

β -arrestin signaling and regulation of transcription

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Summary

β -arrestin 1 and β -arrestin 2 are well-known negative regulators of G-protein-coupled receptor (GPCR) signaling. Upon GPCR activation, β -arrestins translocate to the cell membrane and bind to the agonist-occupied receptors. This uncouples these receptors from G proteins and promotes their internalization, thus causing desensitization. However, accumulating evidence indicates that β -arrestins also function as scaffold proteins that interact with several cytoplasmic proteins and link GPCRs to intracellular signaling pathways such as MAPK cascades. Recent work has also revealed that, in response to activation of certain GPCRs, β -arrestins translocate

from the cytoplasm to the nucleus and associate with transcription cofactors such as p300 and cAMP-response element-binding protein (CREB) at the promoters of target genes to promote transcription. They also interact with regulators of transcription factors, such as I κ B α and MDM2, in the cytoplasm and regulate transcription indirectly. This β -arrestin-mediated regulation of transcription appears to play important roles in cell growth, apoptosis and modulation of immune functions.

Key words: β -arrestins, G-protein-coupled receptors, Transcription, Signal transduction

Introduction

G-protein-coupled receptors (GPCRs) constitute the largest known family of signaling molecules. Indeed, of the 13,779 proteins encoded in the human genome whose functions have been predicted, 616 (>4%) are members of the rhodopsin, secretin or metabotropic glutamate receptor class of GPCR (Venter et al., 2001). Several decades of extensive studies have revealed that these receptors play fundamental roles in regulation of a variety of cellular functions, including cell growth, differentiation and metabolism, by transducing a huge number of extracellular signals from hormones, neurotransmitters, chemokines and other environmental stimuli into the interior of cells (Wess, 1997; Ji et al., 1998).

The classical GPCR signaling pathways involve the activation of heterotrimeric G proteins, stimulation or inhibition of cAMP formation and phosphoinositide turnover as well as regulation of various signaling proteins, such as cAMP-dependent kinase (PKA) and MAPKs, and enzymatic effectors, and the modulation of transcription of target genes (Neves et al., 2002; Shaywitz and Greenberg, 1999). These signals are transmitted through the cytoplasm to the nucleus by a complicated network involving a series of protein-protein interactions and kinase cascades. An important feature is that the activation of the receptors also triggers negative feedback mechanisms that prevent potentially harmful effects resulting from persistent activation of the signaling pathways. Stimulation of GPCRs activates G-protein-coupled receptor kinases (GRKs), a family of receptor serine/threonine kinases, which phosphorylate the agonist-occupied GPCRs at the C-terminus and/or the third intracellular loop. In the case of β 2-adrenergic receptors, PKA also contributes to this agonist-stimulated receptor phosphorylation (Benovic et al., 1985).

Subsequent binding of regulatory proteins called arrestins to the phosphorylated receptor is an important facet of GPCR signaling (Reiter and Lefkowitz, 2006).

The arrestin family consists of four members. Visual arrestins (arrestin 1 and arrestin 4) are expressed almost exclusively in the retina (Kuhn, 1978). β -arrestin 1 and β -arrestin 2 (non-visual arrestins, also known as arrestin 2 and arrestin 3), however, are ubiquitously expressed in most tissues and play an important role in regulating signal transduction by numerous GPCRs (Attramadal et al., 1992; Reiter and Lefkowitz, 2006). Studies of GPCRs such as the β 2-adrenergic receptor revealed that receptor activation promotes the translocation of β -arrestins from the cytoplasm to the cell membrane and the interaction of β -arrestins with the activated receptor. This uncouples the receptor from its cognate G proteins and leads to its internalization, which attenuates signaling (Claing et al., 2002) (Fig. 1).

β -arrestins were initially known as negative regulators of GPCR-mediated signaling. However, new roles of β -arrestins in receptor trafficking and signaling have been discovered in recent years (Lefkowitz and Whalen, 2004; Lefkowitz and Shenoy, 2005). We now know that they also serve as scaffolds and adapters in receptor endocytosis and signal transduction. They recruit endocytic proteins and a variety of signaling molecules to the receptors, thus connecting GPCRs to various pathways, such as MAPK cascades (Lefkowitz and Shenoy, 2005). Moreover, recent studies have demonstrated that β -arrestins bind to I κ B α and MDM2 in the cytoplasm, regulating the NF- κ B and p53 transcription factors, respectively (Gao et al., 2004; Witherow et al., 2004; Shenoy et al., 2001; Wang et al., 2003a). They have further demonstrated that β -arrestin 1 can translocate to the nucleus and induce transcription of

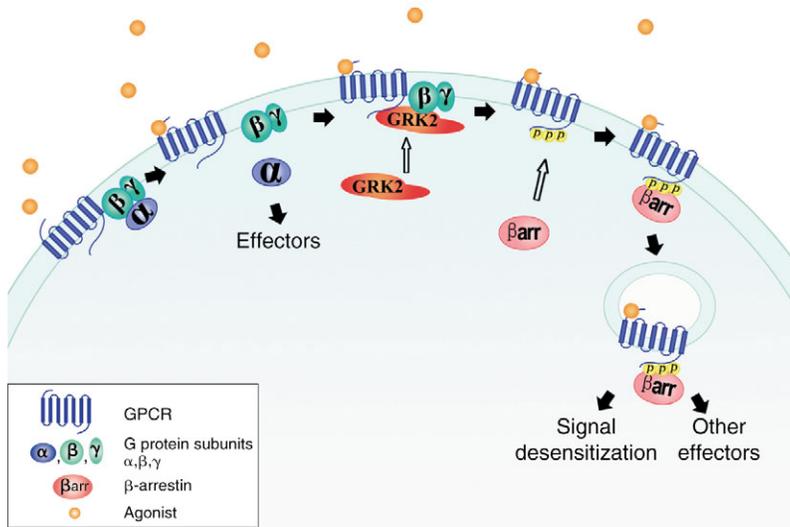


Fig. 1. Basic scheme for β -arrestin negative feedback and GPCR endocytosis. Agonist exposure stimulates activation of GPCR, which leads to the dissociation of G proteins into activated α subunit and $\beta\gamma$ dimers and triggers the activation of various effectors, such as adenylate cyclase and phospholipase C. The agonist-occupied GPCR is phosphorylated by GRKs, leading to signal desensitization, binding of β -arrestin to the activated, phosphorylated GPCR and subsequent endocytosis of the receptor.

various genes by recruiting cofactors such as p300 and increasing histone acetylation (Kang et al., 2005). Here, we review these recent developments in our understanding of the roles of β -arrestins in direct and indirect regulation of gene expression and their implications for GPCR-mediated signal transduction.

β -arrestins as signaling scaffold proteins

Studies over the past few years have shown that β -arrestins function as scaffold proteins and interact with a variety of different signaling molecules in the cytoplasm in an agonist-dependent manner (Fig. 2). During agonist-stimulated receptor endocytosis, β -arrestins function as endocytic adaptors, interacting with a considerable number of endocytic proteins, including trafficking regulators such as clathrin and the clathrin adaptor AP2, N-ethylmaleimide-sensitive fusion protein (NSF), and the GTPase ARF6 and ARNO (reviewed by Lefkowitz and Shenoy, 2005). Luttrell et al. first revealed that β -arrestins function as signaling adaptors, showing that they bind both the agonist-occupied β_2 -adrenergic receptor and Src, a tyrosine kinase involved in the Ras-dependent activation of MAPK pathways, and induce MAPK activation (Luttrell et al., 1999). β -arrestin binding and receptor endocytosis thus also represent critical events for the initiation of a second wave of signal transduction. McDonald and colleagues showed that the MAPK Jun N-terminal kinase 3 (JNK3) is another binding partner of β -arrestin 2. β -arrestins bind to JNK3 and recruit upstream kinases such as apoptosis signal-regulating kinase 1, MEK1 and MKK4 (McDonald et al., 2000). They can also act as scaffolds for Raf, MEK1 and ERK (DeFea et al., 2000; Luttrell et al., 2001). In this way, through the formation of scaffolding complexes with signaling molecules such as Src, Raf-1, Akt, ERK1/2, JNK3, MDM2 and I κ B, β -arrestins act as signal transducers to guide signals from the cell membrane to the MAPK, p53 and NF- κ B pathways (Lefkowitz and Shenoy, 2005).

Regulation of MAPK activation

The role of β -arrestins in the regulation of MAPK cascades is the most extensively studied signaling mechanism. GPCRs are able to activate ERKs through mechanisms involving the

classical G-protein-stimulated production of second-messenger-dependent kinases, such as PKC and PKA, or novel signaling proteins such as β -arrestins (Wei et al., 2003; Ahn et al., 2004; Shenoy et al., 2006; Gesty-Palmer et al., 2006). Activation of ERKs by β_2 -adrenergic receptors has two different components. G-protein-dependent ERK activation appears to be rapid and transient and can be blocked by pertussis toxin or PKA inhibitors. By contrast, β -arrestin-dependent ERK activation is slower in onset and more sustained in duration, and it is sensitive to depletion of β -arrestins but not Gi/PKA inhibition or loss of Gs (Ebisuya et al., 2005; Shenoy et al., 2006). β -arrestin-dependent, G-protein-independent ERK activation has also been demonstrated in the case of inverse agonists of parathyroid hormone receptors (Gesty-Palmer et al., 2006). In addition, β -arrestins can influence ERK activation induced by GPCR stimulation. This appears to occur as a consequence of stimulation of β_2 -adrenergic receptors, for example, where signaling can switch from Gs-coupled adenylyl cyclase activation to Gi-coupled ERK activation (Lefkowitz et al., 2002). β -arrestins recruit phosphodiesterase 4 to the β_2 -adrenergic receptor in response to agonists, which blocks the AKAP79/PKA-mediated switching of β_2 -adrenergic receptor signaling to Gi-coupled activation of ERK (Houslay and Baillie, 2005).

Regulation of subcellular compartmentalization of MAPKs

ERKs are primarily cytoplasmic in most quiescent cells, but they can shuttle between the cytoplasm and the nucleus. Activation of ERKs dissociates them from their cytoplasmic anchoring proteins, such as MEK, and stimulates their translocation from the cytoplasm into the nucleus, where they phosphorylate substrates including many transcription factors. The nuclear translocation of ERKs is essential for proliferation of fibroblasts and differentiation of PC12 cells, and thus the subcellular compartmentalization of the activated ERKs is an important factor controlling cell-fate decisions (Ebisuya et al., 2005). Activation of ERKs does not always induce their nuclear translocation and transcriptional responses. G-protein-dependent ERK activation does stimulate the translocation of

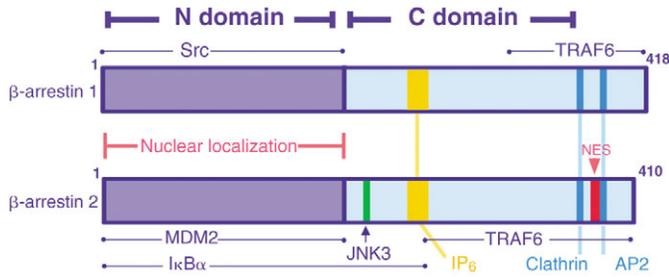


Fig. 2. Domain structure of β -arrestins, showing binding sites and nuclear localization and nuclear export (NES) signals. β -arrestins contain two major domains: an N domain and a C domain. The N domain (1-185) is indispensable for the nuclear localization of both β -arrestins, and a functional NES (VxxxFxxLxL) is located at the C-terminus of β -arrestin 2. The LIEF-binding motif for clathrin and the RxR-binding motif for AP2 within the C-terminus of β -arrestins allow them to initiate clathrin-dependent endocytosis. The IP₆-binding site resides in the C domain (233-251). The binding regions for Src (1-185), MDM2 (1-186), JNK3 (RRS sequence), I κ B α (1-240) and TRAF6 are shown.

activated ERKs from the cytoplasm to the nucleus, but interaction with β -arrestins often restricts activated ERKs to the cytoplasm (Lefkowitz and Shenoy, 2005). The ERK1/2 activated by the β -arrestin scaffold is largely excluded from the nucleus and confined to cytoplasmic locations, such as endocytic vesicles (DeFea et al., 2000; Luttrell et al., 2001; Tohgo et al., 2002; Tohgo et al., 2003; Ebisuya et al., 2005; Jafri et al., 2006). There are many cytosolic ERK substrates, such as p90RSK and cytoskeletal proteins, and several transcription factors translocate to the nucleus following phosphorylation and activation by ERK in the cytoplasm (Ebisuya et al., 2005). Thus, retention of ERKs in the cytoplasm by the β -arrestin scaffold not only attenuates the effect of ERKs on the phosphorylation of substrates in the nucleus, but also actively affects the cellular response through phosphorylation and activation of transcription factors in the cytoplasm and their translocation to the nucleus.

Piu and colleagues have demonstrated that β -arrestin 2 enhances the transcriptional activity of the nuclear receptor RAR- β 2 and this effect is dependent upon the recruitment of ERK2 and its direct interaction with RAR- β 2 in the cytoplasm (Piu et al., 2006). β -arrestin-2-dependent p38 activation provides another example. This is crucially involved in chemokine-receptor-induced chemotaxis (Sun et al., 2002). Accumulating evidence suggests that β -arrestin-mediated activation of MAPKs may induce a distinct profile of gene transcription and thus have different cellular functions to those associated with the classical transcriptional regulation by MAPKs.

Interaction with MDM2 and regulation of p53-mediated transcription

Another way in which β -arrestin regulates transcription is through its binding to the oncoprotein MDM2, an E3 ubiquitin ligase and negative regulator of the tumor suppressor p53 (Shenoy et al., 2001; Wang et al., 2003a). Agonist stimulation of β 2-adrenergic receptors induces ubiquitylation of β -arrestins and the receptors (Shenoy et al., 2001). MDM2 binds

to β -arrestins and catalyzes their ubiquitylation, which leads to receptor internalization (Shenoy et al., 2001). In resting cells, MDM2 is distributed primarily in the nucleus, where it regulates the transcriptional activity of p53 by ubiquitylating it and targeting it for degradation, thereby suppressing p53-mediated apoptosis. Activation of opioid receptors or bradykinin receptors stimulates the formation of a cytoplasmic complex containing MDM2, β -arrestin 2 and the receptor, which retains MDM2 in the cytoplasm. This reduces the concentration of MDM2 in the nucleus, thus decreasing degradation of p53 and increasing p53-dependent transcription and apoptosis. Further studies have revealed that MDM2 binds to the N-domain within β -arrestins (Wang et al., 2003b) (Fig. 2).

Interaction with I κ B α and regulation of NF- κ B-mediated transcription

NF- κ B is a ubiquitously expressed and highly regulated dimeric transcription factor that regulates genes involved in immunity, stress and apoptosis. In the inactive state, NF- κ B dimers are bound to the inhibitor protein I κ B α and thus retained in the cytoplasm. Extracellular signals stimulate phosphorylation of I κ B α , which leads to its degradation and translocation of NF- κ B to the nucleus, where it binds to promoters of target genes and activates transcription. β -arrestins bind directly to I κ B α in yeast two-hybrid assays (Witherow et al., 2004; Gao et al., 2004). Moreover, stimulation of β 2-adrenergic receptors in HEK 293, HeLa and COS-7 cells significantly increases the amount of β -arrestin 2 that is associated with I κ B α , which stabilizes I κ B α (Gao et al., 2004). The interaction with β -arrestin 2 prevents phosphorylation and degradation of I κ B α and thus attenuates activation of NF- κ B and transcription of NF- κ B-target genes (Gao et al., 2004; Witherow et al., 2004) (Fig. 2).

I κ B kinase α , I κ B kinase β and I κ B α -inducing kinase, upstream kinases known to regulate the function of I κ B α , all coimmunoprecipitate with β -arrestin 1 and β -arrestin 2 in HeLa cell lysate (Witherow et al., 2004). β -arrestins thus not only bind to and stabilize I κ B α but also interact with the I κ B kinase complex that regulates its activation and thereby negatively regulate the NF- κ B pathway. More recent work demonstrates that β -arrestin 2 can function as a suppressor of ultraviolet-induced NF- κ B activation through direct interaction with I κ B α (Luan et al., 2005). Moreover, stimulation of β 2-adrenergic receptors promotes dephosphorylation of β -arrestin 2 and its suppression of NF- κ B. These studies suggest that β -arrestins mediate cross-talk between GPCR and NF- κ B signaling pathways, which may play an important role in the regulation of immune functions.

Interaction with TRAF6 and regulation of NF- κ B- and AP-1-mediated transcription

β -arrestins can also affect NF- κ B by modulating signaling by the Toll-like-interleukin-1 receptor (TLR-IL1R). TLR-IL1Rs signal through the adaptor tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6). Upon activation of TLR-IL1R, β -arrestins interact with TRAF6 (Fig. 2). The formation of the β -arrestin-TRAF6 complex prevents autoubiquitylation of TRAF6. This blocks the activation of NF- κ B and the AP-1 transcription factor that normally occurs and thus negatively regulates cytokine production (Wang et al.,

2006). Indeed, β -arrestin-2-deficient mice exhibit higher levels of expression of proinflammatory cytokines and are more susceptible to endotoxic shock. β -arrestins may regulate IL1R signaling through interaction with TRAF6 and/or $\text{I}\kappa\text{B}\alpha$, since IL1R signaling also converges at $\text{I}\kappa\text{B}\alpha$ -NF- κB . However, stimulation of β 2-adrenergic receptors has no effect on the interaction of β -arrestin and TRAF6 (Wang et al., 2006), which suggests that the interaction of β -arrestin and TRAF6 is regulated by IL1R independently of GPCR stimulation. β -arrestin 1 and β -arrestin 2 mutants that can bind to TRAF6 but not $\text{I}\kappa\text{B}\alpha$ inhibit the IL1R signaling as well as their wild-type counterparts do. This suggests that the interaction with TRAF6 is sufficient for β -arrestin-mediated regulation of IL1R signaling.

Thus, β -arrestins appear to interact with a variety of proteins in the cytoplasm to regulate the activity of transcription factors, and this underpins important physiological functions, such as immune responses. Notably, regulation of p53- and NF- κB -mediated transcription by β -arrestins is indirect, involving interactions with a variety of molecules, such as MDM2, $\text{I}\kappa\text{B}\alpha$ and TRAF6, and occurs in response to activation of GPCRs as well as other types of receptors (e.g. IL1R) (Fig. 3).

β -arrestins as nuclear messengers

Signaling by GPCRs can be transmitted to the nucleus through activation of Ca^{2+} -dependent signaling molecules and transcription factors and protein kinase cascades that target transcription factors. For example, GPCRs activate the cAMP-PKA-cAMP-response element-binding protein (CREB) pathway, which produces nuclear responses distinctly different from those initiated by JAKs/STATs, Smads, etc activated by RTK signals. In addition, GPCRs can affect gene expression through the β -arrestin-dependent signaling mechanisms described above. Recent work, however, indicates that β -arrestins can affect gene expression more directly - by entering the nucleus (Kang et al., 2005; Neuhaus et al., 2006). In human embryonic kidney 293, HeLa and osteosarcomal cells, β -arrestin 1 appears to be present in both the nucleus and the

cytoplasm whereas β -arrestin 2 is cytoplasmic at steady state (Oakley et al., 2000; Scott et al., 2002; Wang et al., 2003b). Both β -arrestins shuttle between the cytoplasm and the nucleus in a leptomycin-B-sensitive manner, however (Scott et al., 2002; Wang et al., 2003b). The N-terminal domain is necessary for the nuclear localization of both β -arrestin 1 and β -arrestin 2 (Wang et al., 2003b) (Fig. 2). β -arrestin 2 also contains a classical leucine-rich nuclear export signal (NES) sequence at its C-terminus, which is responsible for its cytoplasmic localization in unstimulated cells (Scott et al., 2002; Wang et al., 2003b) (Fig. 2).

Co-immunoprecipitation of epitope-tagged β -arrestins and resonance energy transfer techniques indicates that β -arrestins form both homo- and hetero-oligomers in living cells (Storez et al., 2005; Milano et al., 2006). β -arrestin 1 oligomers are primarily cytoplasmic whereas β -arrestin 1 monomers display increased nuclear localization (Milano et al., 2006). Coexpression of β -arrestin 2 with β -arrestin 1 prevents accumulation of β -arrestin 1 in the nucleus (Storez et al., 2005). The subcellular distribution of β -arrestin 1 and β -arrestin 2 might thus be affected by the oligomeric state of β -arrestins. Moreover, β -arrestin 1 can interact with inositol hexakisphosphate (IP_6 ; Fig. 2) and mutation of the IP_6 -binding site in β -arrestin 1 disrupts oligomerization while maintaining its interactions with known binding partners, including clathrin, AP-2 and ERK2 (Milano et al., 2006). This suggests that β -arrestin- IP_6 binding regulates the interaction of β -arrestin with the plasma membrane and nuclear signaling proteins by promoting its oligomerization.

Further studies have demonstrated that the nucleocytoplasmic shuttling of β -arrestins and visual arrestin controls the subcellular distribution of MDM2 and JNK3. JNK3 and MDM2 predominantly localize in the nucleus, whereas visual arrestin and β -arrestin 2 localize primarily in the cytoplasm. Coexpression of β -arrestin 2 moves both JNK and MDM2 to the cytoplasm. A point mutation in the NES of β -arrestin 2 (L394Q) not only redistributes β -arrestin 2 from the cytoplasm to the nucleus but also results in the nuclear

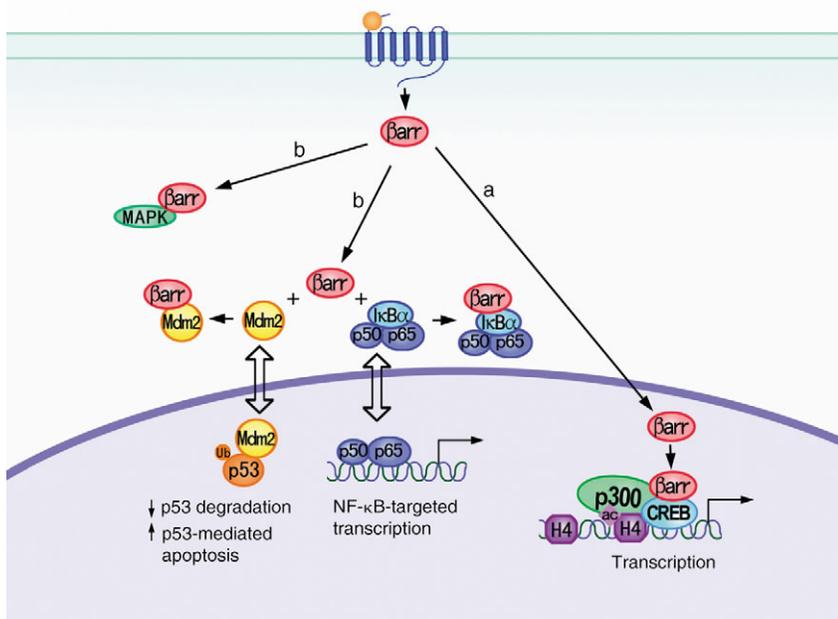


Fig. 3. A model for β -arrestin-mediated regulation of transcription. (a) Direct pathway. In response to receptor activation, β -arrestins translocate from the cytoplasm to the nucleus and associate with transcription cofactors such as p300 and CREB at the promoters of target genes to promote transcription directly. (b) Indirect pathway. β -arrestins interact with regulators of transcription factors such as $\text{I}\kappa\text{B}\alpha$ and MDM2 in the cytoplasm, which results in changes in activity and the subcellular distribution of these binding partners, and thus exert regulatory effects on the activation of transcription factors indirectly. Ub, ubiquitylation.

relocalization of JNK3 and MDM2 (Scott et al., 2002; Wang et al., 2003b; Song et al., 2006) (Fig. 3). Interactions between arrestins and signaling molecules such as JNK3 and MDM2 that regulate subcellular localization of these molecules may thus play an important role in the regulation of cell survival and death.

Recent work has further illustrated the biological significance of the nuclear distribution of β-arrestins. Activation of the δ-opioid receptor, a GPCR, induces translocation of β-arrestin 1 to the nucleus and increases transcription of the *p27* and *FOS* genes in HeLa and SK-N-SH cells to regulate the cell cycle (Kang et al., 2005) (Fig. 3). *p27* encodes the cyclin-dependent kinase inhibitor p27. *FOS* is a transcription factor. Both proteins are involved in the regulation of cell proliferation. β-arrestin 1 accumulates at the *p27* and *FOS* promoters, where it recruits the histone acetyltransferase p300 and thus enhances local histone H4 acetylation and transcription of the corresponding genes. Interaction of β-arrestin 1 and CREB may also be involved in signal-dependent accumulation of β-arrestin 1 at these loci and the subsequent stimulation of transcription of *p27* and *FOS*. Activation of δ-opioid receptors leads to the β-arrestin-1-dependent increase in *p27* transcription and inhibition of growth of human neuroblastoma cells, which underlines the physiological significance of this β-arrestin-mediated epigenetic regulatory pathway.

This type of effect is not restricted to β-arrestin 1 and opioid receptors. Neuhaus and colleagues recently observed that β-arrestin 2 translocates to the nucleus in response to the activation of hOR 17-4, a G-protein-coupled odorant receptor expressed in spermatozoa (Neuhaus et al., 2006). They showed that the nuclear translocation of β-arrestin 2 is dependent on receptor phosphorylation and clathrin-mediated receptor endocytosis. Their data indicate that odorant-receptor-induced nuclear localization of β-arrestin 2 might play a role in the regulation of gene expression during the early processes of fertilization (Neuhaus et al., 2006).

Both β-arrestin 1 and β-arrestin 2 thus seem to serve as nuclear messengers for GPCRs. They probably provide scaffolds, similar to those seen in endocytic pathways, which in this case regulate the local concentration and localization of specific transcription factors at the promoters of target genes (Fig. 3). Further investigations into the mechanism of β-arrestin-mediated gene regulation could reveal novel modes of transcriptional regulation and signaling pathway used by many GPCRs to transduce extracellular signals to the nucleus.

Conclusion and perspectives

The fact that β-arrestins function not only as signal terminators but also as scaffold proteins for GPCR-mediated signal transduction is increasingly recognized. The studies discussed here have revealed further novel functions for these molecules in the regulation of gene expression. β-arrestins appear to be able to modulate gene expression in at least two ways: (1) by translocating from the cytoplasm to the nucleus to form scaffolds for transcription factors at the promoters of target genes and thereby regulate transcription directly, and (2) by binding to regulators of transcription factors in the cytoplasm to alter their activity and subcellular distribution and thus regulate transcription indirectly. β-arrestins thus participate in

the generation of more sustained responses to extracellular stimuli.

There are many interesting questions that remain to be answered, however. Future work should be directed at the identification of more binding partners of β-arrestins in the nucleus and additional genes regulated by β-arrestins. This will further delineate the molecular mechanisms involved as well as the biological significance of β-arrestin-mediated nuclear signal transduction. Other questions concern how many receptors use β-arrestins as nuclear messengers and how β-arrestins translocate to the nucleus in response to receptor activation. Furthermore, since chromatin is a direct target of GPCR- and β-arrestin-mediated signal transduction, it would be interesting to investigate the potential role of β-arrestin-dependent chromatin remodeling in the physiological output of GPCRs.

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