich and Gurevich, 2014)) demonstrate that arrestin-mediated signaling does not necessarily involve GPCR regulation. Each arrestin conformational state preferentially binds certain partners and has its own unique role in cell signaling. Thus, free or microtubule-bound arrestins are just as active as those associated with GPCRs. The situation becomes even more complicated if one takes into account the oligomerization of arrestins-1, -2, and -3, where each subtype self-associates in its own distinctive manner (Chen et al., 2014), and where the functional capabilities of monomeric and oligomeric forms of the same arrestin are quite different (Hanson et al., 2007b; Milano et al., 2006; Storez et al., 2005). It should be noted that conformational preferences (or lack thereof) of the great majority of arrestin binding partners has not yet been established.

In order to understand the biological significance of arrestin interactions with each signaling protein it is necessary to know what is the preference of each partner for different functional and structural forms of arrestins. While the availability of conformationally biased arrestin mutants makes this work feasible and straightforward, it is still a major task requiring considerable experimentation.

Regulation of GPCR-independent arrestin functions

When arrestin-mediated signaling depends on GPCR binding, as in case of ERK1/2 activation (Luttrell et al., 2001), it is obviously regulated by receptor activity. In contrast, the regulation of receptor-independent signaling, e.g., arrestin-3-mediated JNK activation (Breitman et al., 2012; Song et al., 2009), is still poorly understood. Limited evidence suggests mechanisms very similar to GPCR-dependent regulation: an allosteric event where the binding to one protein increases or decreases arrestin affinity for another. Just like GPCR association greatly facilitates arrestin interactions with ERK1/2 (Coffa et al., 2011) (Fig. 2), the binding of JNK3 enhances arrestin affinity for MKK4 (Zhan et al., 2013). Interestingly, JNK3 association decreases arrestin binding to a different upstream kinase, MKK7 (Zhan et

al., 2013), which together with MKK4 is necessary for full activation of JNK family kinases. Importantly, the differential regulation by JNK3 of the binding of MKK4 and MKK7 to arrestin-3 is the first reported example of the interdependence of the binding of MAP kinases from the same signaling cascade to a mammalian scaffolding protein (Zhan et al., 2013) (Fig. 2). Similarly, the association of the E3 ubiquitin ligase, Mdm2 with arrestin is greatly enhanced by the binding of another ubiquitin ligase, parkin (Ahmed et al., 2011) (Fig. 2).

Arrestins enhance the ubiquitination of microtubule-associated proteins (Hanson et al., 2007a). As Mdm2 was the only ubiquitin ligase known at that time to bind arrestins, this effect was ascribed to documented arrestin-mediated recruitment of Mdm2 to microtubules (Hanson et al., 2007a) (Fig. 2). However newer data suggest that the observed increase in ubiquitination may have resulted from arrestin-dependent recruitment to microtubules of other ubiquitin ligases, e.g., parkin (Ahmed et al., 2011) or AIP4 (Bhandari et al., 2007).

Another reported mechanism of regulation of arrestin interactions with other proteins involves proteolysis. Caspase-8 or other initiator caspases in apoptotic cells cleave arrestin-2, generating a truncated arrestin-2-(1-380) form (Kook et al., 2014). The removal of the arrestin-2 C-terminus confers two new functions: in contrast to full-length arrestin-2, arrestin-2-(1-380) fragment localizes to mitochondria and directly binds tBid (Kook et al., 2014), the caspase-generated cleavage product of the pro-apoptotic Bcl family member, Bid. The mitochondrial association of arrestin-2-(1-380) does not depend on its interactions with tBid, suggesting that it likely binds one or more proteins residing in the outer mitochondrial membrane (Kook et al., 2014). tBid is a key player in cytochrome C release from mitochondria, the “point of no return” in apoptotic cell death in vertebrates (Danial and Korsmeyer, 2004). At physiological concentrations (up to 200 nM in neurons (Gurevich et al., 2004)) arrestin-2-(1-380) can double the amount of cytochrome C released by tBid, greatly facilitating apoptotic cell death (Kook et al., 2014). The effects of the other non-visual form, arrestin-3, on apoptosis, as well as possible effects of either non-visual arrestin on other forms of cell death, remain to be elucidated. Strict conservation of the Asp380 targeted by caspases in arrestin-2 of all vertebrate species, as well as its absence in invertebrate arrestins (Kook et al., 2014), suggests that this mechanism emerged early in vertebrate evolution.

Thus two modes of GPCR-independent regulation of arrestin functions have been described to date: i) the binding of certain partners increases or decreases arrestin affinity for others (Ahmed et al., 2011; Zhan et al., 2013), similar to receptor binding-dependent increase in arrestin interactions with signaling proteins (DeWire et al., 2007; Gurevich and Gurevich, 2003), and ii) the proteolytic cleavage of arrestin-2 promotes its interactions with partners that do not bind full-length protein (Kook et al., 2014). Much remains to be learnt regarding the structural basis of other arrestin functions, so it is possible that a greater variety of molecular mechanisms may be involved in the regulation of receptor-independent arrestin signaling.

Visual arrestins have multiple functions

In the last decade it has become increasingly clear that both non-visual subtypes, arrestin-2 and -3 (Table 1), play active roles in the regulation of multiple signaling pathways (DeWire et al., 2007; Gurevich and Gurevich, 2006a), whereas visual arrestin-1 and -4 are still considered to be mono-functional, involved exclusively in terminating photopigment signaling. Recent evidence, although still limited, shows that this is not the case. Both arrestin-1 and -4 (Table 1) bind JNK3 and Mdm2, with the latter showing the same preference for the 7 forms of all four vertebrate arrestin subtypes (Song et al., 2007; Song et al., 2006). Arrestin-1 interacts with clathrin adaptor AP2, and excessive recruitment of this trafficking protein by arrestin-1 bound to constitutively active rhodopsin caused photoreceptor death (Moaven et al., 2013). Phosphorylation-independent enhanced 3A mutant of arrestin-1 caused retinal degeneration with significant early damage to rod synaptic terminals (Song et al., 2013). This effect was shown to be independent of rhodopsin binding, as dark rearing of mice expressing the mutant, which maintains rhodopsin in an inactive state that is not able to bind arrestin-1, did not protect the rods (Song et al., 2013). Thus, arrestin-1 must act via another partner, although the exact mechanism remains to be elucidated. Direct role for arrestin-1 in the modulation of the activity of NEM-sensitive fusion protein (NSF) and neurotransmitter release in rods was recently reported (Huang et al., 2010). Thus, it is reasonable to expect that future studies will identify additional binding partners extending the repertoire of visual arrestin functions, which might even rival their non-visual cousins in terms of versatility.

Conclusions

Arrestins specifically bind to the cytoplasmic side of active phosphorylated GPCRs, blocking the coupling to their cognate G proteins by steric exclusion (Krupnick et al., 1997a; Wilden, 1995). Receptor-bound arrestins recruit the complex to coated pits via direct interactions of the arrestin C-terminus, released upon receptor binding, with clathrin and its adaptor AP2, facilitating GPCR internalization (Goodman et al., 1996; Laporte et al., 1999). The arrestin-receptor complex also initiates a second, G protein-independent wave of GPCR signaling (DeWire et al., 2007; Gurevich and Gurevich, 2006a). However, receptor interaction is only one of numerous biological functions of arrestins, which bind many other partners, including critical members in key cellular signaling pathways. Arrestins exist in the cytoplasm in multiple forms, the best described of which are free monomers, free oligomers, and microtubule-associated arrestins. Recent evidence suggests that arrestins are very flexible and in each functional state assume a distinct subset of conformations. Many binding partners are sensitive to arrestin conformation, preferentially interacting with particular functional and structural states. Thus, each arrestin conformation has a specific set of partners. The ability of arrestins to organize distinct multi-protein complexes in each state translates into key differences in distinct branches of arrestin-mediated signaling, some of which are regulated by arrestin interactions with active GPCRs, while others appear to be receptor-independent.

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Fig. 1. Basal conformation of arrestin proteins

The structure is based on PDB 1CF1 (Hirsch et al., 1999), with the C-tail manually added. Structural elements are color-coded, as follows: N-domain, light blue; C-domain, yellow; inter-domain hinge, dark blue; arrestin C-tail, magenta. Side chains of bulky hydrophobic residues participating in the three-element interaction are shown as CPK models and color-coded: from the C-tail, magenta; from β -strand I, teal; from α -helix I, green.

Fig. 2. Regulation of receptor-dependent and -independent arrestin signaling by protein-protein interactions

Arrestin recruitment to the receptor induces a conformational change that promotes binding of certain partners (kinases of Raf1-MEK1/2-ERK1/2 cascade are shown as an example). Arrestin in free, or microtubule-bound, conformation interacts with microtubules as well as other partners, such as ubiquitin ligase parkin and JNK shown. Arrestins recruit Mdm2 to microtubules and direct its activity to microtubule-associated proteins. Similarly to the effect of the arrestin recruitment to the receptor, arrestin binding to parkin greatly enhances its interactions with another partner, ubiquitin ligase Mdm2. The binding of JNK3 differentially regulates arrestin-3 affinity for the two upstream kinases, promoting the binding of MKK4, but reducing the interaction with MKK7.

Table 1

Vertebrate arrestins and their binding partners

Names	Expressed in	Known binding partners	Known functions
Arrestin-1 (a.k.a. S-antigen; 48 kDa protein; visual arrestin; rod arrestin)	Rod and cone photoreceptors	Rhodopsin; cone opsins; JNK3; Mdm2; NSF; AP2; ERK2; microtubules; parkin; calmodulin	Blocks rhodopsin cone opsin coupling to G proteins; mobilizes Mdm2 to microtubules; supports synaptic function(s)
Arrestin-2 (a.k.a. β -arrestin; β -arrestin1)	Virtually every cell	Numerous non-visual GPCRs; trafficking proteins (clathrin, AP2, NSF); MAP kinases (ASK1, MKK4/7, JNK1/2/3, c-Raf1, MEK1, ERK1/2; p38); ubiquitin ligases (Mdm2, AIP4, parkin), calmodulin and many other signaling proteins	Blocks GPCR coupling to G proteins; promotes GPCR internalization; scaffolds MAP kinase cascades; recruits ubiquitin ligases and deubiquitination enzymes to GPCRs; recruits Src family kinases to GPCRs; recruits Mdm2 and ERK2 to microtubules; scaffolds many other signaling cascades and localizes them to GPCRs or other cellular compartments.
Arrestin-3 (a.k.a. β -arrestin2; hTHY-ARRX)	Virtually every cell	Numerous non-visual GPCRs; trafficking proteins (clathrin, AP2, NSF); MAP kinases (ASK1, MKK4/7, JNK1/2/3, c-Raf1, MEK1, ERK1/2; p38); ubiquitin ligases (Mdm2, AIP4, parkin), calmodulin and many other signaling proteins	Blocks GPCR coupling to G proteins; promotes GPCR internalization; scaffolds MAP kinase cascades; recruits ubiquitin ligases and deubiquitination enzymes to GPCRs; recruits Src family kinases to GPCRs; recruits Mdm2 and ERK2 to microtubules; scaffolds many other signaling cascades and localizes them to GPCRs or other cellular compartments.
Arrestin-4 (a.k.a. X-arrestin; cone arrestin)	Cone photoreceptors	Cone opsins; JNK3; Mdm2; microtubules; parkin; calmodulin	Blocks cone opsin coupling to G proteins