CellPress

Review

Emerging Functional Divergence of β-Arrestin Isoforms in GPCR Function

Ashish Srivastava,[1,2] Bhagyashri Gupta,[1,2] Charu Gupta,[1,2] and Arun K. Shukla[1,*]

G protein-coupled receptors (GPCRs) are tightly regulated by multifunctional protein β-arrestins. Two isoforms of β-arrestin sharing more than 70% sequence identity and overall very similar 3D structures, β-arrestins 1 and 2, were originally expected to be functionally redundant. However, in recent years multiple lines of emerging evidence suggest they have distinct roles in various aspects of GPCR regulation and signaling. We summarize selected examples of GPCRs where β-arrestin isoforms are discovered to display non-overlapping and sometimes even antagonistic functions. We also discuss potential mechanistic basis for their functional divergence and highlight new frontiers that are likely to form the focal points of research in this area in coming years.

GPCRs and β-Arrestins

GPCRs constitute the largest family of cell surface receptors in the human genome with more than 800 different members [1]. A large array of ligands including small molecules, hormones, peptides, and lipids can bind to different GPCRs and activate downstream signaling cascades [2]. GPCR signaling influences a wide range of physiological processes, including olfaction, behavior, cardiovascular regulation, and the immune response, either directly or indirectly [3]. As a result, aberrant signaling and expression of GPCRs lie at the heart of many pathophysiological conditions such as different types of cancer, allergies, asthma, hypertension, and autoimmune diseases [4]. A large repertoire of currently prescribed medicines exert their effects through binding to GPCRs and by turning them 'on' or 'off' [5,6].

Agonist binding activates the receptor, leading to G protein coupling followed by the generation of second messengers such as cAMP, inositol phosphates, and Ca^{++}, and subsequent downstream signaling. Because sustained signaling is detrimental to cell physiology, a **desensitization** mechanism (see Glossary) is in place that involves phosphorylation of activated GPCRs and subsequent binding of β-arrestin proteins (Box 1) [7,8]. Binding of β-arrestins hinders further G protein coupling and results in termination of G protein signaling. There are four different isoforms of arrestins, two of which referred to as visual arrestins are limited primarily to the visual system. The other two isoforms, β-arrestins 1 and 2, are expressed ubiquitously and they play key roles in the function and regulation of non-visual GPCRs.

Considering the high degree of sequence and structural similarity, it is not surprising that β-arrestins 1 and 2 show significant functional overlap. However, several studies document and establish a clear functional specialization for the two isoforms. There have been three major approaches to dissect isoform-specific roles of β-arrestins. These are depletion of individual β-arrestin isoforms in cultured cell lines using siRNAs [9], the generation of isoform-selective

Trends

GPCR functions are crucially regulated by multifunctional scaffold protein β-arrestins.

Emerging evidence suggests that, for many different GPCRs, the two isoforms of β-arrestin (β-arrestins 1 and 2) play distinct roles in downstream functional outcomes.

Despite highly conserved primary sequence and overall very similar 3D structure, there are conformational differences in β-arrestin isoforms that potentially underlie their functional divergence.

Distinct functional outcomes of β-arrestin isoforms add a new dimension to the functional selectivity of GPCRs and offer novel therapeutic possibilities.

[1]Department of Biological Sciences and Bioengineering, Indian Institute of Technology, Kanpur 208016, India
[2]Equal contributions.

*Correspondence: arshukla@iitk.ac.in (A.K. Shukla).

Box 1. Classical Functions of β-Arrestins and their Expanding Functional Repertoire

β-Arrestin 1 was cloned based on its predicted homology with one of the visual arrestins (referred to as 48 kDa protein at the time) and it was found to be approximately 1000-fold more potent than visual arrestin in desensitizing activated β_2AR [79]. Within two years the second isoform, referred to as β-arrestin 2, was cloned and functionally characterized [80]. The two isoforms exhibit similar expression and localization patterns, and in an *in vitro* reconstituted system appeared to be equally potent in promoting β_2AR desensitization [80]. At this point, the primary function of β-arrestins was conceived to be limited to GPCR desensitization and both isoforms were predicted to play similar roles (Figure IA). However, in subsequent years many new functions of β-arrestins have started to emerge. First, β-arrestins were found to scaffold several key components of the clathrin-mediated endocytosis machinery and promote receptor internalization (Panel B) [13]. Subsequently, β-arrestins were found to scaffold various components of MAP kinase modules (ERK, p38, JNK) ultimately leading to the discovery of the G protein-independent β-arrestin-mediated signaling paradigm (Panel C) [81]. More recently, scaffolding of E3 ubiquitin ligases such as MDM2 (panel D) and targeting them to their potential substrates have emerged as another major function of β-arrestins, further broadening the functional reach of β-arrestins [82].

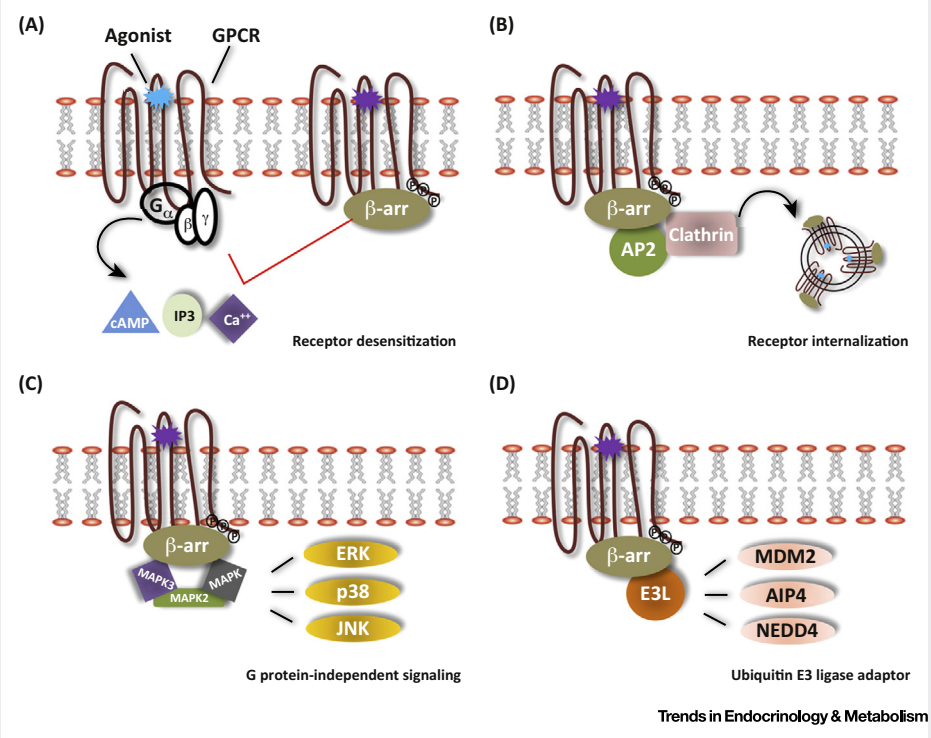

Figure I. Multifunctional Role of β-Arrestins in GPCR Signaling and Regulation. (A) Agonist-induced activation of GPCRs leads to coupling of heterotrimeric G proteins, generation of second messengers, and downstream signaling. Activated receptors are phosphorylated by GRKs, which then leads to recruitment of the cytosolic β-arrestin scaffolding proteins (β-arr 1 and 2). Binding of β-arrestins results in desensitization of the G protein response presumably through a steric hindrance mechanism. Gα, β, γ are three different subunits of the heterotrimeric G proteins. IP3, inositol trisphosphate; cAMP, cyclic adenosine monophosphate. (B) β-Arrestins act as a scaffold protein for various components of clathrin-coated endocytosis machinery, such as clathrin and AP2 (adaptin), and they promote internalization of agonist-activated GPCRs from the cell surface. (C) β-Arrestins also scaffold various components of multiple signaling kinases and phosphatases (e.g., MAP kinase module) to trigger a G protein-independent signaling pathway in the cells. β-Arrestin brings the different kinases of the MAP kinase module in close proximity to facilitate activation of the MAP kinase cascade. MAP kinase, mitogen activated kinase; ERK, extracellular signal regulated kinase; JNK, c-Jun N-terminal kinase. (D) β-Arrestins can also scaffold multiple ubiquitin E3 ligases (E3L) and bring them into close proximity of GPCRs or other non-GPCR substrates and thereby promote ubiquitination of the target proteins. NEDD4, neural precursor cell expressed developmentally downregulated protein 4; AIP4, atrophin-1-interacting protein 4.

knockout (KO) mouse models [10,11], and embryonic fibroblast cultured cell lines generated from these KO mice [12]. We highlight selected examples where the impact of both β-arrestin isoforms has been assessed in parallel with respect to a given functional readout, for example desensitization, **internalization**, and signaling.

Glossary

Aptamer: specific type of nucleic acid (DNA or RNA) that can adopt secondary and tertiary structures and specifically bind to proteins and other biomacromolecules.

Biased agonism: the ability of agonists to trigger biased signaling, in other words the selective activation of one signaling pathway but not another.

Biased signaling: selective activation of one or other signaling pathway (e.g., G protein- or β-arrestin-dependent) downstream of GPCRs. Ligands that preferentially or selectively trigger one or other signaling pathway are referred to as biased ligands.

Desensitization: the inability of receptors to continue to signal following persistent agonist exposure, resulting from functional uncoupling to G proteins. Typically mediated by a β-arrestin-dependent steric hindrance mechanism.

Internalization: removal of receptors from the cell surface upon agonist stimulation. Also referred to as receptor endocytosis.

Pepducins: these are cell-permeable peptides corresponding to the specific intracellular domains of GPCRs that are utilized to modulate GPCR signaling.

Synthetic antibody fragments: antigen-binding fragments of antibodies that are primarily selected from synthetically designed phage-display antibody fragment library and can specifically bind to different conformations of target proteins.