

GRKs and β -arrestins: roles in receptor silencing, trafficking and signaling

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Stimulation of cell-surface seven-transmembrane receptors (7TMRs) elicits biological responses to a wide range of extracellular signals, including many hormones. Classically, heterotrimeric GTP-binding proteins (G proteins) are recruited to the activated conformation of 7TMRs. Only two other families of protein have this remarkable characteristic: G-protein-coupled receptor kinases and β -arrestins. These two protein families have long been known to have a central and coordinated role in the 'desensitization' of G protein activation by 7TMRs. In addition, G-protein-coupled receptor kinases and β -arrestins are involved in an increasing number of interactions with non-receptor proteins, broadening the variety of their cellular functions. These newly appreciated attributes of these two families of protein highlight their unique ability to coordinate the various aspects of 7TMR functions.

Introduction

Given the broad spectrum of extracellular cues to which they are exposed, cells have developed sophisticated systems for the reception and interpretation of extracellular stimuli. Plasma membrane receptors have an important role in the transmission of extracellular signals to the intracellular compartment. Encoded by nearly 1000 genes, the seven-transmembrane receptors (7TMRs) represent the largest and most versatile family of membrane receptors [1]. They are characterized by a conserved structural motif of seven transmembrane-spanning regions and they respond to a wide range of extracellular chemical messengers, including light, odors, ions, neurotransmitters, chemoattractants, lipids, peptides and hormones [2]. As a consequence, they have fundamental roles in virtually all physiological functions and their impact in medicine is huge, with ~40% of currently used therapeutics directly target 7TMRs [3]. Traditionally, upon agonist stimulation, 7TMRs undergo conformational changes that expose intracellular binding sites for the heterotrimeric GTP-binding proteins (G proteins). The recruited G proteins exchange GTP for GDP, leading to the dissociation of G proteins into activated α subunit and $\beta\gamma$ dimers. The activated G proteins trigger the activation of specific effector proteins

(e.g. adenylate cyclase, phospholipase C). Second messengers, including cAMP, inositol trisphosphate and Ca^{2+} , are subsequently generated and induce a range of biological outcomes.

In biological systems, cells are exposed to a complex combination of stimuli. Extracellular stimuli vary by their chemical natures, concentrations and exposure times. The time-scale question is of special interest in endocrinology because target receptors are often exposed to hormones for prolonged periods of time, typically hours to days. By contrast, other 7TMR ligands, such as neurotransmitters, acutely (seconds to minutes) activate their cognate receptors. Over the years, many of the advances in receptor biology have been made by using neurotransmitter receptors and acute stimulations as models. Many of these observations were subsequently found to be broadly applicable to all types of 7TMRs. However, specialized mechanisms, yet to be determined, probably exist for 7TMRs that are physiologically exposed to long-term stimulations.

Typically, multiple intracellular signaling pathways are activated at a given time. For appropriate biological outcomes (e.g. proliferation, differentiation, migration, apoptosis) to occur in physiological situations, efficient orchestrating mechanisms are required. Even though many proteins have been shown to interact directly with 7TMRs (see Bockaert *et al.* for a review [4]), besides heterotrimeric G proteins, only two protein families have the ability specifically to interact generally with the agonist-activated conformation of 7TMRs: the G-protein-coupled receptor kinases (GRKs) and β -arrestins [5]. As a direct consequence of this remarkable attribute, GRKs and β -arrestins have been shown to provide integration, versatility and diversity to a growing number of 7TMR-mediated signaling pathways, increasingly establishing themselves as general 'coordinators' of signal transduction by the 7TMR family.

The GRK family consists of seven different genes. Expression of GRK1 and -7 is confined to retinal rods and cones, respectively, whereas GRK4 has very limited expression in the cerebellum, testis and kidney. By contrast, GRK2, -3, -5 and -6 are widely expressed in mammalian tissues. Based on sequence similarity, the seven GRKs have been divided into three subfamilies: GRK1 and -7; the pleckstrin homology domain-containing GRK2 and -3, whose membrane recruitment depends on

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interaction with G $\beta\gamma$ subunits of G proteins and phosphatidylinositol 4,5-bisphosphate; and GRK4, -5 and -6, which are constitutively associated with membranes [6].

The arrestins constitute a four-member family. The expression of arrestin 1 and arrestin 4 (α -arrestin) is restricted to retinal rods and cones, respectively. By contrast, arrestin 2 and arrestin 3 (better known as β -arrestin 1 and β -arrestin 2) are expressed ubiquitously [7].

Schematically, GRKs and β -arrestins orchestrate 7TMR activities at three different levels: (i) silencing: the functional uncoupling of the receptor from its cognate G protein by a mechanism known as 'homologous desensitization'; (ii) trafficking: receptor internalization, 'resensitization' and/or degradation; and (iii) signaling: the activation or inhibition of intracellular signaling pathways independently of heterotrimeric G proteins. The purpose of the current article is to review the classical and more recent roles of GRKs and β -arrestins, as well as their implications for receptor biology and endocrinology.

Roles of GRKs and β -arrestins in 7TMR silencing

Mechanisms dampening agonist-mediated second messenger generation exist at various levels of the transduction cascade. At the receptor level, GRKs phosphorylate agonist-activated 7TMRs on serine and threonine residues located in the carboxy-terminal tail region and/or the third cytoplasmic loop. β -arrestin is recruited with high affinity only to agonist-occupied, GRK-phosphorylated receptors, and ultimately sterically inhibits G protein coupling [7].

This desensitization process is used by the vast majority of 7TMRs [5,6,8], with some exceptions, such as the $\beta 3$ adrenoreceptor (AR) [9]. An unexpected addition to this paradigm came recently when Perry *et al.* [10] demonstrated that the β -arrestins interact with cAMP phosphodiesterases of the phosphodiesterase 4D family. This remarkable property implies that β -arrestins dually desensitize G α_s -coupled receptors by inhibiting cAMP generation via G α_s uncoupling while simultaneously enhancing the rate of cAMP degradation.

Several lines of evidence have suggested that GRK2 is often the major GRK associated with signal termination (Figure 1). However, the absence of specific inhibitors for GRK subtypes and the fact that GRK2 knockout mice are embryonic lethal have made determining the specificity of its action difficult to approach experimentally. Fortunately, small interfering RNAs (siRNA) have recently enabled the inhibition of each GRK individually. This approach leads to the conclusion for several receptors that GRK2 and -3 are primarily responsible for agonist-dependent receptor phosphorylation, β -arrestin recruitment and functional uncoupling, whereas GRK5 and -6 make lesser contributions to this outcome [11,12]. Another recent siRNA study demonstrated that H1 histamine receptor desensitization is mediated by endogenous GRK2 but not GRK5 [13]. However, GRK5 and -6 have also been implicated in various examples of receptor desensitization *in vivo* and/or *in vitro* [14–18]. Thus, depending on the receptors or the tissues considered, the GRK subtypes

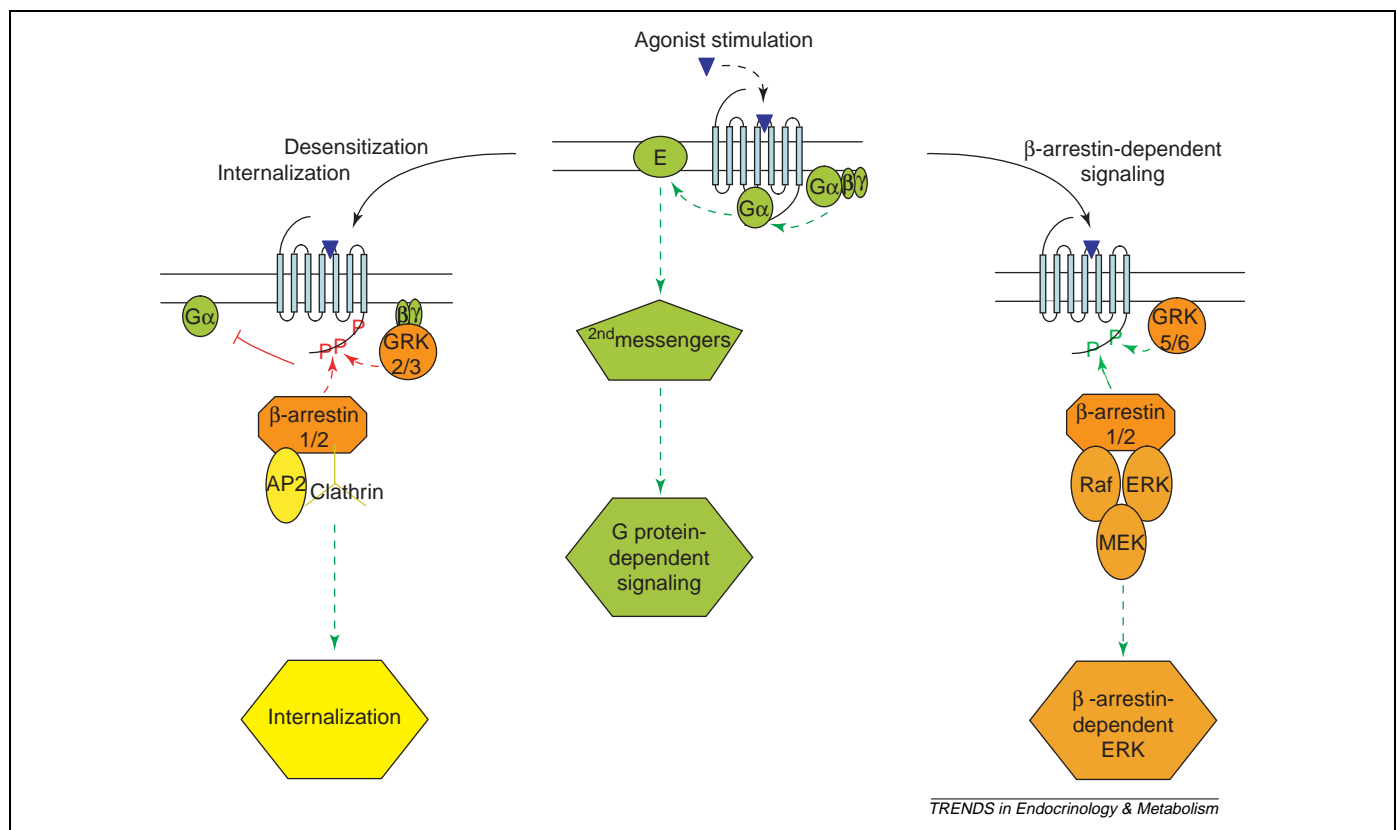


Figure 1. Functional specialization of the different GRKs: a model. Upon agonist stimulation, GRK2 and -3 are recruited to the plasma membrane by interacting with G $\beta\gamma$ subunits. They have a predominant role in receptor phosphorylation, β -arrestin recruitment to the activated receptor, desensitization and internalization. GRK5 and -6 are constitutively associated with the plasma membrane. They are both required for β -arrestin-dependent ERK activation, although the locus of their action might be at the receptor or downstream. In some systems, GRK5 and -6 can also mediate desensitization and internalization. Green-dotted arrows: activation; red-dotted arrows and red lines: inhibition. Abbreviation: E, second messenger-generating enzyme.

involved in desensitization can vary, emphasizing the remarkable versatility of the GRK regulatory system.

A conserved Ser–Thr cluster in the carboxy-terminal tail of certain 7TMRs appears to condition the strength of receptor– β -arrestin interaction, and its presence or absence can be used to define two classes of 7TMRs [19,20]. So-called ‘class A’ receptors (e.g. β 2AR) do not have a Ser–Thr-rich cluster in their carboxy-terminal tail. They transiently recruit β -arrestin 2, traffic with it to clathrin-coated pits and then dissociate. Class A receptors then internalize without β -arrestin and are generally rapidly recycled. Conversely, ‘class B’ 7TMRs [e.g. angiotensin type 1A receptor ($AT_{1A}R$) and vasopressin V2 receptor ($V2R$)] recruit both β -arrestin 1 and -2 equally well, and with higher affinity. They internalize together and form stable complexes in endosomal vesicles. Such a dichotomy suggests that β -arrestin 1 or -2 isoforms might preferentially desensitize certain 7TMRs based on this classification. Isoform-specific β -arrestin-targeted siRNAs have recently been used to compare β 2AR and $AT_{1A}R$, and the results support this hypothesis. It was demonstrated that β -arrestin 2 was necessary for desensitization of second messenger generation and internalization of the class A prototype (β 2AR), whereas both isoforms contribute to internalization of the class B 7TMR ($AT_{1A}R$). Interestingly, ablation of both β -arrestin 1 and -2 is necessary to affect second messenger production by the $AT_{1A}R$ [21–23].

Roles of GRKs and β -arrestins in 7TMR trafficking

Endocytosis of receptors does not seem to be necessary for ‘homologous desensitization’ but nonetheless has a fundamental role in 7TMR biology [24]. Internalization of the receptors can lead to (i) dephosphorylation, resensitization and recycling to the cell surface, (ii) targeting to lysosomes and degradation or (iii) activation of additional intracellular signaling pathways. Three general mechanisms have been described for 7TMR endocytosis: clathrin-coated pits, caveolae or other uncoated vesicles [23,25].

GRK-mediated phosphorylation and subsequent β -arrestin binding are essential to clathrin-dependent internalization [7]. Even though the pathway utilized for endocytosis, as well as the rate of internalization, is receptor- and cell type dependent, β -arrestin-dependent clathrin-mediated endocytosis appears to be used by the majority of 7TMRs. Mechanistically, β -arrestins serve as adaptors linking the receptors to elements of the endocytotic machinery, such as clathrin [26], the clathrin adaptor AP2 [27], the small G protein ADP-ribosylation factor 6 and its guanine nucleotide exchange factor ADP-ribosylation factor nucleotide-binding site opener [28], and *N*-ethylmaleimide-sensitive fusion protein [29].

Recent experiments using siRNA suggest that GRK2 and -3 are significantly more efficient than GRK5 or -6 in triggering receptor endocytosis by the β -arrestin–clathrin pathway [11] (Figure 1). Interestingly, GRKs can also influence other regulatory mechanisms to control receptor endocytosis. For example, GRK2 has been shown to interact with phosphatidylinositol 3-kinase [PtdIns 3-K (PI3K)] through the phosphatidylinositol kinase domain. As a consequence, PtdIns 3-K is targeted to

the agonist-occupied receptor and enhances receptor endocytosis [30,31].

Another important level of regulation for β -arrestin-mediated endocytosis of 7TMRs is ubiquitination. β -arrestins bind mouse double minute 2 protein, a negative regulator of P53 and an E3 ubiquitin ligase [32]. Mouse double minute 2 protein specifically mediates the ubiquitination of β -arrestin recruited to the activated receptor. This ubiquitination of β -arrestin is essential for the subsequent clathrin-mediated endocytosis. The two classes of endocytosis patterns (A and B) appear to reflect differences in the ubiquitination of receptor-bound β -arrestin [33]. Class A receptors induce transient β -arrestin ubiquitination, whereas it is sustained in the case of class B 7TMRs. Interestingly, β -arrestins also serve as adaptors to bring E3 ligases to 7TMRs. Ligand-induced ubiquitination of different 7TMRs is required for their proper sorting to lysosomes and degradation but not for internalization [32,34].

Roles of GRKs and β -arrestins in 7TMR signaling

In recent years, it has become increasingly evident that the functions of GRKs and β -arrestins are not restricted to 7TMR desensitization and internalization. Beyond these now classical functions, GRKs and β -arrestins are involved in a growing number of interactions with signaling proteins. Thus, by their ability to scaffold signaling complexes and transport them to receptors, in an agonist-dependent fashion, they represent a new and generally applicable mechanism for 7TMR signal transduction [5].

This previously unsuspected paradigm was discovered by the demonstration of β -arrestin interaction with proto-oncogene Src (c-Src), a process leading to c-Src recruitment to the occupied receptor and ultimately to extracellular signal-regulated kinase (ERK1 or -2) activation [35,36]. Other non-receptor tyrosine kinases from the c-Src family, such as Hck, Fgr and Yes, are also recruited to 7TMRs via β -arrestin interaction [37,38].

β -arrestins also have the ability to scaffold c-Jun amino-terminal kinase (JNK), as well as ERK1 or -2 mitogen-activated protein kinase (MAPK) signaling modules composed of a MAPK kinase kinase (MAPKKK), a MAPK kinase (MAPKK) and a MAPK. Indeed, apoptosis signal-regulating kinase 1 (a MAPKKK), MKK4 (a MAPKK) and JNK3 (a MAPK) are able to form a unique protein complex in association with β -arrestin [39]. Similarly, β -arrestins simultaneously interact with Raf (MAPKKK), MEK1 (MAPKK) and ERK (MAPK) [40,41]. In both cases, β -arrestins assemble the appropriate kinases of a specific module and lead to its activation under the guidance of an activated 7TMR.

Recently, the list of β -arrestin-dependent signaling pathways has been growing rapidly (Figure 2). For example, β -arrestins also serve to mediate the activation of p38 MAPK via CXCR4 [42] and constitutively via the viral G-protein-coupled receptor US28 [43]. The inhibitor (I κ B) of nuclear factor κ B (NF- κ B) interacts with β -arrestins, resulting in I κ B stabilization and eventually in agonist-dependent NF- κ B inhibition by 7TMR ligands [44,45]. The Akt (protein kinase B) signaling pathway

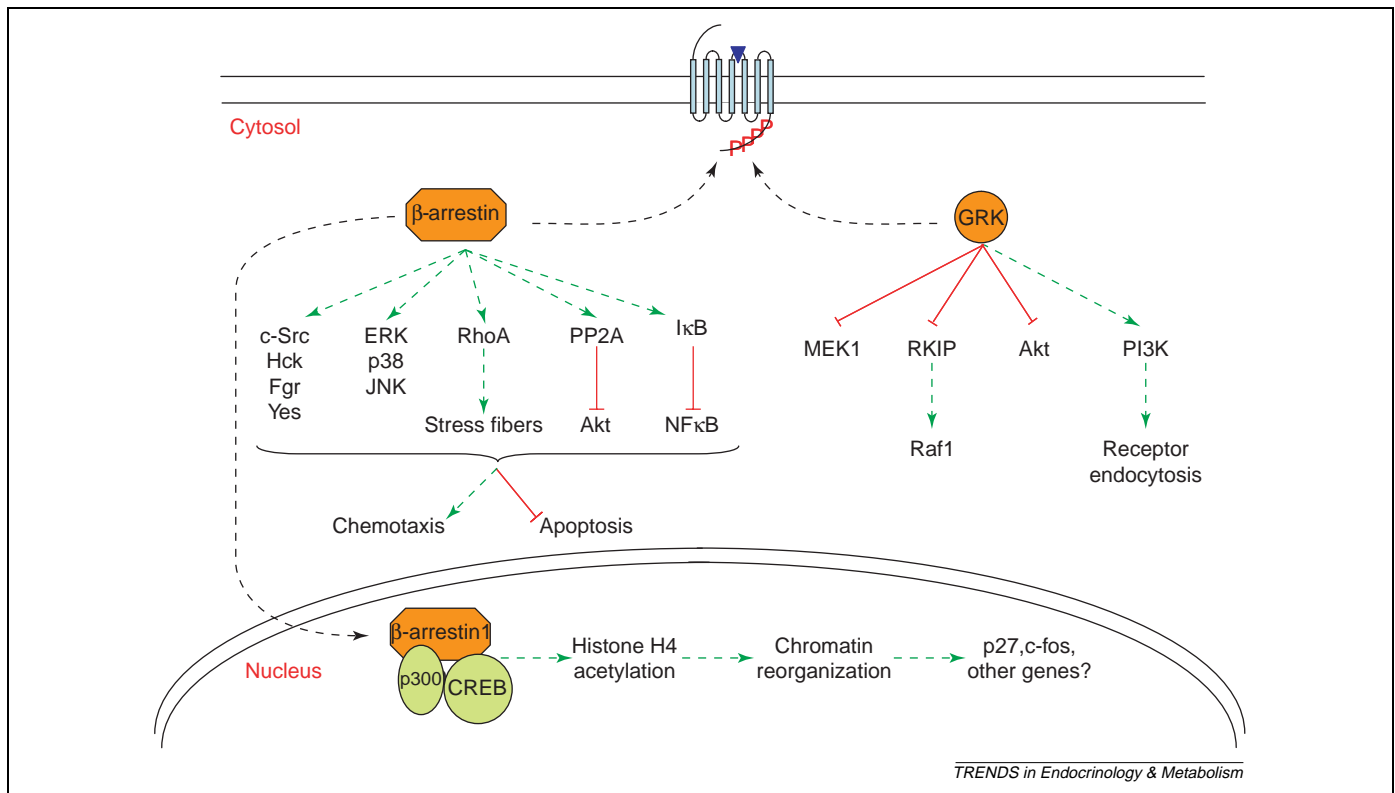


Figure 2. An overview of the current GRK- and β -arrestin-mediated signaling functions and biological activities. Upon agonist stimulation of a 7TMR, β -arrestins and GRKs are recruited and activated. They subsequently activate (green dotted arrows) or inhibit (red plain lines) various signaling pathways. β -arrestin signaling is important for chemotaxis and antiapoptosis. In addition, β -arrestin 1 can translocate to the nucleus and regulate the expression of various genes through reorganization of chromatin. Abbreviations: c-Src, protooncogene SRC, Rous sarcoma; Hck, non-receptor tyrosine protein kinase Hck; Fgr, Gardner Rasheed feline sarcoma viral oncogene homolog; Yes, non-receptor protein tyrosine kinase Yes; ERK, extracellular signal regulated kinase; p38, p38 mitogen activated protein kinase; JNK, c-Jun N-terminal kinase; RhoA, Ras homolog gene family member A; PP2A, protein phosphatase 2A; Akt, protein kinase B; NF κ B, nuclear factor kappa B, I κ B, nuclear factor kappa B inhibitor; MEK1, mitogen activated protein kinase kinase 1; RKIP, Raf kinase inhibitor protein; Raf1, mitogen activated protein kinase kinase kinase 1; PI3K, phosphoinositide 3 kinase. Green-dotted arrows: activation; black-dotted arrows: translocation; red lines: inhibition.

is also under the control of β -arrestin 2 through the formation of a newly discovered kinase–phosphatase scaffold. Indeed, Akt and its negative regulator, protein phosphatase 2A (PP2A), are assembled with β -arrestin 2 upon dopamine activation [46]. Finally, when the AT₁R is activated, β -arrestin 1 stimulates the small guanine triphosphatase Ras homolog gene family member A (RhoA) and induces stress fiber formation, in conjunction with G $\alpha_{q/11}$ [47].

Among these β -arrestin-dependent pathways, that of ERK is the best characterized [5,7,34]. Mutant receptors, modified ligands, siRNA inhibition of β -arrestins and specific inhibitors of protein kinase A or C have all been used to decipher the mechanisms leading to ERK activation by 7TMRs. Sometimes, G proteins and β -arrestins act in concert to activate ERK: several chemokine receptors are sensitive to both pertussis toxin (implicating signaling through G α_i or G α_o) and siRNA-mediated depletion of β -arrestin 2 [42,48,49]. However, most of the receptors characterized so far activate ERK1 or -2 by a dual mechanism involving independent contributions of both G protein and β -arrestins [12,50–52]. The ERK activated via β -arrestins has several particularities that distinguish it from G protein-activated ERK. β -arrestin-mediated ERK activation is slower in onset (~5–10 min to reach maximum), persistent (half-life > 1 h) and sequestered in the cytosol. By contrast, G

protein-mediated ERK activation is rapid, transient, sensitive to second messenger kinase inhibitors and translocates to the nucleus, leading to transcriptional activation and proliferation. These very different characteristics strongly suggest distinct physiological outcomes from the two pathways.

The mechanisms that render β -arrestin ERK activation so protracted are still unknown. At least three explanations can be envisioned: (i) there is an equilibrium between the activation of new signaling modules at the plasma membrane and the inactivation and recycling of cytosolic scaffolds; (ii) the β -arrestin signaling modules are continuously active and an equilibrium is reached between ERK activation and dephosphorylation; or (iii) activation occurs early (i.e. when β -arrestin binds to the receptor and the signaling module is formed) and for a limited amount of time but ERK phosphorylated in the complex is protected from MAPK phosphatase action and, as a consequence, its half-life is greatly increased.

Interestingly, different β -arrestin and GRK isoforms exert specialized and sometimes antagonistic functions on β -arrestin-dependent ERK activation (Figure 1). For the AT₁R, β -arrestin-dependent ERK is mediated by β -arrestin 2, whereas β -arrestin 1 serves as an inhibitor [22]. For the protease-activated receptor 2 [53], neurokinin-1 receptor [36], parathyroid hormone receptor [54] and β 2AR [52], however, both β -arrestin 1 and -2 are

required to promote activation of ERK. Because β -arrestin forms homo- and heterodimers, it might be the case that some receptors use homodimers of β -arrestins to signal, whereas others require heterodimers [55].

Another emerging notion is that GRK5 or -6 are required for β -arrestin-dependent ERK activation by AT₁AR, V2R and β_2 AR [11,12,52]. Indeed, when either GRK5 or -6 were depleted using siRNA, the β -arrestin-dependent ERK activation was completely abolished. Conversely, GRK2 and -3 tend to attenuate the β -arrestin-dependent ERK [11,12]. These data suggest that GRKs of the two subfamilies (i.e. GRK2 and -3 versus GRK5 and -6) are competing with each other at some level. Such a simple competition mechanism would help to not only provide the appropriate balance between desensitization and signaling, but also coordinate the different pathways as a function of extracellular stimuli and constraints. Interestingly, the GRK2 and -3 subfamilies require G protein activation before membrane recruitment, whereas GRK5 and -6 actually reside at the plasma membrane. Discrete receptor populations might preferentially interact with the two GRK subfamilies, possibly in distinct membrane microdomains.

The mechanisms by which the GRKs control β -arrestin-dependent ERK have not yet been elucidated but it is notable how tightly 7TMR transduction can be fine-tuned by the GRKs. One can envisage several possible mechanisms. One is that the specificity of GRK action results from the ability of GRK5 and -6 and GRK2 and -3 to phosphorylate different patterns of serine and threonine residues on the agonist-occupied receptor. The location of these phosphorylation sites might have an impact on the conformation adopted by the bound β -arrestin. According to its conformation, β -arrestin would then be capable of activating different signaling effectors and/or desensitizing G protein coupling. Although details of the preferred phosphorylation consensus motifs for the different GRKs are not clearly defined yet, there are significant differences in the preferences of the various enzymes [6]. Moreover, a recent *in vitro* study showing that a synthetic phosphorylated peptide derived from the carboxy-terminus of the V2R binds to and induces conformational changes, as well as clathrin binding, in β -arrestin 2 supports this hypothesis [56]. Additional data are clearly needed to test this hypothesis further. In particular, the mapping of phosphorylation sites specific to each GRK subtype will be essential.

Another possible mechanism is differential interactions between GRKs and key downstream signaling effectors. Indeed, GRKs might modulate cellular functions in a phosphorylation-independent manner as a result of their ability to interact with a variety of proteins involved in signaling and trafficking, such as G α_q , G $\beta\gamma$, PtdIns 3-K, clathrin, G-protein-coupled receptor kinase-interacting protein and caveolin [57]. Raf kinase inhibitor protein (RKIP) is another newly identified cellular partner for GRK2. Phosphorylation of RKIP by protein kinase C displaces it from Raf and increases its association with GRK2 [58]. Thus, the incoming receptor signal is enhanced both by removing an inhibitor from Raf-1 and by blocking GRK2-dependent receptor desensitization.

Another recently appreciated mechanism is the physical interaction between GRK2 and Akt, leading to the inhibition of Akt activity [59]. Finally, GRK2 and MEK1 have been found in the same multimolecular complex, and this interaction is correlated with an inhibition of MEK activity [60].

The identification of biological functions for β -arrestin signaling is another big challenge. A limited number of biological actions have already been attributed to the signaling functions of β -arrestin. The first is chemotaxis, the directed migration of cells along a gradient of chemoattractant, which is known to be mediated by various chemokine receptors through G α_i coupling. Several recent studies carried out in cell culture [42,53,61,62] or in knockout mouse models [49] have demonstrated that β -arrestins are important transducers of some of these chemotactic signals and might be involved in pathologies such as asthma [63]. Some data suggest that β -arrestins also contribute to antiapoptotic signaling [36,64]. The complex that β -arrestin 2 forms with PP2A and Akt upon dopamine D2 activation appears to be essential for the expression of dopamine-dependent behaviors [46]. Finally, it has been shown recently that β -arrestin 1 translocates to the nucleus in response to the activation of κ - and δ -opioid receptors. Furthermore, translocation of β -arrestin 1 to the nucleus correlates with increased transcription of *c-fos* and the cyclin-dependent kinase inhibitor *p27^{kip1}*, two genes involved in the regulation of cell proliferation. Interestingly, β -arrestin 1 participates in the formation of a nuclear complex, including the transcription factor CREB and the histone acetyltransferase p300, on the promoter regions of these genes. This leads to increased acetylation of histone H4 and the reorganization of chromatin, thereby increasing gene expression [65]. Clearly, a much broader spectrum of biological functions can be anticipated, and the current list is likely to grow significantly in the years to come.

Toward a new era for pharmacology and endocrinology?

In the two-state model of receptor activation, agonists are defined as compounds that stabilize the 'active' receptor conformation and promote G protein activation. The existence of β -arrestin-mediated signaling implies that multiple discrete 'active' receptor conformations coexist (Box 1). As a consequence, specific ligands directing signaling down distinct transduction pathways by stabilizing one of these discrete 'active' conformations might exist. Several examples of β -arrestin-dependent, G protein-independent, signaling directly support this theory, referred to as 'biased agonism' or 'ligand-directed signaling' [66,67]. Some inverse agonists for cAMP generation are capable of recruiting β -arrestin to various 7TMRs (i.e. β_2 AR, V2R and parathyroid hormone receptor) and stimulating ERK activation [54,68]. A mutated angiotensin peptide (SII angiotensin) activates β -arrestin-mediated, but not G protein-mediated, signaling to ERK [50]. The chemokine receptor CCR7 has two physiological ligands, Epstein-Barr virus-induced receptor ligand chemokine (CCL19) and secondary lymphoid tissue chemokine (CCL21). The two ligands have equivalent

Box 1. Impact of β -arrestin-dependent signaling on pharmacological screening

Pharmacological agents acting via 7TMRs have traditionally been discovered through the screening of numerous chemical structures in biological systems. Clearly, the type of receptor screen employed to detect biologically active molecules will greatly define the types of molecules detected. For many years, these functional high-throughput assays have employed second messenger generation, thus G protein activation, as the read-out. The drugs detected by such screens have been agonists, partial agonists, antagonists or inverse agonists for G protein activation. However, the recent demonstration that specific ligands are able to activate β -arrestin-dependent, G protein-independent signaling implies that multiple discrete 'active' receptor conformations coexist [48,50,54,68]. It is now possible to develop assays to screen compound libraries systematically using β -arrestin recruitment (e.g. confocal microscopy, fluorescence resonance energy transfer- or bioluminescence resonance energy transfer-based assays) or β -arrestin-mediated signaling (e.g. activation of a specific gene reporter) as read-outs for 7TMR activation. This new generation of high throughput screens will potentially lead to the identification of new pathway-selective drugs that might have valuable therapeutic properties.

abilities to activate G proteins, but only Epstein–Barr virus-induced receptor ligand chemokine leads to receptor phosphorylation, β -arrestin recruitment, and β -arrestin-dependent ERK activation [48]. Such a physiological example raises the possibility that naturally heterogeneous 7TMR ligands, such as glycoprotein hormones, might also be able to induce multiple receptor conformations and ultimately induce distinct biological outcomes [69].

In conclusion, besides their classical effects, GRKs and β -arrestins are emerging as *bona fide* signal transducers, able to provide diversity and fine-tuning possibilities to 7TMRs signaling. The existence of biased ligands opens promising avenues for the development of pathway-specific drugs with unique therapeutic values and/or limited side effects.

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