Transduction of Receptor Signals by β-Arrestins

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The transmission of extracellular signals to the interior of the cell is a function of plasma membrane receptors, of which the seven transmembrane receptor family is by far the largest and most versatile. Classically, these receptors stimulate heterotrimeric G proteins, which control rates of generation of diffusible second messengers and entry of ions at the plasma membrane. Recent evidence, however, indicates another previously unappreciated strategy used by the receptors to regulate intracellular signaling pathways. They direct the recruitment, activation, and scaffolding of cytoplasmic signaling complexes via two multifunctional adaptor and transducer molecules, β-arrestins 1 and 2. This mechanism regulates aspects of cell motility, chemotaxis, apoptosis, and likely other cellular functions through a rapidly expanding list of signaling pathways.

Transmission of extracellular signals across the plasma membrane by receptor-mediated signaling is one of the most fundamental cellular processes. However, only a small number of paradigmatic, generally operative mechanisms to accomplish this goal have been delineated. Examples include gating of ion channels, stimulation of heterotrimeric G proteins, and activation of receptor tyrosine kinases. However, recent findings have revealed another. A regulatory process, originally discovered as the means by which seven transmembrane receptor (7TMR) activation of G proteins is "desensitized" or turned off, quite surprisingly has been found to serve also as a parallel means of signal transduction. This newly appreciated signaling mechanism involves two families of proteins, the G protein–coupled receptor kinases (GRKs) and β-arrestins. The latter serve as multifunctional adaptor and scaffold proteins that recruit a broad spectrum of signaling molecules and assemblies to the receptors in a strictly activation-dependent fashion.

Historical Perspective and Classical Paradigms

In the mid-1980s, it was discovered that the prototypic adenylyl cyclase–coupled β_2-adrenergic receptor (β_2AR) for catecholamines and the visual sensing protein rhodopsin shared conserved structural and regulatory features, including sequence similarity, a seven transmembrane (TM) architecture, and a mechanism for "desensitization." This immediately suggested that all so-called G protein–coupled receptors (GPCRs) might be members of the same gene family and share these attributes—a hypothesis that was quickly confirmed [see (*1*) and references therein for a review of this historical material].

Today, we know that 7TMRs represent the largest (*2*), most versatile, and most ubiquitous of the several families of membrane receptors. Moreover, they are the most common target of therapeutic drugs (*3*). In response to a remarkable range of stimuli, including neurotransmitters, hormones, ions, and sensory stimuli, these receptors regulate the metabolism, secretory properties, electrical activity, shape, and motility of virtually all mammalian cells.

Studies of rhodopsin and the β_2AR also revealed that G protein–mediated signaling is attenuated or desensitized by a highly conserved process (*4*) that involves phosphorylation of the activated receptors by specific protein kinases, such as rhodopsin kinase (now known

Table 1. A list of β-arrestin–interacting proteins. ARF, ADP ribosylation factor; ARNO, ARF nucleotide exchange factor; IκBα, inhibitor of nuclear factor κB; PDE4D, phosphodiesterase 4D; PP2A, protein phosphatase 2A; Ral, members of the Ras superfamily of small guanosine triphosphatases (GTPases); Ral-GDS, Ral guanosine diphosphate (GDP) dissociation stimulator; RhoA, a small GTPase; small G/GEFs, small GTPase and guanine nucleotide exchange factors.

Binding protein	β-Arrestin isoform	Functional consequence	Ref.
		Trafficking proteins	
Clathrin	β-Arrestin 1, 2	Endocytosis	(*57*)
AP2	β-Arrestin 1, 2	Endocytosis	(*58*)
NSF	β-Arrestin 1	Endocytosis; recycling	(*59*)
		Small G/GEFs	
ARF6	β-Arrestin 2≫1	Endocytosis	(*60*)
ARNO	β-Arrestin 2	Endocytosis	(*60*)
Ral-GDS	β-Arrestin 1, 2	Ral-mediated cytoskeletal changes	(*61*)
RhoA	β-Arrestin 1	Angiotensin II-dependent stress fiber formation	(*62*)
		Signaling proteins	
MAPK cascade components			
ASK1	β-Arrestin 1, 2	JNK3 and p38 activation	(*24*)
c-Raf-1	β-Arrestin 1, 2	ERK activation	(*22, 23*)
JNK3	β-Arrestin 2≫>1	Stabilization of pJNK on endosomes	(*24*)
ERK2	β-Arrestin 1, 2	Stabilization of pERK on endosomes	(*22, 23, 26*)
Nonreceptor tyrosine kinases			
c-Src	β-Arrestin 1, 2	Endocytosis, ERK activation	(*15*)
Yes	β-Arrestin 1	Gαq activation and GLUT4 transport	(*18*)
Hck	β-Arrestin 1	Exocytosis of granules in neutrophils	(*17*)
Fgr	β-Arrestin 1	Exocytosis of granules in neutrophils	(*17*)
Others			
Mdm2	β-Arrestin 1, 2	Ubiquitination, endocytosis	(*10*)
IκBα	β-Arrestin 1, 2	Stabilization of IκBα upon β2AR and TNFR stimulation	(*63, 64*)
PDE4D family	β-Arrestin 1, 2	cAMP degradation	(*65*)
Dishevelled	β-Arrestin 1	Increase in TCF/LEF transcription	(*66*)
Dishevelled	β-Arrestin 2	Endocytosis of Frizzled4	(*42*)
PP2A	β-Arrestin 1	Ser^{412} dephosphorylation	(*49*)

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as GRK1) and the β-adrenergic receptor kinase or βARK (now known as GRK2), respectively. However, the receptor turn-off mechanism was found to require more than stimulus-dependent receptor or rhodopsin phosphorylation. In vitro, rhodopsin kinase–catalyzed phosphorylation of activated rhodopsin led to only partial quenching of rhodopsin signaling. A highly abundant and immunogenic retinal protein, now known as ''arrestin,'' was found to potentiate the signal-dampening effects of rhodopsin kinase phosphorylation on rhodopsin. Simultaneously, purification of βARK was found to lead to progressive loss of its ability to desensitize β_2ARs, in vitro. This suggested that some other factor necessary for the desensitization was being lost. Visual arrestin (also called arrestin 1), albeit at high concentrations, would restore this activity. Because expression of arrestin is limited to the retina, structural and functional homologs were hypothesized to exist in other tissues. Cloning of visual arrestin led to the identification of similar genes encoding β-arrestin 1 and β-arrestin 2 (also known as arrestin 2 and 3).

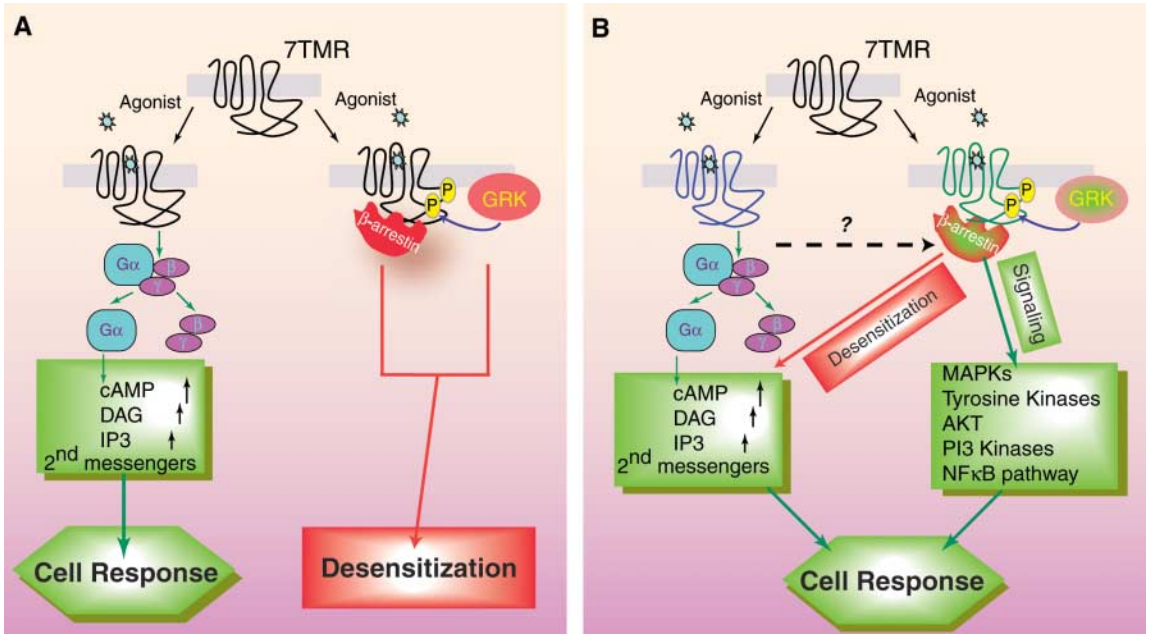


Fig. 1. Signal transduction by seven transmembrane receptors. (**A**) Classical paradigm. The active form of the receptor (R*) stimulates heterotrimeric G proteins and is rapidly phosphorylated by G protein–coupled receptor kinases (GRKs), which leads to β-arrestin recruitment. The receptor is thereby desensitized, and the signaling is stalled. (**B**) New paradigm. β-Arrestins not only mediate desensitization of G protein–signaling but also act as signal transducers themselves.

β-Arrestins 1 and 2 have marked specificity for binding phosphorylated β_2AR as opposed to phospho-rhodopsin, whereas the reverse is true of visual arrestin. Both agonist-induced conformational changes in the receptor and receptor phosphorylation contribute to driving receptor β-arrestin interaction, and β-arrestin competes with G_s for receptor interaction. This ''desensitization'' mechanism appears to be universal for 7TMRs (*4*), although some recent studies suggest that the visual arrestins do not strictly lead to signal termination but rather contribute to adaptation to varying light intensities (*5*).

The arrestins and GRKs are each members of small gene families (tables S1 and S2) (*6*, *7*). There are four arrestin genes and seven GRKs. Retinal rods and cones each have their own dedicated regulatory systems: Arrestin 1 and GRK 1 in the rods regulate rhodopsin; arrestin 4 (X arrestin) and GRK 7 in the cones regulate color opsins. β-Arrestins 1 and 2 and GRKs 2, 3, 5, and 6 are widely expressed and regulate most 7TMRs.

β-Arrestins Are Multifunctional Endocytic Adaptors and Signal Transducers

For signal transduction, 7TMRs propagate a chain of protein conformational changes in response to agonist stimulation. Thus, an essential characteristic of any general transducer of 7TMR signaling is its ability to interact universally with the receptors in an activation-dependent way and, thereby, to undergo conformational changes. Only three families of proteins have this attribute: heterotrimeric G proteins, β-arrestins, and GRKs. It is thus perhaps not surprising that recently β-arrestins have been found to mediate a variety of receptor signaling and regulatory processes and to bind to a growing list of endocytic and signaling proteins (Table 1 and table S3) (*8*).

Endocytosis. Early evidence that β-arrestins have larger roles in 7TMR biology than just desensitization came from the discovery that they also function as endocytic adaptors, linking receptors to the clathrin-coated pit machinery (*8*, *9*). Originally found to bind to clathrin itself, β-arrestins are now known to interact with other endocytic elements, including the adaptor protein AP2, the small guanosine triphosphatase ARF6 and its guanine nucleotide exchange factor ARNO, and the *N*-ethylmaleimide–sensitive fusion protein (NSF). β-Arrestins also bind and are ubiquitinated by the E3 ubiquitin ligase Mdm2 (*10*). This agonist-stimulated ubiquitination event is required for β-arrestin–mediated endocytosis, but precisely how or why is not known.

The avidity with which β-arrestins bind to activated phosphorylated receptors is largely determined by the pattern of GRK-mediated phosphorylation, generally on the C-terminal tails of the receptors (*11*). In the case of some receptors (''class A''), such as the β_2AR, β-arrestins bind relatively weakly, target the receptors to clathrin-coated pits, and then dissociate as the receptors internalize. For other receptors (''class B''), for example, the V_2R vasopressin receptor (V_2R) and the angiotensin II type 1a receptor (AT1aR), because of much tighter binding β-arrestin does not dissociate from the receptor and accompanies it into the cell, where the complex may reside for extended periods in endosomal vesicles before being sorted to lysosomes or slowly recycled. Patterns of 7TMR endocytosis parallel the kinetics of β-arrestin ubiquitination and de-ubiquitination: Transient β-arrestin ubiquitination correlates with class A and more persistent β-arrestin ubiquitination with class B behavior (fig. S1) (*12*). Endocytosis of the receptors plays roles in receptor resensitization by dephosphorylation, receptor recycling, receptor down-regulation, and receptor signaling (*9*).

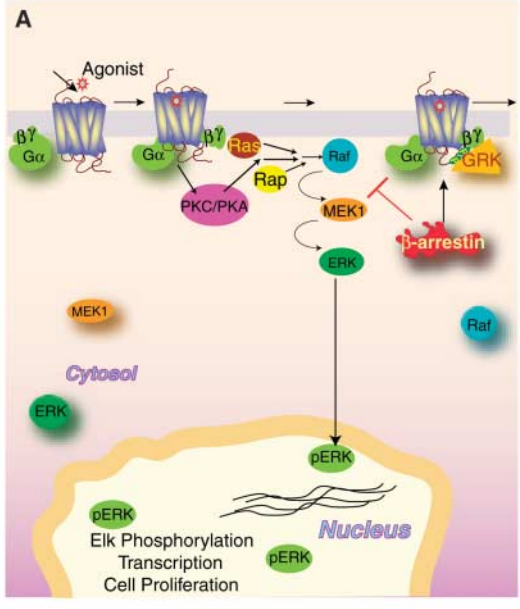
Signaling

The classical paradigms for signaling and desensitization of 7TMRs were developed within the context of providing a molecular

framework for understanding how receptor stimulation leads to regulation of the intracellular concentration of second messengers such as cyclic AMP (cAMP) (Fig. 1A). These events are essentially confined within the plasma membrane. However, the focus of attention has gradually shifted to a group of complex signaling pathways that ultimately link 7TM and other types of plasma membrane receptors to events in the cytoplasm and nucleus.

Initiation of such pathways generally requires that the stimulated receptors nucleate formation and activation of multicomponent signaling complexes and, in some cases, direct them to specific cellular destinations. Much evidence indicates that stimulus-dependent receptor recruitment of β-arrestins provides a general strategy used by 7TM, and perhaps other types of receptors, to accomplish these goals. β-Arrestins serve as adaptors, scaffolds, and/or signal transducers, and they connect the activated receptors with diverse signaling pathways within the cell (Fig. 1B, Table 1; table S3) (*8*, *13*).

Nonreceptor tyrosine kinases. Although 7TMRs and receptor tyrosine kinases were once viewed as completely distinct, nonoverlapping signaling mechanisms, it is now clear that some receptor tyrosine kinases activate G proteins, whereas some 7TMRs "transactivate" receptor tyrosine kinases, such as the epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) receptors (*14*). Other 7TMRs co-opt the activity of nonreceptor tyrosine kinases, and in this process, β-arrestins have positive roles in signal transduction. At the β_2AR (*15*) and the Neurokinin 1 (NK1) receptor for substance P (*16*), β-arrestins mediate agonist-dependent recruitment of c-Src to the receptor, facilitating activation of the mitogen-activated protein (MAP) kinases extracellular signal–regulated kinases (ERK1 and ERK2). Other members of the c-Src family of nonreceptor tyrosine kinases such as Hck (*17*), Fgr (*17*), and Yes (*18*) are recruited to various 7TMRs by β-arrestins. This recruitment regulates such disparate physiological processes as granule release from human neutrophils (CXCR1) (*17*), antiapoptotic effects in response to NK1 receptors (*16*), and GLUT4 (a glucose transmembrane transporter) translocation in response to endothelin A receptors (*18*).



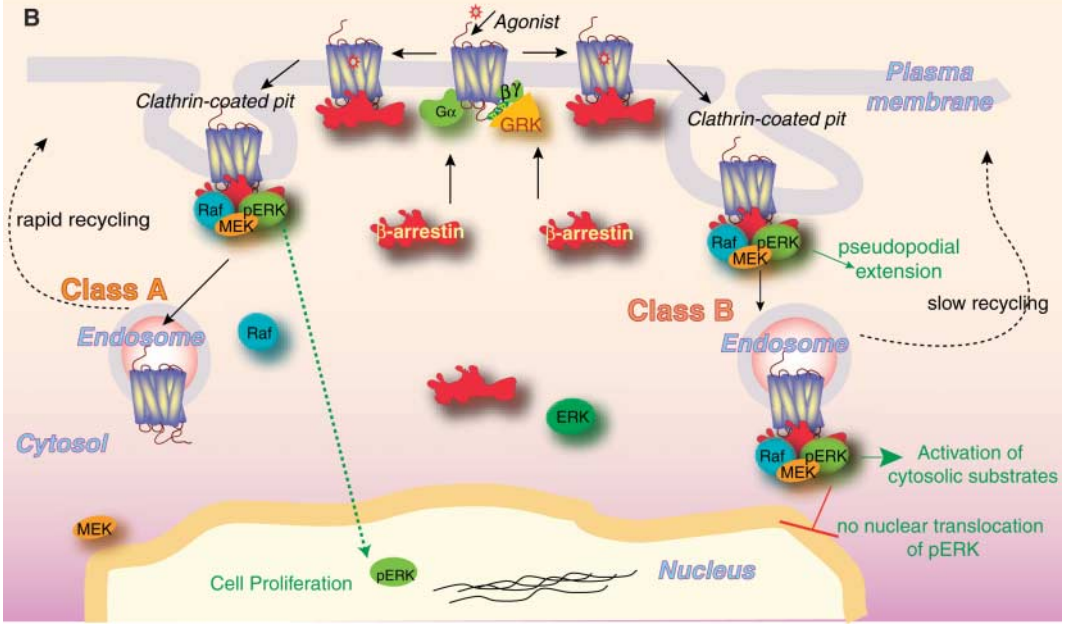


Fig. 2. 7TMR-stimulated pERK. (**A**) G protein–dependent ERK activation. Stimulation of G proteins activates the Raf family proteins through several convergent pathways. Raf translocates to plasma membrane on activation. (**B**) β-Arrestin–dependent ERK activation. β-Arrestin binding to phosphorylated receptors interdicts G protein–dependent signaling, while initiating new waves of signal transduction, for example, by activating ERK1/2.

The example of MAP kinases. A well-studied β-arrestin–dependent signaling system leads to activation of the MAP kinase ERK. MAP kinases are the terminal elements of highly conserved kinase cascades consisting of MAPKKKs (such as Raf), MAPKKs (such as MEK), and the MAP kinases themselves. There are three families of the multifunctional MAP kinases that include five ERKs, three c-Jun NH_2-terminal kinases (JNKs), and four p38s. Each kinase is activated by phosphorylation by the preceding kinase in the cascade. There are a dozen or more enzymes at each level (*19*).

The 7TMRs are connected to these MAPK signaling pathways by classical G protein–stimulated synthesis of second messengers (Fig. 2A) or by nonclassical pathways modulating novel effectors (*20*). MAP kinases activated in this way translocate from the cytosol to the nucleus, where they phosphorylate and activate transcription factors, which regulate programs of transcription that lead to proliferation, differentiation, and many other cellular processes (*19*). However, MAP kinases can also phosphorylate various cytosolic substrates, which leads to distinct but less well characterized consequences such as changes in cell shape and motility (*21*).

A conundrum has been how, in the face of the large number of enzymes in the different MAPK cascades—in which

a single upstream kinase can phosphorylate multiple downstream kinases—the cell is able to organize particular MAPK pathways with any specificity, reproducibility, or efficiency. The answer lies in ''scaffold'' proteins—molecules that tie together the appropriate kinases in a series (*19*). This accomplishes several goals: It forms a discrete signaling module, localizes the grouped components to specific areas in the cell, and insulates the active kinases from dephosphorylation by phosphatases. β-Arrestin 2 acts as a scaffold that binds all the component kinases of both the module that activates ERK1 and ERK2 (Raf, MEK, ERK) (*22*, *23*) and the module that activates JNK3 (ASK1, MKK4, JNK3) (*24*). Of the several mammalian MAP kinase scaffolds, β-arrestin 2 is the only one that is controlled by receptor stimulation.

β-Arrestin–mediated activation of ERK appears to be intimately linked to the function of β-arrestins in mediating endocytosis of receptors in clathrin-coated pits (Fig. 2B). The β-arrestin scaffolded signaling complex is internalized with class B receptors such as the AT1aR and ultimately is found in endocytic vesicles together with the receptors (*22*). Here, its activity persists for prolonged periods, perhaps because the phosphorylated ERK is protected from MAP kinase phosphatases (*21*, *25*). Class A receptors promote much less persistent β-arrestin–mediated activation of ERK (*26*). This apparently relates to the requirement for stable association of β-arrestins with the receptors for this activity to occur (Fig. 2B).

The AT1aR mediates disparate effects of angiotensin on vasoconstriction, smooth muscle cell motility and growth, and aldosterone secretion. Receptor stimulation activates ERK1 and ERK2 by either G protein (G_q)– or β-arrestin–mediated signaling pathways (*27*, *28*). Studies with mutant ligands and receptors that activate one pathway or the other, or with β-arrestin small interfering RNA (siRNA) or specific inhibitors of protein kinase C (PKC), have helped to delineate the characteristics of these two independent pathways (Fig. 3, A and B) (*25*). G protein–mediated activation is rapid and transient and is blocked by PKC inhibitors. It leads to nuclear translocation of the activated ERK, with consequent regulation of transcriptional programs and cellular proliferation. In contrast, β-arrestin–mediated activity is characterized by slower onset, greater persistence, retention of the activated ERK in cytosolic endocytic vesicles, and an absence or paucity of transcriptional regulation. Such distinct characteristics for the ERK activated by these different mechanisms strongly imply distinct physiological consequences. For the β-arrestin pathway, these are likely to include effects on cell motility, chemotaxis, and apoptosis (see below).

For the AT1aR, ERK activation transduced through β-arrestin is mediated almost exclusively by β-arrestin 2, with physiological amounts of β-arrestin 1 serving primarily as an inhibitor (*29*). For the protease-activated receptor 2 (PAR2) (*21*) and NK1 receptors (*16*), however, β-arrestin 1 appears to promote activation of ERK, whereas, for the CCR7 receptor, β-arrestin 2 is implicated (*30*). AT1aR-mediated activation of ERK features independent G protein– and β-arrestin 2–mediated pathways. However, in other cases, G proteins and β-arrestins may act in sequence or in a concerted fashion. For example, with several chemokine receptors, ERK activation and chemotaxis are sensitive to both pertussis toxin (implicating signaling through G protein G_i) and siRNA-mediated depletion of β-arrestin 2 (*30*–*32*).

An interesting implication of the existence of β-arrestin (versus G protein)–mediated signaling is the potential for ligands to selectively activate one or the other pathway. For example, a mutated angiotensin peptide (SII angiotensin) activates β-arrestin–mediated, but not G protein–mediated, signaling to ERK (*25*, *27*). A physiological example of such pathway-selective ligands is provided by the two endogenous ligands, Epstein-Barr virus–induced receptor ligand chemokine (ELC, CCL19) and secondary lymphoid tissue chemokine (SLC, CCL21), of the chemokine receptor CCR7, which regulates the homing and trafficking of T lymphocytes. The ligands have equivalent abilities to activate G proteins, but only ELC leads to receptor phosphorylation, β-arrestin recruitment, and β-arrestin–dependent ERK activation (*30*). The differences in the portfolios of physiological responses evoked by these two different ligands remain to be determined.

Chemotaxis and cell motility. Chemotaxis, the directed migration of cells along a gradient of chemoattractant, relies on signals from 7TMRs for chemokines. The signaling pathways used are heterogeneous and quite complex. Although G proteins (most often G_i) are involved, β-arrestins have recently emerged as important transducers of some of these signals, perhaps through their ability to mediate activation of MAP kinases. Lymphocytes from knockout mice lacking β-arrestin 2, but not from mice lacking β-arrestin 1, are impaired in chemotactic responses to stromal cell–derived factor–1 (SDF-1) mediated by the CXCR4

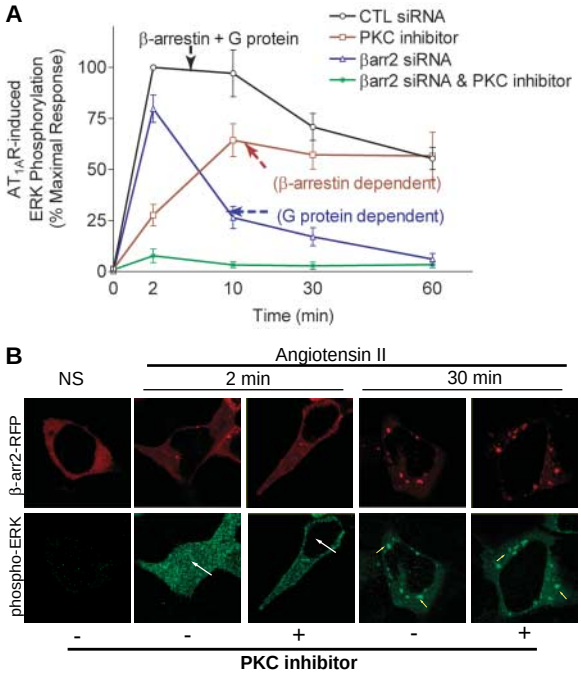


Fig. 3. 7TMR stimulation of ERK by distinct G protein– and β-arrestin–dependent pathways. (**A**) Temporal patterns. Angiotensin II stimulates ERK1 and ERK2 phosphorylation in HEK 293. The black activity curve (with circles) represents both Gαq/11- and β-arrestin–dependent pERK. After transfection of cells with β-arrestin2 siRNA, only a rapid and transient pool of angiotensin II–stimulated pERK is observed; as seen in the G-protein–dependent ERK activity curve (purple, with triangles). This Gαq/11–dependent pathway requires PKC activation, and hence, a PKC inhibitor such as Ro-31-8425 inhibits this mechanism of ERK activation. The red line (with squares) represents β-arrestin–mediated ERK signaling. The combination of β-arrestin 2 siRNA transfection and treatment with PKC inhibitor virtually eliminates angiotensin II–stimulated pERK (as seen in the green line with asterisk). Data taken from (*25*). (**B**) Subcellular distribution. Shown are confocal images of fixed HEK 293 cells expressing the AT1aR (not displayed) and β-arrestin 2-RFP (red). Cellular distribution of pERK was visualized by immunolabeling with a polyclonal antibody against phospho-ERK1/2 followed by a Bodipy fluorescein-conjugated secondary antibody (green). In nonstimulated cells, very little or no pERK is detected by immunostaining (green, bottom row, leftmost panel). The second column of confocal panels displays the pERK detectable in the cytoplasm and nucleus after 2 min of angiotensin II treatment in the absence of the PKC inhibitor (Ro-31-8425). The third column shows that in cells pretreated with the PKC inhibitor, there is marked inhibition of the G protein–stimulated 2-min pERK signal. pERK in the nucleus is completely abolished, whereas some pERK is still detectable in the cytoplasm and at the plasma membrane. The fourth and fifth columns show cells stimulated for 30 min with angiotensin II. At this time point, only β-arrestin–dependent pERK activity persists and is exclusively present on endocytic vesicles. This pERK signal is completely insensitive to the PKC inhibitor, which indicates that it is independent of the G protein pathway. [Data are condensed and reproduced from (*25*) with permission from the *Journal of Biological Chemistry*.]

receptor (*32*). The same is true of human embryonic kidney (HEK) 293 cells transfected with CXCR4 in which expression of β-arrestin 2 has been lowered by siRNA. In these cells, deficiency of β-arrestin 2 also impairs activation of p38 MAP kinase by SDF1, and inhibitors of this kinase, but not of ERK1 and ERK2, block chemotaxis. Thus, in the HEK 293 cells, β-arrestins may mediate the SDF-1 chemotactic signal by activation of p38 (*31*).

Chemotactic responses to PAR2, a 7TMR for trypsin and related proteases that is abundant in highly motile cells such as neutrophils, macrophages, and tumor cells, also require signaling through β-arrestins (*21*). In NIH 3T3 cells transfected with PAR2, the signaling involves ERK1 and ERK2 and β-arrestin–dependent reorganization of the actin cytoskeleton. Increased β-arrestin–mediated assembly of PAR2 and ERK at the leading edge of the cells in the extending pseudopodia leads to prolonged ERK activation (*21*). β-Arrestin–nucleated assembly of activated ERK in pseudopodia is also operative in the highly metastatic human MDA MB-231 breast cancer cell line. These cells, in contrast with the much less metastatic MDA MB-468 line, secrete a trypsin-like PAR2 ligand, which by an autocrine mechanism stimulates cell migration. This process requires both β-arrestin 1 and β-arrestin 2, which may have distinct roles in contributing to the migratory behavior of the metastatic cells by β-arrestin–dependent ERK activation or other mechanisms (*33*).

Inhibition of apoptosis. β-Arrestin contributes to antiapoptotic signaling. In the case of the NK1 receptor for substance P, this may be mediated by ERK activation (*16*). For the insulin-like growth factor 1 (IGF-1) receptor, it is mediated through a β-arrestin 1–dependent pathway leading to activation of phosphatidylinositol 3-kinase (PI3K) and AKT, which operates independently of the tyrosine kinase activity of the IGF-1 receptor (*34*). In mouse embryonic fibroblasts, stimulation of various 7TMRs causes apoptosis in the absence of, but not in the presence of, β-arrestins, which again suggests a role for the β-arrestins in antiapoptotic signaling (*35*).

Conformational changes in β-arrestins. The structure of β-arrestin 2, as modeled on the atomic structure of β-arrestin 1 in its basal state (*36*), reveals an elongated molecule ~70 Å. It has two distinct domains linked by a 12-residue "hinge" region. The domains are held intact by intramolecular interactions defined by buried polar residues, as well as the "three-element interface" that comprises the "buried" C tail, β strand I, and α helix I. β-Arrestins interact with many different protein partners (Fig. 4, A and B), yet they do not have any well-characterized domains specialized for protein-protein interactions. β-Arrestins act as phosphoprotein sensors, which generally display higher affinity for the phosphorylated forms of their binding partners.

Interaction of β-arrestins with activated receptors induces global conformational changes and rapid posttranslational modifications (dephosphorylation and ubiquitination) of the β-arrestin molecule (Fig. 4C) (*37*). Disruption of the polar core by phosphate moieties on receptors and the resulting rearrangement of the "three-element interface" is proposed to induce activating conformational changes in the β-arrestins. Such 7TMR activation of β-arrestin enhances its interaction with several of its binding partners. For example, in some cases, receptor stimulation instigates β-arrestin binding to signaling partners, such as c-Src and phosphorylated ERK1 or ERK2, or to endocytic partners such as clathrin or AP2 (*8*). This reflects the conformational changes in β-arrestin that occur on binding to the phosphorylated domains of activated 7TMRs (Fig. 4C). Moreover, it is possible that multiple conformational states of β-arrestins exist, induced by binding to receptors phosphorylated on different sites or different combinations of sites, which then lead to distinct functional outcomes. Conversely, β-arrestins stabilize conformational changes in the receptors. Thus, in analogy with the high-affinity ternary complex of agonist, receptor, and G protein (*38*), high-affinity ternary complexes of agonist, receptor, and β-arrestin are also formed (*39*).

Variations on a theme. In addition to GRK-dependent interaction of β-arrestins with conventional 7TMRs, arrestins also interact with other receptor types. In mammalian cells, GRK2 and β-arrestin 2 interact with the developmentally important 7TM molecule smooth-

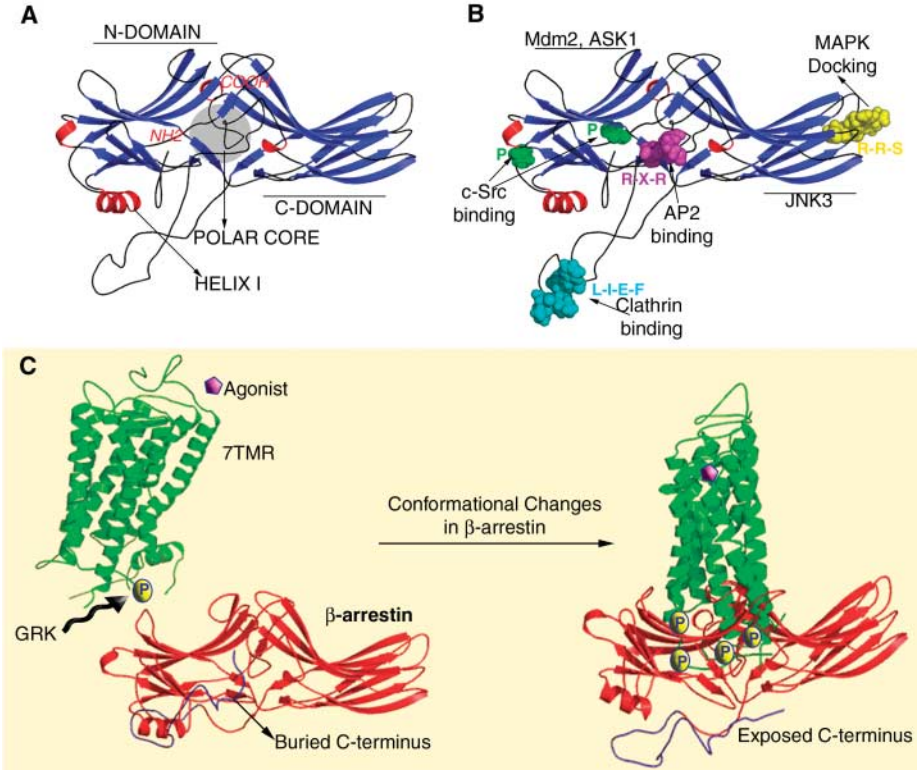


Fig. 4. β-Arrestin 2: Structural model and receptor-dependent conformational changes. (**A**) Basic structural attributes of β-arrestin. Portrayed is a structural model of rat β-arrestin 2 derived by homology modeling using the available structures of arrestin and β-arrestin 1 (PDB files: 1G4R, 1G4M, 1CF1, and 1JSY) and compiled with the program *PyMOL* (*56*). β Sheets are colored blue, helix I (indicated) is red, and the connecting loops and C-tail are black. N-domain and C-domain regions are connected by a hinge region. Both the N-terminal β strand I and the C-terminal β strand XX are juxtaposed to helix I. (**B**) Protein-protein interaction motifs mapped on β-arrestin 2. Prolines at positions 91 and 121 are important for β-arrestin 1–cSrc interaction and are depicted as green space filling spheres. The clathrin-binding domain L-I-E-F and the MAPK docking domain R-R-S are shown as cyan and yellow spheres, respectively. AP2 binding requires the arginine residues at positions 394 and 396, shown as magenta spheres. Mdm2 and ASK1 bind to regions delimited by residues, 160 to 300 and 1 to 185 (N-terminal half), respectively. JNK3 binds to the region 185 to 410 (C-terminal half). Both N- and C-domains of β-arrestin interact with 7TMRs. (**C**) 7TMR-induced conformational changes in β-arrestin. Structural models of 7TMR (rhodopsin: source, PDB file 1L9H) and rat β-arrestin 2 are shown in the basal state on the left side. The C-terminal tail of β-arrestin (blue) is buried and not accessible. Agonist stimulation leads to phosphorylation of serine and threonine residues on the receptor C tail. The charged domain thus created penetrates and disrupts the polar core of β-arrestin and leads to global conformational rearrangements, which cause the β-arrestin C tail to be released and exposed for protein interactions. The C terminus of β-arrestin contains the clathrin- and AP2-binding regions [see (B)].

ened (Smo) in an activity-dependent manner and regulate its endocytosis (*40*). In zebrafish embryos, knockdown of β-arrestin 2 by morpholino antisense leads to a lethal phenotype remarkably similar to that observed after genetic knockout of Smo or its downstream effector Gli (*41*).

Wnts are important morphogenetic and developmental ligands whose actions are mediated by the 7TM frizzled receptors. β-Arrestin 2 mediates endocytosis of the Wnt 5A-Fz-4 complex, but recruitment of β-arrestin is accomplished by an intermediary adaptor, disheveled, which interacts with β-arrestin 2 in a PKC-dependent fashion (*42*).

Even further afield are receptors that are not members of the 7TM superfamily. The tyrosine kinase IGF-1 receptor not only activates G proteins (*43*) but also recruits β-arrestin 1 after IGF-1 stimulation (*44*, *45*). This mediates activation of PI3K (*34*), AKT (*34*), and ERK1/2 (*44*). The transforming growth factor–β (TGF-β) family of ligands signal through heteromeric complexes formed by members of three single membrane–spanning receptor families (TβRI to III). β-Arrestin 2 mediates endocytosis of TβRIII, after it is phosphorylated on threonine (Thr^{841}) by TβRII, which is itself a serine kinase (*46*). This down-regulates antiproliferative signaling.

Future Directions

Research in cellular signal transduction is evolving from a focus on linear pathways to a broader view of signaling networks composed of interacting pathways (*47*). In the terminology of this field, β-arrestins serve both as nodes, which receive signals from multiple receptor inputs, and junctions, which route signals to various effectors. Signaling cross-talk can result from these functions. For example, stimulation of the β_2AR or the insulin receptor leads to activation of ERK, which phosphorylates β-arrestin 1 on Ser^{412} (*48*, *49*), a modification that impairs internalization of several 7TMRs and their consequent activation of ERKs (*49*, *50*).

For both the G_q-coupled AT1aR and the G_s-coupled V_2R, β-arrestin–mediated signaling to ERK requires prior receptor phosphorylation by GRKs 5 and 6 (*51*, *52*). Phosphorylation of the receptors by GRK2, while empowering receptor endocytosis or desensitization, actually antagonizes β-arrestin–mediated activation of ERK. It seems possible that the different GRKs phosphorylate distinct sets of sites on the carboxyl termini of the receptors and thus establish a ''bar code'' that somehow determines the conformation and, hence, the functional potential of the receptor-bound β-arrestin. It will be important to determine the applicability of these principles to other receptors and to other β-arrestin–mediated signaling phenomena.

A provocative issue is the potential role of β-arrestin ubiquitination in regulating its signaling functions. Might it serve as a recognition motif for assembling signaling complexes in analogy with its role in assembling elements of the protein-trafficking machinery? Several considerations are consistent with this hypothesis: the activation (receptor)–dependent nature of the modification (*10*), the correlation of the β-arrestin–mediated endocytic behavior of receptors (class A versus class B) with the stability of the ubiquitin modification (*12*), the correlation of the endocytic behavior of 7TMRs with the robustness of their β-arrestin–mediated activation of ERK (*26*), and the control of several signaling systems by ubiquitination (*53*).

The discovery of β-arrestin–mediated signaling highlights an emerging concept, that of ligand-directed signaling (*54*). In the simplest classical models, receptors exist in two states, active and inactive, with agonists stabilizing the active state, thereby driving activation of effectors such as G proteins. However, the conformation of a receptor that interacts with G protein can be distinct from that which interacts with β-arrestins. Thus, for both the β_2AR and V_2R, inverse agonists for G protein signaling (that is, compounds that actually lower basal adenylyl cyclase) are stimulatory agonists for β-arrestin–mediated signaling (*55*). The ability of ligands to differentially favor one or the other conformation suggests significantly greater diversity and fine-tuning of signaling possibilities for a single receptor than previously imagined. Moreover, such putative β-arrestin– or G protein–specific ligands might have valuable therapeutic properties and perhaps more restricted side effects.

References and Notes

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Supporting Online Material

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